Persistent organic pollutants in tissues of the white-blooded Antarctic fish *Champsocephalus gunnari* and *Chaenocephalus aceratus*

Anneli Strobel[1], Peter Schmid[2], Helmut Segner[3], Patricia Burkhardt-Holm[1,4], Markus Zennegg[2]

Affiliation:

[1] University of Basel, Departement of Environmental Sciences, Programme Man-Society-Environment MGU, Vesalgasse 1, CH-4051 Basel, Switzerland

E-Mail Anneli Strobel: anneli.strobel@unibas.ch

[2] Empa, Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, CH-8600 Dübendorf, Switzerland

E-Mail Markus Zennegg: markus.zenneg@empa.ch

[3] University of Bern, Vetsuisse Faculty, Centre for Fish and Wildlife Health, Länggassstrasse 12, CH-3012 Bern, Switzerland

E-Mail Helmut Segner: helmut.segner@vetsuisse.unibe.ch

[4]Department of Biological Sciences, University of Alberta, Edmonton, AB Canada

E-mail Patricia Burkhardt-Holm: patricia.holm@unibas.ch

Corresponding author:
anneli.strobel@unibas.ch

Abstract

The global occurrence of persistent organic pollutants (POPs) continuously contributes to their accumulation also in remote areas such as the Antarctic Ocean. Antarctic fish, which hold high trophic positions but appear to possess low endogenous elimination rates for chemicals, are expected to bioaccumulate POPs with rising anthropogenic pollution. Using a chemical-analytical method, we measured concentrations of PCBs, PBDEs, HCBs, HCH and DDTs and determined toxic equivalents (TEQs) and bioanalytical equivalents (BEQs) in muscle and ovaries of Antarctic icefish caught in the Southern Ocean around Elephant Island. We used two species with different feeding habits and trophic web positions: the planktivorous *Champsocephalus gunnari* and the piscivorous *Chaenocephalus aceratus*. Our results revealed higher contaminant levels in ovary than in muscle tissues of both species. Most analytes concentrations and the TEQs (0.2-0.5) and BEQs (0.2) were lower as in temperate species. Comparison with literature data points to higher PCB (20-22 ng g^{-1} lipid weight (lw)) and DDT (7-19.5 ng g^{-1} lw) concentrations than those measured in icefish in the 90's. For the other contaminants, we could not identify temporal trends. We found a higher bioaccumulation of contaminants, particularly HCB and DDTs, in *C. aceratus* (6.2 & 19.5 ng g^{-1} lw, respectively) than in *C. gunnari* (3.8 & 7.0 ng g^{-1} lw, respectively). However, there was no general species-specific accumulation pattern of the different toxicant classes between the two icefish. Thus, the expected link between contaminant burdens of *C. aceratus* and *C. gunnari* and their ecological traits was only weakly supported for these species.

Highlights

- PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- POP levels are similar in fish from different sampling sites around the Antarctic Peninsula
- Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs) in icefish

Keywords

Icefish, bioaccumulation, persistent organic pollutants, polychlorinated biphenyls (PCBs), toxic equivalents (TEQs), DR CALUX bioanalytical equivalents (BEQs)

1. Introduction

Antarctica has been less affected by human influences than other continents for a long time, however, contamination with anthropogenic contaminants, in particularly persistent organic pollutants (POPs) has increased progressively (Nash, 2011; UNEP/AMAP, 2011). Nowadays, Antarctica serves as a major sink for highly persistent contaminants. Long-range atmospheric transport, together with global distillation processes and cold condensation, are considered to be the main mechanisms for the progressive contamination of the Antarctic ecosystem, together with local sources such as fishing, tourism and research activities (Simonich and Hites; Feely et al., 2008; Nash, 2011). Particularly POPs such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and amongst them also the formerly used insecticides such as γ-hexachlorcyclohexane (γ-HCH) and *p,p'*-DDT are ubiquitous pollutants with a wide application spectrum. For example, polymer additives such as flame retardants are found globally in building material, furniture, paint, textiles or plastics, which are also used in Antarctic bases and vessels cruising in these regions (Hale et al., 2008; Kohler et al., 2008). This worldwide abundance and the high persistence of POPs continuously contribute to an accumulation in the ice masses and biota of polar regions (Wania and Mackay, 1993; Chiuchiolo et al., 2004; Goerke et al., 2004; Corsolini et al., 2007; Bargagli, 2008; Borghesi et al., 2008; Borghesi et al., 2009; Xie et al., 2011; Wolschke et al., 2015).

Furthermore, those lipophilic organic chemicals have a high potential to bioaccumulate in aquatic biota, and particularly in Antarctic species, which generally possess low endogenous elimination rates for those chemicals (Strobel et al., 2015). Additionally, it is expected that climate warming will lead to the release of those pollutants trapped in glaciers and sea-ice, which additionally contributes to increasing POP levels in the tissues of Antarctic animals (Weber and Goerke, 2003; Bogdal et al., 2010; Schmid et al., 2010; van den Brink et al., 2011; Cabrerizo et al., 2013; Goutte et al., 2013). As global chemical usage continues to grow, including the usage of persistent and bioaccumulative compounds, it is to be expected that contaminant intake into Antarctica will further increase in the future.

The endemic, Antarctic notothenioid fish are evolutionary well-adapted to the cold and stable environment of the Southern Ocean. Adaptations involve e.g. high amounts of tissue lipids, slow growth rates, long life spans and slow metabolism and elimination rates for xenobiotics (Mintenbeck et al., 2012; Strobel et al., 2015). All these factors may favor the bioaccumulation of lipophilic contaminants in the tissues of those fish.

Lipophilic, non-charged contaminants like PCBs can be taken up via a physico-chemically driven, passive partitioning of the chemicals from the water phase into the lipid phase of the organism and thereby accumulate differentially in tissues or fish species, depending on their lipophilicity of the chemical and the lipid content or composition of the tissues (Nichols et al., 2013). In fact, tissue-specific differences of POP levels are reported for notothenioid species (Lana et al., 2014). Furthermore, bioaccumulation and biomagnification can be related to an organism's habitat and its trophic position within the food web (Corsolini et al., 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Particularly for highly lipophilic compounds, oral uptake via the prey contributes by a major extent to the bioaccumulation of toxicants in the tissues of a species. For example, Weber and Goerke (2003) found higher contaminant levels in the piscivorous icefish *Chaenocephalus aceratus* than in the planktivorous icefish *Champsocephalus gunnari*, which was apparently related to the different food spectra of the two notothenioids (Goerke et al., 2004). Such studies highlight that POPs are transferred within the Antarctic food web, leading to increasing POP concentrations along the food chain up to high concentrations in top level predators (Wolschke et al., 2015).

Considering the trends of rising POP concentrations in Antarctica (UNEP/AMAP, 2011 and their high potential to exert toxic effects in marine biota (Nash, 2011), it is very important to keep monitoring body burdens of the fish living in the Southern Ocean. Yet, we are far from having a solid understanding of the contamination status, time trends, the diversity of contaminants or their toxicity potential in Antarctic biota (UNEP, 2002; UNEP/AMAP, 2011). Particularly the bioaccumulation pattern of contaminants such as the polybrominated flame retardants (PBDEs) or insecticides (e.g. DDT) have hardly been measured in icefish.

The aim of this study was to determine levels of selected POPs in muscle and ovary tissue of two white-blooded Antarctic notothenioid species in the study area around Elephant Island,

the South Shetland Islands and the Antarctic Peninsula, and to estimate temporal trends of fish POP levels in this area. The analytically measured contaminant concentrations were converted into toxic equivalents (TEQs) using standardized values. In order to examine whether food web position or tissue lipid content has an influence on chemical concentrations, we analyzed lipid content and POP levels in fish species of the same family but with different feeding habits and trophic web positions , namely the planktivorous *C. gunnari* and the piscivorous *C. aceratus*.

Extending the classical contaminants, this study includes polybrominated flame retardants (PBDEs) and insecticides (i.e. DDT, HCB). In addition to chemical analytics, we also applied bioanalytics using the DR-CALUX assay (Murk et al., 1996; Kuiper et al., 2006) to assess the total accumulated dioxin-like activity in the fish tissues.

2. Methods

2.1 Study species

Two species of the *Channichthyidae* (white-blooded icefish) were fished with bottom trawls down to 500 m, during a cruise with the research vessel 'Polarstern' (ANTXXVIII/4, March 13 to April 9, 2012; http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart) at different, closely located sampling sites around Elephant Island and the South Shetland Islands (61.1°S, 55.1°W). Only fish netted alive and without macroscopically visible damage were used for tissue sampling. The planktivorous mackerel icefish, *C. gunnari*, shows a mainly bentho-pelagic feeding mode, while the piscivorous Scotia Sea icefish *C. aceratus* is predominantly a benthos feeder (Weber and Goerke, 2003). Persistent organic pollutants were analyzed in muscle and ovary tissue of mature (stage III-IV), female fish only. Sex and maturity stage of the fish were verified histologically. Animal weight and length are given in Table 1. All tissue samples were wrapped in aluminum foil and stored immediately at -20 °C until used for analysis.

2.2 Sample preparation

Muscle and gonad samples were defrosted, cut into pieces and lyophilized at 33 Pa for 72 hours until constant weight. Dried tissue of muscle (5-10 g) or gonads (0.5-2 g) was ground

159 with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pistil to obtain a fine
160 powder.

161 This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi,
162 Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and
163 100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml *n*-
164 hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a
165 Syncore evaporator (Büchi, Switzerland) und let dry completely by applying a gentle
166 nitrogen stream. The residue accounted for the fat content of the sample. $^{13}C_{12}$ labeled
167 internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2
168 – 3 mL of *n*-hexane the solution was treated with 3 ml oleum (7% SO_3 in conc. sulfuric acid).
169 After centrifugation for 3 min at 5'000 rpm, the solvent layer with the lipophilic target
170 analytes was removed and the remaining suspension was re-extracted two times more with *n*-
171 hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and
172 300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from
173 top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid
174 and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and
175 eluted with 5 ml *n*-hexane, followed by 5 mL *n*-hexane/dichloromethane 1:1 (v:v). The eluate
176 was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the
177 volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at
178 room temperature. Finally the recovery standard $^{13}C_{12}$ labeled PCB 70 was added. Samples
179 were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical
180 procedure were determined in duplicates (Table 1 and A.2).
181
182 2.3 Chemical analysis
183

184 PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118,
185 123, 126 156, 157, 167, 169, and 189), DDT (*o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE),
186 hexachlorobenzene (HCB), γ-hexachlorocyclohexane (γ-HCH), and PBDEs (BDE 28, 47, 99,
187 100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the
188 target analytes in the extracts was achieved by gas chromatography/high resolution mass
189 spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution
190 mass spectrometer (Thermo Finnigan MAT, Bremen Germany) coupled to a Finnigan Trace

GC Ultra equipped with a Triplus auto sampler (Thermo Electron Corporation, Waltham, MA, USA). Samples were injected in splitless mode (splitless time 30 s) at an injector temperature of 260 °C. For the gas chromatographic separation a RTX5 Sil-MS column (30 m × 0.25 mm, film thickness 0.10 µm) was used with helium as carrier gas at a pressure of 100 kPa. The following temperature programs were used for the different compound classes. For the PCBs, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 20 °C/ min to 180 °C, followed by 3 °C/ min to 250 °C, and 20 °C/ min to 300 °C. For pesticides, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 10 °C/ min to 160 °C, followed by 4 °C/ min to 240 °C, and 20 °C/ min to 300 °C. For the PBDEs, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 20 °C/ min to 220 °C, followed by 6 °C/ min to 300 °C, and 10 °C/ min to 320 °C. The ion source was operated at 220 °C, the electron energy was 70eV, and the mass spectrometer was tuned to a mass resolution of 8000-10000. The two most abundant signals of the molecular ion cluster of the analytes and the $^{13}C_{12}$ labeled internal standards were recorded in the single ion monitoring mode.

The analytes were identified by comparing the retention times with those of the labeled internal standards. Quantification was based on peak areas of the analytes and the labeled reference compounds with known concentrations. More details about the method are available in the literature (Zennegg et al., 2003; Schmid et al., 2007).
For the calculation of TEQs, the dioxin-like PCBs were determined in the same way, but as part of the sample extract was used later on for the analysis by the bioassay (DR-CALUX), no $^{13}C_{12}$-labeled internal dl-PCBs standards could be used for the quantification, as the isotope labeled analogues exhibit similar activity in the DR-CALUX. Therefore, quantitative determination of dl-PCBs was based on the $^{13}C_{12}$ labeled indicator PCBs used as internal standards and previously determined response factors to native dl-PCBs.
The blank values (in ng g^{-1} lipid weight) were all below the compound concentrations and are given in Table 1 and A.1.
The limit of detection (LOD) and the limit of quantification (LOQ) were set by definition at signal to noise ratios of greater than three (s/n ≥3) and ten (s/n ≥10) respectively. All glass ware used were cleaned with strongly alkaline detergents and backed out overnight in a ceramic oven at 450 °C. Directly before use, the glass ware was rinsed with solvents (*n*-hexane, dichloromethane).

2.4 Determination of dioxin-like toxic equivalents (TEQs)

In order to assess total dioxin-like activity in the fish tissues, we calculated the 2,3,7,8-tetrachlorodibezo-*p*-dioxin (TCDD) equivalent (TEQ) concentrations of dl-PCBs in the muscle and gonad extracts of both icefish species using Toxic Equivalency Factors (TEFs) proposed by the World Health Organization (WHO) for fish (Van den Berg et al., 1998). TEQs were calculated as the sum of the TEFs of all dl-PCBs listed in Table A.1. Although the TEF values are not derived from studies with Antarctic fish, since TEFs are only available for salmonids (Van den Berg et al., 1998) they provide a useful tool for a reasonable estimate of toxicity effects of PCBs on Antarctic fish.

2.5 Determination of bioequivalent values (BEQs)

The same extracts that were used for chemical analysis were also used in a standard bioassay, the DR-CALUX (Dioxin Responsive Chemically Activated Luciferase Gene Expression) assay. This cell and receptor based reporter gene bioassay measures the binding of dioxin-like HAHs, e.g. dioxin-like PCBs (dl-PCBs), to the Aryl hydrocarbon receptor (AhR) via activation of a reporter gene. The assay was performed by BioDetection Systems b.v. (Amsterdam, The Netherlands).

Bioassay-derived TEQ values, or bioequivalent values (BEQs), were compared with TEQ values calculated from the chemical analytical data. This comparison revealed if there was a significant higher activity than predicted from the chemical analysis. Such a combined approach has been repeatedly used in studies on chemical contamination of the Arctic region (Letcher et al., 2010), but it has not been used in studies with Antarctic fish so far.

2.5 Calculations and statistics

All analyses include the lipid content of the respective tissue. POP concentrations were normalized against both lipid weight (lw, ng g^{-1}) and fresh weight (fw, ng g^{-1}) of the tissue and provided as mean values ± standard error of the mean (sem). Toxicant concentrations were compared among icefish species and tissues using ANOVA with Tukey post-hoc test.

Influence of sampling site, fish weight and length, maturity stage and tissue lipid content was tested with ANOVA. Data were considered to be statistically significant at $p < 0.05$. Normal distribution of data was tested with Kolmogorov-Smirnov and equality of variances with Bartlett's test. All statistical tests were performed with STATISTICA 12, StatSoft, Inc., and GraphPad Prism 5, GraphPad Software, Inc.

3. Results

3.1 Interspecies comparison of contaminant patterns and levels

In this study, we measured lipid content, PCB, DDT, HCB, γ-HCH, and PBDEconcentrations in both muscle and ovary tissue of female, mature *C. gunnari* and *C. aceratus* from spatially closely located sampling sites around Elephant Island and the South Shetland Islands. We express the compound concentrations on lipid weight and the fresh weight basis.

Sampling site, maturity stage and tissue lipid content of the fish showed no correlation to the accumulation of any of the compounds analysed in this study.

All samples contained detectable levels of the target compounds, which are presented in Table 1. Analytes concentrations were about 15 - 110 times higher when calculated per lipid weight than based on tissue fresh weight.

Overall, the PCBs were the predominant group among all compounds analysed in our study (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 20.0 ± 4.3, *C. gunnari* ovaries 47.8 ± 10.2, *C. aceratus* muscle 21.9 ± 4.9, *C. aceratus* ovaries 31.9 ± 7.3), followed by the DDTs (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 7.0 ± 1.0, *C. gunnari* ovaries 6.9 ± 1.1, *C. aceratus* muscle 19.4 ± 8.1, *C. aceratus* ovaries 17.8 ± 3.9), HCB (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 3.8 ± 0.5, *C. gunnari* ovaries 2.8 ± 0.4, *C. aceratus* muscle 6.2 ± 0.9, *C. aceratus* ovaries 7.3 ± 2.0), the PBDEs (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 4.1 ± 0.5, *C. gunnari* ovaries 0.7 ± 0.1, *C. aceratus* muscle 5.1 ± 0.8, *C. aceratus* ovaries 10.0 ± 1.9) and γ-HCH (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 0.6 ± 0.2, *C. gunnari* ovaries 1.3 ± 0.3, *C. aceratus* muscle 0.6 ± 0.2, *C. aceratus* ovaries 1.2 ± 0.5) (Figure 1).

Within the PCBs, only PCB 28 and 101 were significantly different between the ovaries of *C. gunnari* and *C. aceratus* (Table 1). When we calculated the percentage contribution of the individual PCB congeners to ∑PCBs (lw) of both muscle and ovary tissue, there was no

290 difference in PCB congener composition between the two icefish species (two-way
291 ANOVA).
292 Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs
293 in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%)
294 (Figure 2).
295
296 In the ovaries, levels of *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT (per lw) were significantly
297 higher in *C. aceratus* compared to those of *C. gunnari*. Also *p,p'*-DDT concentrations in the
298 muscle of *C. aceratus* were higher than those in muscle of *C. gunnari* (per lw). ∑DDT (per
299 lw) was significantly higher in muscle and ovaries of *C. aceratus* than in *C. gunnari* (Figure
300 1). Among the DDTs, *p,p'*-DDE showed the highest concentration of up to 57% in muscle of
301 *C. aceratus*. *p,p'*-DDE tended to be higher in muscle tissue, while *p,p'*-DDT was slightly
302 higher in ovary tissue in both species. *o,p'*-DDT was slightly higher in *C. gunnari* with
303 ~22%, while it was only ~16% in *C. aceratus*. Detailed information on the contribution of all
304 individual compounds measured in this study is given in Table A.2 in the appendix.
305 γ-HCH was only different between the muscle tissues of the two icefish (on fw basis).
306 HCB concentrations per lipid weight in both muscle and ovaries were higher in *C. aceratus*
307 than in *C. gunnari* (Figure 1). Except HCB, none of the analysed compounds exhibited a
308 correlation with fish weight or length. HCB showed a significant linear dependence of fish
309 weight and length in *C. aceratus*, except in the ovaries (R^2=0.723).
310
311 In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries),
312 followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197
313 were significantly different between the two tissues types and fish species. Also BDE 99
314 showed different concentrations between the ovaries of *C. aceratus* and *C. gunnari* on the
315 lipid basis (Table 1, Table A.2, Figure 2).
316
317 3.2 Comparison of contaminant patterns and levels in different tissue
318
319 Lipid content was significantly higher in the ovaries of *C. gunnari* than in the ovaries of *C.*
320 *aceratus*. *C. gunnari* exhibited significantly higher lipid content in ovaries than in their
321 muscle tissue, while this was not the case for *C. aceratus* (Table 1).
322 A comparison between the compound concentrations in muscle and ovary tissue for each
323 icefish species revealed higher HCB concentrations in ovary than in muscle tissue of *C.*

gunnari. Concentrations of γ-HCH and DDTs in muscle and ovary tissue (per fresh weight) were significantly different in both species (Table 1).

In *C. aceratus*, all PCB concentrations and most of the PBDEs were different from each other in both muscle and ovary tissue. In case of dl-PCB congeners, the ∑dl-PCBs were higher in muscle of *C. gunnari* than in muscle *C. aceratus* on both lipid and fresh weight basis (Table A.1).

In *C. gunnari*, all PBDEs apart from congener 183 did differ between muscle and ovary tissue. In contrast, only PCB 28 and 180 were different between the tissues of *C. gunnari*, all other PCB congeners showed no significant tissue differences (Table 1, Figure 1).

3.3 Toxic equivalents (TEQs*)*

The toxic equivalent levels of dioxin-like PCBs (WHO_{PCB}-TEQ g^{-1} fw) were higher in the muscle of *C. gunnari* (0.5 ± 0.1) than in *C. aceratus* (0.2 ± 0.1), while *C. aceratus* exhibited higher TEQs (0.5 ± 0.9) in the ovaries than *C. gunnari* (0.1 ± 0.0) (Table 1).

3.4 Bioanalytical equivalents (BEQs)

The bioanalytical equivalents (BEQs) determined by DR-CALUX in the muscle of both species were in a similar range (*C. aceratus*: 0.15 ± 0.07, *C. gunnari*: 0.2 ± 0.14 BEQ g^{-1} fw) to the WHO_{PCB}-TEQs g^{-1} lw, which were calculated using the concentrations of dioxin-like PCBs. In *C. gunnari*, however, the BEQs measured in the ovaries were about 70-fold higher (BEQ: 5.14 ± 3.6, TEQ: 0.07 ± 0.04) than the calculated WHO_{PCB}-TEQ g^{-1} fw (Table 1).

4. Discussion

4.1 Species-specific contaminant patterns and levels

4.1.1. Species-specific patterns

Literature data on present POP concentrations in tissues of Antarctic fish are scarce, and particularly those on contaminant levels in white-blooded icefish.

Earlier studies of the 80's and 90's measured PCB concentrations in the icefish *C. gunnari* and *C. aceratus* around the Antarctic Peninsula (Corsolini et al. 2002a, 2002b, 2009), which

were two to four times lower (0.7 ng g^{-1} fw in muscle) than the ones of the present study. HCB levels of our icefish were within a similar range to those measured previously. It is known for sub-Antarctic fish that PCB levels can follow a seasonal trend, which is related to the release of pollutants from melting snow and ice during summer and the atmospheric transport of loads of pollutants which precipitate in Antarctic regions and are released during warming (Jaffal et al., 2011). Since sampling of our icefish took place in Austral autumn, after the seasonal ice melting in Antarctica, a seasonal release of POPs trapped in sea ice could thereby be one contributor to the comparably high levels of PCBs in our icefish samples. In fact, the PCB concentrations (per fw) of our icefish were within a similar range to those of salmon from the Baltic Sea (Isosaari et al., 2006).

In the early 00's, Borghesi et al. (2008; 2009) sampled *C. hamatus* and *C. gunnari* in the Ross Sea at 74° South. They found ∑PCB concentrations in *C. hamatus* muscle of 0.35 ng g^{-1} fw, ∑non-ortho PCBs of 5 ng g^{-1} fw and ∑PBDEs 0.16 ng g^{-1} fw. In muscle of *C. gunnari*, they report ∑PBDEs of 0.44 ng g^{-1} fw. Those values are about five times higher than the ∑PBDEs in muscle of *C. gunnari* measured in our study. Since they used fish of a similar size class, this difference is likely related to the measurement of different PBDE congeners in the studies.

Another recent study measured contaminant concentrations (in ng g^{-1} lw) in muscle, liver and gonads of three red-blooded Antarctic species, *Notothenia coriiceps, N. rossii* and *Trematomus newnesi*, from Potter Cove, Antarctic Peninsula (Lana et al., 2014). They reported similar ∑DDT values in muscle, but also species differences in the ovaries of their fish: while *N. coriiceps* and *T. newnesi* had similar values to *C. aceratus* measured in our study, *N. rossii* had much higher values than our fish. They also measured γ-HCH values about six-times higher than in our fish. *N. rossii* displays a rather benthic lifestyle, but also feeds on pelagic species, and thus has a prey spectrum similar to *C. gunnari*. Nevertheless, the DDTs and γ-HCH were much higher in *N. rossii* than in *C. gunnari.* In contrast, ∑PCB and ∑BDEs were highly variable among the species and tissues measured by Lana et al. (2014), but were generally within the same order of magnitude compared to our data. Thus, such strong differences in DDT and γ-HCH accumulation patterns between Antarctic fish species could also be related to a selective metabolism for individual contaminant classes between the species (Storelli et al., 2009), and not only to their ecological traits.

Amongst the PBDEs, BDE 47 showed the highest concentration (60% of all congeners) in our icefish amongst all PBDE congeners, followed by BDE 100 and BDE 99. This is in line

392 with the general picture of those congeners being the dominating PBDEs in fish around the
393 globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota
394 (Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).
395 In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight
396 basis were particularly low in icefish. For example, PBDE concentrations range from about
397 1.0 to 8 ng g^{-1} fresh weight, or up to 64 ng g^{-1} lipid weight in various fish species from the
398 Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.
399 Although the production of the former widely used brominated flame retardants penta- and
400 octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in
401 2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still
402 produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and
403 Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).
404 HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per
405 fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere
406 (Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70's, it
407 has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of
408 DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation
409 potential and cold condensation processes, DDTs and its metabolites are nowadays found in
410 biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).
411
412 The TEQs we calculated for *C. aceratus* and *C. gunnari* were in a similar range than TEQs
413 reported for muscle of the icefish *C. hamatus* (TEQ 0.01-0.1 pg g^{-1} wet weight) or red-
414 blooded Antarctic fish (TEQ ~0.1 pg g^{-1} wet weight) (Focardi et al.; Corsolini et al., 2002a;
415 Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other
416 organisms living in less remote parts of the world, e.g. WHO_{PCB}-TEQ (pg g^{-1} fw) of 3 to 15
417 in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit
418 muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).
419 In *C. aceratus*, we found that the BEQs were comparable to the TEQs calculated on the basis
420 on the WHO_{PCB}-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to
421 fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty
422 times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of *C. gunnari*
423 were much higher than the WHO_{PCB}-TEQs calculated for their ovaries. The toxicity effects
424 might thus be actually much higher than expected by the single usage of the calculated
425 WHO_{PCB}-TEQ. In fact, the BEQs in the ovaries of *C. gunnari* were within the same range as

BEQs of fish from temperate latitudes. Also Corsolini et al. (Corsolini et al., 2002a) stated that the TEQ values they measured in Antarctic fish were already half as high as those values which are considered to elicit toxicological effects, such as reproductive and immunological disorders, in marine mammals or birds (Kannan et al., 2000).
Rising PCB concentrations as observed by us in tissues of Antarctic fish will thus increasingly have the potential to exert their toxic effects on those fish.

4.1.2. Temporal trends in contaminant levels

Long-term observations on contaminant levels in Antarctic biota are scarce. Yet, Weber and Goerke (2003; Goerke et al., 2004) measured contaminant levels in the liver of *C. aceratus* and *C. gunnari* in the same sampling area as in the present study. From 1987 to 1996, the authors measured an increase of PCB 153 and PCB 180 levels in *C. aceratus*, but not in *C. gunnari.* In the present study, we found about three times higher concentrations of those PCB congeners in the tissues of our two icefish species than in the previous study from 1996 (Weber and Goerke, 2003). In contrast, the HCB concentrations show a declining trend from the 1987 study to our current survey. A similar trend of stable or declining HCB levels by up to 2.5% per year has also been observed in Arctic biota, such as birds, fish or marine mammals, since the late 80's (Barber et al., 2005; Rigét et al., 2010).

In liver of *C. aceratus*, concentrations of *p,p'*-DDE had already increased from 1987 to 1996, and the values we measured in muscle and ovaries of *C. aceratus* were almost twice as high as in 1996 (Weber and Goerke, 2003). In contrast, *p,p'*-DDE concentrations in *C. gunnari* remained at similar levels from 1987 over 1996 (study by Weber and Goerke (2003)) to the present study.
Nevertheless, overall DDT concentrations were increasing from 1987 to 1996 in both icefish species, and our values are also slightly higher than those in 1996, suggesting an increasing trend of DDTs in icefish around the Antarctic Peninsula. Despite a general global reduction of DTT as an insecticide, DDT is still produced at high volumes (see above, (UNEP, 2008)). Furthermore, climate warming on the one hand leads to an increased volatility and worldwide distribution of DDTs, and on the other hand local sources such as melting glaciers may additionally contribute to increasing DDT concentrations in tissues of Antarctic fish around the Antarctic Peninsula (van den Brink et al., 2009; van den Brink et al., 2011).

4.2 Correlation of contaminant concentrations with ecological traits

4.2.1 Tissue-specific patterns

Since all POPs analyzed in this study are highly lipophilic substances, tissue differences in the contaminant levels in *C. aceratus* and *C. gunnari* on fresh weight basis at first instance should correlate to tissue lipid concentrations (Corsolini et al., 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Indeed, *C. gunnari,* which possesses a clearly higher lipid content in the ovaries than *C. aceratus*, accordingly showed significantly higher PCB concentrations in its ovaries based on fresh weight compared to *C. gunnari*. Also HCB was two times more concentrated in the fat-rich ovaries of *C. aceratus* than in *C. gunnari*, but only on the lipid basis. Accordingly, the BEQs were higher in the ovaries of *C. aceratus* and *C. gunnari* than in their muscle tissue. Also Lana et al. (2014), investigating POP accumulation patterns in notothenioid species, reported that the highest levels were found in the gonads of the fish.

4.2.2 Ecological-related patterns

In addition to body lipid contents, our results point to an influence of habitat and trophic level on POP levels in white-blooded icefish. The benthic-living *C. aceratus* had two-times higher concentrations of almost all DDT congeners and HCB than the bentho-pelagic *C. gunnari.* Also the previous study by Weber and Goerke (2003) report higher (lipid-based) contaminant burdens in *C. aceratus* than in *C. gunnari* and highlight the higher tendency of *C. aceratus* to accumulate DDTs in its tissues than *C. gunnari* over the time.

Since POPs accumulate in sediments, benthic fish species are generally thought have a higher exposure and uptake of lipophilic contaminants (Goerke et al., 2004; Borghesi et al., 2008). In addition to uptake from water or sediment, also the feeding habit thus plays a role, with species at higher tropic levels tending to show higher contaminant accumulation due to biomagnification. A recent study by Wolschke et al. (2015) also highlights the biomagnification of POPs from lower to higher trophic levels in the Antarctic food chain, which can be attributed to the diets of the animals.

From these observations, the benthic, piscivorous *C. aceratus* was expected to have higher contaminant burdens than the bentho-pelagic, planktivorous *C. gunnari*. However, only HCB

and DDTs, but none of the other congener classes, were higher in the predominantly benthic *C. aceratus* than in the bentho-pelagic *C. gunnari* on the lipid weight basis.

Conclusion

Overall, PCB and DDT concentrations tend to rather increase than decrease in tissues of the two white-blooded icefish species *C. aceratus* and *C. gunnari* around Elephant Island and the South Shetland Islands, when compared to earlier studies. Our results thereby support the global transportation of POPs to the Southern Ocean and their bioaccumulation in the local marine fish, and point to a trend of increasing concentrations of POPs in Antarctic icefish. Our data also suggest that worldwide climate change effects may contribute to an increased volatilization and release of POPs trapped in glaciers, sea- or pack-ice, thereby leading to an ongoing contamination of the Southern Ocean and its biota.

Furthermore, we found differences in POP accumulation patterns between the two icefish species, which were weakly correlated to their trophic position. The piscivorous *C. aceratus* showed a higher potential to accumulate contaminants in its tissue than the planktivorous *C. gunnari*. This species difference highlights the influence of intake of POPs via the specific prey of individual fish species. However, the expected link between the contaminant burdens of *C. aceratus* and *C. gunnari* and their ecological traits could not be fully supported. Additional factors, such species differences in toxicant metabolism rates and selective metabolism for single contaminant classes, may also play an important role in defining chemical bioaccumulation patterns in Antarctic fish species in the long term. In the end, Antarctic fish are a central link between the benthic community and top level predators, concomitantly POPs bioaccumulated in their tissues are likely to contribute to a progressive biomagnification of POPs along Antarctic food webs.

Acknowledgements

This work was funded by the Swiss National Science Foundation (SNSF 31003A_149964/1) and the Freiwillige Akademische Gesellschaft Basel. We thank Donatella Perrone and Melanie Senn (Empa) for their helpful assistance in the laboratory.

527 **Tables & Figures**

528

529 **Tables**

530

531 **Table 1**: Lipid content (%) and levels of organic contaminants (ng g^{-1} lipid weight; ng g^{-1} fresh weight) (mean ± sem) in tissues of two Antarctic
532 icefish species.

533

	C. aceratus (*n*=10)								*C. gunnari* (*n*=11)								
Length (cm)	47-66								33-50								
Weight (g)	636-3620								252-888								
	lipid weight (ng g^{-1} lw)				fresh weight (ng g-^{1} fw)				lipid weight (ng g^{-1} lw)				fresh weight (ng g^{-1} fw)				Blank
Tissue	muscle		ovaries		muscle		ovaries		muscle		ovaries		muscle		ovaries		
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	
TEQs					**0.16**	**0.1**	**0.53**	**0.94**					**0.47**	**0.14***	**0.07**	**0.04***	
BEQs					**0.15**	**0.07**	**0.57**	**0.75**					**0.20**	**0.14***	**5.14**	**3.60***	

HCB	6.22	0.85[#]	7.37	1.98[#]	0.09	0.02	0.16	0.04	3.82	0.55[#]	2.84	0.37[#]	0.07	0.01[*]	0.18	0.04[*]	0.06
γ-HCH	0.58	0.21[*]	1.18	0.49[*]	0.01	<0.01[#,*]	0.03	0.01[*]	0.59	0.20	1.25	0.31	0.01	<0.01[#,*]	0.07	0.02[*]	0.07
p,p′-DDE	13.11	5.99	8.76	2.46[#]	0.14	0.04[#,*]	0.19	0.03[*]	3.19	0.58	2.36	0.38[#]	0.06	0.01[#,*]	0.16	0.04[*]	0.52
o,p′-DDT	3.20	0.98	2.83	0.65[#]	0.04	0.01[*]	0.07	0.01[*]	1.65	0.36	1.64	0.38[#]	0.03	<0.01[*]	0.09	0.02[*]	0.07
p,p′-DDT	5.31	1.61[#]	6.21	1.10[#]	0.06	0.02[*]	0.15	0.02[*]	2.19	0.42[#]	2.98	0.52[#]	0.04	0.01[*]	0.19	0.04[*]	0.32
Σ DDTs	**19.45**	**8.14** [#]	**17.80**	**3.91**[#]	**0.22**	**0.07**	**0.40**	**0.05**	**7.04**	**1.00**[#]	**6.98**	**1.14**[#]	**0.12**	**0.02**[*]	**0.44**	**0.10**[*]	
28	0.95	0.06[*]	1.50	0.26[*]	0.01	<0.01[*]	0.04	0.01[#,*]	1.23	0.11[*]	2.84	0.53[*]	0.04	0.01[*]	0.18	0.05[#,*]	0.12
52	2.15	0.79[*]	3.29	0.98[*]	0.02	0.01[*]	0.09	0.03[*]	1.56	0.38	7.72	2.31	0.36	0.34	0.37	0.09	0.25
101	5.12	1.48[*]	7.10	1.69[*]	0.06	0.01[*]	0.19	0.05[#,*]	3.99	0.97	10.74	2.72	0.68	0.63	0.60	0.12[#]	0.26
138	5.74	1.21[*]	8.20	1.83[*]	0.07	0.01[*]	0.22	0.05[*]	5.47	1.23	11.06	2.69	0.60	0.52	0.68	0.18	0.40
153	6.00	1.26[*]	8.72	2.10[*]	0.07	0.01[*]	0.23	0.06[*]	5.94	1.40	11.96	2.52	0.41	0.33	0.76	0.20	0.49
180	1.94	0.31[*]	3.06	0.67[*]	0.02	<0.01[*]	0.08	0.02[*]	1.86	0.35[*]	3.51	0.50[*]	0.07	0.04	0.25	0.08	0.17
Σ PCBs	**21.91**	**4.98**[*]	**31.87**	**7.26**[*]	**0.26**	**0.03**[*]	**0.85**	**0.21**[#,*]	**20.04**	**4.27**	**47.82**	**10.24**	**2.17**	**1.86**	**2.85**	**0.63**[#]	
28	0.21	0.09	0.83	0.37	<0.01	<0.01[*]	0.02	0.01[*]	0.22	0.08[*]	0.87	0.33[*]	<0.01	<0.01[*]	0.05	0.02[*]	<0.01

47	2.90	0.50*	5.32	1.10*	0.04	<0.01*	0.15	0.03*	2.46	0.39*	10.14	1.41*	0.04	0.01*	0.70	0.26*	0.33
100	0.59	0.12*	1.27	0.27*	0.01	<0.01*	0.04	0.01*	0.45	0.10*	2.17	0.42*	0.01	<0.01*	0.15	0.05*	0.11
99	0.99	0.47*	1.97	0.49#,*	0.01	0.02*	0.06	0.02*	0.86	0.12*	5.28	1.01#,*	0.02	<0.01*	0.39	0.19*	0.05
153	0.11	0.02	0.21	0.07	<0.01	<0.01*	0.01	<0.01*	0.07	0.01*	0.53	0.09*	<0.01	<0.01*	0.04	0.02*	0.01.
183	0.08	0.01#	0.16	0.07	<0.01	<0.01#,*	<0.01	<0.01#,*	0.04	0.01#	0.53	0.09	<0.01	<0.01#,*	0.04	0.02#,*	0.01
197	0.13	0.03#,*	0.27	0.08#,*	<0.01	<0.01*	0.01	<0.01#,*	0.07	0.01#,*	0.33	0.10#,*	<0.01	<0.01*	0.02	0.01#,*	0.01
Σ PBDEs	**5.01**	**0.82***	**10.03**	**1.86***	**0.07**	**0.01***	**0.29**	**0.06***	**4.08**	**0.54***	**0.72**	**0.14***	**0.08**	**0.01***	**0.05**	**0.02***	
%lipid					1.45	0.18	2.64	0.23#					2.13	0.35*	6.93	1.32#,*	

In **bold**: sum (Σ) of all DDT, PCB & PBDE congeners. Lw: lipid weight, fw: fresh weight. TEQ: toxic equivalents, pg WHO-TEQ $^{-1}$g fw, calculated by using the toxic equivalency factors recommended by Van den Berg (1998). BEQ: bioequivalent values, BEQ g^{-1} fw. The # denotes a significant difference between *C. gunnari* and *C. aceratus* in the given tissue at $p \leq 0.05$. The * denotes a significant difference between tissues for each species at $p \leq 0.05$.

538 **Figure Captions**

539

540 **Figure 1** Mean (± sem) concentration of Σ PCBs, Σ BDEs, HCBs, γ-HCHs and Σ DDTs (ng
541 g^{-1} lipid weight), in muscle and ovaries of the two icefish species, *C. gunnari* (*n*=11) and *C.*
542 *aceratus* (*n*=10). The # denotes a significant difference between *C. gunnari* and *C. aceratus* in
543 the given tissue at $p \leq 0.05$. The * denotes a significant difference between tissues for each
544 species at $p \leq 0.05$.

545

546 **Figure 2:** Congener composition (percentage) of PCBs, DDTs and BDEs in muscle (m) and
547 ovaries (ov) of *C. gunnari* (*Cg*) and *C. aceratus* (*Ca*).

548

549

550

551 **References**

552 Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the
553 global environment: emissions, levels, distribution, trends and processes. Sci. Total. Environ.
554 349, 1-44.
555 Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. Sci. Total.
556 Environ. 400, 212-226.
557 Bogdal, C., Ni oli c, D., L thi , M.P., Schen e r, ., Scheringer, M., Hungerb hle r, K., 2010.
558 Release of legacy pollutants from melting glaciers: model evidence and conceptual
559 understanding. Environ. Sci. Technol. 44, 4063-4069.
560 Borghesi, N., Corsolini, S., Focardi, S., 2008. Levels of polybrominated diphenyl ethers
561 (PBDEs) and organochlorine pollutants in two species of Antarctic fish (*Chionodraco*
562 *hamatus* and *Trematomus bernacchii*). Chemosphere 73, 155-160.
563 Borghesi, N., Corsolini, S., Leonards, P., Brandsma, S., de Boer, J., Focardi, S., 2009.
564 Polybrominated diphenyl ether contamination levels in fish from the Antarctic and the
565 Mediterranean Sea. Chemosphere 77, 693-698.
566 Cabrerizo, A., Dachs, ., Barcel , D., Jones, K.C., 2013. Climatic and biogeochemical
567 controls on the remobilization and reservoirs of persistent organic pollutants in Antarctica.
568 Environ. Sci. Technol. 47, 4299-4306.
569 Chiuchiolo, A.L., Dickhut, R.M., Cochran, M.A., Ducklow, H.W., 2004. Persistent organic
570 pollutants at the base of the Antarctic marine food web. Environ. Sci. Technol. 38, 3551-
571 3557.
572 Corsolini, S., Borghesi, N., Schiamone, A., Focardi, S., 2007. Polybrominated diphenyl
573 ethers, polychlorinated dibenzo-dioxins,-furans, and-biphenyls in three species of Antarctic
574 penguins. Environ. Sci. Pollut. R. 14, 421-429.
575 Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., Giesy, J.P., 2002a.
576 Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food
577 webs. Environ. Sci. Technol. 36, 3490-3496.
578 Corsolini, S., Ademollo, N., Mariottini, M., Fossi, S., Guerranti, C., Perra, G., Duhamel, G.,
579 Focardi, S., 2002b. Polychlorinated biphenyls, polychlorinated-dibenzodioxinds and –
580 dibenzofurans in mackerel icefish and marbled rockcod from the Kerguelen Islands
581 (Antarctica). Food and Feed I, 57, 161 – 164.
582 Cox, P., Efthymiou, P., 2003. Directive 2003/11/EC of the European parliament and of the
583 council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC
584 relating to restrictions on the marketing and use of certain dangerous substances and
585 preparations (pentabromodiphenyl ether, octabromodiphenyl ether). Off. J. Eur. Union 42, 45-
586 46.
587 Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for
588 upwelling of corrosive "acidified" water onto the continental shelf. Science 320, 1490-1492.
589 Focardi, S., Fossi, M., Lari, L., Casini, S., Leonzio, C., Meidel, S., Nigro, M., 1995. Induction
590 of MFO activity in the antarctic fish *Pagothenia bernacchii*: Preliminary results. Mar.
591 Environ. Res. 39, 97-100.
592 Goerke, H., Weber, K., Bornemann, H., Ramdohr, S., Plötz, J., 2004. Increasing levels and
593 biomagnification of persistent organic pollutants (POPs) in Antarctic biota. Mar. Pollut. Bull.
594 48, 295-302.
595 Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013.
596 Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica.
597 Mar. Pollut. Bull. 77, 82-89.

598 Hale, R.C., Kim, S.L., Harvey, E., La Guardia, M.J., Mainor, T.M., Bush, E.O., Jacobs, E.M.,
599 2008. Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame
600 retardants. Environ. Sci. Technol. 42, 1452-1457.
601 Hartmann, R., 2013. Extraction of PCDD/Fs and PCBs in fish using SpeedExtractor E-914.
602 Proc. Environ. Sci. 18, 875-881.
603 Husain, A., Gevao, B., Dashti, B., Brouwer, A., Behnisch, P.A., Al-Wadi, M., Al-Foudari,
604 M., 2014. Screening for PCDD/Fs and dl-PCBs in local and imported food and feed products
605 available across the State of Kuwait and assessment of dietary intake. Ecotox. Environ. Safe.
606 100, 27-31.
607 Isosaari, P., Hallikainen, A., Kiviranta, H., Vuorinen, P.J., Parmanne, R., Koistinen, J.,
608 Vartiainen, T., 2006. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls,
609 naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea
610 and lakes in Finland. Environ. Pollut. 141, 213-225.
611 Jaffal, A., Paris-Palacios, S., Jolly, S., Thailly, A., Delahaut, L., Beall, E., Roche, H.,
612 Biagianti-Risbourg, S., Betoulle, S., 2011. Cadmium and copper contents in a freshwater fish
613 species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands. Polar
614 Biol. 34, 397-409.
615 Kannan, K., Blankenship, A., Jones, P., Giesy, J., 2000. Toxicity reference values for the
616 toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk Assess. 6,
617 181-201.
618 Kohler, M., Zennegg, M., Bogdal, C., Gerecke, A.C., Schmid, P., V. Heeb, N., Sturm, M.,
619 Vonmont, H., E. Kohler, H.-P., Giger, W., 2008. Temporal trends, congener patterns, and
620 sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and
621 hexabromocyclododecanes (HBCD) in Swiss lake sediments. Environ. Sci. Technol. 42,
622 6378-6384.
623 Kuiper, R.V., Murk, A., Leonards, P.E., Grinwis, G., Van den Berg, M., Vos, J.G., 2006. In
624 vivo and in vitro Ah-receptor activation by commercial and fractionated
625 pentabromodiphenylether using zebrafish (*Danio rerio*) and the DR-CALUX assay. Aquat.
626 Toxicol. 79, 366-375.
627 Lana, N.B., Berton, P., Covaci, A., Ciocco, N.F., Barrera-Oro, E., Atencio, A., Altamirano,
628 J.C., 2014. Fingerprint of persistent organic pollutants in tissues of Antarctic notothenioid
629 fish. Sci. Total Environ. 499, 89-98.
630 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault,
631 J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent
632 organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995-3043.
633 Mintenbeck, K., Barrera-Oro, E.R., Brey, T., Jacob, U., Knust, R., Mark, F.C., Moreira, E.,
634 Strobel, A., Arntz, W.E., 2012. 5 - Impact of Climate Change on Fishes in Complex Antarctic
635 Ecosystems. in: Ute, J., Guy, W. (Eds.). Adv. Ecol. Res. Academic Press, 351-426.
636 Mirmigkou, S., de Boer, J., 2015. DDT and Metabolites. The Handbook of Environmental
637 Chemistry, 1-24.
638 Murk, A., Legler, J., Denison, M., Giesy, J., Van de Guchte, C., Brouwer, A., 1996.
639 Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah
640 receptor active compounds in sediments and pore water. Toxicol. Sci. 33, 149-160.
641 Nash, S.B., 2011. Persistent organic pollutants in Antarctica: current and future research
642 priorities. J. Environ. Monitor. 13, 497-504.
643 Nichols, J.W., Huggett, D.B., Arnot, J.A., Fitzsimmons, P.N., Cowan‐Ellsberry, C.E., 2013.
644 Toward improved models for predicting bioconcentration of well‐metabolized compounds
645 by rainbow trout using measured rates of in vitro intrinsic clearance. Environmental
646 Toxicology and Chemistry 32, 1611-1622.
647 Renner, R., 2004. Small fish for healthy babies. Environ. Sci. Technol. 38, 262-263.

Schmid, P., Bogdal, C., Bl t hgen, N., Anselmetti, .S., wyssig, A., Hungerb hle r, K., 2010. The Missing Piece: Sediment Records in Remote Mountain Lakes Confirm Glaciers Being Secondary Sources of Persistent Organic Pollutants. Environ. Sci. Technol. 45, 203-208.
Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. Chemosphere 67, 16-21.
Sharma, C.M., Rosseland, B.O., Almvik, M., Eklo, O.M., 2009. Bioaccumulation of organochlorine pollutants in the fish community in Lake Årungen, Norway. Environ. Pollut. 157, 2452-2458.
Simonich, S.L., Hites, R.A., 1995. Global distribution of persistent organochlorine compounds. Science 269, 1851.
Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs in Arctic biota, an update. Sci. Total Environ. 408, 2874-2884.
Storelli, M., Losada, S., Marcotrigiano, G., Roosens, L., Barone, G., Neels, H., Covaci, A., 2009. Polychlorinated biphenyl and organochlorine pesticide contamination signatures in deep-sea fish from the Mediterranean Sea. Environ. Res. 109, 851-856.
Strobel, A., Burkhardt-Holm, P., Schmid, P., Segner, H., 2015. Benzo (a) pyrene metabolism and EROD and GST biotransformation activity in the liver of red-and white-blooded Antarctic fish. Environ. Sci. Technol. 49, 8022-8032.
UNEP, 2002. Antarctica Regional Report. In: Chemicals, U.N.E.P. (Ed.). Regionally Based Assessment Of Persistent Toxic Substances.
UNEP, 2008. Global Status Of DDT And Its Alternatives For Use In Vector Control To Prevent Disease. In: UNEP/POPS/DDTBP.1/2 (Ed.). Stockholm Convention on Persistent Organic Pollutants. UNEP, Geneva.
UNEP/AMAP, 2011. Climate Change and POPs: Predicting The Impacts. Report of the UNEP/AMAP Expert Group. Geneva.
Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Persp. 106, 775.
van den Brink, N., Riddle, M., Heuvel-Greve, M.v.d., Allison, I., Snape, I., van Franeker, J.A., 2009. Correspondence on Geisz et al. Melting glaciers: a probable source of DDT to the Antarctic marine ecosystem. Environ. Sci. Technol. 43, 3976-3977.
van den Brink, N.W., Riddle, M.J., van den Heuvel-Greve, M., van Franeker, J.A., 2011. Contrasting time trends of organic contaminants in Antarctic pelagic and benthic food webs. Mar. Pollut. Bull. 62, 128-132.
Vives, I., Grimalt, J.O., Fernandez, P., Rosseland, B., 2004. Polycyclic aromatic hydrocarbons in fish from remote and high mountain lakes in Europe and Greenland. Sci. Total Environ. 324, 67-77.
Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio, 10-18.
Weber, K., Goerke, H., 2003. Persistent organic pollutants (POPs) in Antarctic fish: levels, patterns, changes. Chemosphere 53, 667-678.
Wolschke, H., Meng, X.-Z., Xie, Z., Ebinghaus, R., Cai, M., 2015. Novel flame retardants (N-FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. Mar. Pollut. Bull. 96, 513.518.
Xie, ., M l ler, A., Ahrens, L., Sturm, R., Ebinghaus, R., 2011. Brominated flame retardants in seawater and atmosphere of the Atlantic and the Southern Ocean. Environ. Sci. Technol. 45, 1820-1826.
Zennegg, M., Kohler, M., Gerecke, A.C., Schmid, P., 2003. Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout. Chemosphere 51, 545-553.

Manuscript No. CHEM38908

Highlights

- PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- POP levels are similar in fish from different sampling sites around the Antarctic Peninsula
- Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs) in icefish

Persistent organic pollutants in tissues of the white-blooded Antarctic fish *Champsocephalus gunnari* and *Chaenocephalus aceratus*

Anneli Strobel[1], Peter Schmid[2], Helmut Segner[3], Patricia Burkhardt-Holm[1,4], Markus Zennegg[2]

Affiliation:

[1] University of Basel, Departement of Environmental Sciences, Programme Man-Society-Environment MGU, Vesalgasse 1, CH-4051 Basel, Switzerland

E-Mail Anneli Strobel: anneli.strobel@unibas.ch

[2] Empa, Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, CH-8600 Dübendorf, Switzerland

E-Mail Markus Zennegg: markus.zenneg@empa.ch

[3] University of Bern, Vetsuisse Faculty, Centre for Fish and Wildlife Health, Länggassstrasse 12, CH-3012 Bern, Switzerland

E-Mail Helmut Segner: helmut.segner@vetsuisse.unibe.ch

[4]Department of Biological Sciences, University of Alberta, Edmonton, AB Canada

E-mail Patricia Burkhardt-Holm: patricia.holm@unibas.ch

Corresponding author:
anneli.strobel@unibas.ch

Abstract

The global occurrence of persistent organic pollutants (POPs) continuously contributes to their accumulation also in remote areas such as the Antarctic Ocean. Antarctic fish, which hold high trophic positions but appear to possess low endogenous elimination rates for chemicals, are expected to bioaccumulate POPs with rising anthropogenic pollution. Using a chemical-analytical method, we measured concentrations of PCBs, PBDEs, HCBs, HCH and DDTs and determined toxic equivalents (TEQs) and bioanalytical equivalents (BEQs) in muscle and ovaries of Antarctic icefish caught in the Southern Ocean around Elephant Island. We used two species with different feeding habits and trophic web positions: the planktivorous *Champsocephalus gunnari* and the piscivorous *Chaenocephalus aceratus*. Our results revealed higher contaminant levels in ovary than in muscle tissues of both species. Most analytes concentrations and the TEQs (0.2-0.5) and BEQs (0.2) were lower as in temperate species. Comparison with literature data points to higher PCB (20-22 ng g^{-1} lipid weight (lw)) and DDT (7-19.5 ng g^{-1} lw) concentrations than those measured in icefish in the 90's. For the other contaminants, we could not identify temporal trends. We found a higher bioaccumulation of contaminants, particularly HCB and DDTs, in *C. aceratus* (6.2 & 19.5 ng g^{-1} lw, respectively) than in *C. gunnari* (3.8 & 7.0 ng g^{-1} lw, respectively). However, there was no general species-specific accumulation pattern of the different toxicant classes between the two icefish. Thus, the expected link between contaminant burdens of *C. aceratus* and *C. gunnari* and their ecological traits was only weakly supported for these species.

Highlights

- PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- POP levels are similar in fish from different sampling sites around the Antarctic Peninsula
- Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs) in icefish

Keywords

Icefish, bioaccumulation, persistent organic pollutants, polychlorinated biphenyls (PCBs), toxic equivalents (TEQs), DR CALUX bioanalytical equivalents (BEQs)

1. Introduction

Antarctica has been less affected by human influences than other continents for a long time, however, contamination with anthropogenic contaminants, in particularly persistent organic pollutants (POPs) has increased progressively (Nash, 2011; UNEP/AMAP, 2011). Nowadays, Antarctica serves as a major sink for highly persistent contaminants. Long-range atmospheric transport, together with global distillation processes and cold condensation, are considered to be the main mechanisms for the progressive contamination of the Antarctic ecosystem, together with local sources such as fishing, tourism and research activities (Simonich and Hites; Feely et al., 2008; Nash, 2011). Particularly POPs such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and amongst them also the formerly used insecticides such as γ-hexachlorcyclohexane (γ-HCH) and *p,p'*-DDT are ubiquitous pollutants with a wide application spectrum. For example, polymer additives such as flame retardants are found globally in building material, furniture, paint, textiles or plastics, which are also used in Antarctic bases and vessels cruising in these regions (Hale et al., 2008; Kohler et al., 2008). This worldwide abundance and the high persistence of POPs continuously contribute to an accumulation in the ice masses and biota of polar regions (Wania and Mackay, 1993; Chiuchiolo et al., 2004; Goerke et al., 2004; Corsolini et al., 2007; Bargagli, 2008; Borghesi et al., 2008; Borghesi et al., 2009; Xie et al., 2011; Wolschke et al., 2015).

Furthermore, those lipophilic organic chemicals have a high potential to bioaccumulate in aquatic biota, and particularly in Antarctic species, which generally possess low endogenous elimination rates for those chemicals (Strobel et al., 2015). Additionally, it is expected that climate warming will lead to the release of those pollutants trapped in glaciers and sea-ice, which additionally contributes to increasing POP levels in the tissues of Antarctic animals (Weber and Goerke, 2003; Bogdal et al., 2010; Schmid et al., 2010; van den Brink et al., 2011; Cabrerizo et al., 2013; Goutte et al., 2013). As global chemical usage continues to grow, including the usage of persistent and bioaccumulative compounds, it is to be expected that contaminant intake into Antarctica will further increase in the future.

The endemic, Antarctic notothenioid fish are evolutionary well-adapted to the cold and stable environment of the Southern Ocean. Adaptations involve e.g. high amounts of tissue lipids, slow growth rates, long life spans and slow metabolism and elimination rates for xenobiotics (Mintenbeck et al., 2012; Strobel et al., 2015). All these factors may favor the bioaccumulation of lipophilic contaminants in the tissues of those fish.

Lipophilic, non-charged contaminants like PCBs can be taken up via a physico-chemically driven, passive partitioning of the chemicals from the water phase into the lipid phase of the organism and thereby accumulate differentially in tissues or fish species, depending on their lipophilicity of the chemical and the lipid content or composition of the tissues (Nichols et al., 2013). In fact, tissue-specific differences of POP levels are reported for notothenioid species (Lana et al., 2014). Furthermore, bioaccumulation and biomagnification can be related to an organism's habitat and its trophic position within the food web (Corsolini et al., 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Particularly for highly lipophilic compounds, oral uptake via the prey contributes by a major extent to the bioaccumulation of toxicants in the tissues of a species. For example, Weber and Goerke (2003) found higher contaminant levels in the piscivorous icefish *Chaenocephalus aceratus* than in the planktivorous icefish *Champsocephalus gunnari*, which was apparently related to the different food spectra of the two notothenioids (Goerke et al., 2004). Such studies highlight that POPs are transferred within the Antarctic food web, leading to increasing POP concentrations along the food chain up to high concentrations in top level predators (Wolschke et al., 2015).

Considering the trends of rising POP concentrations in Antarctica (UNEP/AMAP, 2011 and their high potential to exert toxic effects in marine biota (Nash, 2011), it is very important to keep monitoring body burdens of the fish living in the Southern Ocean. Yet, we are far from having a solid understanding of the contamination status, time trends, the diversity of contaminants or their toxicity potential in Antarctic biota (UNEP, 2002; UNEP/AMAP, 2011). Particularly the bioaccumulation pattern of contaminants such as the polybrominated flame retardants (PBDEs) or insecticides (e.g. DDT) have hardly been measured in icefish.

The aim of this study was to determine levels of selected POPs in muscle and ovary tissue of two white-blooded Antarctic notothenioid species in the study area around Elephant Island,

the South Shetland Islands and the Antarctic Peninsula, and to estimate temporal trends of fish POP levels in this area. The analytically measured contaminant concentrations were converted into toxic equivalents (TEQs) using standardized values. In order to examine whether food web position or tissue lipid content has an influence on chemical concentrations, we analyzed lipid content and POP levels in fish species of the same family but with different feeding habits and trophic web positions , namely the planktivorous *C. gunnari* and the piscivorous *C. aceratus*.

Extending the classical contaminants, this study includes polybrominated flame retardants (PBDEs) and insecticides (i.e. DDT, HCB). In addition to chemical analytics, we also applied bioanalytics using the DR-CALUX assay (Murk et al., 1996; Kuiper et al., 2006) to assess the total accumulated dioxin-like activity in the fish tissues.

2. Methods

2.1 Study species

Two species of the *Channichthyidae* (white-blooded icefish) were fished with bottom trawls down to 500 m, during a cruise with the research vessel 'Polarstern' (ANTXXVIII/4, March 13 to April 9, 2012; http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart) at different, closely located sampling sites around Elephant Island and the South Shetland Islands (61.1°S, 55.1°W). Only fish netted alive and without macroscopically visible damage were used for tissue sampling. The planktivorous mackerel icefish, *C. gunnari*, shows a mainly bentho-pelagic feeding mode, while the piscivorous Scotia Sea icefish *C. aceratus* is predominantly a benthos feeder (Weber and Goerke, 2003). Persistent organic pollutants were analyzed in muscle and ovary tissue of mature (stage III-IV), female fish only. Sex and maturity stage of the fish were verified histologically. Animal weight and length are given in Table 1. All tissue samples were wrapped in aluminum foil and stored immediately at -20 °C until used for analysis.

2.2 Sample preparation

Muscle and gonad samples were defrosted, cut into pieces and lyophilized at 33 Pa for 72 hours until constant weight. Dried tissue of muscle (5-10 g) or gonads (0.5-2 g) was ground

159 with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pistil to obtain a fine
160 powder.

161 This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi,
162 Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and
163 100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml *n*-
164 hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a
165 Syncore evaporator (Büchi, Switzerland) und let dry completely by applying a gentle
166 nitrogen stream. The residue accounted for the fat content of the sample. $^{13}C_{12}$ labeled
167 internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2
168 – 3 mL of *n*-hexane the solution was treated with 3 ml oleum (7% SO_3 in conc. sulfuric acid).
169 After centrifugation for 3 min at 5'000 rpm, the solvent layer with the lipophilic target
170 analytes was removed and the remaining suspension was re-extracted two times more with *n*-
171 hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and
172 300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from
173 top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid
174 and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and
175 eluted with 5 ml *n*-hexane, followed by 5 mL *n*-hexane/dichloromethane 1:1 (v:v). The eluate
176 was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the
177 volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at
178 room temperature. Finally the recovery standard $^{13}C_{12}$ labeled PCB 70 was added. Samples
179 were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical
180 procedure were determined in duplicates (Table 1 and A.2).
181
182 2.3 Chemical analysis
183

184 PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118,
185 123, 126 156, 157, 167, 169, and 189), DDT (*o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE),
186 hexachlorobenzene (HCB), γ-hexachlorocyclohexane (γ-HCH), and PBDEs (BDE 28, 47, 99,
187 100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the
188 target analytes in the extracts was achieved by gas chromatography/high resolution mass
189 spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution
190 mass spectrometer (Thermo Finnigan MAT, Bremen Germany) coupled to a Finnigan Trace

GC Ultra equipped with a Triplus auto sampler (Thermo Electron Corporation, Waltham, MA, USA). Samples were injected in splitless mode (splitless time 30 s) at an injector temperature of 260 °C. For the gas chromatographic separation a RTX5 Sil-MS column (30 m × 0.25 mm, film thickness 0.10 µm) was used with helium as carrier gas at a pressure of 100 kPa. The following temperature programs were used for the different compound classes. For the PCBs, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 20 °C/ min to 180 °C, followed by 3 °C/ min to 250 °C, and 20 °C/ min to 300 °C. For pesticides, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 10 °C/ min to 160 °C, followed by 4 °C/ min to 240 °C, and 20 °C/ min to 300 °C. For the PBDEs, the initial column temperature was 100 °C. After 0.5 min, the temperature was ramped at 20 °C/ min to 220 °C, followed by 6 °C/ min to 300 °C, and 10 °C/ min to 320 °C. The ion source was operated at 220 °C, the electron energy was 70eV, and the mass spectrometer was tuned to a mass resolution of 8000-10000. The two most abundant signals of the molecular ion cluster of the analytes and the $^{13}C_{12}$ labeled internal standards were recorded in the single ion monitoring mode.

The analytes were identified by comparing the retention times with those of the labeled internal standards. Quantification was based on peak areas of the analytes and the labeled reference compounds with known concentrations. More details about the method are available in the literature (Zennegg et al., 2003; Schmid et al., 2007).
For the calculation of TEQs, the dioxin-like PCBs were determined in the same way, but as part of the sample extract was used later on for the analysis by the bioassay (DR-CALUX), no $^{13}C_{12}$-labeled internal dl-PCBs standards could be used for the quantification, as the isotope labeled analogues exhibit similar activity in the DR-CALUX. Therefore, quantitative determination of dl-PCBs was based on the $^{13}C_{12}$ labeled indicator PCBs used as internal standards and previously determined response factors to native dl-PCBs.
The blank values (in ng g^{-1} lipid weight) were all below the compound concentrations and are given in Table 1 and A.1.
The limit of detection (LOD) and the limit of quantification (LOQ) were set by definition at signal to noise ratios of greater than three (s/n ≥3) and ten (s/n ≥10) respectively. All glass ware used were cleaned with strongly alkaline detergents and backed out overnight in a ceramic oven at 450 °C. Directly before use, the glass ware was rinsed with solvents (*n*-hexane, dichloromethane).

2.4 Determination of dioxin-like toxic equivalents (TEQs)

In order to assess total dioxin-like activity in the fish tissues, we calculated the 2,3,7,8-tetrachlorodibezo-*p*-dioxin (TCDD) equivalent (TEQ) concentrations of dl-PCBs in the muscle and gonad extracts of both icefish species using Toxic Equivalency Factors (TEFs) proposed by the World Health Organization (WHO) for fish (Van den Berg et al., 1998). TEQs were calculated as the sum of the TEFs of all dl-PCBs listed in Table A.1. Although the TEF values are not derived from studies with Antarctic fish, since TEFs are only available for salmonids (Van den Berg et al., 1998) they provide a useful tool for a reasonable estimate of toxicity effects of PCBs on Antarctic fish.

2.5 Determination of bioequivalent values (BEQs)

The same extracts that were used for chemical analysis were also used in a standard bioassay, the DR-CALUX (Dioxin Responsive Chemically Activated Luciferase Gene Expression) assay. This cell and receptor based reporter gene bioassay measures the binding of dioxin-like HAHs, e.g. dioxin-like PCBs (dl-PCBs), to the Aryl hydrocarbon receptor (AhR) via activation of a reporter gene. The assay was performed by BioDetection Systems b.v. (Amsterdam, The Netherlands).

Bioassay-derived TEQ values, or bioequivalent values (BEQs), were compared with TEQ values calculated from the chemical analytical data. This comparison revealed if there was a significant higher activity than predicted from the chemical analysis. Such a combined approach has been repeatedly used in studies on chemical contamination of the Arctic region (Letcher et al., 2010), but it has not been used in studies with Antarctic fish so far.

2.5 Calculations and statistics

All analyses include the lipid content of the respective tissue. POP concentrations were normalized against both lipid weight (lw, ng g^{-1}) and fresh weight (fw, ng g^{-1}) of the tissue and provided as mean values ± standard error of the mean (sem). Toxicant concentrations were compared among icefish species and tissues using ANOVA with Tukey post-hoc test.

Influence of sampling site, fish weight and length, maturity stage and tissue lipid content was tested with ANOVA. Data were considered to be statistically significant at $p < 0.05$. Normal distribution of data was tested with Kolmogorov-Smirnov and equality of variances with Bartlett's test. All statistical tests were performed with STATISTICA 12, StatSoft, Inc., and GraphPad Prism 5, GraphPad Software, Inc.

3. Results

3.1 Interspecies comparison of contaminant patterns and levels

In this study, we measured lipid content, PCB, DDT, HCB, γ-HCH, and PBDEconcentrations in both muscle and ovary tissue of female, mature *C. gunnari* and *C. aceratus* from spatially closely located sampling sites around Elephant Island and the South Shetland Islands. We express the compound concentrations on lipid weight and the fresh weight basis.

Sampling site, maturity stage and tissue lipid content of the fish showed no correlation to the accumulation of any of the compounds analysed in this study.

All samples contained detectable levels of the target compounds, which are presented in Table 1. Analytes concentrations were about 15 - 110 times higher when calculated per lipid weight than based on tissue fresh weight.

Overall, the PCBs were the predominant group among all compounds analysed in our study (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 20.0 ± 4.3, *C. gunnari* ovaries 47.8 ± 10.2, *C. aceratus* muscle 21.9 ± 4.9, *C. aceratus* ovaries 31.9 ± 7.3), followed by the DDTs (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 7.0 ± 1.0, *C. gunnari* ovaries 6.9 ± 1.1, *C. aceratus* muscle 19.4 ± 8.1, *C. aceratus* ovaries 17.8 ± 3.9), HCB (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 3.8 ± 0.5, *C. gunnari* ovaries 2.8 ± 0.4, *C. aceratus* muscle 6.2 ± 0.9, *C. aceratus* ovaries 7.3 ± 2.0), the PBDEs (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 4.1 ± 0.5, *C. gunnari* ovaries 0.7 ± 0.1, *C. aceratus* muscle 5.1 ± 0.8, *C. aceratus* ovaries 10.0 ± 1.9) and γ-HCH (mean values ± sem (ng g^{-1} lw): *C. gunnari* muscle 0.6 ± 0.2, *C. gunnari* ovaries 1.3 ± 0.3, *C. aceratus* muscle 0.6 ± 0.2, *C. aceratus* ovaries 1.2 ± 0.5) (Figure 1).

Within the PCBs, only PCB 28 and 101 were significantly different between the ovaries of *C. gunnari* and *C. aceratus* (Table 1). When we calculated the percentage contribution of the individual PCB congeners to ∑PCBs (lw) of both muscle and ovary tissue, there was no

290 difference in PCB congener composition between the two icefish species (two-way
291 ANOVA).
292 Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs
293 in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%)
294 (Figure 2).
295
296 In the ovaries, levels of *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT (per lw) were significantly
297 higher in *C. aceratus* compared to those of *C. gunnari*. Also *p,p'*-DDT concentrations in the
298 muscle of *C. aceratus* were higher than those in muscle of *C. gunnari* (per lw). ∑DDT (per
299 lw) was significantly higher in muscle and ovaries of *C. aceratus* than in *C. gunnari* (Figure
300 1). Among the DDTs, *p,p'*-DDE showed the highest concentration of up to 57% in muscle of
301 *C. aceratus*. *p,p'*-DDE tended to be higher in muscle tissue, while *p,p'*-DDT was slightly
302 higher in ovary tissue in both species. *o,p'*-DDT was slightly higher in *C. gunnari* with
303 ~22%, while it was only ~16% in *C. aceratus*. Detailed information on the contribution of all
304 individual compounds measured in this study is given in Table A.2 in the appendix.
305 γ-HCH was only different between the muscle tissues of the two icefish (on fw basis).
306 HCB concentrations per lipid weight in both muscle and ovaries were higher in *C. aceratus*
307 than in *C. gunnari* (Figure 1). Except HCB, none of the analysed compounds exhibited a
308 correlation with fish weight or length. HCB showed a significant linear dependence of fish
309 weight and length in *C. aceratus*, except in the ovaries (R^2=0.723).
310
311 In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries),
312 followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197
313 were significantly different between the two tissues types and fish species. Also BDE 99
314 showed different concentrations between the ovaries of *C. aceratus* and *C. gunnari* on the
315 lipid basis (Table 1, Table A.2, Figure 2).
316
317 3.2 Comparison of contaminant patterns and levels in different tissue
318
319 Lipid content was significantly higher in the ovaries of *C. gunnari* than in the ovaries of *C.*
320 *aceratus*. *C. gunnari* exhibited significantly higher lipid content in ovaries than in their
321 muscle tissue, while this was not the case for *C. aceratus* (Table 1).
322 A comparison between the compound concentrations in muscle and ovary tissue for each
323 icefish species revealed higher HCB concentrations in ovary than in muscle tissue of *C.*

gunnari. Concentrations of γ-HCH and DDTs in muscle and ovary tissue (per fresh weight) were significantly different in both species (Table 1).

In *C. aceratus*, all PCB concentrations and most of the PBDEs were different from each other in both muscle and ovary tissue. In case of dl-PCB congeners, the ∑dl-PCBs were higher in muscle of *C. gunnari* than in muscle *C. aceratus* on both lipid and fresh weight basis (Table A.1).

In *C. gunnari*, all PBDEs apart from congener 183 did differ between muscle and ovary tissue. In contrast, only PCB 28 and 180 were different between the tissues of *C. gunnari*, all other PCB congeners showed no significant tissue differences (Table 1, Figure 1).

3.3 Toxic equivalents (TEQs*)*

The toxic equivalent levels of dioxin-like PCBs (WHO_{PCB}-TEQ g^{-1} fw) were higher in the muscle of *C. gunnari* (0.5 ± 0.1) than in *C. aceratus* (0.2 ± 0.1), while *C. aceratus* exhibited higher TEQs (0.5 ± 0.9) in the ovaries than *C. gunnari* (0.1 ± 0.0) (Table 1).

3.4 Bioanalytical equivalents (BEQs)

The bioanalytical equivalents (BEQs) determined by DR-CALUX in the muscle of both species were in a similar range (*C. aceratus*: 0.15 ± 0.07, *C. gunnari*: 0.2 ± 0.14 BEQ g^{-1} fw) to the WHO_{PCB}-TEQs g^{-1} lw, which were calculated using the concentrations of dioxin-like PCBs. In *C. gunnari*, however, the BEQs measured in the ovaries were about 70-fold higher (BEQ: 5.14 ± 3.6, TEQ: 0.07 ± 0.04) than the calculated WHO_{PCB}-TEQ g^{-1} fw (Table 1).

4. Discussion

4.1 Species-specific contaminant patterns and levels

4.1.1. Species-specific patterns

Literature data on present POP concentrations in tissues of Antarctic fish are scarce, and particularly those on contaminant levels in white-blooded icefish.

Earlier studies of the 80's and 90's measured PCB concentrations in the icefish *C. gunnari* and *C. aceratus* around the Antarctic Peninsula (Corsolini et al. 2002a, 2002b, 2009), which

were two to four times lower (0.7 ng g^{-1} fw in muscle) than the ones of the present study. HCB levels of our icefish were within a similar range to those measured previously. It is known for sub-Antarctic fish that PCB levels can follow a seasonal trend, which is related to the release of pollutants from melting snow and ice during summer and the atmospheric transport of loads of pollutants which precipitate in Antarctic regions and are released during warming (Jaffal et al., 2011). Since sampling of our icefish took place in Austral autumn, after the seasonal ice melting in Antarctica, a seasonal release of POPs trapped in sea ice could thereby be one contributor to the comparably high levels of PCBs in our icefish samples. In fact, the PCB concentrations (per fw) of our icefish were within a similar range to those of salmon from the Baltic Sea (Isosaari et al., 2006).

In the early 00's, Borghesi et al. (2008; 2009) sampled *C. hamatus* and *C. gunnari* in the Ross Sea at 74° South. They found ∑PCB concentrations in *C. hamatus* muscle of 0.35 ng g^{-1} fw, ∑non-ortho PCBs of 5 ng g^{-1} fw and ∑PBDEs 0.16 ng g^{-1} fw. In muscle of *C. gunnari*, they report ∑PBDEs of 0.44 ng g^{-1} fw. Those values are about five times higher than the ∑PBDEs in muscle of *C. gunnari* measured in our study. Since they used fish of a similar size class, this difference is likely related to the measurement of different PBDE congeners in the studies.

Another recent study measured contaminant concentrations (in ng g^{-1} lw) in muscle, liver and gonads of three red-blooded Antarctic species, *Notothenia coriiceps, N. rossii* and *Trematomus newnesi*, from Potter Cove, Antarctic Peninsula (Lana et al., 2014). They reported similar ∑DDT values in muscle, but also species differences in the ovaries of their fish: while *N. coriiceps* and *T. newnesi* had similar values to *C. aceratus* measured in our study, *N. rossii* had much higher values than our fish. They also measured γ-HCH values about six-times higher than in our fish. *N. rossii* displays a rather benthic lifestyle, but also feeds on pelagic species, and thus has a prey spectrum similar to *C. gunnari*. Nevertheless, the DDTs and γ-HCH were much higher in *N. rossii* than in *C. gunnari.* In contrast, ∑PCB and ∑BDEs were highly variable among the species and tissues measured by Lana et al. (2014), but were generally within the same order of magnitude compared to our data. Thus, such strong differences in DDT and γ-HCH accumulation patterns between Antarctic fish species could also be related to a selective metabolism for individual contaminant classes between the species (Storelli et al., 2009), and not only to their ecological traits.

Amongst the PBDEs, BDE 47 showed the highest concentration (60% of all congeners) in our icefish amongst all PBDE congeners, followed by BDE 100 and BDE 99. This is in line

392 with the general picture of those congeners being the dominating PBDEs in fish around the
393 globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota
394 (Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).
395 In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight
396 basis were particularly low in icefish. For example, PBDE concentrations range from about
397 1.0 to 8 ng g^{-1} fresh weight, or up to 64 ng g^{-1} lipid weight in various fish species from the
398 Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.
399 Although the production of the former widely used brominated flame retardants penta- and
400 octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in
401 2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still
402 produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and
403 Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).
404 HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per
405 fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere
406 (Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70's, it
407 has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of
408 DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation
409 potential and cold condensation processes, DDTs and its metabolites are nowadays found in
410 biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).
411
412 The TEQs we calculated for *C. aceratus* and *C. gunnari* were in a similar range than TEQs
413 reported for muscle of the icefish *C. hamatus* (TEQ 0.01-0.1 pg g^{-1} wet weight) or red-
414 blooded Antarctic fish (TEQ ~0.1 pg g^{-1} wet weight) (Focardi et al.; Corsolini et al., 2002a;
415 Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other
416 organisms living in less remote parts of the world, e.g. WHO_{PCB}-TEQ (pg g^{-1} fw) of 3 to 15
417 in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit
418 muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).
419 In *C. aceratus*, we found that the BEQs were comparable to the TEQs calculated on the basis
420 on the WHO_{PCB}-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to
421 fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty
422 times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of *C. gunnari*
423 were much higher than the WHO_{PCB}-TEQs calculated for their ovaries. The toxicity effects
424 might thus be actually much higher than expected by the single usage of the calculated
425 WHO_{PCB}-TEQ. In fact, the BEQs in the ovaries of *C. gunnari* were within the same range as

BEQs of fish from temperate latitudes. Also Corsolini et al. (Corsolini et al., 2002a) stated that the TEQ values they measured in Antarctic fish were already half as high as those values which are considered to elicit toxicological effects, such as reproductive and immunological disorders, in marine mammals or birds (Kannan et al., 2000).
Rising PCB concentrations as observed by us in tissues of Antarctic fish will thus increasingly have the potential to exert their toxic effects on those fish.

4.1.2. Temporal trends in contaminant levels

Long-term observations on contaminant levels in Antarctic biota are scarce. Yet, Weber and Goerke (2003; Goerke et al., 2004) measured contaminant levels in the liver of *C. aceratus* and *C. gunnari* in the same sampling area as in the present study. From 1987 to 1996, the authors measured an increase of PCB 153 and PCB 180 levels in *C. aceratus*, but not in *C. gunnari.* In the present study, we found about three times higher concentrations of those PCB congeners in the tissues of our two icefish species than in the previous study from 1996 (Weber and Goerke, 2003). In contrast, the HCB concentrations show a declining trend from the 1987 study to our current survey. A similar trend of stable or declining HCB levels by up to 2.5% per year has also been observed in Arctic biota, such as birds, fish or marine mammals, since the late 80's (Barber et al., 2005; Rigét et al., 2010).

In liver of *C. aceratus*, concentrations of *p,p'*-DDE had already increased from 1987 to 1996, and the values we measured in muscle and ovaries of *C. aceratus* were almost twice as high as in 1996 (Weber and Goerke, 2003). In contrast, *p,p'*-DDE concentrations in *C. gunnari* remained at similar levels from 1987 over 1996 (study by Weber and Goerke (2003)) to the present study.
Nevertheless, overall DDT concentrations were increasing from 1987 to 1996 in both icefish species, and our values are also slightly higher than those in 1996, suggesting an increasing trend of DDTs in icefish around the Antarctic Peninsula. Despite a general global reduction of DTT as an insecticide, DDT is still produced at high volumes (see above, (UNEP, 2008)). Furthermore, climate warming on the one hand leads to an increased volatility and worldwide distribution of DDTs, and on the other hand local sources such as melting glaciers may additionally contribute to increasing DDT concentrations in tissues of Antarctic fish around the Antarctic Peninsula (van den Brink et al., 2009; van den Brink et al., 2011).

4.2 Correlation of contaminant concentrations with ecological traits

4.2.1 Tissue-specific patterns

Since all POPs analyzed in this study are highly lipophilic substances, tissue differences in the contaminant levels in *C. aceratus* and *C. gunnari* on fresh weight basis at first instance should correlate to tissue lipid concentrations (Corsolini et al., 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Indeed, *C. gunnari,* which possesses a clearly higher lipid content in the ovaries than *C. aceratus*, accordingly showed significantly higher PCB concentrations in its ovaries based on fresh weight compared to *C. gunnari*. Also HCB was two times more concentrated in the fat-rich ovaries of *C. aceratus* than in *C. gunnari*, but only on the lipid basis. Accordingly, the BEQs were higher in the ovaries of *C. aceratus* and *C. gunnari* than in their muscle tissue. Also Lana et al. (2014), investigating POP accumulation patterns in notothenioid species, reported that the highest levels were found in the gonads of the fish.

4.2.2 Ecological-related patterns

In addition to body lipid contents, our results point to an influence of habitat and trophic level on POP levels in white-blooded icefish. The benthic-living *C. aceratus* had two-times higher concentrations of almost all DDT congeners and HCB than the bentho-pelagic *C. gunnari.* Also the previous study by Weber and Goerke (2003) report higher (lipid-based) contaminant burdens in *C. aceratus* than in *C. gunnari* and highlight the higher tendency of *C. aceratus* to accumulate DDTs in its tissues than *C. gunnari* over the time.

Since POPs accumulate in sediments, benthic fish species are generally thought have a higher exposure and uptake of lipophilic contaminants (Goerke et al., 2004; Borghesi et al., 2008). In addition to uptake from water or sediment, also the feeding habit thus plays a role, with species at higher tropic levels tending to show higher contaminant accumulation due to biomagnification. A recent study by Wolschke et al. (2015) also highlights the biomagnification of POPs from lower to higher trophic levels in the Antarctic food chain, which can be attributed to the diets of the animals.

From these observations, the benthic, piscivorous *C. aceratus* was expected to have higher contaminant burdens than the bentho-pelagic, planktivorous *C. gunnari*. However, only HCB

and DDTs, but none of the other congener classes, were higher in the predominantly benthic *C. aceratus* than in the bentho-pelagic *C. gunnari* on the lipid weight basis.

Conclusion

Overall, PCB and DDT concentrations tend to rather increase than decrease in tissues of the two white-blooded icefish species *C. aceratus* and *C. gunnari* around Elephant Island and the South Shetland Islands, when compared to earlier studies. Our results thereby support the global transportation of POPs to the Southern Ocean and their bioaccumulation in the local marine fish, and point to a trend of increasing concentrations of POPs in Antarctic icefish. Our data also suggest that worldwide climate change effects may contribute to an increased volatilization and release of POPs trapped in glaciers, sea- or pack-ice, thereby leading to an ongoing contamination of the Southern Ocean and its biota.

Furthermore, we found differences in POP accumulation patterns between the two icefish species, which were weakly correlated to their trophic position. The piscivorous *C. aceratus* showed a higher potential to accumulate contaminants in its tissue than the planktivorous *C. gunnari*. This species difference highlights the influence of intake of POPs via the specific prey of individual fish species. However, the expected link between the contaminant burdens of *C. aceratus* and *C. gunnari* and their ecological traits could not be fully supported. Additional factors, such species differences in toxicant metabolism rates and selective metabolism for single contaminant classes, may also play an important role in defining chemical bioaccumulation patterns in Antarctic fish species in the long term. In the end, Antarctic fish are a central link between the benthic community and top level predators, concomitantly POPs bioaccumulated in their tissues are likely to contribute to a progressive biomagnification of POPs along Antarctic food webs.

Acknowledgements

This work was funded by the Swiss National Science Foundation (SNSF 31003A_149964/1) and the Freiwillige Akademische Gesellschaft Basel. We thank Donatella Perrone and Melanie Senn (Empa) for their helpful assistance in the laboratory.

527 **Tables & Figures**

528

529 **Tables**

530

531 **Table 1**: Lipid content (%) and levels of organic contaminants (ng g^{-1} lipid weight; ng g^{-1} fresh weight) (mean ± sem) in tissues of two Antarctic
532 icefish species.

533

	C. aceratus (*n*=10)								*C. gunnari* (*n*=11)								
Length (cm)	47-66								33-50								
Weight (g)	636-3620								252-888								
	lipid weight (ng g^{-1} lw)				fresh weight (ng g-^{1} fw)				lipid weight (ng g^{-1} lw)				fresh weight (ng g^{-1} fw)				Blank
Tissue	muscle		ovaries		muscle		ovaries		muscle		ovaries		muscle		ovaries		
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	
TEQs					**0.16**	**0.1**	**0.53**	**0.94**					**0.47**	**0.14***	**0.07**	**0.04***	
BEQs					**0.15**	**0.07**	**0.57**	**0.75**					**0.20**	**0.14***	**5.14**	**3.60***	

HCB	6.22	0.85[#]	7.37	1.98[#]	0.09	0.02	0.16	0.04	3.82	0.55[#]	2.84	0.37[#]	0.07	0.01[*]	0.18	0.04[*]	0.06
γ-HCH	0.58	0.21[*]	1.18	0.49[*]	0.01	<0.01[#,*]	0.03	0.01[*]	0.59	0.20	1.25	0.31	0.01	<0.01[#,*]	0.07	0.02[*]	0.07
p,p′-DDE	13.11	5.99	8.76	2.46[#]	0.14	0.04[#,*]	0.19	0.03[*]	3.19	0.58	2.36	0.38[#]	0.06	0.01[#,*]	0.16	0.04[*]	0.52
o,p′-DDT	3.20	0.98	2.83	0.65[#]	0.04	0.01[*]	0.07	0.01[*]	1.65	0.36	1.64	0.38[#]	0.03	<0.01[*]	0.09	0.02[*]	0.07
p,p′-DDT	5.31	1.61[#]	6.21	1.10[#]	0.06	0.02[*]	0.15	0.02[*]	2.19	0.42[#]	2.98	0.52[#]	0.04	0.01[*]	0.19	0.04[*]	0.32
Σ DDTs	**19.45**	**8.14** [#]	**17.80**	**3.91**[#]	**0.22**	**0.07**	**0.40**	**0.05**	**7.04**	**1.00**[#]	**6.98**	**1.14**[#]	**0.12**	**0.02**[*]	**0.44**	**0.10**[*]	
28	0.95	0.06[*]	1.50	0.26[*]	0.01	<0.01[*]	0.04	0.01[#,*]	1.23	0.11[*]	2.84	0.53[*]	0.04	0.01[*]	0.18	0.05[#,*]	0.12
52	2.15	0.79[*]	3.29	0.98[*]	0.02	0.01[*]	0.09	0.03[*]	1.56	0.38	7.72	2.31	0.36	0.34	0.37	0.09	0.25
101	5.12	1.48[*]	7.10	1.69[*]	0.06	0.01[*]	0.19	0.05[#,*]	3.99	0.97	10.74	2.72	0.68	0.63	0.60	0.12[#]	0.26
138	5.74	1.21[*]	8.20	1.83[*]	0.07	0.01[*]	0.22	0.05[*]	5.47	1.23	11.06	2.69	0.60	0.52	0.68	0.18	0.40
153	6.00	1.26[*]	8.72	2.10[*]	0.07	0.01[*]	0.23	0.06[*]	5.94	1.40	11.96	2.52	0.41	0.33	0.76	0.20	0.49
180	1.94	0.31[*]	3.06	0.67[*]	0.02	<0.01[*]	0.08	0.02[*]	1.86	0.35[*]	3.51	0.50[*]	0.07	0.04	0.25	0.08	0.17
Σ PCBs	**21.91**	**4.98**[*]	**31.87**	**7.26**[*]	**0.26**	**0.03**[*]	**0.85**	**0.21**[#,*]	**20.04**	**4.27**	**47.82**	**10.24**	**2.17**	**1.86**	**2.85**	**0.63**[#]	
28	0.21	0.09	0.83	0.37	<0.01	<0.01[*]	0.02	0.01[*]	0.22	0.08[*]	0.87	0.33[*]	<0.01	<0.01[*]	0.05	0.02[*]	<0.01

47	2.90	0.50*	5.32	1.10*	0.04	<0.01*	0.15	0.03*	2.46	0.39*	10.14	1.41*	0.04	0.01*	0.70	0.26*	0.33
100	0.59	0.12*	1.27	0.27*	0.01	<0.01*	0.04	0.01*	0.45	0.10*	2.17	0.42*	0.01	<0.01*	0.15	0.05*	0.11
99	0.99	0.47*	1.97	0.49#,*	0.01	0.02*	0.06	0.02*	0.86	0.12*	5.28	1.01#,*	0.02	<0.01*	0.39	0.19*	0.05
153	0.11	0.02	0.21	0.07	<0.01	<0.01*	0.01	<0.01*	0.07	0.01*	0.53	0.09*	<0.01	<0.01*	0.04	0.02*	0.01.
183	0.08	0.01#	0.16	0.07	<0.01	<0.01#,*	<0.01	<0.01#,*	0.04	0.01#	0.53	0.09	<0.01	<0.01#,*	0.04	0.02#,*	0.01
197	0.13	0.03#,*	0.27	0.08#,*	<0.01	<0.01*	0.01	<0.01#,*	0.07	0.01#,*	0.33	0.10#,*	<0.01	<0.01*	0.02	0.01#,*	0.01
Σ PBDEs	**5.01**	**0.82***	**10.03**	**1.86***	**0.07**	**0.01***	**0.29**	**0.06***	**4.08**	**0.54***	**0.72**	**0.14***	**0.08**	**0.01***	**0.05**	**0.02***	
%lipid					1.45	0.18	2.64	0.23#					2.13	0.35*	6.93	1.32#,*	

In **bold**: sum (Σ) of all DDT, PCB & PBDE congeners. Lw: lipid weight, fw: fresh weight. TEQ: toxic equivalents, pg WHO-TEQ $^{-1}$g fw, calculated by using the toxic equivalency factors recommended by Van den Berg (1998). BEQ: bioequivalent values, BEQ g^{-1} fw. The # denotes a significant difference between *C. gunnari* and *C. aceratus* in the given tissue at $p \leq 0.05$. The * denotes a significant difference between tissues for each species at $p \leq 0.05$.

538 **Figure Captions**

539

540 **Figure 1** Mean (± sem) concentration of Σ PCBs, Σ BDEs, HCBs, γ-HCHs and Σ DDTs (ng
541 g^{-1} lipid weight), in muscle and ovaries of the two icefish species, *C. gunnari* (*n*=11) and *C.*
542 *aceratus* (*n*=10). The # denotes a significant difference between *C. gunnari* and *C. aceratus* in
543 the given tissue at $p \leq 0.05$. The * denotes a significant difference between tissues for each
544 species at $p \leq 0.05$.

545

546 **Figure 2:** Congener composition (percentage) of PCBs, DDTs and BDEs in muscle (m) and
547 ovaries (ov) of *C. gunnari* (*Cg*) and *C. aceratus* (*Ca*).

548

549

550

551 **References**

552 Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the
553 global environment: emissions, levels, distribution, trends and processes. Sci. Total. Environ.
554 349, 1-44.
555 Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. Sci. Total.
556 Environ. 400, 212-226.
557 Bogdal, C., Ni oli c, D., L thi , M.P., Schen e r, ., Scheringer, M., Hungerb hle r, K., 2010.
558 Release of legacy pollutants from melting glaciers: model evidence and conceptual
559 understanding. Environ. Sci. Technol. 44, 4063-4069.
560 Borghesi, N., Corsolini, S., Focardi, S., 2008. Levels of polybrominated diphenyl ethers
561 (PBDEs) and organochlorine pollutants in two species of Antarctic fish (*Chionodraco*
562 *hamatus* and *Trematomus bernacchii*). Chemosphere 73, 155-160.
563 Borghesi, N., Corsolini, S., Leonards, P., Brandsma, S., de Boer, J., Focardi, S., 2009.
564 Polybrominated diphenyl ether contamination levels in fish from the Antarctic and the
565 Mediterranean Sea. Chemosphere 77, 693-698.
566 Cabrerizo, A., Dachs, ., Barcel , D., Jones, K.C., 2013. Climatic and biogeochemical
567 controls on the remobilization and reservoirs of persistent organic pollutants in Antarctica.
568 Environ. Sci. Technol. 47, 4299-4306.
569 Chiuchiolo, A.L., Dickhut, R.M., Cochran, M.A., Ducklow, H.W., 2004. Persistent organic
570 pollutants at the base of the Antarctic marine food web. Environ. Sci. Technol. 38, 3551-
571 3557.
572 Corsolini, S., Borghesi, N., Schiamone, A., Focardi, S., 2007. Polybrominated diphenyl
573 ethers, polychlorinated dibenzo-dioxins,-furans, and-biphenyls in three species of Antarctic
574 penguins. Environ. Sci. Pollut. R. 14, 421-429.
575 Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., Giesy, J.P., 2002a.
576 Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food
577 webs. Environ. Sci. Technol. 36, 3490-3496.
578 Corsolini, S., Ademollo, N., Mariottini, M., Fossi, S., Guerranti, C., Perra, G., Duhamel, G.,
579 Focardi, S., 2002b. Polychlorinated biphenyls, polychlorinated-dibenzodioxinds and –
580 dibenzofurans in mackerel icefish and marbled rockcod from the Kerguelen Islands
581 (Antarctica). Food and Feed I, 57, 161 – 164.
582 Cox, P., Efthymiou, P., 2003. Directive 2003/11/EC of the European parliament and of the
583 council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC
584 relating to restrictions on the marketing and use of certain dangerous substances and
585 preparations (pentabromodiphenyl ether, octabromodiphenyl ether). Off. J. Eur. Union 42, 45-
586 46.
587 Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for
588 upwelling of corrosive "acidified" water onto the continental shelf. Science 320, 1490-1492.
589 Focardi, S., Fossi, M., Lari, L., Casini, S., Leonzio, C., Meidel, S., Nigro, M., 1995. Induction
590 of MFO activity in the antarctic fish *Pagothenia bernacchii*: Preliminary results. Mar.
591 Environ. Res. 39, 97-100.
592 Goerke, H., Weber, K., Bornemann, H., Ramdohr, S., Plötz, J., 2004. Increasing levels and
593 biomagnification of persistent organic pollutants (POPs) in Antarctic biota. Mar. Pollut. Bull.
594 48, 295-302.
595 Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013.
596 Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica.
597 Mar. Pollut. Bull. 77, 82-89.

598 Hale, R.C., Kim, S.L., Harvey, E., La Guardia, M.J., Mainor, T.M., Bush, E.O., Jacobs, E.M.,
599 2008. Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame
600 retardants. Environ. Sci. Technol. 42, 1452-1457.
601 Hartmann, R., 2013. Extraction of PCDD/Fs and PCBs in fish using SpeedExtractor E-914.
602 Proc. Environ. Sci. 18, 875-881.
603 Husain, A., Gevao, B., Dashti, B., Brouwer, A., Behnisch, P.A., Al-Wadi, M., Al-Foudari,
604 M., 2014. Screening for PCDD/Fs and dl-PCBs in local and imported food and feed products
605 available across the State of Kuwait and assessment of dietary intake. Ecotox. Environ. Safe.
606 100, 27-31.
607 Isosaari, P., Hallikainen, A., Kiviranta, H., Vuorinen, P.J., Parmanne, R., Koistinen, J.,
608 Vartiainen, T., 2006. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls,
609 naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea
610 and lakes in Finland. Environ. Pollut. 141, 213-225.
611 Jaffal, A., Paris-Palacios, S., Jolly, S., Thailly, A., Delahaut, L., Beall, E., Roche, H.,
612 Biagianti-Risbourg, S., Betoulle, S., 2011. Cadmium and copper contents in a freshwater fish
613 species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands. Polar
614 Biol. 34, 397-409.
615 Kannan, K., Blankenship, A., Jones, P., Giesy, J., 2000. Toxicity reference values for the
616 toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk Assess. 6,
617 181-201.
618 Kohler, M., Zennegg, M., Bogdal, C., Gerecke, A.C., Schmid, P., V. Heeb, N., Sturm, M.,
619 Vonmont, H., E. Kohler, H.-P., Giger, W., 2008. Temporal trends, congener patterns, and
620 sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and
621 hexabromocyclododecanes (HBCD) in Swiss lake sediments. Environ. Sci. Technol. 42,
622 6378-6384.
623 Kuiper, R.V., Murk, A., Leonards, P.E., Grinwis, G., Van den Berg, M., Vos, J.G., 2006. In
624 vivo and in vitro Ah-receptor activation by commercial and fractionated
625 pentabromodiphenylether using zebrafish (*Danio rerio*) and the DR-CALUX assay. Aquat.
626 Toxicol. 79, 366-375.
627 Lana, N.B., Berton, P., Covaci, A., Ciocco, N.F., Barrera-Oro, E., Atencio, A., Altamirano,
628 J.C., 2014. Fingerprint of persistent organic pollutants in tissues of Antarctic notothenioid
629 fish. Sci. Total Environ. 499, 89-98.
630 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault,
631 J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent
632 organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995-3043.
633 Mintenbeck, K., Barrera-Oro, E.R., Brey, T., Jacob, U., Knust, R., Mark, F.C., Moreira, E.,
634 Strobel, A., Arntz, W.E., 2012. 5 - Impact of Climate Change on Fishes in Complex Antarctic
635 Ecosystems. in: Ute, J., Guy, W. (Eds.). Adv. Ecol. Res. Academic Press, 351-426.
636 Mirmigkou, S., de Boer, J., 2015. DDT and Metabolites. The Handbook of Environmental
637 Chemistry, 1-24.
638 Murk, A., Legler, J., Denison, M., Giesy, J., Van de Guchte, C., Brouwer, A., 1996.
639 Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah
640 receptor active compounds in sediments and pore water. Toxicol. Sci. 33, 149-160.
641 Nash, S.B., 2011. Persistent organic pollutants in Antarctica: current and future research
642 priorities. J. Environ. Monitor. 13, 497-504.
643 Nichols, J.W., Huggett, D.B., Arnot, J.A., Fitzsimmons, P.N., Cowan‐Ellsberry, C.E., 2013.
644 Toward improved models for predicting bioconcentration of well‐metabolized compounds
645 by rainbow trout using measured rates of in vitro intrinsic clearance. Environmental
646 Toxicology and Chemistry 32, 1611-1622.
647 Renner, R., 2004. Small fish for healthy babies. Environ. Sci. Technol. 38, 262-263.

Schmid, P., Bogdal, C., Bl t hgen, N., Anselmetti, .S., wyssig, A., Hungerb hle r, K., 2010. The Missing Piece: Sediment Records in Remote Mountain Lakes Confirm Glaciers Being Secondary Sources of Persistent Organic Pollutants. Environ. Sci. Technol. 45, 203-208.
Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. Chemosphere 67, 16-21.
Sharma, C.M., Rosseland, B.O., Almvik, M., Eklo, O.M., 2009. Bioaccumulation of organochlorine pollutants in the fish community in Lake Årungen, Norway. Environ. Pollut. 157, 2452-2458.
Simonich, S.L., Hites, R.A., 1995. Global distribution of persistent organochlorine compounds. Science 269, 1851.
Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs in Arctic biota, an update. Sci. Total Environ. 408, 2874-2884.
Storelli, M., Losada, S., Marcotrigiano, G., Roosens, L., Barone, G., Neels, H., Covaci, A., 2009. Polychlorinated biphenyl and organochlorine pesticide contamination signatures in deep-sea fish from the Mediterranean Sea. Environ. Res. 109, 851-856.
Strobel, A., Burkhardt-Holm, P., Schmid, P., Segner, H., 2015. Benzo (a) pyrene metabolism and EROD and GST biotransformation activity in the liver of red-and white-blooded Antarctic fish. Environ. Sci. Technol. 49, 8022-8032.
UNEP, 2002. Antarctica Regional Report. In: Chemicals, U.N.E.P. (Ed.). Regionally Based Assessment Of Persistent Toxic Substances.
UNEP, 2008. Global Status Of DDT And Its Alternatives For Use In Vector Control To Prevent Disease. In: UNEP/POPS/DDTBP.1/2 (Ed.). Stockholm Convention on Persistent Organic Pollutants. UNEP, Geneva.
UNEP/AMAP, 2011. Climate Change and POPs: Predicting The Impacts. Report of the UNEP/AMAP Expert Group. Geneva.
Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Persp. 106, 775.
van den Brink, N., Riddle, M., Heuvel-Greve, M.v.d., Allison, I., Snape, I., van Franeker, J.A., 2009. Correspondence on Geisz et al. Melting glaciers: a probable source of DDT to the Antarctic marine ecosystem. Environ. Sci. Technol. 43, 3976-3977.
van den Brink, N.W., Riddle, M.J., van den Heuvel-Greve, M., van Franeker, J.A., 2011. Contrasting time trends of organic contaminants in Antarctic pelagic and benthic food webs. Mar. Pollut. Bull. 62, 128-132.
Vives, I., Grimalt, J.O., Fernandez, P., Rosseland, B., 2004. Polycyclic aromatic hydrocarbons in fish from remote and high mountain lakes in Europe and Greenland. Sci. Total Environ. 324, 67-77.
Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio, 10-18.
Weber, K., Goerke, H., 2003. Persistent organic pollutants (POPs) in Antarctic fish: levels, patterns, changes. Chemosphere 53, 667-678.
Wolschke, H., Meng, X.-Z., Xie, Z., Ebinghaus, R., Cai, M., 2015. Novel flame retardants (N-FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. Mar. Pollut. Bull. 96, 513.518.
Xie, ., M l ler, A., Ahrens, L., Sturm, R., Ebinghaus, R., 2011. Brominated flame retardants in seawater and atmosphere of the Atlantic and the Southern Ocean. Environ. Sci. Technol. 45, 1820-1826.
Zennegg, M., Kohler, M., Gerecke, A.C., Schmid, P., 2003. Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout. Chemosphere 51, 545-553.

Figure 1
Click here to download high resolution image

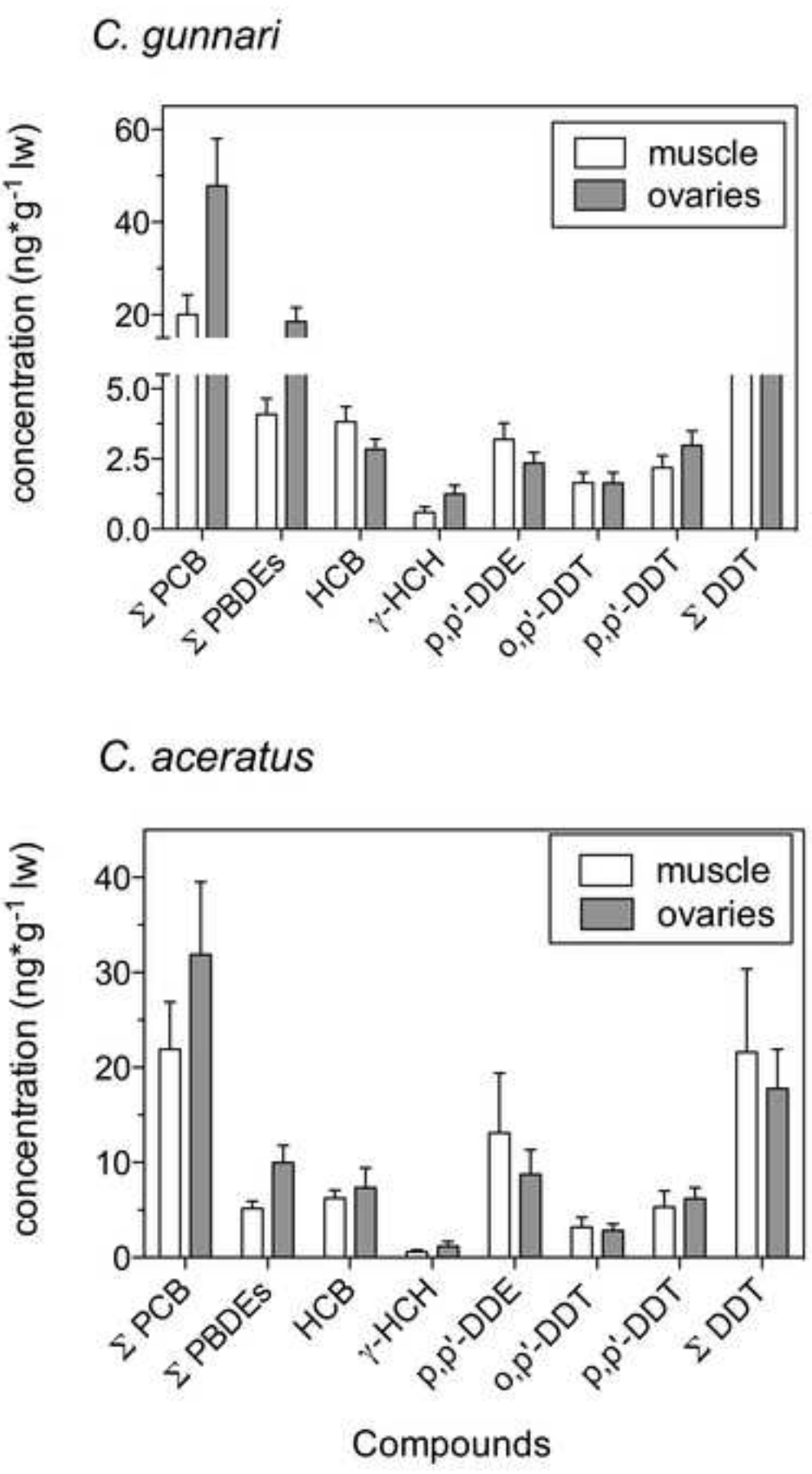
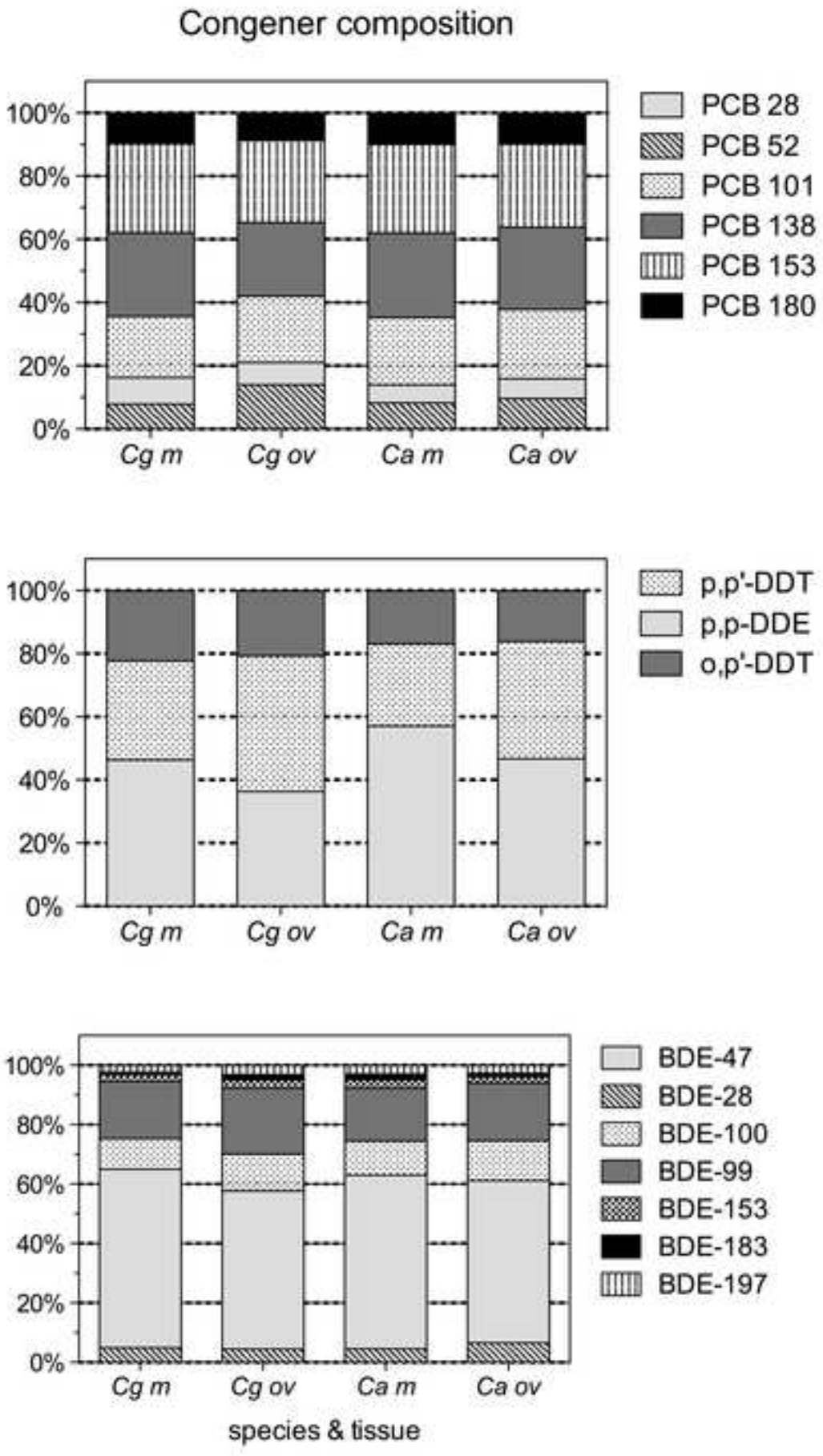


Figure 2
Click here to download high resolution image



Supplementary Material

Click here to download Supplementary Material: CHEM38908R1 Appendix A_Chemosphere.docx