Persistent organic pollutants in tissues of the white-blooded Antarctic fish

Champsocephalus gunnari and Chaenocephalus aceratus

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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 Champsoscephalus 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 benthic-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
with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pestle to obtain a fine powder.

This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi, Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and 100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml n-hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a Syncore evaporator (Büchi, Switzerland) and let dry completely by applying a gentle nitrogen stream. The residue accounted for the fat content of the sample. $^{13}$C$_{12}$ labeled internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2 – 3 mL of n-hexane the solution was treated with 3 ml oleum (7% SO$_3$ in conc. sulfuric acid). After centrifugation for 3 min at 5’000 rpm, the solvent layer with the lipophilic target analytes was removed and the remaining suspension was re-extracted two times more with n-hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and 300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and eluted with 5 ml n-hexane, followed by 5 mL n-hexane/dichloromethane 1:1 (v:v). The eluate was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at room temperature. Finally the recovery standard $^{13}$C$_{12}$ labeled PCB 70 was added. Samples were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical procedure were determined in duplicates (Table 1 and A.2).

2.3 Chemical analysis

PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118, 123, 126 156, 157, 167, 169, and 189), DDT ($o,p'$-DDT, $p,p'$-DDT, and $p,p'$-DDE), hexachlorobenzene (HCB), $\gamma$-hexachlorocyclohexane ($\gamma$-HCH), and PBDEs (BDE 28, 47, 99, 100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the target analytes in the extracts was achieved by gas chromatography/high resolution mass spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution 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 $^{13}\mathrm{C}_{12}$ 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 $^{13}\mathrm{C}_{12}$-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 $^{13}\mathrm{C}_{12}$ 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 ($s/n \geq 3$) and ten ($s/n \geq 10$) respectively. All glassware 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-tetrachlorodibenzo-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 PBDE concentrations 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 ΣPCBs (lw) of both muscle and ovary tissue, there was no
difference in PCB congener composition between the two icefish species (two-way ANOVA).

Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%) (Figure 2).

In the ovaries, levels of \( p,p' \)-DDE, \( o,p' \)-DDT and \( p,p' \)-DDT (per lw) were significantly higher in *C. aceratus* compared to those of *C. gunnari*. Also \( p,p' \)-DDT concentrations in the muscle of *C. aceratus* were higher than those in muscle of *C. gunnari* (per lw). \( \Sigma \)DDT (per lw) was significantly higher in muscle and ovaries of *C. aceratus* than in *C. gunnari* (Figure 1). Among the DDTs, \( p,p' \)-DDE showed the highest concentration of up to 57% in muscle of *C. aceratus*. \( p,p' \)-DDE tended to be higher in muscle tissue, while \( p,p' \)-DDT was slightly higher in ovary tissue in both species. \( o,p' \)-DDT was slightly higher in *C. gunnari* with ~22%, while it was only ~16% in *C. aceratus*. Detailed information on the contribution of all individual compounds measured in this study is given in Table A.2 in the appendix. \( \gamma \)-HCH was only different between the muscle tissues of the two icefish (on fw basis).

HCB concentrations per lipid weight in both muscle and ovaries were higher in *C. aceratus* than in *C. gunnari* (Figure 1). Except HCB, none of the analysed compounds exhibited a correlation with fish weight or length. HCB showed a significant linear dependence of fish weight and length in *C. aceratus*, except in the ovaries (\( R^2=0.723 \)).

In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries), followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197 were significantly different between the two tissues types and fish species. Also BDE 99 showed different concentrations between the ovaries of *C. aceratus* and *C. gunnari* on the lipid basis (Table 1, Table A.2, Figure 2).

3.2 Comparison of contaminant patterns and levels in different tissue

Lipid content was significantly higher in the ovaries of *C. gunnari* than in the ovaries of *C. aceratus*. *C. gunnari* exhibited significantly higher lipid content in ovaries than in their muscle tissue, while this was not the case for *C. aceratus* (Table 1).

A comparison between the compound concentrations in muscle and ovary tissue for each icefish species revealed higher HCB concentrations in ovary than in muscle tissue of *C.
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
with the general picture of those congeners being the dominating PBDEs in fish around the
globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota
(Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).

In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight
basis were particularly low in icefish. For example, PBDE concentrations range from about
1.0 to 8 ng g\(^{-1}\) fresh weight, or up to 64 ng g\(^{-1}\) lipid weight in various fish species from the
Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.

Although the production of the former widely used brominated flame retardants penta- and
octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in
2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still
produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and
Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).

HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per
fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere
(Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70’s, it
has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of
DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation
potential and cold condensation processes, DDTs and its metabolites are nowadays found in
biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).

The TEQs we calculated for \textit{C. aceratus} and \textit{C. gunnari} were in a similar range than TEQs
reported for muscle of the icefish \textit{C. hamatus} (TEQ 0.01-0.1 pg g\(^{-1}\) wet weight) or red-
blooded Antarctic fish (TEQ ~0.1 pg g\(^{-1}\) wet weight) (Focardi et al.; Corsolini et al., 2002a;
Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other
organisms living in less remote parts of the world, e.g. WHO\textsubscript{PCB}-TEQ (pg g\(^{-1}\) fw) of 3 to 15
in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit
muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).

In \textit{C. aceratus}, we found that the BEQs were comparable to the TEQs calculated on the basis
on the WHO\textsubscript{PCB}-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to
fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty
times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of \textit{C. gunnari}
were much higher than the WHO\textsubscript{PCB}-TEQs calculated for their ovaries. The toxicity effects
might thus be actually much higher than expected by the single usage of the calculated
WHO\textsubscript{PCB}-TEQ. In fact, the BEQs in the ovaries of \textit{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 benthopelagic *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 benthopelagic, 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

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and the Freiwillige Akademische Gesellschaft Basel. We thank Donatella Perrone and
Melanie Senn (Empa) for their helpful assistance in the laboratory.
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 icefish species.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C. aceratus (n=10)</th>
<th>C. gunnari (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (cm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47-66</td>
<td>33-50</td>
</tr>
<tr>
<td></td>
<td>Weight (g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>636-3620</td>
<td>252-888</td>
</tr>
<tr>
<td></td>
<td>lipid weight (ng g(^{-1}) lw)</td>
<td>fresh weight (ng g(^{-1}) fw)</td>
</tr>
<tr>
<td>muscle</td>
<td>0.16 0.1</td>
<td>0.47 0.14*</td>
</tr>
<tr>
<td>ovaries</td>
<td>0.53 0.57</td>
<td>0.07 0.04*</td>
</tr>
<tr>
<td>muscle</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>ovaries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ovaries</td>
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</table>

TEQs

BEQs

Blank

Mean

SEM

*
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<tr>
<th></th>
<th>28</th>
<th>52</th>
<th>101</th>
<th>138</th>
<th>153</th>
<th>180</th>
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</thead>
<tbody>
<tr>
<td>HCB</td>
<td>6.22</td>
<td>0.85</td>
<td>7.37</td>
<td>1.98</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>0.58</td>
<td>0.21</td>
<td>1.18</td>
<td>0.49</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>13.11</td>
<td>5.99</td>
<td>8.76</td>
<td>2.46</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>3.20</td>
<td>0.98</td>
<td>2.83</td>
<td>0.65</td>
<td>0.04</td>
<td>0.07</td>
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<tr>
<td>Σ DDTs</td>
<td>19.45</td>
<td>8.14</td>
<td>17.80</td>
<td>3.91</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td>Σ PCBs</td>
<td>21.91</td>
<td>4.98</td>
<td>31.87</td>
<td>7.26</td>
<td>0.26</td>
<td>0.03</td>
</tr>
<tr>
<td>Σ PCBs</td>
<td>21.91</td>
<td>4.98</td>
<td>31.87</td>
<td>7.26</td>
<td>0.26</td>
<td>0.03</td>
</tr>
</tbody>
</table>

- HCB: Hexachlorobenzene
- γ-HCH: Gamma-Hexachlorocyclohexane
- p,p'-DDE: p,p'-Dichlorodiphenyl ether
- o,p'-DDT: o,p'-Dichlorodiphenyl trichloroethane
- p,p'-DDT: p,p'-Dichlorodiphenyl dichloroethane
- Σ DDTs: Sum of DDTs
- Σ PCBs: Sum of PCBs

Units: ng/g wet weight
<table>
<thead>
<tr>
<th>Species</th>
<th>Lw</th>
<th>fw</th>
<th>TEQ (pg WHO-TEQ g⁻¹ fw)</th>
<th>BEQ (g⁻¹ fw)</th>
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<tbody>
<tr>
<td>C. gunnari</td>
<td>5.01</td>
<td>0.82</td>
<td>10.03</td>
<td>1.86</td>
</tr>
<tr>
<td>C. aceratus</td>
<td>1.45</td>
<td>0.18</td>
<td>2.64</td>
<td>0.23</td>
</tr>
</tbody>
</table>

In bold: sum (Σ) of all DDT, PCB & PBDE congeners. Lw: lipid weight, fw: fresh weight. TEQ: toxic equivalents, pg WHO-TEQ g⁻¹ 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 ≤ 0.05. The * denotes a significant difference between tissues for each species at p ≤ 0.05.
Figure Captions

Figure 1 Mean (± sem) concentration of $\Sigma$ PCBs, $\Sigma$ BDEs, HCBs, $\gamma$-HCHs and $\Sigma$ DDTs (ng g$^{-1}$ lipid weight), in muscle and ovaries of the two icefish species, *C. gunnari* ($n=11$) and *C. aceratus* ($n=10$). 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 $p \leq 0.05$.

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


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*

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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 particular, 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-hexachlorocyclohexane (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 benthic-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
with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pistil to obtain a fine
powder.

This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi,
Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and
100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml n-
hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a
Syncore evaporator (Büchi, Switzerland) and let dry completely by applying a gentle
nitrogen stream. The residue accounted for the fat content of the sample. 13C12 labeled
internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2
– 3 mL of n-hexane the solution was treated with 3 ml oleum (7% SO3 in conc. sulfuric acid).

After centrifugation for 3 min at 5'000 rpm, the solvent layer with the lipophilic target
analytes was removed and the remaining suspension was re-extracted two times more with n-
hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and
300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from
top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid
and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and
eluted with 5 ml n-hexane, followed by 5 mL n-hexane/dichloromethane 1:1 (v:v). The eluate
was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the
volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at
room temperature. Finally the recovery standard 13C12 labeled PCB 70 was added. Samples
were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical
procedure were determined in duplicates (Table 1 and A.2).

2.3 Chemical analysis

PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118,
123, 126 156, 157, 167, 169, and 189), DDT (o,p'-DDT, p,p'-DDT, and p,p'-DDE),
hexachlorobenzene (HCB), γ-hexachlorocyclohexane (γ-HCH), and PBDEs (BDE 28, 47, 99,
100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the
target analytes in the extracts was achieved by gas chromatography/high resolution mass
spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution
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 glassware used were cleaned with strongly alkaline detergents and backed out overnight in a ceramic oven at 450 °C. Directly before use, the glassware was rinsed with solvents ($n$-hexane, dichloromethane).
In order to assess total dioxin-like activity in the fish tissues, we calculated the 2,3,7,8-tetrachlorodibenzop-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.

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.

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, $\gamma$-HCH, and PBDE concentrations 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 $\gamma$-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 $\Sigma$PCBs (lw) of both muscle and ovary tissue, there was no
difference in PCB congener composition between the two icefish species (two-way ANOVA).

Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%) (Figure 2).

In the ovaries, levels of \( p,p' \)-DDE, \( o,p' \)-DDT and \( p,p' \)-DDT (per lw) were significantly higher in \( C. \) aceratus compared to those of \( C. \) gunnari. Also \( p,p' \)-DDT concentrations in the muscle of \( C. \) aceratus were higher than those in muscle of \( C. \) gunnari (per lw). \( \sum \)DDT (per lw) was significantly higher in muscle and ovaries of \( C. \) aceratus than in \( C. \) gunnari (Figure 1). Among the DDTs, \( p,p' \)-DDE showed the highest concentration of up to 57% in muscle of \( C. \) aceratus. \( p,p' \)-DDE tended to be higher in muscle tissue, while \( p,p' \)-DDT was slightly higher in ovary tissue in both species. \( o,p' \)-DDT was slightly higher in \( C. \) gunnari with ~22%, while it was only ~16% in \( C. \) aceratus. Detailed information on the contribution of all individual compounds measured in this study is given in Table A.2 in the appendix. \( \gamma \)-HCH was only different between the muscle tissues of the two icefish (on fw basis).

HCB concentrations per lipid weight in both muscle and ovaries were higher in \( C. \) aceratus than in \( C. \) gunnari (Figure 1). Except HCB, none of the analysed compounds exhibited a correlation with fish weight or length. HCB showed a significant linear dependence of fish weight and length in \( C. \) aceratus, except in the ovaries (\( R^2 = 0.723 \)).

In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries), followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197 were significantly different between the two tissues types and fish species. Also BDE 99 showed different concentrations between the ovaries of \( C. \) aceratus and \( C. \) gunnari on the lipid basis (Table 1, Table A.2, Figure 2).

3.2 Comparison of contaminant patterns and levels in different tissue

Lipid content was significantly higher in the ovaries of \( C. \) gunnari than in the ovaries of \( C. \) aceratus. \( C. \) gunnari exhibited significantly higher lipid content in ovaries than in their muscle tissue, while this was not the case for \( C. \) aceratus (Table 1).

A comparison between the compound concentrations in muscle and ovary tissue for each icefish species revealed higher HCB concentrations in ovary than in muscle tissue of \( C. \)
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\(^{\circ}\) South. They found \(\sum\)PCB concentrations in \(C.\) hamatus muscle of 0.35 ng g\(^{-1}\)

fw, \(\sum\)non-ortho PCBs of 5 ng g\(^{-1}\) fw and \(\sum\)PBDEs 0.16 ng g\(^{-1}\) fw. In muscle of \(C.\) gunnari,

they report \(\sum\)PBDEs of 0.44 ng g\(^{-1}\) fw. Those values are about five times higher than the

\(\sum\)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 \(\sum\)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 \(\gamma\)-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 \(\gamma\)-HCH were much higher in \(N.\) rossii than in \(C.\) gunnari. In contrast, \(\sum\)PCB

and \(\sum\)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 \(\gamma\)-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
with the general picture of those congeners being the dominating PBDEs in fish around the

with the general picture of those congeners being the dominating PBDEs in fish around the
globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota
(Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).

In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight
basis were particularly low in icefish. For example, PBDE concentrations range from about
1.0 to 8 ng g\(^{-1}\) fresh weight, or up to 64 ng g\(^{-1}\) lipid weight in various fish species from the
Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.

Although the production of the former widely used brominated flame retardants penta- and
octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in
2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still
produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and
Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).

HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per
fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere
(Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70’s, it
has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of
DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation
potential and cold condensation processes, DDTs and its metabolites are nowadays found in
biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).

The TEQs we calculated for *C. aceratus* and *C. gunnari* were in a similar range than TEQs
reported for muscle of the icefish *C. hamatus* (TEQ 0.01-0.1 pg g\(^{-1}\) wet weight) or red-
blooded Antarctic fish (TEQ ~0.1 pg g\(^{-1}\) wet weight) (Focardi et al.; Corsolini et al., 2002a;
Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other
organisms living in less remote parts of the world, e.g. WHO\(_{PCB}\)-TEQ (pg g\(^{-1}\) fw) of 3 to 15
in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit
muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).

In *C. aceratus*, we found that the BEQs were comparable to the TEQs calculated on the basis
on the WHO\(_{PCB}\)-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to
fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty
times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of *C. gunnari*
were much higher than the WHO\(_{PCB}\)-TEQs calculated for their ovaries. The toxicity effects
might thus be actually much higher than expected by the single usage of the calculated
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.

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Melanie Senn (Empa) for their helpful assistance in the laboratory.
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 icefish species.

<table>
<thead>
<tr>
<th></th>
<th>C. aceratus (n=10)</th>
<th>C. gunnari (n=11)</th>
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<tbody>
<tr>
<td><strong>Length (cm)</strong></td>
<td>47-66</td>
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<tr>
<td><strong>Weight (g)</strong></td>
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<td><strong>Muscle</strong></td>
<td><strong>Ovaries</strong></td>
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<td>fresh weight (ng g(^{-1}) fw)</td>
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<td>HCB</td>
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18
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 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 $p \leq 0.05$. 
Figure Captions

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

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


Borghesi, N., Corsolini, S., Focardi, S., 2008. Levels of polybrominated diphenyl ethers (PBDEs) and organochlorine pollutants in two species of Antarctic fish (*Chionodraco hamatus* and *Trematomus bernacchii*). Chemosphere 73, 155-160.


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