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Implications of biological factors on accumulation of persistent organic pollutants in Antarctic notothenioid fish

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ABSTRACT

In the present study, the possible associations between selected persistent organic pollutants (POPs) and biological factors were assessed in different tissues of two Antarctic notothenioid fish: *Notothenia rossii* (NOR) and *Trematomus newnesi* (TRN) collected at Potter Cove, King George Island/Isla 25 de Mayo, South Shetland Islands. Specifically, association patterns between biological factors (body size, lipid content, body condition) and POP concentrations (polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and metabolites, polybrominated diphenyl ethers (PBDEs), and hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), chlordanes (CHLs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs)), were explored by using two approaches: multivariate analyses (principal component analysis: PCA) and intraspecific correlations. Integrating results suggest that biological factors such as size, KI and tissue type seemed to be associated to selective accumulation of POPs for immature specimens of *N. rossii*, and KI and tissue type for mature specimens of *T. newnesi*. Each particular factor should be considered when choosing *N. rossii* or *T. newnesi* as sentinels for POPs pollution in Antarctic marine environments. Further, both nototheniids showed a selective accumulation pattern in their gonads of penta-chlorinated biphenyls (*penta*-CBs; 55.5 and 29 ng g^{-1} lw for *N. rossii* and *T. newnesi*, respectively) and organochlorine pesticides such as DDTs (199 and 13.3 ng g^{-1} lw, for *N. rossii* and *T. newnesi* respectively), and of polybrominated diphenyl ethers (PBDEs) in gills (97.2 and 22.1 for ng g^{-1} lw, for *N. rossii* and *T. newnesi*, respectively), highlighting the importance of these tissues in monitoring studies of pollution in fish. The current study expands the knowledge concerning the biological factors to be investigated when specific pollutants are monitored and supports the importance of tissue type for the selective accumulation of POPs in Antarctic fish. Additionally, a contribution to the scarce data on concentration of MeO-PBDEs in Antarctic marine organisms, particularly in the highly diverse perciform suborder Notothenioidei is provided.

1. Introduction

The perciform suborder Notothenioidei is the dominant group of the Antarctic ichthyofauna in terms of diversity (35%), abundance and biomass, containing 97% of endemic species (DeWitt et al., 1990; Eastman and Eakin, 2000). Notothenioid fish have developed a variety of feeding types and feeding behaviors on a wide range of preys such as krill (*Euphausia superba*), fish and a diversity of benthic, epibenthic, nektonic, and planktonic organisms (Daniels, 1982; Barrera-Oro, 2002). The Antarctic Notothenіids, *Notothenia rossii* (NOR) and *Trematomus newnesi* (TRN) are circum-Antarctic and typical representatives of the western Antarctic Peninsula ichthyofauna (Kock et al., 2012). They have similar ecological habits in the fjords, living commonly in shallow inshore waters from 20 to 25 m deep on rocky bottoms with macroalgae beds, to offshore shelf waters down to depths of 450 m (Kock, 1982; Tiedtke and Kock, 1989; Barrera-Oro et al., 2012). Their relative

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abundance, feeding ecology, and biological characteristics, including size and lipids content among other factors, but mainly their wide Antarctic distribution, support their usefulness as sentinels of pollution in the Antarctic marine environment.

The increasing number of reports about environmental research focused on the analysis of persistent organic pollutants (POPs) in Antarctic fish, especially in the suborder Notothenioidei, shows that this global theme is gaining concern in recent years (Goerke et al., 2004; Corsolini et al., 2006; Borghesi et al., 2008; Cipro et al., 2013; Ghosh et al., 2013; Goutte et al., 2013; Lana et al., 2014). However, it is still unclear which biological variables are associated with POP concentrations in Antarctic fish. Biological factors, like body size, body condition, tissue type, and lipids content are related to POPs accumulation in fish species (Tricklebank et al., 2002; Ríos et al., 2015). Size (either length or weight) is a measure of the time that an organism has been exposed to a contaminant and therefore, might influence the body burden of POPs (Gewurtz et al., 2011). Another important biological factor is the body condition index, which indicates how well the fish is coping with the environment (Tricklebank et al., 2002). Condition index (KI), liver index (LI), and gonadosomatic index (GI) are used to assess the physiological state of the body, the energetic reserves available for liver metabolism, and the degree of gonad development, respectively (Fechhelm et al., 1995). Lipid content in tissues is another factor associated with accumulation of POP concentrations in fish (Gewurtz et al., 2011). POPs concentrations reported in lipid-rich tissues were generally higher than those reported in other tissues owing to the apolar character of these compounds [log K_{ow} > 5.5, (Goutte et al., 2013)]. However, lipids content of tissues are dynamic and therefore could differ among season (pre- and post-spawning time) and habitat localities in terms of food availability and aquatic environment characteristics (Gewurtz et al., 2011). In fact, a recent report on Antarctic notothenioid species suggests that the accumulation pattern of POPs in different tissue types might be simultaneously conditioned by multiple factors, including physicochemical characteristic of the target POPs, tissue type and ecological characteristics of the studied species (Lana et al., 2014).

In the present study, we analyzed *N. rossii* and *T. newnesi* specimens with the aim to determine whether there are biological factors associated with POPs accumulation capability. In this sense, POP concentrations in muscle, liver, gonads, and gills tissue together with biological factors were assessed using a multivariate methodology (principal component analysis = PCA) and an intraspecific correlation approach to address this objective. It was expected that both exploratory methods will provide information on which biological factor is key when choosing notothenioid fish species as sentinels for POPs pollution in Antarctic marine environments. In addition, data of tissue distribution patterns of hexachlorobenzene (HCB), chlordanes (CHL), and the metabolite oxychlordane (OxC), and two methoxylated polybrominated diphenyl ethers (MeO-PBDEs) are reported in the present study as new data. This unpublished data set was obtained together with the previously reported data about polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and metabolites, polybrominated diphenyl ethers (PBDEs), and hexachlorocyclohexane (HCH) in the studied species (Lana et al., 2014). Our results add new information to the scarce data on POP concentrations in Antarctic marine organisms, particularly MeO-PBDEs, which is here firstly reported for notothenioid species.

2. Materials and methodology

Collection, preservation, chemicals and sample preparation, POPs analysis, and quality assurance were previously described (Lana et al., 2014), therefore each methodological subsection is briefly described below.

2.1. Collection, preservation and fish morphometry

Fish were collected at Potter Cove, King George Island/Isla 25 de Mayo, South Shetland Islands (62°14' S; 58°40' W) in summer from years 2008–2011. Specimens of *N. rossii* and *T. newnesi* were collected with trammel nets (length 25, 35 and 50 m; width 1.5 m; inner mesh 2.5 cm; outer mesh 12 cm) set for 6–96 h at rocky, macro algae beds at 5–50 m depths at three sites in the outer portion of the cove. Each specimen of *N. rossii* (n = 8) and *T. newnesi* (n = 21) was wrapped and kept in single aluminum foil and taken to the laboratory where they were measured, weighed and stored at − 20 °C until analysis. The length and weight of the fishes and their organs were measured before freezing them. The length of the fish was measured from the front-tip of the mouth to the end of the caudal fin (total length). Body condition indexes were chosen to reflect several vital physiological functions that can be affected by POPs intake and bioaccumulation (Hanson and Larsson, 2009). Hence, condition index (KI), liver index (LI), and gonadosomatic index (GI) assess the physiological state of the body, the food reserves available for hepatic metabolism and the degree of gonad development, respectively (Fechhelm et al., 1995). Indexes were calculated as follows: condition index: KI = $(W_{fish}/L_{fish}^3) \times 100$; liver index: LI = $(W_{liver} / W_{fish}) \times 100$; and gonadosomatic index: GI = $(W_{gonad}/W_{fish}) \times 100$ (Ondarza et al., 2011, 2012), where W_{fish} and L_{fish} represent the fish weight and length, respectively; and W_{liver} and W_{gonad} are the wet weight of both organs. Values of these indexes higher than 1 indicate healthy fish conditions (KI, LI), and a sexual activity stage (GI) (Tricklebank et al., 2002; Ondarza et al., 2011, 2012).

2.2. Chemicals and sample preparation

The following compounds were included in the analysis: 23 PCB congeners (*penta*-CBs: 99, 101, 105, 118; *hexa*-CBs: 128, 138, 146, 149, 151, 153, 156; *hepta*-CBs: 170, 171, 174, 177, 180, 183, 187; *octa*-CBs: 194, 195, 199; *nona*-CB: 206; *deca*-CB: 209), 7 PBDE congeners (*tri*-BDE: 28; *tetra*-BDE: 47; *penta*-BDEs: 99, 100; *hexa*-BDEs: 153, 154; *hepta*-BDE:183), HCH isomers (α-, β-, γ-HCH), DDT and metabolites (*p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *p,p′*-DDT), HCB, 3 CHLs (CN, TN, OxC); and 2 MeO-PBDEs (6-MeO-BDE-47, 2′-MeO-BDE-68). All individual standards were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany), with the exception of PBDE standard mixtures that were purchased from Wellington Laboratories (Guelph, Ontario, Canada). General chemicals, such as acetone, n-hexane, dichloromethane (DCM), isooctane (all pesticide grade), and sulfuric acid (analytical grade) were purchased from Merck (Darmstadt, Germany). Silica gel 60 (63–230 mesh) and anhydrous Na_2SO_4 (Merck, Germany) were pre-washed with hexane aliquots and dried afterwards. Before use, silica gel and Na_2SO_4 were heated at 150 °C for 24 h. Extraction thimbles were pre-extracted (1 h) with the solvent-extraction mixture used for the samples and dried at 100 °C for 1 h.

Specimens and dissected organs were lyophilized before analysis. Methods used for the extraction and clean-up were previously validated (Covaci et al., 2006). Muscle, liver, gonads, and gills tissues were freeze-dried at − 55 °C and 33 Pa until constant weight (ca. 72 h). Dried tissue aliquot of muscle (~ 2 g), liver (~ 0.8 g), gonads (~ 0.8 g) or gills (~ 1 g) was homogenized in an agate mortar, mixed with Na_2SO_4, and spiked with internal standards (IS): 10 ng CB-143, 2 ng ε-HCH and 1 ng BDE-77. The homogenate was then Soxhlet-extracted with 100 mL n-hexane: acetone (3:1, v/v) for 2 h. An aliquot (ca. 1/10) of the resulting extract was taken and weighed for the determination of lipid content by gravimetric technique (Roosens et al., 2008). The remaining extract was further cleaned up on ~ 8 g acidified silica (H_2SO_4 44%, w/w) column; and analytes were eluted with 20 mL hexane and 15 mL DCM. The eluent was rotary evaporated to ~ 2 mL, further evaporated to incipient dryness under a gentle N_2 stream, and finally reconstituted with 150 μL isooctane.