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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 Notothenioids, *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.

Table 1
Mean ± standard deviation, and range (in brackets) of size, and body condition indexes in *Notothenia rossii* (NOR) and *Trematomus newnesi* (TRN). KI: condition index; LI: liver index; GI: gonadosomatic index.

Species	Total length (mm)	Total weight (g)	KI	LI	GI
NOR	302 ± 46 (240–380)	452.04 ± 178.06 (204–736)	1.42 ± 0.11 (1.26–1.57)	1.05 ± 0.20 (0.85–1.30)	0.67 ± 0.32 (0.44–0.9)
TRN	207 ± 14 (186–240)	109.61 ± 24.72 (66.20–158)	1.20 ± 0.12 (1.01–1.49)	2.44 ± 0.82 (1.53–4.58)	2.49 ± 0.80 (1.48–4.01)

2.3. POPs analysis

Detection and quantification of analytes were carried out by using an Agilent 6890-5973 GC-MS instrument (Agilent, USA) equipped with an electron capture negative ionization (ECNI) source, and a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J & W Scientific, Folsom, USA). Ion source, quadrupole, and interface temperatures were set at 170, 150, and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 mL min^{-1}), with methane as moderating gas. The electron multiplier voltage was set at 2200 V. An aliquot of the extract (1 μL) was injected in solvent vent mode (vent time 1.25 min, vent flow 54.2 mL min^{-1}, splitless time 1.50 min; initial injector temperature at 92 °C, maintained for 0.03 min, then heated at 700 °C min^{-1} to 300 °C and maintained for 30 min). Temperature of the DB-5 column was programmed from 90 °C (1.25 min) to 310 °C at a rate of 10 °C min^{-1} holding for 6.75 min. Bromine isotope ions (*m/z* 79 and 81) were acquired in selected ion monitoring (SIM) mode for the whole run time. For PCBs and organochlorine pesticides, two most intense characteristic ions were monitored in specific time segments according to elution characteristics (Ali et al., 2013; Jaspers et al., 2013). Dwell times were 20–25 ms.

2.4. Quality assurance

A procedural blank was analyzed every ten samples. This was carried out for each type of tissue analyzed. Procedural blanks were consistent among them (relative standard deviations -RSD < 30%, Table S1). These values were used for correcting compounds concentrations by subtraction of the average blank value. Method limits of quantification (LOQ) were calculated as 3*SD of the procedural blanks. For compounds not detectable in blanks, LOQs were calculated from a signal to noise ratio of 10 (Ael et al., 2012). LOQs for the analyzed POPs and main metabolites ranged from 0.005 to 3.50 ng g^{-1} dry weight (dw) for muscle, from 0.01 to 5.50 ng g^{-1} dw for liver, from 0.02 to 11.50 ng g^{-1} dw for gonads and, from 0.01 to 3.20 ng g^{-1} dw for gills. Recovery of internal standards was above 70%. A standard reference material SRM 1945 (PCBs, organochlorine pesticides: OCPs, PBDEs, and MeO-BDEs in whale blubber) was used to test the accuracy of the method. The results demonstrated good agreement with the certified values and suitable repeatability for individual compounds (RSD between 1% and 16%, Table S2). Recovery values were above of 92%.

2.5. Statistical analysis

Statistical analyses were carried out using the software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). For calculations of sums and means, a value of *f* *LOQ was assigned to concentrations of compounds < LOQ, where '*f*' is the proportion of determinations with concentrations above the LOQ (or the detection frequency) (Covaci et al., 2008a). PCA were carried out with the statistical software InfoStat® and used to identify POPs distribution patterns among the tissue types in both fish species. In order to address this exploratory approach systematically, three separate PCA were accomplished by categorizing the determined POPs into three groups: PCBs, PBDEs, and OCPs (HCHs, DDTs, HCB, CHLs). Factor loadings > 0.45 were considered significant (Hair et al., 2010) and used for interpreting PCA patterns. The non-parametric Spearman's Rho correlations were used to explore for possible intraspecific associations among fish morphometry, body condition status and POP concentrations in each specific tissue. The relationship between contaminant concentrations, expressed as ng g^{-1} wet weight (ww), and lipid content in each specific tissue was also assessed using Spearman's correlations. A p value < 0.05 was considered significant.

3. Results and discussion

3.1. Morphometry, physiological condition and lipid content of N. rossii and T. newnesi

Details of fish morphometry and body condition are included in Table 1. Both nototheniids, *N. rossii* and *T. newnesi*, showed good body condition (KI > 1), with relatively high energetic reserves in liver [LI > 1; (Ondarza et al., 2011)]. Gonadosomatic index for *N. rossii* had on average a value of GI < 1, indicating immature gonadal development, and therefore, a non-reproductive stage. In the case of *T. newnesi*, the specimens presented a GI > 1, on average, indicating that they were in developing maturity stages. Literature data on the gonadal reproductive stages in similar sizes of *N. rossii* and *T. newnesi* in the same site and season give support to these findings (Eastman and Barrera-Oro, 2010; Eastman et al., 2011).

Lipid contents in tissues of *N. rossii* and *T. newnesi* specimens are showed in Table 2 and Fig. S3. Specimens of *T. newnesi* report the higher levels of lipids in all sample tissue. In this species, muscle reported the highest levels of lipids (8.3%), while in liver, gills and gonads, the lipid content were 6.2%, 5.1% and 1.9%, respectively (Fig. S3). For *N. rossii*, the highest lipid content was found in liver (4.2%), followed by muscle, gills and gonads (2.7%, 2.1%, and 1.1%, respectively). Significant differences in lipid content were found between *N. rossii* and *T. newnesi* in muscle, gonads and gills (P < 0.05, Table 2 and Fig. S3) while no differences were found for liver tissue (Table 2, Fig. S3).

3.2. POP concentrations in Antarctic notothenioids

This work continues the research line of a recent publication by our group (Lana et al., 2014), where *N. rossii* and *T. newnesi* are nototheniid species considered in both studies. In the previous study, concentrations of PCBs, PBDEs, DDT and metabolites, and HCH were determined in muscle, liver, gonads, and gills tissue. The following compounds were not included before because they are minority and therefore are reported here for the first time: HCB, CHL related compounds: cis-nonachlor (CN), trans-nonachlor (TN), and oxychlordane (OxC), and two MeO-PBDEs (6-MeO-BDE-47 and 2′-MeO-BDE-68). All analyzed samples indicated detectable concentrations of the target POPs. The contribution of target POP families to the total load was leaded by PCBs and DDTs, while the minority compounds were MeO-PBDEs for *N. rossii* and *T. newnesi* (Fig. S1). The HCB concentrations ranged from 0.72 (*T. newnesi,* liver) to 15.31 ng g^{-1} lw (*N. rossii,* gills), and the values were in the same order of magnitude for the two nototheniid species. HCB showed comparable distribution patterns in different tissues of *N. rossii*

Table 2
Mean and standard deviation (SD) of the not previously published POP concentrations (ng g^{-1} lw), mean lipid content in percentage and minimum and maximum values of lipids (between brackets) in *Notothenia rossii* (NOR) and *Trematomus newnesi* (TRN).

	NOR (n = 8)						**TRN (n = 21)**					
	Muscle		Liver		Gonads		Gills		Muscle		Liver		Gonads		Gills	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lipids (%)	**2.71*** (1.99–3.93)	**0.74**	**4.19^{ns}** (1.72–6.33)	**1.62**	**1.16*** (0.56–1.65)	**0.41**	**2.14*** (1.64–3.31)	**0.57**	**8.28** (1.82–15.55)	**3.83**	**6.18** (2.91–13.98)	**2.80**	**1.95** (1.31–2.42)	**0.37**	**5.15** (1.24–15.94)	**3.66**
HCB	**8.31^{ns}**	**2.01**	**1.13^{ns}**	**1.17**	**13.79^{ns}**	**2.29**	**15.31^{ns}**	**4.62**	**7.32**	**3.16**	**0.72**	**0.72**	**11.45**	**5.34**	**11.38**	**3.9**
TN	1.38* (60.5%)	0.41	3.79^{ns} (70.6%)	2.2	3.766^{ns} (92.3%)	2.5	3.11^{ns} (77.9%)	1.07	2.48 (67.5%)	1.55	2.5 (71.8%)	1.76	3.39 (85.6%)	0.93	3.77 (75.5%)	1.74
OxC	0.6^{ns} (26.3%)	0.36	0.51^{ns} (9.5%)	0.43	< LOD	a	0.43^{ns} (10.7%)	0.46	0.58 (15.8%)	0.43	0.22 (6.3%)	0.23	< LOD	a	0.56 (11.2%)	0.43
CN	0.29* (12.7%)	0.11	1.07^{ns} (19.9%)	0.62	0.32* (7.7%)	0.11	0.45^{ns} (11.4%)	0.3	0.6 (16.3%)	0.4	0.76 (21.8%)	0.54	0.567 (14.3%)	0.21	0.66 (13.2%)	0.5
ΣCHL	**2.28***	**0.88**	**5.37^{ns}**	**3.26**	**4.08^{ns}**	**2.61**	**3.99^{ns}**	**1.83**	**3.67**	**2.38**	**3.48**	**2.53**	**3.96**	**1.14**	**4.99**	**2.67**
2-MeO-BDE68	< LOQ	a	< LOQ	a	< LOQ	a	< LOQ	a	0.07	0.09	< LOD	a	< LOQ	a	0.07	0.1
6-MeO-BDE47	1.13*	0.83	3.77^{ns}	2.58	0.78*	0.44	1.07*	1.02	3.08	1.39	2.47	1.81	2.55	0.95	3.10	2.07
ΣMeO-BDE	**1.13***	**0.83**	**3.77^{ns}**	**2.58**	**0.78***	**0.44**	**1.07***	**1.02**	**3.15**	**1.48**	**2.47**	**1.81**	**2.55**	**0.95**	**3.17**	**2.1**

Total (in bold) = ΣCHL = Σ(TN, CN, OxC); ΣMeO-PBDE = (2-MeO-BDE68, 6-MeO-BDE47); < LOD: below detection limit; < LOQ: below quantification limit. [a] no standard error could be calculated since only one value was available or values are < LOD and < LOQ. Asterisks (*) indicate significant differences between species for lipid and contaminant concentrations for the same tissue type (*$P < 0.05$; ns: not significant, Mann–Whitney *U*test). Relative contribution (%) of TN, OxC, CN to the ΣCHLs for each tissue sample, is provided between brackets.

and *T. newnesi*. The highest HCB concentrations were found in gills (15.31, and 11.45 ng g^{-1} lw for *N. rossii* and *T. newnesi*, respectively) followed by gonads (13.79 and 11.45 ng g^{-1} lw for *N. rossii* and *T. newnesi*, respectively) and muscle (8.31 and 7.32 ng g^{-1} lw for *N. rossii* and *T. newnesi*, respectively). The lowest HCB value was found in liver tissue of both species (Table 2). Concerning ΣCHL (sum of TN, CN, and OxC), the two notothenids showed comparable total concentrations (15.7 and 16.1 ng g^{-1} lw, for *N. rossii* and *T. newnesi*, respectively) and the relative contribution of each CHL components to the ΣCHL load showed the following pattern: TN > CN > OxC. Among the analyzed tissues, liver, gonads and gills showed comparable ΣCHL concentrations (Table 2).

To our best knowledge, this is the first report of MeO-PBDEs in muscle, liver, gonads and gills of *N. rossii* and *T. newnesi*. Unlike the other POPs considered in this study (PCBs, OCPs, and PBDEs), MeO-PBDEs are not commercially produced and they are not reported as by-products of industrial processes (Vetter, 2006). The predominant MeO-PBDEs in the environment are the tetrabrominated 6-MeO-BDE-47 and 2′-MeO-BDE-68 (Vetter et al., 2011). Total MeO-PBDE concentration (hereinafter MeO-PBDEs) ranged from 0.78 in gonads to 3.77 ng g^{-1} lw in liver of *N. rossii*. For *T. newnesi,* the highest concentrations were found in gonads and muscle (3.17 and 3.15 ng g^{-1} lw, respectively). Considering MeO-PBDE concentrations for all tissues combined, it was almost double in *T. newnesi* (11.4 ng g^{-1} lw) than that in *N. rossii* (6.8 ng g^{-1} lw). The congener 6-MeO-BDE-47 mostly contributed (98%) to the MeO-PBDEs, while 2′-MeO-BDE-68 had a minor contribution in the analyzed tissues of both species. MeO-PBDEs profiles with predominance of 6-MeO-BDE-47 were reported also in other marine fish species with different position in the food web, including, hollowsnout grenadier (*Trachyrinchus trachyrinchus*), roughsnout grenadier (*Coelorhynchus coelorynchus*), Atlantic salmon (*Salmo salar*), and arctic cod (*Cadus callarias*) (Sinkkonen et al., 2004; Vetter et al., 2007; Weijs et al., 2009; Covaci et al., 2008b). MeO-PBDEs can be considered as PBDEs metabolites, but are also known as natural halogenated compounds produced by algae, sponges and other marine organisms, and can biomagnify through the food web (Vetter et al., 2002; Losada et al., 2009). However, the mechanism of MeO-PBDE uptake in the fish and their metabolism is not yet fully understood. A plausible explanation regarding the differences in the concentrations observed between the two species could be the differences in the MeO-PBDE profiles and concentrations in the prey species and in the water column (Covaci et al., 2008b).

3.3. Multivariate statistical analysis

PCA was carried out to examine possible relationships between concentrations of POPs in different tissue samples and biological factors of both studied species. In order to address this exploratory approach systematically, three separate PCA were carried out by categorizing the analyzed POPs into three groups: PCBs, PBDEs, and OCPs (DDTs, HCHs, HCB, and CHLs). A specific PCA for MeO-PBDEs was carried out. This analysis included six variables, considering the determined analytes (6-MeO-BDE-47 and 2′-MeO-BDE-68) and four biological factors (total weight, total length, condition index, and lipid content). The result of this PCA did not show any association patterns among the data (Fig. S2). This results could be attributed to the lack of robustness in the data ordering due to the small number of variables available for carrying out the PCA (Hair et al., 2010). The biological factors LI and GI were excluded as variables to run PCA due to the lack of data of POPs in tissue samples of liver and gonads for some of the fish specimens collected. PCA results for PCBs showed three components that explained 76% of the variability of the data set (Table S3). The first component accounted for 55% of the data variability, and was positively associated with concentrations of PCB congeners in Antarctic notothenids, *N. rossii* and *T. newnesi*. The second component accounted for 13% of the data variability, and showed positive loadings for *penta*-CBs (CB-99, 101,

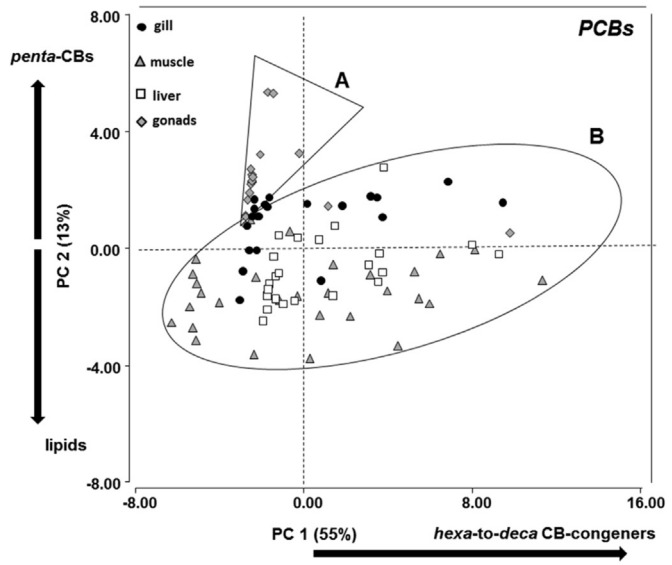

Fig. 1. Position of *Notothenia rossii* and *Trematomus newnesi* data in the plane defined by the first two principal components carried out with concentrations of 23 PCB congeners, size, condition factor (KI), and lipid content. The PCB concentration concentrations in each tissue were previously reported in Lana et al. (2014).

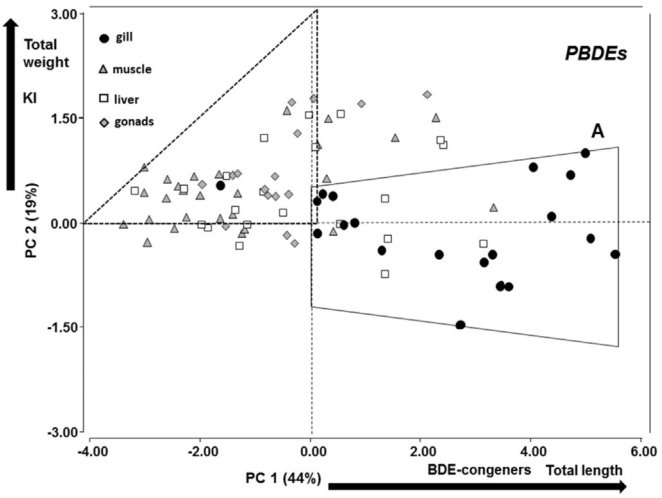

Fig. 2. Position of *Notothenia rossii* and *Trematomus newnesi* data in the plane defined by the first two principal components carried out with concentrations of 7 PBDE congeners, size, condition factor (KI), and lipid content. The PBDE concentrations in each tissue were previously reported in Lana et al. (2014).

-105, and -118) and a negative loading for lipid content. The heaviest fish specimens scored high on the third component, representing 8% of data variance (Table S3). Principal component (PC) 1 was plotted against PC 2 with the four types of tissue indicated by different color symbols (Fig. 1). Data points located on the upper left side showed a group for gonads tissue of both fish species (cluster A), separated from the overlapped data for muscle, liver and gill tissue (cluster B). Gonads are a low-fat tissue (Fig. S3) with relatively high concentrations of *penta*-CBs homologues (CB-99, -101, -105, and -118) and low concentrations of *hexa*-CB to *deca*-CB congeners. This suggested a selective tissue-dependent accumulation of the low-chlorinated PCBs, which have a log K_{ow} < 6.6 (Goutte et al., 2013). On the other hand, higher chlorinated congeners readily accumulate in lipid-rich tissue and therefore, were mainly reported in liver and muscle tissues (Storelli et al., 2009). Taking into account the results, it can be assumed that diet is the main uptake route of PCBs into the fish rather than water column. The higher the K_{ow} of a compound is, the lower its water solubility and its concentration in water is (Goutte et al., 2013). On the other hand, the higher the K_{ow}, the higher the bioaccumulation factor is (Gobas et al., 2009), and therefore a higher concentration in the prey items of the study specimens than in the water column, could be expected. This assumption is supported by previous studies conducted on two other Antarctic notothenioid species, crocodile icefish (*Chionodraco hamatus*) and Emerald rockcod (*Trematomus bernacchii*) (Corsolini et al., 2006; Borghesi et al., 2008; Cipro et al., 2013; Ghosh et al., 2013). For both species, authors proposed that concentrations of PCBs reported in lipid-rich tissues, such as liver, were the result of biomagnification process of these substances through the diet.

The second PCA carried out for PBDEs identified three components that explained 78% of the variability of the data set (Table S4). The PC 1 accounted for 44% of the variation and was positively associated with BDE congener concentrations (except BDE-183) and fish total length. The second component accounted for 19% of the variation and was positively associated with fish total weight and KI (Fig. 2). Specimens with fatty tissues scored high on the third component, which represented 15% of total variance (Table S4). In both nototheniids, *N. rossii* and *T. newnesi*, gills showed the highest PBDE concentrations and clustered together (Fig. 2, cluster A), suggesting a key role of this organ to accumulate PBDEs for the two studied species. Gills are in continuously contact with the external medium and were thus, the main uptake route of pollutants from the water column (Playle, 1998; Ondarza et al., 2011). The observed results were expected considering the benthonic habits of *N. rossii* and *T. newnesi* (Barrera-Oro, 2002), thus, both species might be exposed to PBDEs adsorbed to suspended particles, as well as seabed (Lana et al., 2014). Additionally, it was considered the fact that bioconcentration and biomagnification processes are directly related to the fish size. Furthermore, in general, the largest fish are piscivorous (Waltham et al., 2013), which involves a gradual increase in contaminant intake (Gobas et al., 2009). Therefore, it was expected that the highest PBDE concentrations were found in larger specimens of *N. rossii* and *T. newnesi*, as reported for other fish species (Kuo et al., 2009; Waltham et al., 2013; Ríos et al., 2015). Muscle and gonads samples of the heaviest specimens with a healthier body condition seem to be weakly grouped (Fig. 2, connected with dashed line) and associated with low PBDE concentrations. This clustering pattern are consistent with previous reports where is also shown that the healthiest studied fish had the lowest concentrations of contaminants in their tissues (Podolska et al., 2016; Gundersen et al., 2008), and are in agreement with the correlational approach presented and discussed below, Section 3.4.

The PCA conducted for OCPs identified three components that explained 64% of the variability of the data set (Table S5). The first component showed that liver had the highest concentrations of OxC for both fish species (cluster A, Fig. 3). Since OxC is the major metabolite of chlordanes generally reported in vertebrates, a plausible explanation could be related to the action of hepatic cytochrome P450 oxidase enzymes involved in phase I of CHL metabolism (Corsolini et al., 2006). In the right side of Fig. 3, gonad data was grouped (cluster B) reporting the highest concentrations of HCH, HCB, TN and *p,p′*-DDE. Organochlorine compounds, such as HCHs, can be transferred from the liver to ovaries during the fish breeding season (Singh and Singh, 2008). This could partially explain the high concentrations of OCPs found in gonads and could be a potential threat to the reproductive success for notothenioid species.

Subsequently, in order to identify interspecific differences in POP accumulation patterns among fish species by using the same multivariate statistical methodology, four supplementary PCAs were performed setting by species as partition tool (Fig. S4). Except for MeO-PBDEs, no clear differences among species (i.e., species-specific clusters) were identified for PCB, PBDE, and organochlorine compound concentrations (Fig. S4a–c; respectively). Interspecific differences in the association patterns of MeO-PBDEs were identified among species: higher concentrations 6-MeO-BDE-47, and 2′-MeO-BDE-68 were

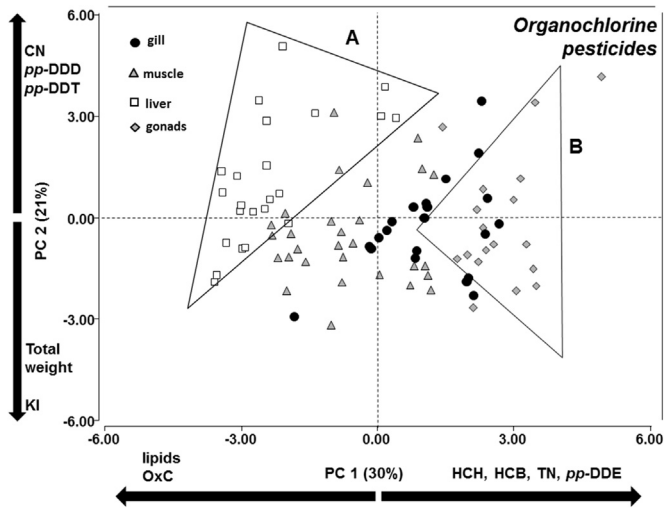

Fig. 3. Position of *Notothenia rossii* and *Trematomus newnesi* data in the plane defined by the first two principal components carried out with concentrations of organochlorine pesticides (and metabolites), size, condition factor (KI), and lipid content. The DDT and metabolites, and HCH concentrations in each tissue were previously reported in Lana et al. (2014). The HCB, and CHL related compounds concentrations in each tissue are new data.

associated with the lipid content in tissues for *T. newnesi* (Fig. S4d, bottom cluster), separated from low concentrations of both compounds for *N. rossii* (Fig. S4d, top cluster). Two plausible explanations on the difference in the MeO-PBDE concentrations detected between species could be attributable to species-specific differences in lipid content (Table 2, Fig. S3) or differences in MeO-PBDE concentrations in their prey as stated above (Covaci et al., 2008b).

3.4. Intraspecific associations between POP levels and biological factors

Results of Spearman correlations carried out to explore intraspecific associations among POP concentrations in fish tissues and biological factors (lipid content, total weight, total length, and body condition status indexes: KI, LI, GI) are summarized below:

Significant positive correlations were found among the total weight of *N. rossii* and: i) the concentrations of HCB and BDE-47 in gonads; ii) the concentrations of CN in muscle; iii) the concentrations of 6-MeO-BDE-47 in gill (Fig. 4a). Also significant positive correlations were found between the total length of *N. rossii* and the concentrations of BDE-100 in liver and 6-MeO-BDE-47 in gill (Fig. 4b). KI values were negatively associated with the concentrations of PCB congeners (CB-101, -118, -151, -180, -187, -194, -195, and -199) and PBDE congeners (BDE-28) in gills (Fig. 5). Spearman correlation coefficients indicated that there were no significant associations among POP concentrations and the remaining biological factors (lipid content, LI, and GI) in *N. rossii*. Concerning *T. newnesi*, significant negative correlations were found among KI and concentrations of TN, BDE-99, BDE-28, 6-MeO-

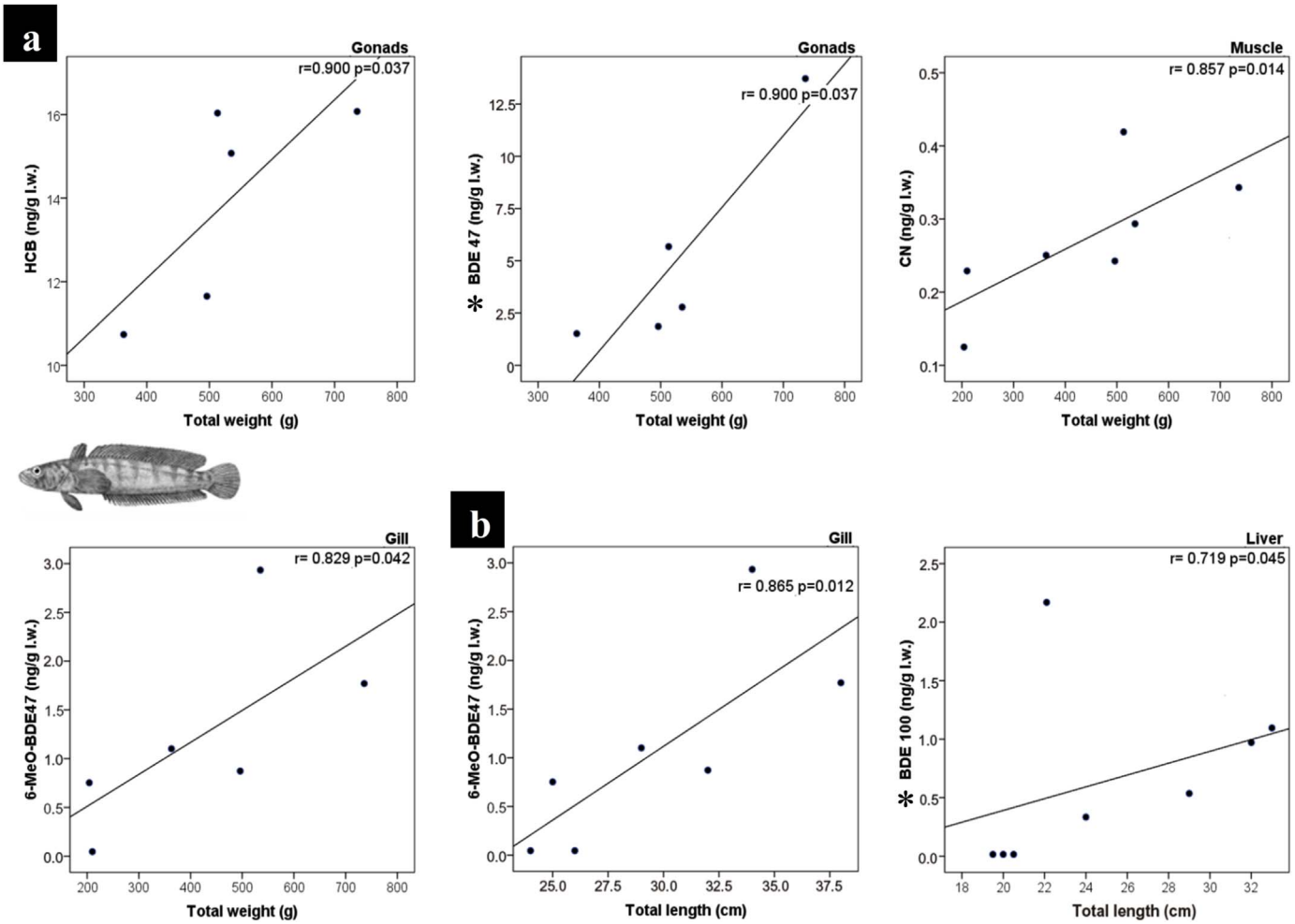

Fig. 4. Relationships between POP concentrations in tissues and a) total weight and b) total length in *Notothenia rossii*. Asterisk (*) in the *y* axis indicates previously reported POP concentrations in a particular tissue (Lana et al., 2014).

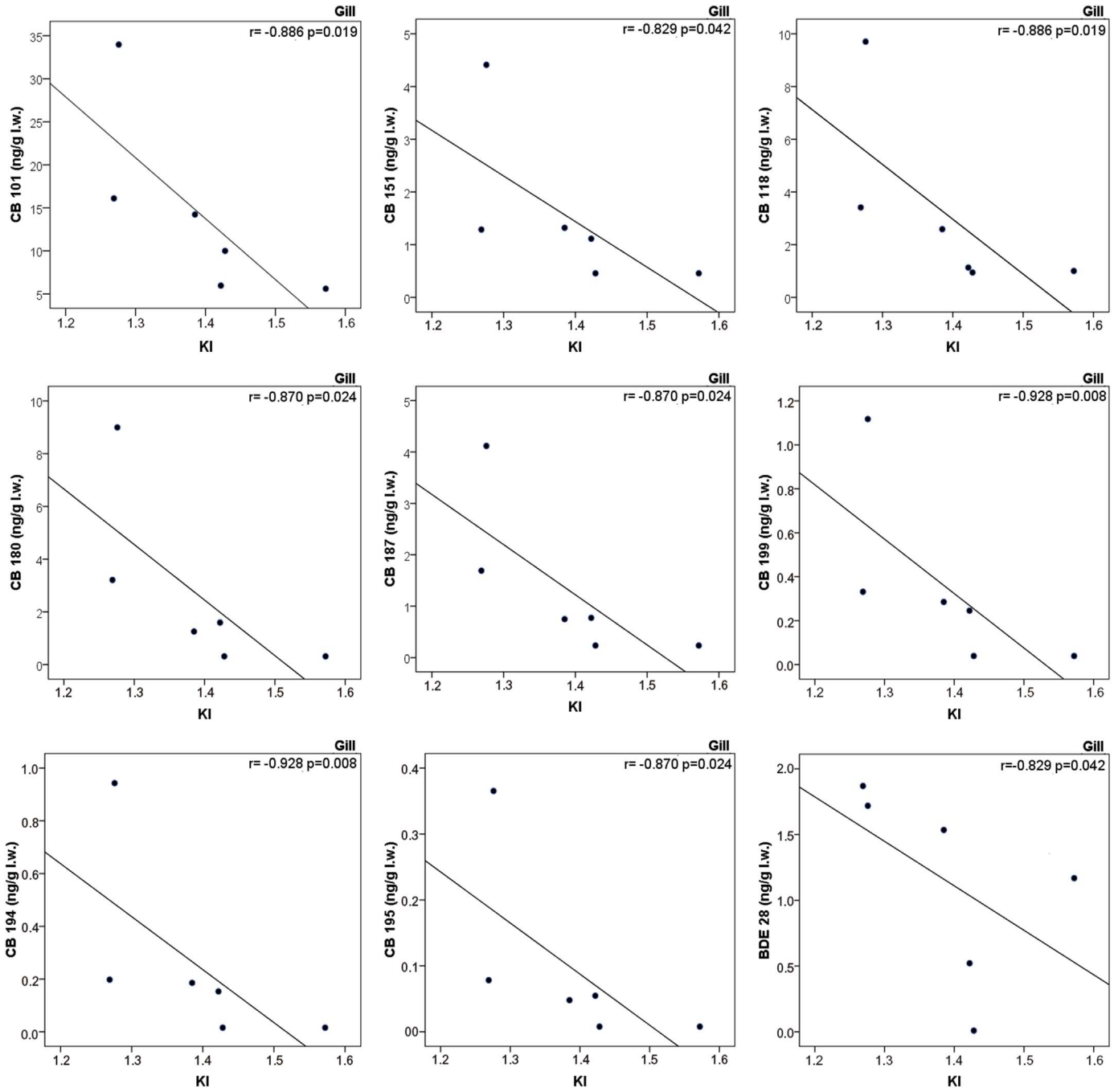

Fig. 5. Relationships between PCB congener concentrations in gill and condition index in *Notothenia rossii*. The PCB concentrations in a particular tissue were previously reported in Lana et al. (2014).

BDE-47, and *p,p′*-DDT in gonads; and concentrations of CN and 6-MeO-BDE-47 in gill (Fig. 6). Furthermore, significant negative correlations were found among GI and concentrations of CB-99, and *p,p′-DDE* in gonads of *T. newnesi* (Fig. 6). For remaining biological factors (lipid content, size and LI) of *T. newnesi*, no other associations were found.

By using the correlational approach, the intraspecific analysis showed that in *N. rossii*, fish size (weight and length) was clearly associated with the concentrations of HCB, BDE-47, BDE-100, 6-MeO-BDE-47 and CN. The influence of fish size on POPs accumulation capacity was previously reported (Kuo et al., 2009; Waltham et al., 2013; Ríos et al., 2015). The increased concentration of POPs with size could be due to differences in food habits, pollutants uptake and detoxification rates, as well as to POPs exposure over time (Tricklebank et al., 2002). For example, during the juvenile stage, *N. rossii* have a broad diet with lower-energetic value food items (e.g. algae). A dietary shift occurs in this species during their adult stage, when smaller fish and krill constitute their main prey (Barrera-Oro, 2002). Fish tend to consume only what they can swallow, so larger fish will usually eat larger prey and thus may feed at a higher trophic level than smaller fish (Ríos et al., 2015). In this sense, the computed positive associations could be explained by the feeding behavior of the large fish, which involves a higher intake of pollutants through diet.

The intraspecific correlational approach also showed negative relationships among KI and several POPs in gill tissue of *N. rossii* (CB-101, -118, -151, -180, -187, 194, -195, -199; and BDE-28) and *T. newnesi* (CN and 6-MeO-BDE-47). This association could be linked with morpho-physiological key functions of this organ. Gills have a wide diffusion surface for gaseous exchange, osmotic and ionic regulation, acid–base

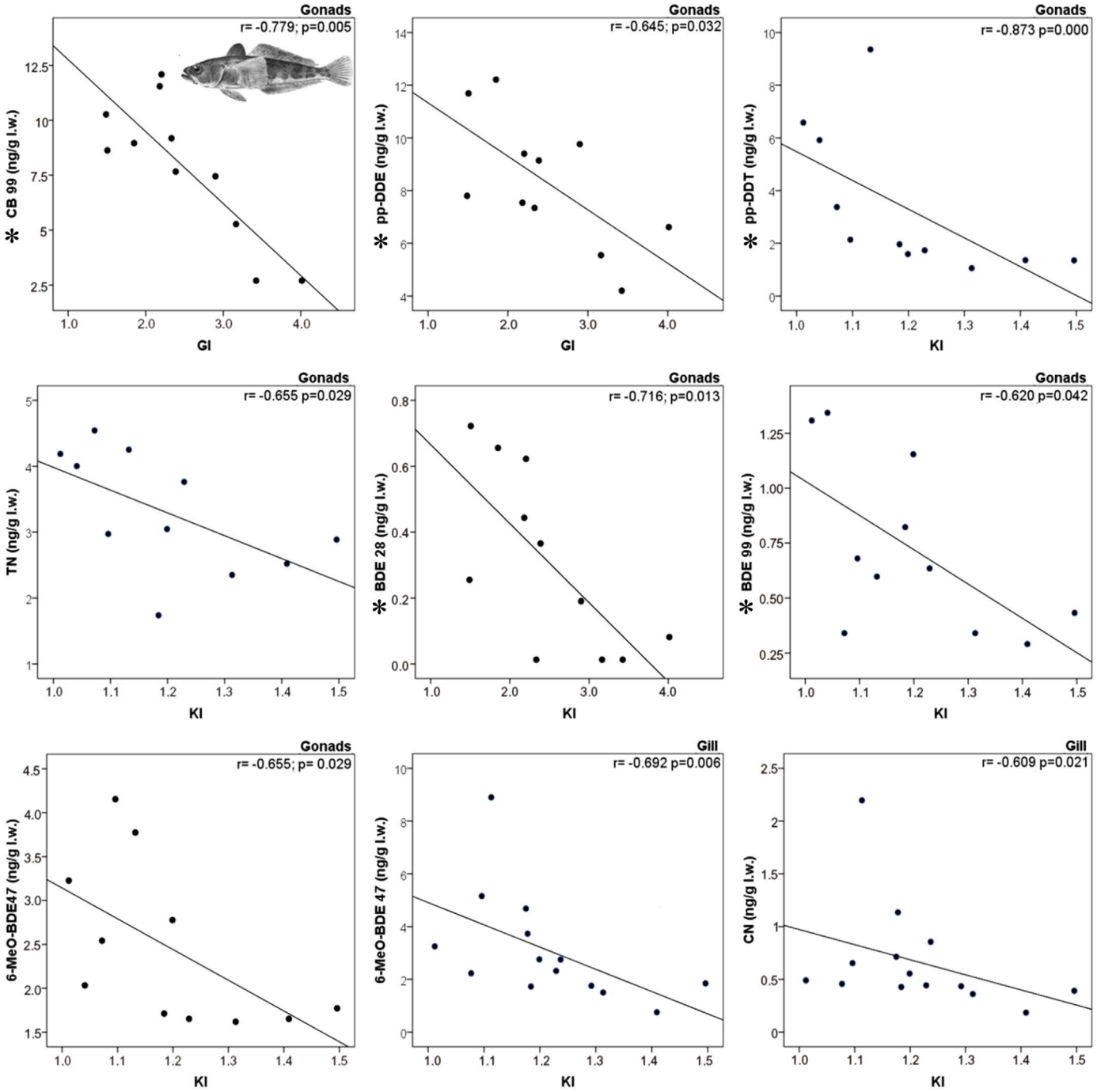

Fig. 6. Relationships between POP concentrations in gonads and gills and body condition indexes in *Trematomus newnesi*. Asterisk (*) in the *y* axis indicates previously reported POP concentrations in a particular tissue (Lana et al., 2014).

balance and nitrogenous waste excretion (Ahmad et al., 2008), contributing with homeostasis maintenance. Therefore, gills are in continuously contact with the external medium and thus are an uptake route of pollutants from the water column as well as seabed (Playle, 1998). Furthermore, the position of the gills between the venous and arterial circulation allows the accumulation of chemicals on it, which were absorbed by other route of exposure as food intake (Levine and Oris, 1999). Condition index, such as KI, provides simple and rapid indications on overall fish health status and may change in response to environmental stress. In general, pollutants have a negative effect on KI by reducing food availability and/or by increasing the energy required to maintain homeostasis (Goede and Barton, 1990). In this sense, it is expected that the healthiest specimens (i.e., with higher KI values) are those with lower concentrations of pollutants in an organ exposed to the environment conditions such as gills.

Several studies have shown that fish reproduction is an important path for excretion of POPs (Bodiguel et al., 2009; Arai, 2013; Ríos et al., 2015), due to loose of pollutants of the gonads during spawning. The specimens of *T. newnesi* analyzed in this study were mature, indicating a stage of sexual activity (Table 1). In situations where fish are in their mature stage, spawning is expected (Arai, 2013). In this study, individuals of *T. newnesi* with a better body condition (i.e., higher KI value) were those who showed lower load of certain contaminants in their gonads (Fig. 6). In this sense, the negative associations found between KI and concentrations of p,p'-DDT, TN, BDE-28, BDE-99, and 6-MeO-BDE-47 in gonad of *T. newnesi*, could be due to spawning events

occurred in the previous reproductive season, although more studies on the reproductive ecology of this species are needed to affirm this assertion. *T. newnesi* also showed negative associations between GI and the concentrations of CB-99, as well as p,p'-DDE in their gonads. This pattern was expected to occur as result of gonad disruption in response to exposure to toxins (Tricklebank et al., 2002). For example, in wild marine fish species as swordfish (*Xiphias gladius*), European hake (*Merluccius merluccius*), flatfish (*Limanda limanda*), and black sea turbot (*Scophthalmus maximus*, var. *maeotica*), an abnormal gonad development was associated to the concentrations of TN, PCBs and *p,p'*-DDE in this tissue (Stefanelli et al., 2004; Stentiford and Feist, 2005; Bodiguel et al., 2009; Malakhova et al., 2014). Particularly, male summer flounder (*Paralichthys dentatus*) showed lower GI values and histopathological changes indicative of regressed gonads when exposed to *p,p'*-DDE in laboratory experiments (Mills et al., 2001). Further, Monteiro et al. (2015) elucidated the mechanisms underlying endocrine effects during gonad development in zebrafish (*Danio rerio*) when exposed to sublethal concentrations of *p,p'*-DDE. They demonstrated the mode of action of *p,p'*-DDE to cause endocrine disruption in zebrafish during gonad differentiation of juvenile specimens (Monteiro et al., 2015).

4. Conclusions

The implication of biological factors involved in POP concentrations in tissues was assessed by multivariate analyses and intraspecific correlations in two Antarctic notothenioid species, *N. rossii* and *T. newnesi*. Integrating results of both exploratory approaches helped to identify association patterns among biological factors and POP concentrations according to the tissue type. Multivariate approach identified the following patterns for both species: gonad tissue is a representative organ for monitoring PCBs (mainly *penta*-CBs), HCHs, HCB, TN and *p,p'*-DDE; and gill tissue for monitoring PBDEs. The highest OxC concentration was found in the liver of both nototheniids and was also associated with total lipid content. The importance of several biological factors on the accumulation of POPs was also corroborated by the intraspecific correlation approach: the biological factors that showed to be associated with selective accumulation of POPs for immature specimens of *N. rossii* were size, KI and tissue type; while for mature specimens of *T. newnesi*, were KI and tissue type. Each particular factor should be considered when choosing *N. rossii* or *T. newnesi* as sentinels for POPs pollution in Antarctic marine environments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.08.009.

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