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Lipid and Fatty Acid Composition, and Persistent Organic Pollutant Levels in Tissues of Migrating Atlantic Bluefin Tuna (*Thunnus thynnus,* L.) Broodstock

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Abstract

Lipid class, fatty acid and POP levels were measured in migrating Atlantic bluefin tuna (ABT) tissues caught off the Barbate coast, Spain. Tissue lipids were largely characterized by triacylglycerol, reflecting large energy reserves accumulated prior to reproductive migration. Fatty acid compositions of muscle, liver and adipose exhibited similar profiles, whereas gonads showed a higher affinity for docosahexaenoic acid. Tissue POP concentrations correlated positively with percentage triacylglycerol and negatively with polar lipids. Highest POP concentrations were in adipose and lowest in gonads, reflecting lipid content. DL-PCBs contributed most to total PCDD/F+DL-PCB levels, with mono-*ortho* concentrations higher in tissues, whereas non-*ortho* PCBs contributed greater WHO-TEQs due to differences in TEFs. PBDE47 was the most prominent BDE congener in tissues, probably through biotransformation of BDE99 and other higher brominated congeners. The perceived POP risk from ABT consumption should be balanced by the well-established beneficial effects on human health of omega-3 fatty acids.

Key words: Atlantic bluefin tuna, *Thunnus thynnus*, Persistent organic pollutants (POPs), PCDD/Fs, DL-PCBs, PBDEs, Lipids, Fatty acids

Capsule

Lipids, fatty acids (particularly omega-3) and POPs were determined in Atlantic Bluefin tuna to examine tissue distribution, and assess the risk-benefit to human health.

Introduction

Lipids are an integral nutrient of marine ecosystems with structural and energy storage roles (Sargent et al., 1989). However, persistent organic pollutants (POPs) including dioxins [polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)], dioxin-like polychlorinated biphenyls (DL-PCBs) and polybrominated diphenyl ethers (PBDEs) have a high affinity for lipids. These ubiquitous environmental contaminants partition between the storage (neutral) and structural (polar) lipid compartments of aquatic organisms where they are deposited, and subsequently have the potential to accumulate through the food chain (Elskus et al., 2005). Several physiological mechanisms, such as lipid mobilisation during starvation periods, may affect POP tissue concentrations as the trophic transfer of POPs and lipids may follow similar pathways (Kainz et al., 2009).

Fish, like other animals, store energy primarily in the form of triacylglycerols (TAG) in tissues such as muscle, liver and mesenteric adipose tissue (Ackman, 1980). Lipid, and thus TAG levels within fish tissues varies according to nutritional state based upon age, sex and developmental or reproductive status. Seasonal variations in fish lipid levels are generally related to the reproductive cycle and, prior to sexual maturation, large lipid deposits are accumulated and subsequently mobilized to support gonadal development and spawning migration (Bell, 1998).

The Atlantic bluefin tuna (ABT; *Thunnus thynnus*) is an important ecological, recreational and commercial species within the Atlantic and Mediterranean ecosystems (Fromentin and Powers, 2005; Rodríguez-Roda, 1964). The ABT fishery has become highly profitable with the sushi-sashimi markets of Japan increasing demand for high quality fish, stimulating high market prices that have led to increased regulated and unregulated fishing (Fromentin and Powers, 2005). From late-April to mid-June, ABT pass through the Strait of Gibraltar on their way to spawning grounds located around the Balearic Islands and Tyrrhenian Sea (Medina et al. 2002; Mourente et al., 2002; Rodríguez-Roda, 1964). Local

fishermen take advantage of this migration by using traditional tuna traps (almadraba) off the southwest coast of Spain.

From an ecotoxicological viewpoint, ABT are of interest since they can accumulate lipophilic POPs due to their high trophic position, long lifespan and continuous migratory habits (Corsolini et al., 2005). Furthermore, fish consumption is a source of human exposure to environmental contaminants including POPs (Sidhu, 2003; Storelli et al., 2003), as well as being the main source of the highly beneficial omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which have important roles in human health, including promoting cardiovascular health and protecting against neurological and inflammatory conditions (Calder and Yaqoob, 2009; Dewailly et al., 2001). Although POP levels in Mediterranean ABT have been reported (Borghesi et al., 2009; Corsolini et al., 1995, 2005, 2007; Di Bella et al., 2006; Kannan et al., 2002; Pena-Abaurrea, 2009; Porte and Albaigés, 1993; Stefanelli et al., 2002; Storelli et al., 2008; Vizzini et al., 2010), different POPs and congener profiles were analysed which often result in data between studies not being directly comparable. However, in most cases the more toxic congeners, *i.e.* those assigned toxic equivalency factors (TEF) underscored by the World Health Organisation (WHO) based on relative toxicity to 2,3,7,8-TCDD (Van den Berg et al., 1998), were determined. Through TEF inclusion the concentrations of less toxic compounds can be weighted to 2,3,7,8-TCDD and expressed as toxic equivalents (TEQ), subsequently used in risk assessment and regulatory control.

The present study examined POP levels, total lipid contents, lipid class and fatty acid compositions in tissues of ABT caught in traditional tuna traps during spawning migration. The risk-benefit associated with POP and n-3 LC-PUFA intake from ABT consumption is discussed.

Materials and Methods

Sample collection

Wild ABT were caught using traditional tuna traps (Almadraba) off the Barbate coast (Cádiz, Spain) during May 2006. Six randomly selected individuals (2 male, 4 female), weighing between 180-310 kg with lengths of 211-254 cm, were dissected and samples of muscle (skinned and deboned), liver, gonad and adipose tissue removed for analysis. Samples were wrapped in aluminium foil and stored at -70°C until analysis. Fish age was estimated according to Rodríguez-Roda (1964). Fish condition (K) was calculated using Fulton's coefficient K=(W/L)^{3}×100 (Ricker, 1979), where W is wet weight (g) and L total length (cm). These fish were representative of maturing ABT broodstock migrating to Mediterranean spawning grounds according to age and length data (Corriero et al. 2005; Medina et al. 2002), and histological data from previous studies using the same sample site showing fish of non-spawning mature status (Abascal et al. 2004; Medina et al. 2002; Mourente et al. 2002). Individual sample analysis was performed for lipid content, class and fatty acid composition, whereas samples of similar lipid contents from same sexed fish were pooled and analysed for POPs (see Table 1).

Solvents and chemicals

Solvents were HPLC or Pesticide grade and 95-97% sulphuric acid of analytical reagent grade (Fisher Scientific, Loughborough, UK). Concentrated sulphuric acid (Aristar®, sp. gr. 1.84) was purchased from BDH (Poole, UK). Anhydrous sodium sulphate and nonane puriss p.a. standard for GC were obtained from Sigma-Aldrich (Poole, UK). Nanopure water was collected from a Milli-Q ultrapure purification system (0.22 µm; Millipore, UK). Hydromatrix (Varian Inc., USA) was used after heating overnight at 400°C in a muffle furnace.

The 29 PCDD/F and DL-PCB congeners with WHO-TEF values (Van den Berg et al., 1998) and nine PBDE congeners (IUPAC numbers 28, 47, 49, 66, 99, 100, 153, 154, 183)

were targeted in samples. PBDE119 and ^{13}C-labelled PCDD/F (EPA-1613LCS) and PCB (WP-LCS) were used as internal standards, PCDD/F (EPA-1613ISS) and PCB (68A-IS) as recovery standards and PCDD/F (EPA-1613CVS) and PCB (WP-CVS) as calibration standard solutions (Wellington Laboratories, Ontario, Canada).

Lipid content, lipid class and total lipid fatty acid composition

Total lipid was extracted from tissues by homogenizing in 20 volumes of ice-cold chloroform/methanol (2:1 v/v) using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) according to Folch et al. (1957) and quantified gravimetrically.

Lipid classes were separated by double development high-performance thin-layer chromatography (HPTLC) using methyl acteate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) and isohexane/diethyl ether/acetic acid (85:15:1, by vol.) as first and second development systems, respectively (Henderson and Tocher 1992). Classes were visualized by charring at 160°C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (Version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with winCATS Planar Chromatography Manager. Identities of individual classes were confirmed by comparison with reference to Rf values of authentic standards run alongside samples.

Fatty acid methyl esters (FAMEs) from total lipid were prepared by acid-catalyzed transmethylation at 50°C for 16 h (Christie, 1993). FAME were extracted and purified as described previously (Tocher and Harvie, 1988) and separated and quantified by GC using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionization detection. Hydrogen was used as carrier gas with initial oven thermal gradient from 50°C to 150°C at 40°C.min^{-1} to a final temperature of 230°C at 2°C.min^{-1}. Individual FAME were identified by comparison to known standards (Supelco™ 37-FAME mix;

Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A., Milan, Italy). Fatty acid content per g of tissue was calculated using heptadecanoic acid (17:0) as internal standard.

POP extraction and clean-up

Homogenates of 25-50 g wet weight tissue were freeze-dried (12 h minimum) prior to accelerated solvent extraction (ASE™100; Dionex, Camberley, UK). Freeze-dried tissue was mixed with hydromatrix, transferred to an ASE cell and 5 $ng.ml^{-1}$ PBDE119 and 2 $ng.ml^{-1}$ ^{13}C-labelled PCDD/F and PCB internal standards added. Samples were extracted by ASE for 20 min x 2 cycles with isohexane under pressure (1500 psi) and temperature (125°C). The 120 ml extracts were mixed with 95-97% sulphuric acid in separation funnels and left for 72 h. The separated solvent layer was rinsed with nanopure water and dried by passing through anhydrous sodium sulphate.

Further clean-up and fractionation of analytes was performed using the automated Power-Prep™ system (Fluid Management Systems, USA). Extracts were loaded for lipid removal and isolation of interfering compounds following conditioning of the disposable column series, consisting of multi-layered silica (4 g acid, 2 g base, 1.5 g neutral), basic alumina (8 g) and carbon (2 g). A high capacity silica column (28 g acid, 16 g base, 6 g neutral) was used for high lipid samples. Total run time was 150 min followed by a 40 min decontamination programme. The mono-*ortho* PCB and PBDE fraction (F1) was eluted in 120 ml isohexane/dichloromethane (1:1 v/v) and the PCDD/F and non-*ortho* PCB fraction (F2) in 120 ml toluene. Fractions underwent further clean up with F1 transferred to silanized vials containing 150 µl nonane as keeper and evaporated to 500 or 100 µl prior to analysis for PBDE and mono-*ortho* PCBs respectively, and F2 transferred to conical GC autosampler vials containing 10 µl of nonane as keeper and evaporated to 50 or 10 µl prior to analysis for non-*ortho* PCB and PCDD/F respectively.

Instrumental analysis

Mono-, non-*ortho* PCBs and PCDD/Fs were analysed using a PolarisQ™ ion trap MS/MS coupled to a Trace GC-2000 (Thermo Finnigan, Bremen, Germany) equipped with a 30 m x 0.25 mm i.d. x 0.25 μm Rxi®-5ms (5% diphenyl, 95% dimethyl polysiloxane) fused-silica capillary column (Thames Restek Ltd., Saunderton, UK). GC oven temperatures were; Mono-*ortho* PCBs: initial temperature of 140°C (2 min hold) to 180°C at 25°C.min^{-1}, 205°C at 2°C.min^{-1} (11 min hold), 235°C at 3°C.min^{-1} to final temperature of 280°C at 6°C.min^{-1} (4 min hold). Non-*ortho* PCBs: initial temperature of 140°C (2 min hold) to 220°C at 15 °C.min^{-1}, 250°C at 1.2°C.min^{-1}, 270°C at 4°C.min^{-1} (6 min hold) to final temperature of 300°C at 10°C.min^{-1} (4 min hold). PCDD/Fs: initial temperature of 140°C (2 min hold) to 230°C at 40°C.min^{-1}, 250°C at 1.2°C.min^{-1}, 270°C at 4°C.min^{-1} (6 min hold) ramped to final temperature of 300°C at 10°C.min^{-1} (4 min hold). Helium was used as carrier gas at constant flow (0.8 ml.min^{-1}). Injector temperature was 250°C, with samples and standards (2 μl) injected in the splitless injection mode. MS operating conditions were in positive electron ionisation (EI+) mode using automatic gain control with electron energy of 70 eV and emission current 250 μA. Transfer line and ion source were maintained at 305°C and 250°C, respectively. Quantification of PCDD/F and DL-PCBs was based on US Environmental Protection Agency isotopic dilution methods (EPA, 1994, 1999). Relative response factors (RRFs) for individual 2,3,7,8-chlorosubstituted PCDD/F and DL-PCB congeners were determined using calibration standards. Xcalibar™ version 1.3 was used for data acquisition and results processing.

For PBDE analysis, 1 μl was injected in splitless mode (225°C, 1.5 min) with surge (240 kPa) on a Trace GC-Ultra™ equipped with a 30 m x 0.25 mm i.d. x 0.25 μm ZB5-MS column (Phenomenex, Cheshire UK) coupled to a Trace DSQ™ MS (Thermo Finnigan, Bremen, Germany) operating in negative chemical ion mode (CI^{-}). Initial GC temperature

was 100°C (2 min hold) to 200°C at 15°C.min^{-1}, 300°C at 4°C.min^{-1} (5 min hold) to final temperature of 325°C at 30°C.min^{-1} (15 min hold). Ion source and interface temperatures were 200 and 320°C respectively. Helium was used as carrier gas at constant flow (1.2ml.min^{-1}) and methane as reagent gas (2.0ml.min^{-1}). The MS operated in selective ion monitoring (SIM) mode by monitoring bromide isotope ions (*m/z* 81 and 79) with dwell time of 80 ms. Quantification of PBDE congeners was performed by congener-specific linear calibration curves (r^2>0.99). Xcalibar™ version 1.4 was used for data acquisition and results processing.

Quality assurance (QA) and quality control (QC)

Samples were ran with a procedural blank, standard reference material (CRM-0641 PCDD/Fs and PCBs in cod liver oil, FAPAS®; CSL, UK) and 'in-house' reference material, consisting of pooled salmon flesh. Limits of detection (LOD) were determined using a software option for estimating signal-to-noise (S/N) ratio, where limit of quantification (LOQ) was three times LOD (nine times S/N ratio). LOQs were in the range of 0.01-0.03 pg.g^{-1} ww for PCDD/Fs, 0.1-0.5 pg.g^{-1} ww for non-*ortho* PCBs, 2.1-3.9 pg.g^{-1} ww for mono-*ortho* PCBs and 18-63 pg.g^{-1} ww for PBDEs. Recovery values for PCDD/Fs and DL-PCBs, based on congener-specific response factors of ^{13}C internal surrogate standard relative to ^{13}C performance standards (EPA, 1994), were in the range of 78-114%. Percentage recoveries for PBDEs, based on spiked sample matrix with internal standards for all congeners, were in the range of 79-118%. Method performance was further validated through annual participation in 'Interlaboratory Comparison on Dioxin in Food' proficiency tests organized by the Norwegian Institute of Public Health (Liane et al., 2011), with good results for PCDD/F, DL-PCB and PBDE congeners.

Statistical analysis

Results are presented as mean and standard deviation. Statistical analyses were performed using Minitab® v.16.1.0 statistical software (Minitab Inc.). Data were assessed for normality with Kolmogorov-Smirnov test and for homogeneity of variances by Bartlett's test and examination of residual plots and, where necessary, transformed using the natural logarithm or arcsine transformation. Data were compared by one-way analysis of variance (ANOVA), with post hoc comparisons using Tukey's test (Zar, 1999). Relationships between variables were assessed using Pearson's correlation coefficient (r). Runs test was used to check linearity, with failed data indicating non-linear relationships (GraphPad Instat® v.3.01). A significance of $P<0.05$ was applied to all statistical tests performed.

Results and Discussion

Lipid content, class and total lipid fatty acid composition

Adipose tissue presented the highest lipid content (73%), followed by liver, muscle, testes and ovary (Table 2). Total polar lipids, predominantly phosphatidylcholine and phosphatidylethanolamine, ranged from 7% in adipose to 36% in testes, with intermediate levels of about 21% in muscle, liver and ovary, but TAG predominated in the tissues of migrating ABT. Adipose tissue showed the highest TAG proportion (89%), followed by muscle, liver and gonads. Tissue TAG levels were similar to those reported in early maturational stages (I and II) of migrating female ABT sampled from the Straits of Gibraltar (Mourente et al., 2002) and Messina (Salvo et al., 1998). Tuna entering spawning grounds through the Strait of Gibraltar often arrive with empty stomachs (Rodríguez-Roda, 1964), relying upon accumulated lipid stores (TAG) to fuel gonadal development and spawning migration. Wax/steryl esters (WE/SE) were not detected in muscle and adipose tissue and were minor components in liver and testes but, in ovaries, they comprised almost 27% of total lipid, similar to other marine fish species from temperate waters (Bell, 1998). It is unclear why marine fish store both TAG and WE/SE in the ovaries although it is suggested

they may provide metabolic energy and components for biomembrane formation during ABT embryogenesis (Mourente et al., 2002).

Although fatty acid compositions have been studied in numerous marine fish species, there is surprisingly few data on ABT tissues. The most abundant fatty acids in muscle lipid were oleic (18:1n-9) and palmitic (16:0), accounting for 28% and 21% of total fatty acids, respectively, with the major PUFA being the n-3 LC-PUFA, DHA (12%) and EPA (5%) (Table 2). Fatty acid compositions of liver and adipose closely resembled that of muscle. Similar profiles were reported in both ABT muscle (Mourente et al., 2002; Salvo et al., 1998; Popovic et al., 2012) and liver (Mourente et al., 2002), regardless of capture site. Fatty acids such as 16:0 and 18:1n-9, together with 20:1n-9 and 22:1n-11 are presumed major sources of metabolic energy in fish, being catabolized during growth and gonad formation (Sargent et al., 1989). In contrast, fatty acid compositions of gonads were largely characterized by 16:0, 18:1n-9 and DHA in equal portions of 20-25% (Table 2). This significant accumulation of PUFA, particularly DHA, in gonads relative to other tissues is considered important in fish for physiological functions including sperm motility and function (Jeong et al., 2002) and biomembrane formation during embryonic and larval development (Sargent et al., 1989, 2002). In general, n-3 PUFA are important for somatic growth of marine fish with DHA the most highly retained PUFA in a variety of species (Sargent et al., 2002). Tuna tissues consistently contain relatively high levels of DHA resulting in high DHA:EPA ratios (Mourente et al., 2002; Mourente and Tocher, 2009). In the present study, DHA:EPA ratios were significantly greater in gonads (approx. 4:1) compared to around 2.5:1 in other tissues, further highlighting the importance of DHA in gonad production. As with other marine fish, tuna have very limited capability for biosynthesis of LC-PUFA suggesting that the high proportion of DHA represents selective accumulation and retention of dietary DHA (Mourente et al., 2002).

Concentrations, TEQs and congener profiles of PCDD/Fs and DL-PCBs

Mean ∑PCDD/F levels on a wet weight basis (ww) were 0.83 $pg.g^{-1}$ ww in muscle, 1.46 $pg.g^{-1}$ in liver, 0.46 and 0.27 $pg.g^{-1}$ in ovary and testes respectively, and 5.57 $pg.g^{-1}$ in adipose (Table 3). DL-PCB concentrations in tissues were higher with mean ∑DL-PCBs levels of 5.16, 11.95, 2.14, 0.96 and 40.19 $ng.g^{-1}$ ww in muscle, liver, ovary, and adipose respectively. The WHO-TEQ concentrations mirrored wet weight concentrations with ∑PCDD/F+DL-PCBs levels lowest in gonads (0.54 and 1.19 $pgTEQ.g^{-1}$ ww, testes and ovaries respectively) and highest in adipose (20.96 $pgTEQ.g^{-1}$ ww) (Table 3). Muscle and liver presented levels of 2.39 and 5.15 $pgTEQ.g^{-1}$ ww respectively. For comparison to TEQ levels published in the scientific literature, the 1998 TEF system was used (Van den Berg et al., 1998). However, TEQ values were also calculated using 2005 TEFs (Van den Berg et al., 2006) resulting in an 18-24% reduction for ΣPCDD/F+DL-PCB WHO-TEQ_{2005} for muscle, liver, ovary, testis and gonad of 1.84 ± 0.28, 3.97 ± 0.80, 0.94 ± 0.26, 0.41 and 17.25 ± 4.30, respectively. This was consistent with Van den Berg et al. (2006) who concluded that "changes in 2005 values have a limited impact on the total TEQ of these [biotic] samples with an overall decrease in TEQ ranging between 10% and 25%". Variability between tissue concentrations was related to total lipid content. Linear regression analysis between POP concentrations and total lipid content revealed, as expected, highly significant positive relationships (r^2 0.74-0.89, $P<0.001$; Table 4) attributable to the lipophilic nature of the POPs. Thus, lipid normalized concentrations were highest in liver (94.89 $ng.g^{-1}$ lipid weight), intermediary in muscle (64.50 $ng.g^{-1}$ lw) and adipose (51.57 $ng.g^{-1}$ lw) and lowest in gonads (47.60 and 41.47 $ng.g^{-1}$ lw in ovary and testes, respectively).

In addition to total lipid, tissue POP distribution is also affected by the relative proportion of different lipid classes (Elskus et al., 2005). In the present study, tissue POP concentrations were positively correlated to TAG levels (r^2 0.73-0.83, $P<0.001$) and negatively correlated to cholesterol (r^2 0.60-0.75, $P<0.001$), total polar lipids (r^2 0.76-0.81, $P<0.001$), phosphatidylcholine (r^2 0.391-0.481, $P<0.002$) and phosphatidylinositol (r^2 0.469-0.546, $P<0.001$) (Table 4). Similar relationships were observed with another POP,

octachlorostyrene, in Arctic charr, *Salvelinus alpinus* (Jørgensen et al., 1997). In both ABT and charr the highest proportion of TAG was in adipose tissue and muscle, the most important energy storage compartments in these species and, consequently, the main tissues POPs accumulate in. Unlike some other marine fish, the liver in migratory fish such as ABT is not a lipid storage site, instead functioning as a high capacity site for lipid processing and *de novo* lipid synthesis (Abascal et al., 2004; Mourente et al., 2002). During migration, lipid stores are depleted and used for gonadal development and energy metabolism and, whereas physiologically required lipids are selectively regulated in animals, lipophilic POPs are passively bioaccumulated and their concentrations not regulated. The high lipid normalized POP content in the liver may therefore be a result of accumulation after transport of lipid from other tissues for physiological and metabolic processes. With respect to the gonads, evidence suggests that POP distribution is influenced by maternal lipid content and deposited in the developing oocytes (Elskus et al., 2005). In the present study, ovary POP levels may be lower than expected considering that lipid redistribution to gonads can be dramatic in sexually maturing fish. Histological analyses of female ABT captured at the same site in previous years showed they were sexually mature, albeit of non-spawning status (Medina et al., 2002). In the earlier study, the gonadal-somatic index and number of highly-yolked (high lipid) oocytes were greater in ABT caught around spawning grounds, suggesting that the incipient stage of maturation occurs at the Strait of Gibraltar, later than in the present study. Conversely, relatively few POPs are redistributed to testes in any fish species (Elskus et al., 2005). The higher proportion of polar lipids in the testes compared with other tissues explains the lower POP levels since polar lipids have a lower sequestering affinity for POPs.

Chain length and unsaturation of fatty acids in lipids can also influence the solubility and bioavailability of ingested PCBs (Elskus et al., 2005), and so similar correlations between individual fatty acids in total lipid and POPs were carried out in the present study. However, there were few significant results limited to DL-PCBs showing positive

relationships with 22:0, 18:4n-3 and 16:3 fatty acids (r^2 0.358-0.404, $P<0.04$), and PCDD/Fs positively correlated with 22:0, 18:3n-6 18:4n-3 and 16:3 (r^2 0.334-0.438, $P<0.003$), and negatively correlated to 16:0, 20:2n-6 and 20:4n-6 (r^2 0.327-0.338, $P<0.004$) and so the full data are not presented. The biological significance of these data is doubtful as many of these fatty acids are quantitatively rather minor. Given that percentage TAG explains 73-78% of the variation in POP levels between tissues, further consideration of the role of fatty acid composition should perhaps be based on individual lipid classes.

The DL-PCBs were the most abundant of the PCDD/F and DL-PCB groups with mono-*ortho* PCBs representing greater than 99% of total concentration levels in all tissues (Table 3). Of these, PCB118 accounted for around 60-65%, PCB105 17-23% and PCB156 5-9% of total measured levels. Mid-chlorinated, penta- through hepta-CBs, have been reported to be the dominant PCBs in tuna tissues (Corsolini et al., 1995, 2005, 2007; Gómara et al., 2005; Kannan et al., 2002; Phua et al., 2008; Storelli et al., 2008; Ueno et al., 2005; Vizzini et al., 2010). Their predominance is due to their stability, as PCB biotransformation is largely dependent upon the degree of chlorination and chlorine position on the biphenyl ring. In PCB188, chlorine atoms in the 2,4,5- positions in one or both rings are particularly resistant to metabolic degradation by cytochrome P450 isoenzymes (Storelli et al., 2008). Non-*ortho* PCBs represented less than 0.9%, in rank order PCB77>PCB126>PCB169>PCB81. Increased prevalence of PCB77 compared to PCB126 and PCB 169 was reported in skipjack tuna (*Katsuwonus pelamis*) muscle with a shift towards the northern hemisphere, indicating preferential deposition and accumulation of this congener at higher latitudes (Ueno et al., 2005).

The most prominent PCDD/F congener in all tissues was 2,3,7,8-TCDF, although this represented less than 0.01% of total PCDD/F+DL-PCB levels (Table 3). This was followed by 1,2,3,7,8,9-HxCDD>2,3,4,7,8-PeCDF in muscle; 2,3,4,7,8-PeCDF>1,2,3,7,8-PeCDF in adipose and liver; and 2,3,4,7,8-PeCDF>OCDD in gonads. The identical profiles in liver and adipose tissues may reflect the utilization of adipose tissue lipid and the role of liver in lipid

processing. A significant reduction in PCDD/F levels has been observed in commercially available fish and seafood in Spanish markets between 1995-2003 (Gómara et al., 2005). DL-PCBs also decreased over the same timescale, but not to the same extent as PCDD/Fs.

The DL-PCBs contributed 92-94% of total PCDD/F+DL-PCB TEQ levels in ABT tissues, with non-*ortho* PCBs contributing more (62-67%) than mono-*ortho* PCBs (26-30%) (Table 3). This was largely credited to PCB126 that, whilst amounting to minor concentration levels (0.3-0.4%), presented the largest TEQ concentration (61-65%) owing to its higher TEF value. Additionally, both mono-*ortho* PCB118 and PCB156 made significant contributions to total TEQ levels (11-15% and 6-9%, respectively), weighted largely by their high concentration levels. The DL-PCB TEQ contribution presented here is greater than the 76% reported in farmed Southern bluefin tuna (SBT), *T. maccoyii* (Phua et al., 2008), but similar to tuna found in Spanish markets (Gómara et al., 2005). However, there is clear evidence that PCB concentrations in marine surface waters in the northern hemisphere are much higher than southern hemisphere levels (Fowler *et al*., 1990). The less chlorinated PCDD/Fs, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDD, were the main contributors to total WHO-TEQ values as reported elsewhere (Gómara et al., 2005).

The WHO-TEQ levels were within the maximum levels set by the European Union of 8 pgWHO-TEQ PCDD/F+DL-PCB.g^{-1} ww for muscle and gonads, and 25 pgWHO-TEQ PCDD/F+DL-PCB.g^{-1} ww for liver (EC, 2006a, 2006b). The concentrations and WHO-TEQs of PCDD/Fs and or DL-PCBs found in the present study were comparable to ABT sampled from the Ionian Sea (Corsolini et al., 2005; Storelli et al., 2008), Tyrrhenian Sea (Corsolini et al., 2007), Strait of Messina (Di Bella et al., 2006; Kannan et al., 2002; Stefanelli et al., 2002), western Mediterranean coast (Porte and Albaigés, 1993), as well as tuna from Spanish commercial markets (Gómara et al., 2005) and farmed SBT (Padula et al., 2008; Phua et al., 2008), but are higher than found in wild SBT (Phua et al., 2008) (Table 5). Additionally, PCDD/F levels were similar to those previously reported in the muscle of skipjack tuna collected from offshore waters and open seas worldwide, although DL-PCB

concentrations were higher (Ueno et al., 2005). However, data between studies are often difficult to compare due to various factors including size and age of fish sampled, although differences in the number and types of congeners examined is usually the most influential.

Concentrations and congener profiles of PBDEs

All measured congeners, except BDE183 found only in adipose, were detected in all tissues. Mean ΣBDE tissue concentrations were 0.8 and 1.9 $ng.g^{-1}$ ww in testes and ovary respectively, 3.9 $ng.g^{-1}$ ww in muscle, 5.5 $ng.g^{-1}$ ww in liver, and 10.8 $ng.g^{-1}$ ww in adipose tissue (Table 6). As with PCDD/Fs and DL-PCBs, PBDEs showed a significant positive relationship with percentage lipid and TAG (r^2~0.74, P<0.001). In terms of lipid weight, concentrations were 14.7 $ng.g^{-1}$ lw in adipose, 25.1 and 41.2 in testes and ovary respectively, 43.5 $ng.g^{-1}$ lw in liver, and 48.5 $ng.g^{-1}$ lw in muscle.

The PBDE concentrations measured in the present study were comparable to those for wild and farmed ABT from the South Tyrrhenian Sea (Pena-Abaurrea et al., 2009), and similar or higher than those found in skipjack tuna from open sea areas worldwide (Ueno et al., 2004), and tuna from Spanish markets (Domingo et al., 2006), but lower than those reported in ABT off the Northern Sicilian coast (Borghesi et al., 2009) (Table 7). However, 23 BDE congeners were analyzed in the latter study (compared to 9 in the present study), although only BDE126 contributed significantly to total PBDE levels in addition to the congeners measured here. The European Food Safety Authority (EFSA) currently recommends monitoring BDE congeners #28, 47, 99, 100, 153, 154, 183 and 209 in feed and food, congeners typically prevalent in penta-, octa- and decaBDE commercial mixtures (EFSA, 2006). Compared to other Mediterranean species, PBDE levels in ABT were higher than those found in liver and muscle of swordfish, *Xiphias gladius* (Corsolini et al., 2008) and 2.5-10 x higher than concentrations measured in livers of deep-sea species, hollowsnout (*Trachyrinchus trachyrinchus*) and roughsnout (*Coelorhynchus coelorynchus*) grenadiers, when expressed in terms of lipid weight but within range when expressed in terms of wet

weight reflecting the difference in lipid storage between species (Covaci et al., 2008) (Table 7).

The most abundant PBDE congeners in ABT tissues were BDE47 and BDE100, accounting for 50% and 15% respectively of the total congeners measured (Table 6). These were generally followed in order by BDE154 (8-11%) >BDE49 (6-8%) >BDE66 (6-7%) >BDE28 (4%) >BDE99 (2-4%) >BDE153 (1-4%). Similar profiles have been reported for tuna (Domingo et al., 2006; Borghesi et al., 2009) and other Mediterranean fish species (Corsolini et al., 2008; Covaci et al., 2008). The level of BDE47 may reflect its high inclusion in commercial pentaBDE mixtures and hence its increased bioavailability. Additionally, higher levels of the more stable BDE47 have been suggested to occur through biotransformation from *meta*- and/or *para*-debromiantion of BDE99 and other higher brominated congeners (Borghesi et al., 2009; Roberts et al., 2011; Stapleton et al., 2004; Wan et al., 2008). Equally, the lower level of BDE99 may be a consequence of its preferential excretion (Isosaari et al., 2005; Kannan et al., 2000). Additionally, the absence or trace levels of BDE183 in tuna tissues may be a result of its biotransformation to BDE154 (Stapleton et al., 2004).

Concentration ratios between PBDE congeners have been studied in a variety of matrices as a way of understanding accumulation mechanisms. The BDE99:BDE100 ratio in abiotic samples (80:20) is similar to that in the industrial product Bromkal 70-5DE (84:16), but averages 30:70 in fish and marine mammals (Christensen et al., 2002). This may be a result of the higher bioavailability of BDE100 compared to BDE99, or a low biotransformation of BDE100. The BDE99:BDE100 ratios within tissues in the present study were similar, 17:83 in muscle and ovary, 20:80 in liver and adipose, and 9:91 in testes. These ratios are in agreement with those found in wild (21:79) (Borghesi et al., 2009), farmed (23:77) and wild longline- or trap-caught ABT (40:60 and 28:72) (Pena-Abaurrea et al., 2009), and in muscle and liver of swordfish (26:74 and 33:67) (Corsolini et al., 2008). Large differences in ratios have been noted amongst a variety of fish species (Wan et al.,

2008). Moreover, a significant decrease in BDE99:100 ratio with an increase in trophic level has been observed (Voorspoels et al., 2003; Wan et al., 2008). The same study found that the BDE99:BDE47 ratio also decreased with an increase in trophic level, resulting in high BDE47 concentration levels as BDE 99 is biotransformed whilst simultaneously increasing the dominance of BDE100 over BDE99. The high BDE47 levels and low BDE99:BDE100 ratios in ABT are therefore consistent with its high trophic level.

Health benefit-risk association of tuna consumption

Fish consumption is an important source of the marine n-3 LC-PUFA, EPA and DHA, which are known to benefit human health (Calder and Yaqoob, 2009; Dewailly et al., 2001). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) currently recommend a weekly intake of 3.5 g EPA+DHA for optimal cardiac health in adults (ISSFAL, 2004). Nevertheless, oily fish are also a major dietary exposure route for human POP intake (Sidhu, 2003; Storelli et al., 2003). Consequently, the European Scientific Committee on Food (SCF) have set a tolerable weekly intake level (TWI), the weekly dose to which humans can be exposed without harm, of 14 pgWHO-TEQ.kg^{-1} body wt. for PCDD/F+DL-PCBs (SCF, 2001), equivalent to 980 pgWHO-TEQ.kg^{-1} for an adult of 70kg. From the present study, a typical 130 g portion based on EFSA guidelines (EFSA, 2005) would mean ABT muscle contributing 1.22 g EPA+DHA and 310.7 pgWHO-TEQ PCDD/F+DL-PCB intake, or 34.9% and 31.7% of the weekly intake for a 70 kg adult (Fig. 1). This compares to 2.56 g (72.3%) EPA+DHA and 134.6 (13.7%) pgWHO-TEQ PCDD/F+DL-PCB in commercial farmed Atlantic salmon flesh, *Salmo salar*, analysed in our laboratory between 2006-2011. Thus, ABT muscle contains approximately half the amount of EPA+DHA and twice as much PCDD/F+DL-PCB levels as salmon flesh. Additionally, tuna roe is also popular in the Mediterranean diet, thus 130 g portions would provide 1.04 (29.7%) and 0.62 g (17.7%) EPA+DHA and 154.7 (15.8%) and 70.2 (7.2%) pgWHO-TEQ PCDD/F+DL-PCB intake for ovaries and testes, respectively. The importance

of identifying and labelling fish products in terms of risk and benefits, together with region of origin, has been suggested in order to allow consumer based choices and avoid unnecessary exposure to highly contaminated fish (Shaw et al., 2006). Whilst the above approach assesses the actual risk-benefit (POPs-omega 3 intake) from tuna consumption based on recommended limits, other methods have taken a more weighted approach in assessing human health by factoring in disease risks and consumption rates and fitting to a model (e.g. Hoekstra et al., 2012; Sirot et al., 2012).

Typical dietary guidelines recommend two portions of fish per week, with at least one portion of oily fish. In Mediterranean countries where the diet is richer in fish, reaching an average of 640 g per week (Domingo et al., 2008), the risk of exceeding TWI is greater. Although unlikely that all fish would be of ABT origin, ABT is estimated to contribute 67% of the total TEQ intake (Bocio et al., 2007). Simple trade-offs between EPA+DHA and POP intake can therefore be made through careful consumption of a mixture of edible portions of ABT and other species. Unlike PCDD/Fs and DL-PCBs, PBDEs are not currently limited by legislation although EFSA have an advisory TWI of 0.7 μg kg^{-1}.body $wt.week^{-1}$ or 49.0 μg $PBDE.week^{-1}$ for a 70 kg adult (EFSA, 2005). Correspondingly, 130 g of ABT muscle, ovary and testes would contain 504.4, 240.8 and 107.8 ng PBDE respectively, less than 1% of the PBDE advisory TWI.

Although ABT contains higher POPs than other species, e.g. farmed salmon, levels are below the current safe limits set by food safety organisations. Furthermore, POP risks arising from ABT consumption are largely perceived and should be considered in conjunction with the clear and well researched beneficial effects on human health of high quality protein and levels of essential n-3 LC-PUFA.

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Legend to Figure

Fig.1. Risk-benefit association (mean EPA+DHA and pgTEQ intake) for people consuming 130 g per week of Atlantic bluefin tuna muscle or roe (ovary and testes) in comparison to Atlantic salmon muscle. Liver and adipose tissue values are shown for comparison. Dotted line represents the 980 pgTEQ TWI for an adult of 70 kg and the recommended 3.5 g EPA+DHA intake for optimal cardiac health.

Table 1. Biological data and total lipid content (%) in tissues from six individual Atlantic bluefin tuna (*Thunnus thynnus*) sampled from the Almadraba de Barbate tuna trap (SW coast Spain) in May 2006.

Fish No.	Pool No.[1]	Sex	Body weight (kg)	Body length (cm)	Condition (*K*)	Estimated age (years)[2]	Lipid (%)			
							Muscle	Liver	Gonad	Adipose[3]
ABT 1	1	Male	310	254	1.89	14	8.5	11.7	3.6	-
ABT 2	1	Male	281	245	1.91	13	9.9	16.2	2.9	73.8
ABT 3	2	Female	264	240	1.91	12	9.2	8.8	2.6	-
ABT 4	2	Female	209	222	1.91	10	9.8	14.2	6.0	66.2
ABT 5	3	Female	180	211	1.92	9	4.5	12.6	1.9	-
ABT 6	3	Female	183	213	1.89	9	6.3	11.9	7.5	79.0

[1]Samples pooled as indicated for POP (PCDD/F, DL-PCB, PBDE) analysis only, all other analyses performed on individual fish samples

[2]Rodríguez-Roda, 1964

[3]Adipose tissue removed from ABT 2, 4 and 6 only

Table 2. Lipid content, lipid class (% of total lipid) and fatty acid compositions (% total fatty acids) of total lipid from muscle, liver, gonad and adipose tissues from Atlantic bluefin tuna (*Thunnus thynnus*) broodstock during reproductive migration. Means within rows bearing different superscript letters are significantly different ($P<0.05$).

	Muscle	**Liver**	**Ovary**	**Testes**	**Adipose**
n	6	6	4	2	3
Lipid content (%)	8.0 ± 2.2[ab]	12.6 ± 2.5[a]	4.5 ± 2.7[b]	3.3 ± 0.5[b]	73.0 ± 6.4[c]
Lipid class (% of total lipid)					
Phosphatidylethanolamine	1.3 ± 0.8[a]	2.6 ± 1.1[ab]	3.7 ± 0.8[b]	12.4 ± 1.7[c]	1.8 ± 1.3[ab]
Phosphatidic acid/cardiolipin	1.2 ± 0.9	0.7 ± 0.4	1.0 ± 0.2	1.5 ± 0.7	0.9 ± 0.6
Phosphatidylinositol	1.1 ± 0.5[ab]	1.6 ± 0.3[bc]	2.0 ± 0.3[b]	1.4 ± 0.4[abc]	0.5 ± 0.1[b]
Phosphatidylserine	1.3 ± 0.5[a]	1.4 ± 0.4[a]	1.2 ± 0.4[a]	4.5 ± 1.3[b]	0.7 ± 0.1[a]
Phosphatidylcholine	3.5 ± 1.2[ac]	4.9 ± 1.0[a]	9.6 ± 1.4[b]	11.9 ± 2.2[b]	1.5 ± 0.9[c]
Sphingomyelin	0.8 ± 0.3[ab]	1.1 ± 0.4[a]	1.8 ± 0.3[a]	1.6 ± 0.7[a]	0.3 ± 0.4[b]
Lysophosphatidylcholine	0.6 ± 0.2[a]	2.2 ± 0.5[b]	1.3 ± 0.3[ab]	1.3 ± 0.4[ab]	0.7 ± 0.6[a]
Pigmented material	12.5 ± 4.1[a]	6.0 ± 2.3[b]	1.2 ± 1.1[c]	1.5 ± 0.5[bc]	0.3 ± 0.3[c]
Total Polar	***22.2 ± 4.4[a]***	***20.4 ± 3.7[a]***	***21.9 ± 2.3[a]***	***35.9 ± 7.0[b]***	***6.7 ± 1.9[c]***
Cholesterol	8.6 ± 2.1[a]	10.9 ± 1.3[ab]	11.3 ± 1.4[ab]	15.2 ± 1.2[b]	2.0 ± 0.9[c]
Free fatty acid	9.9 ± 0.9[a]	20.6 ± 2.9[b]	9.1 ± 0.7[a]	15.0 ± 3.8[c]	2.6 ± 0.3[d]
Triacylglycerol	59.3 ± 6.5[a]	45.5 ± 7.9[b]	30.7 ± 3.6[c]	32.7 ± 13.7[bc]	88.7 ± 1.0[d]
Wax/Steryl ester	nd	2.6 ± 0.5[a]	27.1 ± 2.3[b]	1.3 ± 1.8[a]	nd
Total Neutral	***77.8 ± 4.4[a]***	***79.6 ± 3.7[a]***	***78.1 ± 2.3[a]***	***64.1 ± 7.0[b]***	***93.3 ± 1.9[c]***
Fatty acid (% total fatty acid)					
14:0	3.2 ± 0.6[a]	1.7 ± 0.5[b]	1.5 ± 0.2[b]	1.7 ± 0.2[bc]	3.0 ± 0.5[ac]
16:0	20.6 ± 1.2	23.3 ± 3.2	20.6 ± 1.5	22.2 ± 4.0	18.5 ±0.9
18:0	7.1 ± 0.4[ab]	8.2 ± 1.5[a]	6.0 ± 0.6[b]	8.5 ± 0.4[a]	6.2 ± 0.4[ab]
Total Saturates[1]	**31.9 ± 2.1[ab]**	**34.0 ± 3.9[a]**	**28.8 ± 1.8[b]**	**32.9 ± 3.4[ab]**	**28.8 ± 1.0[ab]**
16:1n-7	4.8 ± 0.7[a]	3.5 ± 0.5[bc]	3.1 ± 0.4[c]	2.7 ± 0.1[c]	4.6 ± 0.2[ab]
18:1n-9	27.7 ± 2.6[a]	29.1 ± 5.7[a]	20.3 ± 2.0[b]	19.7 ± 0.1[ab]	29.4 ± 1.4[a]
18:1n-7	3.2 ± 0.2[a]	3.8 ± 0.5[b]	3.1 ± 0.2[a]	2.9 ± 0.1[a]	3.0 ± 0.1[a]
20:1n-9	3.2 ± 0.7[a]	2.5 ± 0.4[ab]	1.5 ± 0.2[c]	2.0 ± 0.3[bc]	2.9 ± 0.3[ab]
22:1n-11	2.3 ± 1.2[a]	1.0 ± 0.5[ab]	0.7 ± 0.2[b]	0.8 ± 0.5[ab]	1.7 ± 0.4[ab]
24:1n-9	1.1 ± 0.1[a]	0.9 ± 0.2[ab]	0.8 ± 0.2[b]	1.2 ± 0.1[a]	1.0 ± 0.2[ab]
Total monoenes[2]	**43.9 ± 3.0[a]**	**42.0 ± 5.5[a]**	**30.3 ± 2.2[b]**	**30.1 ± 0.5[b]**	**43.9 ± 1.7[a]**

18:2n-6	1.2 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	0.9 ± 0.4	1.0 ± 0.1
20:4n-6	0.9 ± 0.3[a]	1.4 ± 0.5[ab]	2.9 ± 0.3[c]	2.8 ± 1.2[bc]	0.8 ± 0.1[a]
22:5n-6	0.5 ± 0.3	0.4 ± 0.3	0.8 ± 0.2	1.3 ± 1.1	0.4 ± 0.1
Total n-6 PUFA[3]	**3.1 ± 1.0**	**3.4 ± 1.1**	**5.5 ± 0.5**	**5.7 ± 3.2**	**2.7 ± 0.2**
18:3n-3	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.1
18:4n-3	1.2 ± 0.3[ac]	0.5 ± 0.2[b]	0.5 ± 0.0[b]	0.6 ± 0.1[bc]	1.3 ± 0.3[a]
20:5n-3	4.9 ± 1.1	5.0 ± 1.8	6.7 ± 0.9	5.5 ± 0.6	6.0 ± 1.1
22:5n-3	1.3 ± 0.1	1.5 ± 0.5	1.8 ± 0.3	2.2 ± 0.5	1.4 ± 0.1
22:6n-3	12.1 ± 1.5[a]	12.4 ± 3.6[a]	25.0 ± 1.9[b]	22.0 ± 0.6[b]	14.1 ± 1.0[a]
Total n-3 PUFA[4]	**20.7 ± 1.6[a]**	**20.5 ± 6.2[a]**	**35.0 ± 2.8[b]**	**30.9 ± 0.7[bc]**	**24.2 ± 1.0[ab]**
Total PUFA[5]	**24.2 ± 1.9[a]**	**23.9 ± 6.7[a]**	**40.9 ± 3.0[b]**	**37.1 ± 3.9[bc]**	**27.3 ± 0.9[ac]**
n-3/n-6	**6.7 ± 1.8**	**6.0 ± 2.2**	**6.4 ± 0.6**	**5.4 ± 3.5**	**9.0 ± 1.0**
DHA:EPA	**2.5 ± 0.8[a]**	**2.5 ± 0.4[a]**	**3.7 ± 0.4[b]**	**4.0 ± 0.6[b]**	**2.4 ± 0.6[a]**

[1]includes 15:0, 20:0, 22:0, 24:0
[2]includes 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-9
[3]includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6
[4]includes 20:3n-3, 20:4n-3
[5]includes 16:2, 16:3, 16:4
nd – not detected

Table 3. Concentration ranges of PCDD/Fs and mono- and nono-*ortho* PCBs (pg.g^{-1} wet wt) and their corresponding WHO-TEQ$_{1998}$ upperbound values (mean ± SD; pg.g^{-1} wet wt) in tissues of migrating Atlantic bluefin tuna (n=3 for muscle, liver and adipose, n=2 for ovary and n=1 for testes). ΣTEQ values within a row with different superscripts are statistically different (P<0.05)

	TEF[a]	Muscle		Liver		Ovary		Testes		Adipose	
		Conc	**TEQ**	**Conc**	**TEQ**	**Conc**	**TEQ**	**Conc**	**TEQ**	**Conc**	**TEQ**
PCDDs											
2378-TCDD	1.0000	0.011-0.035	0.025 ± 0.009	0.067-0.105	0.082 ± 0.020	0.011-0.022	0.016 ± 0.007	0.011	0.011	0.263-0.367	0.330 ± 0.058
12378-PeCDD	1.0000	0.040-0.040	0.040 ± 0.000	0.040-0.103	0.067 ± 0.031	0.040-0.040	0.040 ± 0.000	0.040	0.040	0.185-0.374	0.284 ± 0.095
123478-HxCDD	0.1000	nd-0.009	0.002 ± 0.000	0.009-0.020	0.002 ± 0.000	0.009-0.009	0.002 ± 0.000	nd	nd	0.009-0.025	0.002 ± 0.000
123678-HxCDD	0.1000	0.020-0.020	0.002 ± 0.000	0.020-0.020	0.002 ± 0.000	0.020-0.020	0.002 ± 0.000	0.020	0.002	0.037-0.142	0.009 ± 0.005
123789-HxCDD	0.1000	0.070-0.162	0.010 ± 0.005	0.007-0.007	0.001 ± 0.000	0.007-0.007	0.001 ± 0.000	0.007	0.001	0.010-0.022	0.001 ± 0.001
1234678-HpCDD	0.0100	0.015-0.030	<0.0002	0.015-0.015	<0.0002	0.015-0.015	<0.0002	0.015	<0.0002	0.019-0.041	<0.0003
OCDD	0.0001	0.012-0.081	<0.0001	0.012-0.029	<0.0001	0.023-0.077	<0.0001	0.022	<0.0001	0.032-0.101	<0.0001
ΣPCDD		**0.25±0.02**	**0.08±0.02^{a}**	**0.21±0.05**	**0.15±0.05^{b}**	**0.14±0.04**	**0.06±0.01^{a}**	**0.12**	**0.05^{ab}**	**0.83±0.19**	**0.63±0.16^{c}**
PCDFs											
2378-TCDF	0.1000	0.188-0.493	0.032 ± 0.016	0.589-1.082	0.079 ± 0.026	0.134-0.151	0.014 ± 0.001	0.024	0.002	2.328-3.599	0.299 ± 0.064
12378-PeCDF	0.0500	0.030-0.062	0.002 ± 0.001	0.064-0.104	0.004 ± 0.001	0.012-0.032	0.001 ± 0.001	0.012	0.001	0.311-0.509	0.022 ± 0.005
23478-PeCDF	0.5000	0.051-0.137	0.042 ± 0.023	0.278-0.365	0.156 ± 0.023	0.081-0.083	0.041 ± 0.001	0.021	0.009	0.833-1.219	0.512 ± 0.096
123478-HxCDF	0.1000	0.006-0.014	0.001 ± 0.000	0.006-0.006	0.001 ± 0.000	0.006-0.006	0.001 ± 0.000	0.006	0.001	0.026-0.093	0.006 ± 0.003
123678-HxCDF	0.1000	0.023-0.023	0.002 ± 0.000	0.023-0.023	0.002 ± 0.000	0.023-0.023	0.002 ± 0.000	0.023	0.002	0.029-0.099	0.007 ± 0.004
234678-HxCDF	0.1000	0.015-0.015	0.002 ± 0.000	0.015-0.015	0.002 ± 0.000	0.015-0.015	0.002 ± 0.000	0.015	0.002	0.018-0.076	0.004 ± 0.003
123789-HxCDF	0.1000	0.005-0.005	0.001	nd-0.005	0.001	0.005-0.005	0.001	0.005	0.001	0.017-0.020	0.002 ± 0.000
1234678-HpCDF	0.0100	0.030-0.111	0.001 ± 0.000	0.013-0.017	<0.0002	0.002-0.002	<0.0001	0.014	<0.0002	0.021-0.028	<0.0003
1234789-HpCDF	0.0100	0.007-0.016	<0.0002	0.007-0.007	0.0001	0.007-0.015	<0.0002	0.007	0.0001	0.007-0.019	<0.0002
OCDF	0.0001	0.013-0.030	<0.0001	0.010-0.019	<0.0001	0.021-0.026	<0.0001	0.02	<0.0001	0.031-0.082	<0.0001
ΣPCDF		**0.58±0.16**	**0.08±0.04^{a}**	**1.25±0.30**	**0.25±0.05^{b}**	**0.32±0.02**	**0.06±0.00^{a}**	**0.15**	**0.02^{c}**	**4.73±0.99**	**0.85±0.17^{d}**
Mono-ortho PCBs											
PCB 105	0.0001	923-982	0.096 ± 0.003	1680-3626	0.275 ± 0.099	388-560	0.047 ± 0.012	167	0.017	5626-7888	0.701 ± 0.121
PCB 114	0.0005	47.4-55.9	0.025 ± 0.002	82.6-156.9	0.062 ± 0.019	22.3-28.4	0.013 ± 0.002	10.8	0.005	215-336	0.139 ± 0.030
PCB 118	0.0001	2954-4109	0.327 ± 0.073	5244-8760	0.754 ± 0.199	1043-1485	0.126 ± 0.031	586	0.059	16285-31220	2.610 ± 0.850
PCB 123	0.0001	36.1-62.1	0.005 ± 0.001	87.6-164	0.014 ± 0.004	21.9-33.8	0.003 ± 0.001	22.5	0.002	497-698	0.058 ± 0.011
PCB 156	0.0005	332-470	0.200 ± 0.035	456-902	0.323 ± 0.115	140-189	0.082 ± 0.017	86.3	0.043	2098-3487	1.384 ± 0.348
PCB 157	0.0005	108-260	0.060 ± 0.006	158-326	0.127 ± 0.043	55.4-70.8	0.032 ± 0.005	30.1	0.015	800-1348	0.531 ± 0.137
PCB 167	0.00001	208-260	0.002 ± 0.000	253-488	0.004 ± 0.001	77.8-99.3	0.001 ± 0.000	44.3	<0.0005	1313-2288	0.018 ± 0.005
PCB 189	0.0001	27.3-49.0	0.004 ± 0.001	59.1-116	0.009 ± 0.003	15.1-17.6	0.002 ± 0.000	9.81	0.001	224-371	0.032 ± 0.008
Σmono-ortho PCBs		**5115±878**	**0.72±0.12^{a}**	**11886±3347**	**1.57±0.44^{a}**	**2124±509**	**0.31±0.07^{b}**	**957**	**0.14^{b}**	**39891±11175**	**5.47±1.46^{c}**
Non-ortho PCBs											
PCB 77	0.0001	22.0-27.7	0.002 ± 0.000	18.8-50.7	0.003 ± 0.002	5.52-10.8	0.001 ± 0.000	2.95	<0.0003	81.1-135	0.011 ± 0.003
PCB 81	0.0001	0.99-3.14	<0.0002	0.84-2.57	<0.0002	0.61-0.66	<0.0001	0.21	<0.0001	5.77-9.31	0.001 ± 0.000
PCB 126	0.1000	12.4-16.2	1.474 ± 0.207	24.2-37.1	3.129 ± 0.652	5.71-9.08	0.739 ± 0.238	3.12	0.312	103-172	13.609 ± 3.466
PCB 169	0.0100	1.99-4.55	0.030 ± 0.014	4.00-6.39	0.050 ± 0.012	1.78-1.84	0.018 ± 0.000	0.94	0.009	29.1-49.1	0.388 ± 0.100
Σnon-ortho PCBs		**44.25±3.66**	**1.51±0.21^{a}**	**68.25±20.67**	**3.18±0.67^{b}**	**17.97±6.01**	**0.76±0.24^{ac}**	**7.22**	**0.32^{c}**	**296.36±73.71**	**14.01±3.57^{d}**

ΣPCDDs + PCDFs	**0.83±0.17**	**0.16±0.05[a]**	**1.46±0.34**	**0.40±0.10[b]**	**0.46±0.02**	**0.12±0.01[c]**	**0.27**	**0.07[c]**	**5.57±1.15**	**1.48±0.30[a]**
ΣPCBs (MO + NO)	**5159±878**	**2.23±0.32[a]**	**11954±3363**	**4.75±1.10[b]**	**2142±515**	**1.07±0.31[ac]**	**964**	**0.46[c]**	**40188±11245**	**19.48±4.96[d]**
ΣPCDD/Fs + PCBs	**5160±878**	**2.39±0.35[a]**	**11956±3363**	**5.15±1.12[b]**	**2142±515**	**1.19±0.32[ac]**	**964**	**0.54[c]**	**40193±11246**	**20.96±5.21[a]**

[a]TEFs (Van den Berg et al.,1998)
nd – not detected
concentration values lower than LOQ were set at ½ LOQ
for upperbound TEQ reporting, values below LOQ were set at LOQ

Table 4. Relationships between concentrations of DL-PCBs, PCDD/Fs, (pg.g^{-1} ww) and PBDEs (ng.g^{-1} ww), and the percentage of total lipid, TAG, FFA, sterol and total polar lipid in tissues of migrating Atlantic bluefin tuna (n=12; 3 pools, 4 tissues). Relationships are described by the linear regression $y = a + bx$, where y is DL-PCB, PCDD/F or PBDE, a is the regression constant (intercept), b is the regression coefficient (slope) and x is percentage lipid or lipid class (TAG, FFA, Sterol, Total Polar).

	a	*b*	r^2	*P* value
Lipid v				
ΣDL-PCB	2168.4	515.7	0.848	<0.001
ΣMono-ortho	2157.0	511.9	0.847	<0.001
ΣNon-ortho	11.442	3.864	0.892	<0.001
ΣPCDD	0.125	0.010	0.869	<0.001
ΣPCDF	0.215	0.061	0.891	<0.001
ΣPCDD/F	0.340	0.071	0.894	<0.001
ΣBDE	2.643	0.113	0.740	<0.001
TAG v				
ΣDL-PCB	-21522	643.7	0.737	<0.001
ΣMono-ortho	-21354	638.9	0.736	<0.001
ΣNon-ortho	-168.1	4.860	0.787	<0.001
ΣPCDD	-0.343	0.012	0.824	<0.001
ΣPCDF	-2.547	0.0755	0.759	<0.001
ΣPCDD/F	-2.890	0.0879	0.773	<0.001
ΣBDE	-3.075	0.1504	0.732	<0.001
FFA v				
ΣDL-PCB	29344	-1324.6	0.303	ns
ΣMono-ortho	29119	-1313.7	0.302	ns
ΣNon-ortho	226.0	-10.914	0.385	0.031
ΣPCDD	0.691	-0.030	0.476	0.013
ΣPCDF	3.537	-0.167	0.357	0.040
ΣPCDD/F	4.228	-0.197	0.375	0.034
ΣBDE	8.099	-0.245	0.189	ns
Sterol v				
ΣDL-PCB	41222	-3122.1	0.683	<0.001
ΣMono-ortho	40917	-3098.6	0.683	<0.001
ΣNon-ortho	305.2	-23.523	0.727	<0.001
ΣPCDD	0.862	-0.060	0.749	<0.001
ΣPCDF	4.847	-0.371	0.721	<0.001
ΣPCDD/F	5.709	-0.430	0.730	<0.001
ΣBDE	11.223	-0.6871	0.603	0.003
Total Polar v				
ΣDL-PCB	46538	-1671.7	0.769	<0.001
ΣMono-ortho	46197	-1659.3	0.769	<0.001
ΣNon-ortho	341.6	-12.406	0.794	<0.001
ΣPCDD	0.932	-0.030	0.758	<0.001
ΣPCDF	5.385	-0.194	0.772	<0.001
ΣPCDD/F	6.317	-0.224	0.776	<0.001
ΣBDE	13.039	-0.402	0.810	<0.001

ns – not significant (P>0.05)

Table 5. Recent literature concerning levels of PCDD/Fs and/or PCBs in Mediterranean ABT and similar species. Data are presented as mean ± sd (range).

Sampling Site	Species	Levels of PCDD/Fs and/or PCBs					Reference
		Congeners analysed	Tissues analysed	ww (ng.g^{-1})	lw (ng.g^{-1})	TEQ (pg.g^{-1} ww)	
Barbate Coast	*T. thynnus*	Σ29 PCDD/Fs+DL-PCBs	Muscle	51.6 ± 0.88	64.50 ± 10.97	2.37 ± 0.25	This Study
			Liver	11.96 ± 3.36	94.89 ± 26.69	5.14 ± 1.12	
			Ovary	2.14 ± 0.51	47.6 ± 11.44	1.18 ± 0.32	
			Testes	0.96	41.47	0.54	
			Adipose	40.19 ± 11.25	51.57 ± 12.81	20.96 ± 5.21	
Tyrrhenian Sea	*T. thynnus*	Σ13 PCBs	Muscle	(170-2200)	-	(17-200)	Corsolini et al. 1995
Ionian Sea	*T. thynnus*	Σ57 PCBs	Muscle	80 ± 86 (12-229)	-	1.97	Corsolini et al. 2005
			Liver	233 ± 76 (117-357)	-	-	
			Gonad	269 ± 305 (17-838)	-	4.65	
	X. gladius	Σ57 PCBs	Muscle	89 ± 82 (11-267)	-	2.71	
			Liver	69 ± 68 (13-194)	-	-	
			Gonad	75 ± 98 (16-221)	-	4.65	
Southern Tyrrhenian Sea	*T. thynnus*	Σ43 PCBs	Muscle	(5-1327)	(17-16839)	-	Corsolini et al. 2007
Straits of Messina	*T. thynnus*	Σ7 PCBs	Muscle	(nd-188.5)	(nd-2324)	-	Di Bella et al. 2006
			Liver	(nd-191.3)	(nd-4203)	-	
			Adipose	(nd-426)	(nd-2321)	-	
Spanish markets	*T. thynnus*	Σ29 PCDD/Fs+DL-PCBs	Edible parts	53.2 (19.1-288)	-	3.24 (0.69-7.53)	Gómara et al., 2005
		Σ23 PCBs	Edible parts	100.4 (10-176)	-	-	
Straits of Messina	*T. thynnus*	Σ29 PCDD/Fs+DL-PCB	Muscle	280 (197-363)	-	3.06*	Kannan et al, 2002
			Liver	934 (224-1660)	(5670-14400)	23.51*	
			Fat	817	-	28.5*	
	X. gladius	Σ29 PCDD/Fs+DL-PCB	Muscle	329 (258-399)	-	1.57*	
			Liver	745	-	3.55*	
Spencer Gulf region	*T. maccoyii*	Σ29 PCDD/Fs+DL-PCBs	Wild	-	-	0.27 (0.18-0.45)	Padula et al. 2008
			Farmed	-	-	1.0 (0.23-4.3)	
		Σ45 PCBs	Wild	0.47 (0.4-5.5)	100 (24-200)	-	
			Farmed	6.6 (0.81-4)	64 (7.7-240)	-	
Spencer Gulf region	*T. maccoyii*	Σ29 PCDD/Fs+DL-PCBs	Farmed	0.86 ± 0.46 (0.08-1.58)	-	0.80 ± 0.39 (0.17-1.5)	Phua et al. 2008
Western Mediterranean	*T. thynnus*	Σ7 PCBs	Muscle	(3.1-25.8)	-	-	Porte and Albaigés 1993
			Liver	(112-275)	-	-	
Ionian Sea	*T. thynnus*	Σ17 PCBs	Liver	15.9 ± 8.0 (5.3-35)	526 ± 183.9 (325-812)	0.55	Storelli et al. 2008
Straits of Messina	*T. thynnus*	Σ35 PCBs	Muscle	74.9 (21-325)	-	-	Stefanelli et al. 2002
			Liver	263 (13-603)	-	-	
			Gonad	174 (9-539.67)	-	-	
			Blubber	2861 (1521-5409)	-	-	
Offshore waters and open	*K. pelamis*	Σ29 PCDD/Fs+DL-PCBs	Muscle	-	(nd-62000)	(0.092-2.4)	Ueno et al. 2005

seas							
Tyrrhenian Sea	*T. thynnus*	Σ43 PCBs	Wild Muscle	-	2751.73 ± 1276.83	-	Vizzini et al. 2010
			Wild Liver	-	337 ± 94.28	-	
			Farmed Muscle	-	1916.98 ± 1446.57	-	
			Farmed Liver	-	371.74 ± 78.38	-	

*includes contribution of TEQs from polychlorinated-naphthalenes

Table 6. Concentrations of nine PBDE congeners (ng.g^{-1} wet wt) in tissues of migrating Atlantic bluefin tuna (*n*=3 for muscle, liver and adipose, *n*=2 for ovary and *n*=1 for testes). Means bearing identical superscripts within same row are not statistically different (P>0.05).

	Muscle			Liver			Ovary			Testes			Adipose		
	Mean ± SD	**Range**	**%**	**Mean ± SD**	**Range**	**%**	**Mean ± SD**	**Range**	**%**	**Mean ± SD**	**Range**	**%**	**Mean ± SD**	**Range**	**%**
PBDE 28	0.162±0.022	0.148-0.187	4.2	0.241±0.095	0.167-0.348	4.4	0.086±0.029	0.065-0.107	4.6	0.029	-	3.4	0.457±0.124	0.360-0.597	4.3
PBDE 47	2.025±0.261	1.780-2.298	52.2	2.970±1.160	1.893-2.819	54.2	1.010±0.367	0.750-1.269	54.5	0.429	-	50.3	5.119±1.793	4.038-7.189	47.7
PBDE 49	0.338±0.090	0.249-0.428	8.7	0.393±0.189	0.252-0.608	7.2	0.150±0.050	0.115-0.185	8.1	0.050	-	5.9	0.758±0.243	0.583-1.036	7.1
PBDE 66	0.267±0.034	0.235-0.304	6.9	0.300±0.094	0.197-0.378	5.5	0.119±0.032	0.096-0.142	6.4	0.055	-	6.5	0.823±0.189	0.654-1.026	7.7
PBDE 99	0.116±0.048	0.082-0.171	3.0	0.213±0.172	0.104-0.412	3.9	0.064±0.042	0.035-0.094	3.5	0.016	-	1.9	0.422±0.157	0.304-0.600	3.9
PBDE 100	0.569±0.042	0.524-0.608	14.7	0.841±0.275	0.525-1.026	15.3	0.264±0.069	0.215-0.313	14.3	0.162	-	19	1.682±0.501	1.355-2.259	15.7
PBDE 153	0.080±0.002	0.078-0.082	2.1	0.072±0.026	0.052-0.101	1.3	0.019±0.007	0.014-0.024	1.0	0.031	-	3.6	0.224±0.037	0.184-0.256	2.1
PBDE 154	0.323±0.068	0.250-0.385	8.3	0.449±0.084	0.352-0.506	8.2	0.140±0.020	0.126-0.154	7.6	0.080	-	9.4	1.243±0.085	1.152-1.321	11.6
PBDE 183	nd	nd	0	nd	nd	0	nd	nd	0	nd	nd	0	0.009±0.000	0.009-0.009	<0.1
Σ9 PBDE	**3.88±0.44^{a}**			**5.48±2.01^{a}**			**1.85±0.62^{b}**			**0.83^{bc}**			**10.74±3.08^{d}**		

nd – not detected

values lower than LOQ were set at ½ LOQ

10 **Table 7**. Recent literature concerning levels of PBDEs in Mediterranean ABT and similar species. Data are presented as mean ± sd (range).
11

Sampling Site	Species	Congeners analysed	Tissues analysed	ww ($ng.g^{-1}$)	lw ($ng.g^{-1}$)	Reference
Barbate Coast	*T. thynnus*	Σ9 PBDE	Muscle	3.88 ± 0.44	48.50 ± 5.53	This Study
			Liver	5.48 ± 2.01	43.49 ± 15.97	
			Ovary	1.85 ± 0.62	41.16 ± 13.70	
			Testes	0.83	25.12	
			Adipose	10.76 ± 3.08	14.70 ± 4.22	
Tyrrhenian Sea	*T. thynnus*	Σ23 PBDE	Muscle	15 ± 3	-	Borghesi et al. 2009
South Tyrrhenian Sea	*X. gladius*	Σ19 PBDE	Muscle	0.61 ± 0.60	6.69 ± 6.54	Corsolini et al. 2008
			Liver	2.22 ± 3.33	13.86 ± 20.57	
Mediterranean Sea	*T. trachyrinchus*	Σ10 PBDE	Liver		5.1 ± 1.1 (3.2-7.0)	Covaci et al. 2008
	C. coelorynchus		Liver		16.9 ± 5.5 (11.8-27.3)	
Spanish Markets	*T. thynnus*	Σtetra-octa PBDE	Edible parts	0.56 (0.07-1.50)	-	Domingo et al. 2006
	X. gladius		Edible parts	0.98 (0.26-1.92)	-	
South Tyrrhenian Sea	*T. thynnus*	Σ9 PBDE	Muscle	(1-52)	(17-219)	Pena-Abaurrea et al. 2009
Open sea areas	*K. pelamis*	Σ11 PBDE	Muscle	-	(nd-53)	Ueno et al. 2004

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13 **Figure 1.**
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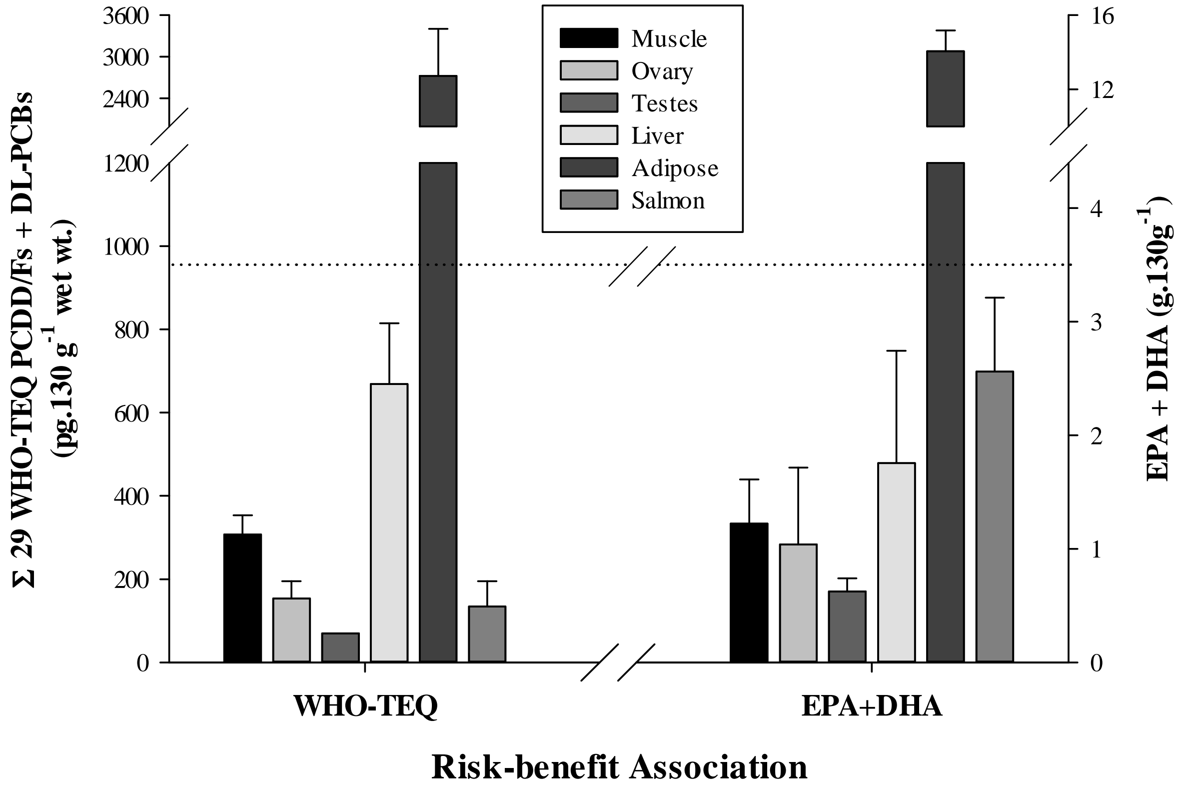

18