Introduction

The bluefin tuna, *Thunnus thynnus* (Linnaeus 1758), is biologically and ecologically important in the Atlantic–Mediterranean ecosystems. Bluefin tuna feed on diverse food items depending on their age, thus they occupy different trophic levels during their lifespan. Hexachlorobenzene (HCB), *p,p*-DDE and polychlorinated biphenyls (PCBs) are well-known persistent organic pollutants (POPs) in the Mediterranean basin. The relationship between stable isotopes of nitrogen (N) and the POP residue levels in tissues has recently increased knowledge on the link between the trophic levels and the contaminant accumulation. Trophic levels were estimated by using *δ*¹⁵N/*δ*¹⁴N ratio (*δ*¹⁵N) and HCB, *p,p*-DDE, and forty-three PCBs were quantified in bluefin tuna from the southern Tyrrhenian Sea. Results showed that changes in PCB and *p,p*-DDE concentrations were a function of size and trophic level, while no correlations were observed for HCB. Apart from HCB and PCB nos. 101, 207, 95, 158, and 60 with *p,p*-DDE, and forty-three PCBs and the *p,p*-DDE increased significantly. The ontogenic magnification factor of PCBs was 6.6 ± 0.5, which was significantly (12 times) higher (*p < 0.05) than the values found for *p,p*-DDE (1.4) and HCB (1.4).

HCB, *p,p*-DDE and PCB Ontogenetic Transfer and Magnification in Bluefin Tuna (*Thunnus thynnus*) from the Mediterranean Sea


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<table>
<thead>
<tr>
<th>TABLE 1. Mean Weight (kg), Age (Years), Carbon and Nitrogen Isotopic Composition (%), and Estimated Trophic Level (TL) Classes of Bluefin Tuna Specimens Analyzed in the Present Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>class</strong></td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>VI</td>
</tr>
<tr>
<td>VII</td>
</tr>
<tr>
<td>All</td>
</tr>
</tbody>
</table>

F-value *** *** ***

* Data expressed as average ± standard deviation; **n** = sample size; **TW** = mean total weight of each class expressed in kg; **TL** = estimated trophic level according to Post (10); **F** = Fisher value; * = *p* < 0.05; ** = *p* < 0.01; *** = *p* < 0.001; **ns** = not significant difference (*p > 0.05*).

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Bluefin tuna show interesting and peculiar features that may affect their contaminant bioaccumulation. In fact, bluefin tuna are the best example of a fast-growing, long-lived, wide-ranging fish, capable of migrating from the Mediterranean Sea to the Atlantic Ocean and back (1, 3, 7). Thus they need to exploit a great variety of food resources (7–9), and their diet changes as they grow (7, 10). They are top predators of the benthic-pelagic trophic web from the time they are yearlings, feeding on several species of small fish, crustaceans, and cephalopods; once adults, their diet becomes more specific, relying on large cephalopods and pelagic fish (7).

Theory affirms that the trophic levels of an organism, because of diet, can vary according to ontogeny, resulting in a shift of position within the food web as they mature (11, 12). The ecological features of bluefin tuna make them very interesting from an ecotoxicological point of view; due to predator feeding behavior, long life, and migrating habit, they accumulate lipophilic persistent organic pollutants (POPs). The study of bioaccumulation in the different size classes, that is the ontogenetic variation, may give interesting information on the bioaccumulation and biomagnification of lipophilic POPs in relation to their migratory habits.

Hexachlorobenzene (HCB), *p,p*-DDE and polychlorinated biphenyls (PCBs) are well-known POPs of the Mediterranean basin (2, 13, 14). Large concentrations of POPs have already been detected in bluefin tuna (2, 4), highlighting a potential risk for this species. Although the dynamics of POPs through food webs have been previously studied (9), data on size-transfer patterns in aquatic organisms are scarce. This is particularly true in *k*-strategy long-lived fish such as tuna, which show ontogenetic changes in their trophic position (7, 10). Once the trophic level for each size class of an organism has been obtained, usually from *δ*¹⁵N data (10, 15, 16), the evaluation of POP concentrations in its tissues allows investigation of the link between contaminant accumulation and life history. This approach has been successfully used in temperate and cold ecosystems of the northern hemisphere to investigate POP biomagnification (16, 17), but little is known about the ontogenetic changes of bioaccumulation in fish.

HCB, *p,p*-DDE, and forty-three PCBs were quantified in bluefin tuna collected from the southern Tyrrhenian Sea (37°9’ N, 12°5’ E and 38°2’ N, 13°1’ E); results were used to...
address the following questions: (i) whether the transfer of $^{15}$N and trophic level shift depended on ontogeny and size; (ii) whether changes of concentrations of POPs were a function of age, size, and trophic levels; and last (iii) how each POP compound showed differential food web magnification, depending on trophic levels.

Materials and Methods

Study Areas and Sample Collection. Thirty-three samples of bluefin tuna muscle were collected in the southern Tyrrhenian Sea. Tuna weighing 15–225 kg ($n = 25$) (Table 1) were collected on May 3, 2003 by a traditional western Mediterranean fixed trap (called tonnara; 1). The tuna trap was positioned off the western coast of Sicily (San Cusumano, Trapani; 37°9′ N, 12°5′ E; see Sara et al. (7) for details).

Young-of-the-year (YOY) tunas weighing 0.7–2.2 kg ($n = 8$) (18) were collected by purse seine in the Gulf of Palermo (about 30 nm eastward of San Cusumano; 38°2′ N, 13°1′ E), between September third and October first, 2003. We were forced to collect tuna in different periods of the year due to the asynchronous presence of different aged tuna in the Mediterranean Sea (Figure S1 given in the Supporting Information) (1, 8). Small juveniles (i.e., YOY, ref 18) are widely present in the Mediterranean only from late summer to autumn after spawning (late spring–early summer; Figure S1). Adults travel from the eastern Atlantic through the Gibraltar Strait (19) to reach their Mediterranean spawning grounds from May to late July (8, 18–19). Subadults remain in the Mediterranean basin until they reach 30–50 kg (for information on the length-age relationships and growth dynamics, see at www.fishbase.org); in autumn and winter they roam far from the coasts and they feed on the offshore resources, more homogeneous than the coastal ones. In spring, they aggregate with the adults coming from the Atlantic Ocean and approach the coasts where tuna traps are located (8, 19–20). Hence, sampling is very difficult; catches of small juveniles are very rare in winter and spring and occasionally carried out only by sport fishermen; adult spawners are usually fished in the south Tyrrhenian Sea from May to late July.

Analytical Methods. Estimates of $\delta^{15}$N in specimens from each class were carried out according to methods reported in Sara and Sara (7), while estimates of trophic levels according to Post (10). Briefly, $\delta^{15}$N values were used to estimate trophic levels (TL) according to the following equation:

$$\delta^{15}N = 8.7 (+0.18) + 0.62 (+0.001) \times TW$$

($n = 7$, $r = 0.91$, $p = 0.003$ [**]).

FIGURE 1. Relationships between (a) total weight (TW, kg) and trophic level (TL) including the seven classes of age (regression line, its model and SNK test are also reported), and the weight class concentrations pattern of (b) $\Sigma$PCBs and $p,p'$-DDE (ng/g wet wt).

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TABLE 2. Average Concentrations of POPs (ng/g Wet Weight and ng/g Lipids) of Bluefin Tuna Specimens Analyzed in the Present Study

<table>
<thead>
<tr>
<th>class</th>
<th>n</th>
<th>HCB</th>
<th>p,p'-DDE</th>
<th>∑PCBs</th>
<th>lip. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng/g wet weight</td>
<td>ng/g lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>&lt;0.001 ± 1.4 (0.001 – 1.8)</td>
<td>2.9 ± 1.2 (0.8 – 1.1)</td>
<td>10 ± 7.4 (5 – 21)</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003 ± 4.3 (0.003 – 5.7)</td>
<td>9.1 ± 3.9 (2.5 – 3.5)</td>
<td>31 ± 23 (17 – 65)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>1.9 ± 0.5 (1.3 – 2.4)</td>
<td>6.2 ± 5.6 (1.2 – 13.5)</td>
<td>32 ± 28 (6.8 – 67)</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.4 ± 1.4 (3.6 – 6.9)</td>
<td>18.0 ± 16.2 (3.5 – 39.2)</td>
<td>94 ± 80 (19.6 – 194)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>1.1 ± 1.1 (0.2 – 2.7)</td>
<td>24 ± 20 (5.6 – 49)</td>
<td>150 ± 164 (30 – 388)</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.4 ± 5.5 (1.1 – 13.5)</td>
<td>120.7 ± 99.5 (27.9 – 245.8)</td>
<td>751 ± 822 (148 – 1942)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>1.2 ± 0.8 (0.1 – 1.9)</td>
<td>50 ± 19 (26 – 68)</td>
<td>255 ± 91 (87 – 383)</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.2 ± 3.5 (0.6 – 8.7)</td>
<td>226 ± 85 (117 – 308)</td>
<td>1147 ± 408 (392 – 1725)</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>0.9 ± 0.9 (0.001 – 1.9)</td>
<td>56 ± 36 (14.9 – 112)</td>
<td>475 ± 482 (194 – 1327)</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 ± 4.3 (0.005 – 9.5)</td>
<td>275 ± 177 (73 – 551)</td>
<td>2326 ± 1360 (951 – 6505)</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>6</td>
<td>1.8 ± 1.5 (0.001 – 3.5)</td>
<td>73 ± 25 (40 – 97)</td>
<td>611 ± 429 (149 – 1290)</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.2 ± 7.5 (0.005 – 18)</td>
<td>373 ± 128 (205 – 492)</td>
<td>3114 ± 1890 (761 – 6580)</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>0.7 ± 0.5 (0.2 – 3.5)</td>
<td>20 ± 1 (18.9 – 97)</td>
<td>195 ± 74 (112 – 1290)</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6 ± 2.7 (1.1 – 18.8)</td>
<td>105 ± 5.2 (100 – 512)</td>
<td>1036 ± 394 (596 – 16839)</td>
<td></td>
</tr>
</tbody>
</table>

* Data expressed as average ± standard deviation; minimum and maximum values in brackets; n = sample size; Lip.% = lipid percentage.

FIGURE 2. PCB homologue series of the seven tuna age classes.

Chemical analyses of HCB, p,p’-DDE, forty-three PCB congeners were determined in all specimens from each class. All chemicals were analyzed following the method described elsewhere, with some modifications (4, 21). Samples were homogenized with sodium sulfate and spiked with 50 ng of CBs 30 and 209 as internal standards; they were Soxhlet extracted for 16 h with n-hexane and dichloromethane (1:3). The extract was rotary evaporated to 11 mL and an aliquot was used for the determination of fat content by gravimetry. Interfering substances were removed by fractionation with multilayer silica gel column; it was prepared by packing a glass column (20 mm i.d.) with a series of layers of silica gel in the following order: 2 g silica gel, 6 g 40% acidic-silica gel, 2 g silica gel and a thin layer of sodium sulfate at the top. The column was cleaned with 150 mL of hexane (discarded fraction), prior to the transfer of sample extracts. Samples were then eluted with 200 mL of hexane and rotary evaporated to 1 mL for the HR-GC analysis. HCB, p,p’-DDE, and PCB congeners were identified and quantified (4, 21) using a gas chromatograph (Perkin-Elmer mod. Autosystem) equipped with 63Ni electron capture detector (HRGC-ECD). The capillary column was coated with DB-5 [(5%-phenyl)-methyl-polysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco Inc]. For GC-ECD conditions see Corsolini et al. (4). Results are given on a wet weight basis (wt wt), and PCBs are identified by their IUPAC numbers. Average concentration of each forty-three congeners were reported; moreover, the sum of congeners was also reported and expressed as ∑PCB concentration.

The methodological limit of detection (MDL) of individual compounds was evaluated as mean blank + 3SD and the values ranged from 0.001 to 0.003 pg. Blanks were run with each set of samples and chemical values ranged from 0.1 pg to 10 pg. Recovery rates were evaluated by adding known amounts (25, 50, 100, 250 ppb; internal standard volume = 200 µL) of PCB congeners (CBs 153, 138, 170, 194, 101, 118, 195) to a set of samples (n = 6) prior the analyses. Recovery rates were PCB138 = 97 ± 12%; PCB153 = 93 ± 18%; PCB101 = 86 ± 19%; PCB194 = 92 ± 14%; PCB118 = 75 ± 39%; PCB195 = 87 ± 19%; PCB156 = 93 ± 12%. The standard solutions used for identification and quantification of single chemicals and for the recovery rate experiments were obtained by formulas: \( TL_{ni} = \frac{[\delta^{15}N_i - \delta^{15}N_{soil}]/3.4} + 2 \), where \( TL_{ni} \) is the trophic level of size class \( i \), \( \delta^{15}N_{soil} \) is the mean \( \delta^{15}N \) of size class \( i \), and \( \delta^{15}N_{ref} \) is the mean \( \delta^{15}N \) of the zooplankton (trophic baseline), which were assumed to have trophic level 2 (10).
Supelco, Inc. (Sigma-Aldrich, U.S.). The procedure’s accuracy and precision were tested through the IAEA-MEL intercomparison exercise for the determination of organohalogen compounds in mussel homogenate samples (IAEA-432, 2002). Our laboratory is in compliance with the standard ISO9001: 2000 for the ecotoxicological analyses of sediments and organisms (reg. no. IT33804).

**Statistical Analyses.** ANOVA was used to test the null hypothesis of no difference among size classes of isotope and POP content in tuna samples. Size classes (class, 7 levels) were treated as a fixed factor. The heterogeneity of variance was tested using Cochran’s C test prior to the ANOVA. The Student–Newman–Keuls (SNK) test enabled the appropriate means comparison. The software GMAV 5.0 (University of Sidney, licensed to G. Sara) was used to run the ANOVA, while Statistica rel. 5.0/99 edition (StatSoft) has been used for regressions between size classes and trophic levels. Furthermore, we ran linear regressions using size and $\delta^{15}N$ as independent and POP values as dependent for testing ontogenetic relationships among POPs, size, and trophic levels (trophic levels as estimated by $\delta^{15}N$).

**Results and Discussions**

**Changes in $\delta^{15}N$ and Trophic Levels as a Function of Ontogeny.** Mediterranean bluefin tuna varied their $^{15}N$ accumulation and trophic level with size (Table 1, Figure 1a) following a linear model ($\delta^{15}N = 8.7 \pm 0.18 + 0.02 \pm 0.001$ Total weight; ($n = 7$; $r = 0.91$; $p=0.003$; ***)). Thus, it was evident that $\delta^{15}N$ concentrations increased with size, as shown by the significant linear increase from small juveniles to giants. Class I and II had similar $^{15}N$ concentrations and occupied the same trophic level (about 3.1 $\pm 0.0$), significantly different from greater tuna (from class III to VI, see SNK test outcome reported in Figure 1a). Class III–VI were rather homogeneous and were positioned at trophic level 3.9 $\pm 0.1$; they were significantly different from class VII, including giant.
tunas, which was the top-level class showing the highest $\delta^{15}N$ and TL values (about $13.0 \pm 0.4\%$ and $4.8 \pm 0.1$, respectively). The relationship between nitrogen and size was notable, as younger tuna displayed nitrogen-depleted values ($\sim 7.0 - 8.0\%$), while values in older tuna were more enriched ($\sim 13.0\%$). This pattern indicates a considerable accumulation of $^{15}N$, which can be explained either by physiological changes or different habitat exploitation and resource use (12, 18, 22). The relationship between stable isotope signatures and size theoretically occurs as a result of the changing allocation of isotopes or from changes in tissue turnover rates during ontogeny (7, 23, 24). However, the marked retention of the heavier isotope ($^{15}N$) in bluefin tuna in relation to size was probably due to shifts in diet and habitat, rather than to physiological changes (25).

The size effect in resource use has already been well documented in fish (26–28), and it is thought to be associated with/or caused by changes in feeding habitat (12). Size differences were not evident for tuna caught in North Western Atlantic, where isotopic composition was quite constant ($\delta^{15}N$ ca. $14.0\%$, ranging from 35 to 196 kg) (29).

PCB, $p,p'$-DDE and HCB Concentrations. The concentrations of $\Sigma$PCBs increased significantly with size and trophic levels (Table 2, Figure 1b). PCBs showed increasing concentrations from class I to class VI, ranging from $10 \pm 7.4$ to $611 \pm 429$ ng/g wet wt (31 $\pm 23$ to $3114 \pm 2190$ ng/g lipids). PCB concentrations were three time smaller in Class VII than in younger tuna of class VI (we aware that class VII included only three samples; nevertheless, considering the huge difficulties of collecting this size of tuna in the Mediterranean Sea, we reported data anyway). Tuna grouped in classes I–V were all juveniles, while those grouped in the classes VI–VII were adults (7). They were collected at the beginning of the reproductive period (May–June); during this period adults use their stored lipids as an energy reservoir to produce gametes (tuna usually reduce their feeding at the beginning of the reproductive season, (7)). For this reason, concentrations of POPs in muscle likely decreased, as a consequence of the decreasing presence of stored lipids in muscle. On the other hand, concentrations in gonads may be very high (4), and gonad formation may be an important excretion route for tuna; this excretion route was already demonstrated for dolphins and birds (30–32) and it may be possible for tuna as well.

Corsolini et al. (4) found $0.4 \pm 0.2$, 31 $\pm 38$, and $80 \pm 86$ ng/g wet wt of HCB, $p,p'$-DDE, and PCBs, respectively, in bluefin tuna (weight 46–250 kg) muscle collected off the Ionian coasts of Sicily (Italy). Giant bluefin tuna (300–400 kg) collected off the Tyrrhenian coasts of Sicily in 1992 showed greater concentrations of PCBs, viz. $170 - 2200$ ng/g wet wt (2); these high values can be attributed both to the large size of the specimens collected in 1992 (not comparable to any of our size classes), and to a decreasing release after the banning of PCB use and production in the 1970s.

Figure 2 shows the PCB homologue series in the tuna age classes. All classes showed a similar pattern, that was hexa-CBs > hepta-CBs > penta-CBs; they made up 43–46%, 27–34%, and 13–18% of the PCB residue, respectively. Hexa- and hepta-CBs were reported to be the most abundant PCBs in tuna (3, 33) and other Mediterranean fish (34–35). Tetra- and octa-CBs were very low in the muscle of all the specimens (less than 1.6%); their small contribution to the PCB residue was likely due to the elevated exchange rate of low-chlorinated congeners with the environment through the gills (36).

Among the most abundant classes of isomers there were the hexa-CBs 153 > 149 + 118 > 138, the hepta-CBs 180 > 170 > 187 (Figure 3); those congeners, together with the octa-CBs 196, 201, 207, 189, 195, 194, and 205 have a chlorine atom in the 2, 4, or 5 position on one or both of the biphenyl rings. Such chemical structures make the PCB congeners resistant to the metabolic degradation in invertebrates and fish, and therefore, they tend to accumulate mostly in tissues (37–38). The fingerprint patterns in the tuna age classes were similar, with few differences (Figure 3), thus it was not possible to characterize different sources of contamination and metabolism in relation to size. CBs 153 > 138 + 118 + 149 > 180 > 170 were predominant congeners in the fingerprints of tuna (Figure 3). All congeners except CBs 60 + 56, 85, 101, 158, and 207 showed a significant increase in concentration with trophic levels (Figure 4). Their contribution to the total PCB residue was very small (Figure 3), likely in relation to their small concentration in the environment. It is also

![FIGURE 4. Ontogenetic magnification factor (OMF) of each POP ordered by ranks (significant increasing with TL is indicated by asterisk).](Image 402x0 to 612x210)
possible that degradation/elimination in tuna fish may occur; in fact, among these congeners, only CBs 101 and 207 have chlorines in the 2, 4, 5 positions only (37, 39).

Concentrations of p,p′-DDE showed a significant increase (Tables 2 and Figure 1b) with size and trophic level, ranging from 2.9 ± 1.2 in class I (9.1 ± 3.9 ng/g lipids), to 73 ± 25 ng/g in class VI (373 ± 128 ng/g lipids). The concentrations followed the same pattern of PCBs, viz it increased from class I to class VI, and decreased in class VII tunas.

The reduction in concentration of PCBs and p,p′-DDE observed in class VII compared to class VI may be attributed to the age of specimens; in fact, tuna belonging to class VII are mature and they were collected just at the beginning of the reproductive period (May). Gonad formation involves the use of the stored lipids to produce gametes (39–40). The use of those lipids entails that of contaminants bonded to them; the consequence of this mobilization may be the contaminant accumulation in the gonads that show higher concentrations of PCBs than liver and muscle (4). Further analyses on all size classes (including the giant tuna weighing 400 kg or more), in different periods of the year will be carried out to understand if the concentrations of POPs in tissues of tuna may vary with seasons and the period of their lifecycle, as it would be expected. For instance, male cetaceans are more contaminated than females because the latter eliminate some contaminants through gestation and lactation (e.g., refs 41, 42). Moreover, females show an increase or decrease of PCB concentrations during their life depending on their status (immature, mature pregnant, mature non-pregnant) (41, 42).

Hexachlorobenzene concentrations ranged from ~0.001 ± 1.4 ng/g wet wt in class I (0.003 ± 4.3 ng/g lipids) to 1.9 ± 0.5 ng/g wet wt (5.4 ± 1.4 ng/g lipids) and 1.8 ± 1.5 ng/g wet wt (9.2 ± 7.5 ng/g lipids) of class II and VI, respectively (Table 2). There was no significant difference between concentration of HCB and age class, and no magnification pattern was observed for this chemical (Table 1–2; pattern not reported). Highest average concentration was found in class II (1.9 ± 0.5 ng/g wet wt or 5.4 ± 1.4 ng/g lipids) and class VI (1.8 ± 1.5 ng/g wet wt or 9.2 ± 7.5 ng/g lipids), but the maximum values were detected in tuna of classes VI and VII (3.5 ng/g wet wt or 18.8 ng/g lipids); class VII showed approximately 2.5 lower contamination level than class VI. HCB is more volatile than PCBs and it is spread worldwide; it is less lipophilic than PCBs and, therefore, it reaches a quicker equilibrium between aquatic organism tissues and water (36, 43).

Changes in Ontogenetic Magnification Factor (OMF). The formula reported in Fisk et al. (44) allowed us to extrapolate the magnification factor according to ontogenetic changes of trophic levels (OMF). Apart from HCB and CBs 101, 207, 95, 158, and 60 + 56, which did not show any significant increase per trophic level (Figure 4), the other PCBs and the p,p′-DDE increased significantly. OMF of PCBs was 6.6 ± 0.5, which was significantly (12 times) higher (p < 0.05) than the values found for p,p′-DDE (1.4) and HCB (1.4).

Our results agreed with those by Ueno et al. (45) that reported an increasing trend with body length of PCBs and p,p′-DDE in bluefin tuna collected from Japanese coastal waters. Bioaccumulation and biomagnification processes are strongly correlated with the physical-chemical properties of the molecules; therefore, p,p′-DDE and PCBs and, among them, those with a high persistency in fish (the 2,4,5 substituted congeners) are expected to increase their concentrations with age and trophic level. Moreover, HCB and low-chlorinated PCB congeners can reach a quicker equilibrium between the body (lipid components), and water because they show a lower partition coefficient (K_{ow}) between polar and nonpolar phases (46). A similar p,p′-DDE and PCB concentration increase with body length was also reported for another Scombridae fish, the mackerel Scomber scombrus (40).

The wide range of POP concentrations found in tuna samples can be explained by the differences in feeding habits and metabolic rates, that depend on their disparity in size (2, 4, 45). Results showed that the concentrations of PCBs and p,p′-DDE were a function of size and trophic level, while no correlations were observed for HCB. Apart from HCB and CBs 101, 207, 95, 158, and 60 + 56, which did not show any significant increase per trophic level, the other PCBs and the p,p′-DDE increased significantly. Our study showed the reliability of using δ^{13}N to characterize trophic level and POP trophic transfer as a function of ontogeny in fish.

Acknowledgments
We are indebted and deeply grateful to Prof. Raimondo Sarà (Palermo, Italy) for providing the ecological information of Mediterranean bluefin tuna.

Supporting Information Available
The distribution of bluefin tuna in the Mediterranean Sea around the year; the n-MDS ordination on the basis of Bray–Curtis dissimilarities carried out for comparing PCB congener concentrations varying among classes. This material is available free of charge via the Internet at http://pubs.ac.org.

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