Ontogenetic Transfer of Chlorinated Hydrocarbons in Mediterranean Bluefin Tuna (Thunnus thynnus)

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Introduction

The bluefin tuna *Thunnus thynnus* (Linnaeus 1758) has a relevant importance for the Mediterranean ecosystems not only by an ecological but also by an economic point of view. Fishermen use the long-line fishing and the centuries-old *mattanza* technique since ancient time and they were the first to observe changes of population features and a decreasing trend in the size of captured specimens.[1] This situation might depend both on industrial fishing impact and contamination effects. Hexachlorobenzene (HCB), *p,p'*-DDE and polychlorinated biphenyls (PCBs) are well known persistent organic compounds (POPs) of the Mediterranean basin. High POP levels as well as dioxin-like compound toxic equivalents (TEQs) have been already detected in bluefin.[2,3]

Stable isotopes of nitrogen (N) combined with the POP residue levels in tissues has recently gained ground to understand the link between the trophic levels and the contaminant accumulation. The $^{15}N/^{14}N$ ratio ($d^{15}N$) estimates the trophic level of an organism.[4] This approach has been successfully used in temperate and cold ecosystems of the Northern hemisphere to investigate biomagnification.[5,6]

Bluefin tunas feed on diverse food items depending on their age, thus they occupy different trophic levels during their lifetime span.[1] HCB, *p,p'*-DDE and forty-three PCBs were quantified in bluefin tuna from the Southern Tyrrhenian Sea; results were tested to assess whether bluefin tuna show: i) transfer of ^{15}N and trophic level shift as a function of ontogeny; ii) changes in POP concentrations as a function of the disparity of age, size and trophic level; iii) magnification magnitude of each POP compound.

Material and Methods

Study area and sample collection. Thirty-three samples of bluefin tunas muscle were caught in the Southern Tyrrhenian Sea (Western Mediterranean). Tuna fish weighing 15-225 kg (n = 24) were collected on 3 May 2003 by traditional western Mediterranean *tonnara*.[1] The *tonnara* was positioned off the western coasts of Sicily (San Cusumano, Trapani; 37°9' N, 12°5' E). Juveniles (n = 8) were collected by purse seine in the Gulf of Palermo (about 40 nm eastward of San Cusumano; 38°2' N, 13°1' E), between September 3rd and October 1st, 2003. We were forced to collect tunas in different periods of the year, due to the asynchronous presence of different age tuna fish in the Mediterranean Sea.[1,7]

Stable isotopic analysis and estimates of trophic levels. Muscle samples from tunas were analyzed for $d^{15}N$ by means of a Finnigan Delta-S isotope ratio mass spectrometer. $\delta^{15}N$ values were used to estimate trophic levels (TL) according to the following formula: $TL_{Ni} = [(\delta^{15}N_i - \delta^{15}N_{ref})/3.4] + 2$, where TL_{Ni} is the trophic level of size class *i*, $\delta^{15}N_i$ 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 be herbivorous–detritivorous and feeding at trophic level 2.[8] A constant per-trophic-level fractionation of 3.4‰ was assumed.[8,9]

Analytical methods for chlorinated chemicals. PCB congeners and pesticides were analyzed following the method described elsewhere, with some modifications.[3,10] PCB congeners were identified and quantified using a gas chromatograph (Perkin Elmer mod. Autosystem) equipped with ^{63}Ni electron capture detector (GC-ECD). A

fused silica capillary column coated with DB-5 (Supelco Inc.) was used. Procedural blanks were analyzed through the whole analytical procedure. Recoveries and detection limits were previously described and validated.[3] PCB congeners are represented by their IUPAC numbers throughout the text. Results are given on a wet weight basis (wet wt).

Statistical analyses. ANOVA was used to test the null hypothesis of no difference among size class of isotope and POP content in tuna samples. The heterogeneity of variances was tested using Cochran's C test prior to the analysis of variance and Student-Newman-Keulls (SNK) test allowed the appropriate mean comparison.

Results and Discussion

Changes in $\delta^{15}N$ and trophic levels as a function of ontogeny. Mediterranean tuna fish varied their ^{15}N accumulation and trophic levels with size (Figure 1) according to a significant linear law [$\delta^{15}N = 8.7 (\pm 0.18) + 0.02 (\pm 0.001) * \text{Total Weight}$; (n = 7; r = 0.91; p=0.003; ***)]. $\delta^{15}N$ concentrations increased positively with size, as showed by the significant linear increasing from small juveniles to giants. Class I and II had similar ^{15}N concentrations and occupied the same trophic level (about 3.1±0.0) meaningfully different from greater tunas (from class III to VI, see SNK test outcome reported in Figure 1). Class III-VI were rather homogeneous and were positioned on trophic level of about 3.9±0.1; they were significantly different from class VII, including the giant tunas, which was the top-level class showing the highest $\delta^{15}N$ and TL values (about 13.0±0.4 ‰ and 4.8±0.1, respectively).

POP concentrations. The *p,p'*-DDE and SPCB concentrations increased significantly with size and trophic levels (Table 1). There was no significant difference between HCB values and age class and no magnification pattern was observed for this chemical. Highest average concentration was found in class II (1.9±0.5 ng/g) and class VI (1.8±1.5 ng/g), even if the maximum values were detected in tuna of classes VI and VII (3.5 ng/g). *p,p'*-DDE and PCBs showed increasing concentrations from class I to class VI, ranging from 2.9±1.2 to 73±25 ng/g, and from 10±7.4 to 611±429 ng/g, respectively. Class VII showed a three time lower *p,p'*-DDE and PCB concentrations respect to younger tuna. Tuna grouped in classes I to V were all juveniles, while those grouped in the class VI-VII were adult tuna fish. Moreover, they were collected at the beginning of the reproductive period (May-June), when they use their lipid *reservoir* to produce gametes; in fact gametes show high HCB, *p,p'*-DDE and PCB concentrations.[1,3] On the other hand, the maternal transfer may be an important excretion route for tuna, as already demonstrated for birds and marine mammals.[11-13]

Figure 2 shows the PCB class of isomer patterns in the tuna age classes. The classes III-VII showed a similar pattern, that was octa-CBs > hepta-CBs > hexa-CBs; they differed from classes I and classes II. Those classes of isomers include the hexa-CBs nos. 149, 138, 153, the hepta-CBs nos. 170, 180, 187 and the octa-CBs nos. 196, 201, 189, 195, 207, 194 205, that have a chlorine atom in the 2, 4 or 5 position of one or both the biphenyl ring. 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.[14,15]

Corsolini et al. (2005) found 0.4±0.2, 31±38 and 80±86 ng/g wet wt of HCB, *p,p'*-DDE and PCBs, respectively, in bluefin tuna muscle collected off the Ionian coasts of Sicily (Italy); those levels were of the same order of magnitude as our samples belonging to classes III-VII.[3]

Changes in Ontogenetic Magnification Factor (OMF) according to trophic levels. The formula reported in Fisk et al. (2001) allowed us to extrapolate the magnification factor according to ontogenetic changes of trophic levels (OMF).[16] Apart from HCB and PCB nos. 101, 207, 95, 158 and 60+56, which did not show any significant increasing *per* trophic level (Figure 1b), 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 and HCB (1.4).

The wide range of POP concentrations found in tuna samples can be explained by the differences in feeding habits and rates, depending on their disparity of age.[2,3,17] Our preliminary study showed the reliability of using $\delta^{15}N$ to characterize trophic level and POP trophic transfer as a function of ontogeny in fish.

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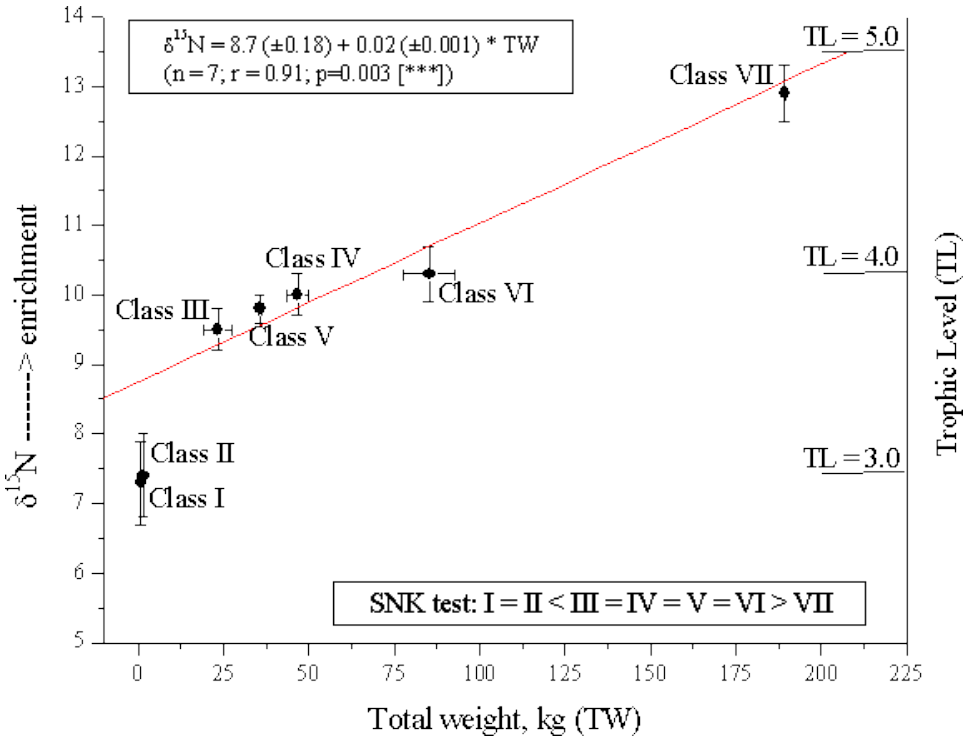
Table 1: HCB, *p,p'*-DDE and SPCB concentrations in the various tuna age class (ng/g wet wt; average±standard deviation, minimum value = min, maximum value = max; weight in Kg).

	class I	class II	class III	class IV	class V	class VI	class VII
weight	0.8±0	1.6±0.5	23.3±7.2	35.7±1.2	46.7±5.8	85.3±13.1	189.3±0.6
min- max	0.7-0.9	1.0-2.2	15-30	31-40	41-50	75-100	180-200
n	4	4	4	7	5	6	3
HCB	0.001±1.4	1.9±0.5	1.1±1.1	1.2±0.8	0.9±0.9	1.8±1.5	0.7±0.5
min- max	0.001-1.8	1.3-2.4	0.2-2.7	0.1-1.9	0.001-1.9	0.001-3.5	0.2-3.5

p,p'-DDE	2.9±1.2	6.2±5.6	24±20	50±19	56±36	7325	20±1
min- max	0.8-1.1	1.2-13.5	5.6-49	26-68	14.9-112	40-97	18.9-97
SPCBs	10±7.4	32±28	150±164	255±91	475±482	611±429	195±74
min- max	5-21	6.8-67	30-388	87-383	194-1327	149-1290	112-1290

a)



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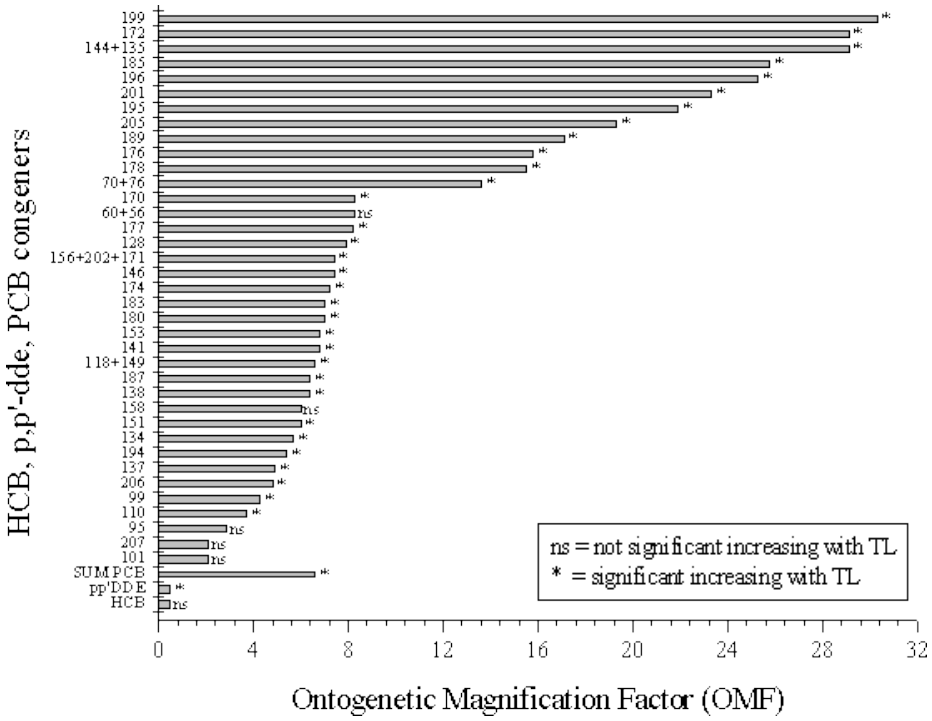

Figure 1: a) Relationship between total weight (TW, kg) and trophic level (TL) including the seven weight classes of tuna sampled for this study (regression line and its model is also reported); b) Ontogenetic Magnification Factor of each POP ordered by ranks (significant increasing with TL is indicated by asterisk).

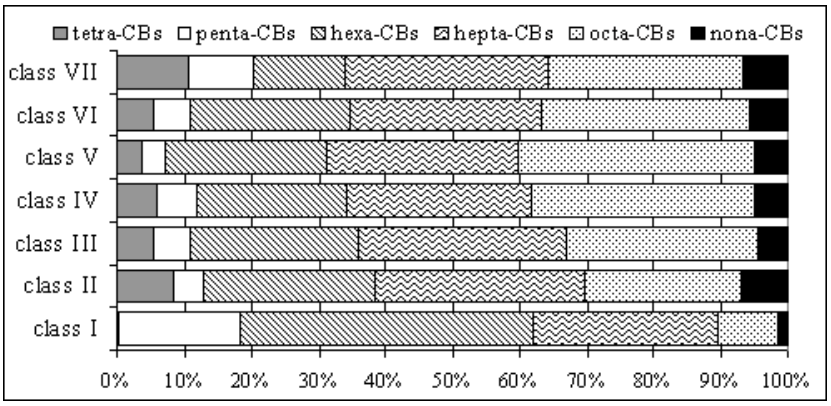

Figure 2: PCB class of isomer pattern in the tuna age class.