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Comparison of stable isotope composition and inorganic and organic contaminant levels in wild and farmed bluefin tuna, *Thunnus thynnus*, in the Mediterranean Sea

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ABSTRACT

Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) and persistent pollutants, including heavy metals (Hg, Cd, Pb, As, Cu, Zn) and organochlorine compounds (PCBs, HCB and *p,p′*-DDE), were measured in muscle and liver tissues of wild and farmed bluefin tunas to investigate the changes occurring during the farming period and to assess the quality, in terms of contaminants, of the final product. At the end of farming, the food supplied was clearly integrated into the tuna tissues as derived from stable isotope signatures, and, contrarily to the literature findings obtained for other species, farmed tunas showed slight variations in persistent elements and chemical compounds in comparison with wild fish. The low tissue turnover of long-lived adult tunas together with the short farming period appeared to act to preserve the initial low contamination levels in the absence of new, elevated contamination sources (feed and environment), determining an acceptable quality of the final aquaculture product.

1. Introduction

The bluefin tuna, *Thunnus thynnus* L. 1758, is a long-lived and fast-growing pelagic species with spawning migratory behavior. Worldwide, bluefin tunas are grouped into three stocks for management purposes: the western Atlantic, the eastern Atlantic and Mediterranean, and the Pacific stock (Majkowsky, 2007). Eastern Atlantic tunas enter the Mediterranean, where their spawning ground is restricted, through the Strait of Gibraltar (Rey, 1999).

The high commercial value and increasing demand make the bluefin tuna a suitable species for farming. Since the 1990s a cage fattening industry has developed in the Mediterranean. After capture in the wild with purse seine fleets, tunas are transferred to floating cages where they are kept for a short period (5–6 months) to increase their fat content. During fattening, tunas are fed with frozen food, predominantly round sardinella, mackerel and pilchard. They are then marketed almost exclusively for the Japanese sashimi and sushi restaurant industry. The quality of farmed tunas arises from several factors to do with farming (type, quantity and quality of food, stocking density, etc.) and the environment (physical–chemical variables, hydrodynamics, contaminant levels, etc.). Most farms are sited in coastal marine areas which, as a direct consequence of their vicinity to inshore environments, can be seriously subject to anthropogenic pollution. In particular, heavy metals and organochlorine substances are among the most diffuse pollutants affecting near-shore waters (Shahidul and Tanaka, 2004).

Heavy metals are often toxic, persistent and not easily degradable elements (Ikem and Egiebor, 2005; Khansari et al., 2005). A few, such as zinc (Zn) and copper (Cu), are essential elements for organisms, but excessive levels become detrimental. Others, such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) are not essential and even low concentrations are toxic for many organisms. Their toxic effect is a consequence of stable chemical bonds (often covalent) with functional groups of organic molecules; indeed the proteins bind metal ions nearly exclusively via coordination by three types of atoms: sulfurs (Cys and Met), nitrogens (His) and oxygens (Asp and Glu) (Pennella and Giedroc, 2005), altering metabolic processes.

Organochlorine substances, such as PCBs, HCB and DDTs, belong to a group of stable and persistent compounds and function as chemical indicators of anthropogenic pressure and pollution (Bayarri et al., 2001; Storelli et al., 2004). Their lipophilic feature makes these molecules able to bioaccumulate in organisms, especially in lipid-rich tissues and organs, and be transferred up marine food webs causing biomagnification processes (Fuoco and Colombini, 1995; Calamari, 2002). Human exposure to these pollutants occurs mainly through ingestion of contaminated food (Schecter et al., 1997; Bordajandi et al., 2004). The most important contribution

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to the dietary intake of PCBs is fish and other seafood (Domingo and Bocio, 2007).

Bluefin tuna, being long-lived and at the top of marine food webs, is a fragile species because of bioaccumulation and biomagnification (Ueno et al., 2002; Corsolini et al., 2005). Captive specimens kept in near-shore cages in potentially polluted coastal ecosystems and fed with fresh food that often comes from non-Mediterranean areas, can be exposed to contaminants and be negatively affected in terms of flesh quality.

In recent years, contaminant determination has been coupled to the analysis of carbon and mainly nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) (Domi et al., 2005; Bodin et al., 2008). This method is a reliable tool to investigate sources of nutrition for consumers, allowing discernment of the origin and pathways of organic matter from primary producers to intermediate and top-level consumers (Vizzini and Mazzola, 2003; Smit et al., 2005). While δ^{13}C signatures primarily reveal the main sources of carbon for consumers, δ^{15}N is indicative of the trophic distance of consumers from the food web base. The coupling of isotopic and contaminant determinations provides a tool to assess the trophic transfer of pollutants within food webs and to analyse bioaccumulation and biomagnification processes (e.g. Tittlemier et al., 2002).

In this study we compared heavy metal levels (Hg, Cd, Pb, As, Cu and Zn), organochlorine compound concentrations (PCBs, HCB and *p,p'*-DDE) and stable isotope composition (δ^{13}C and δ^{15}N) in wild and farmed bluefin tuna, to assess the changes occurring during farming and the quality, in terms of contaminants, of the final product.

2. Materials and methods

2.1. Sample collection

A total of 15 individuals of *T. thynnus* were collected: nine captured in the wild and six sampled from a tuna farm. The wild fish were caught by means of traditional tuna fishing traps (i.e. "tonnara") at Favignana (western Sicily, Italy) in May 2005 (n = 9, total length TL = 126.26 ± 11.56 cm, standard length SL = 101.08 ± 5.41, total weight TW = 28.06 ± 8.41 kg). The reared individuals were sampled in a tuna farm sited in the Gulf of Castellammare (north-western Sicily, Italy) in November 2005 (n = 6, total length TL = 138.17 ± 9.37 cm, standard length SL = 118.67 ± 7.23, total weight TW = 35.67 ± 7.87 kg). The cycle of production of the tuna farm started in May 2005 and lasted until November 2005. Tunas (about 6–7 thousand) were fed about 6–8% of their average overall body weight, two times a day with fresh fish. Individuals of the round sardinella, *Sardinella aurita*, which made up almost exclusively the feed for reared tunas, were also analysed (n = 10, total length TL = 33.08 ± 2.59 cm, standard length SL = 26.06 ± 1.96, total weight TW = 0.35 ± 0.06 kg).

2.2. Laboratory analyses

Samples were kept at −80 °C until analysis. Total lipids, heavy metals, organochlorine compounds and isotopic determinations were carried out on liver and muscle tissues (collected near the caudal fin) of wild and farmed tunas and on the whole homogenised individual for the round sardinella, apart from isotopic analyses, which for the tuna food were performed only on muscle.

All the variables were determined in 15 tunas (nine wild and six farmed) and 10 round sardinella.

2.2.1. Total lipids

Extraction and determination of lipids were carried out in triplicate following Bligh and Dyer (1959) and Marsh and Weinstein (1966) respectively. The lipid content was expressed as percentage of ash-free dry matter. Liver and muscle ash content was determined by burning tissues in a muffle furnace at 450 °C for 36 h (Lucas, 1996).

2.2.2. Heavy metals

The heavy metals determined were: cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg) and arsenic (As). A Varian Vista MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) was used to analyse digested samples.

Liver and muscle samples (about 0.2 g) were mineralized in Teflon digestion vessels with 5 ml HNO_3 67–70% Suprapur, 1 ml 30% H_2O_2 Suprapur and 4 ml deionized water MilliQ.

After cooling, each sample was transferred to a 25 ml volumetric flask and diluted to the mark with distilled water. For each cycle of mineralization an analytical blank was prepared. The analytical procedure was checked using a standard reference material (Dogfish muscle DORM-2) provided by the National Research Council of Canada.

Concentrations of arsenic and mercury were determined using a hydride generation system (VGA-77 linked ICP-OES). For mercury determinations, a reductant solution, consisting of 0.3% $NaBH_4$ and 0.5% NaOH, and 5 M HCl as carrier, were used. For arsenic determinations, 4 ml 5% KI, 4 ml 5% ascorbic acid and 4 ml 5% HCl were added in that sequence to 4 ml of the digested sample to convert total As to As^{3+}. After that, As^{3+} was transformed to AsH_3, by adding 0.6% $NaBH_4$ and 0.5% NaOH as reductants and 5–10 M HCl as carrier. All reagents were Suprapur. Results were given in mg kg^{-1} wet weight (w.w.).

2.2.3. Organochlorine compounds

PCBs (sum of 43 congeners), HCB and *p,p'*-DDE were analysed following the method described by Kannan et al. (2002). Samples were homogenised with sodium sulfate. For the Soxhlet extraction, 300 ml of methylene chloride and 100 ml of hexane were used for 12–16 h. The elution column (diameter 10 mm) was prepared in the following order with: 2 g silica, 2 g acidic-silica (60% silica and 40% H_2SO_4), 2 g silica and a thin layer of sodium sulfate.

Organochlorine compounds were analysed using a gas chromatograph (Shimadzu, GC-2010) equipped with an electron capture detector (GC-ECD) and a fused silica capillary coated with DB-5 (length = 30 m; thickness of film = 0.25 μ; bore = 0.25 mm; Supelco Inc.). PCB congeners were determined against a standard mixture of Aroclor 1260 (Supelco Inc.), certified by the US Environmental Protection Agency. The standard of HCB and *p,p'*-DDE was supplied by Supelco Inc. Results were given in ng g^{-1} wet weight (w.w.) and ng g^{-1} lipid.

2.2.4. Isotopic composition

Muscle and liver samples were oven dried (60 °C) to constant weight, ground with a mortar and pestle and weighed (0.8 mg) in tin capsules. Carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) were analysed using an Isotope Ratio Mass Spectrometer (IRMS; Thermo-Electron Delta Plus XP) coupled to an Elemental Analyser (EA; Thermo-Electron 1112). Results were expressed in δ notation according to this equation:

$$^{x}\delta = \delta^{x}E = [(R_A - R_r)/R_r](10^3)$$

where: E = element, x = heavy isotope mass, R = ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$.

Analytical precision was 0.2‰ for both isotopic ratios.