A representative sampling of tuna muscle for mercury control

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Abstract

The mechanisms of mercury accumulation and distribution in fish tissues are related to its high affinity for sulfhydryl groups in proteins. There is evidence that mercury is distributed unevenly based on the different reactivity of these groups in the various muscle proteins. Tuna fish also shows numerous specialized anatomical features including the structure of the swimming muscles and some form of endothermy, which generates variations in the mercury content between dark and white muscle and between muscle tissues with different lipid content. The aim of the study is to verify, through a suitable sub lot of *Thunnus thynnus* caught by a static trap in south-western Sardinia, the effective uneven distribution of mercury in the various muscles and also identify the sites representative of the entire carcass. In agreement with other authors, the results show that even in the Bluefin tuna of the Mediterranean, the site "anterior extremity of upper loin (*schienale* in Italian)" is representative of the mercury average content of muscle tissues as a whole.

Introduction

Mercury (Hg) is a global pollutant, released in aquatic ecosystems by anthropogenic actions or natural causes. This toxic element is transferred to aquatic organisms in different ways, leading to bioaccumulation and biomagnification phenomena (De Almeida Rodrigues *et al.*, 2019). This is of great concern in fish, especially for top-level aquatic predators and for species of large human consumption and high nutritional value, such as tunas (EFSA 2012). Of particular interest to the authors of the present study is to determine Hg concentrations in *Thunnus thynnus* caught in a specific area of the Mediterranean, as already reported in other studies on farmed and wild tunas (Annibaldi *et al.*, 2019). In a previous article (Piras *et al.*, 2019), a case study on the muscle sampling procedure for a lot of *Thunnus thynnus* caught by a static trap in a Mediterranean area was presented. The purpose of this study was to evaluate the representative levels of Hg in the lot, assuming that, on the basis of the data available in the bibliography for another large size Scombrids (Balshaw *et al.*, 2008; Bosch *et al.*, 2016), also in the species under study Hg distribution was not uniform for each carcass, as well as between the individual specimens of the lot. Based on the indications reported in this study on the corrected sampling procedure for Hg determination in Tuna, the aim is to verify the different Hg distribution in the various carcass sites. In addition to ecosystem characteristics that contribute to the variability in Hg concentration in aquatic food webs, also correlated with species (Camilleri *et al.*, 2018) and latitude (Houssard *et al.*, 2019; Lavoie *et al.*, 2013), there is a further factor related to the mechanisms of accumulation and distribution of Hg in fish tissues (Balshaw *et al.*, 2007). It is known that Hg has a strong binding property to the sulfhydryl (SH) groups and that cysteine is by a large margin the most likely candidate as the predominant biological thiol, though it is likely part of a larger protein (Balshaw *et al.*, 2008; Bradley *et al.*, 2017; De Almeida Rodrigues *et al.*, 2019; Harris *et al.*, 2003; Itano and Sasaki, 1983; Kumar, 2017). So, the Hg that comes into the muscle cells may unevenly distribute itself according to the reactivity of relative SH groups among muscle proteins (actomyosin, sarcoplasmic protein, subactomyosin and myofibrillar protein). Consider also that among the high diversity of fish, there are some, such as Tuna, which are considered specialized in their swimming performance (Altringham and Shadwick, 2001). Their particular anatomical features include the structure of their swimming muscles and some forms of endothermy (Katz, 2002). Tunas are predators that have evolved numerous adaptations necessary for their exceptionally active lifestyle that requires large energy consumption (Olson *et al.*, 2016); in particular cruising tunas species that have specialized anatomical and physiological features like a dark muscle, deeper than white muscle and the regional endothermy (Altringham and Block, 1997). These features ensure a substantial slow muscle power reserve, sufficient to supply a significantly higher and sustainable swimming speed, at an energetic cost lower if fast fibers intrinsically less efficient, were recruited. The capacity to retain metabolic heat in dark muscle and the elevated rate of metabolic and other physiological functions distinguish therefore tunas from most other teleosts (Shadwick *et al.*, 2013). The differences in muscle function and development muscle rate cause variations in Hg content. It has been observed that in ecologically groupable tuna among the intertropical cosmopolite species of Scombrids, higher Hg concentrations were found in dark muscles than in white muscles as observed in *Thunnus albacares* or "Yellowfin" tuna (Bosch *et al.,* 2016). This is due to the higher activity of dark muscle would cause a higher rate of muscle fiber development and consequently higher Hg accumulation (De Almeida Rodrigues *et al.*, 2019; Kumar, 2017). A similar difference in Hg concentration has also been observed between the white and dark muscles of *Katsuwonus pelamis* or "Skipjack tuna" (Vieira *et al.*, 2017).

These differences could be explained by the pathways of absorption and accumulation in the fish muscles. Hg adsorbed into fish tissue is bind to thiol groups of the protein fraction; since the distinct muscle types (white and dark muscle) have a different protein composition, that produces a variation in the total Hg accumulation. Finally, consider that these muscles specializations also support continuous, relatively fast swimming by tunas and minimize thermal barriers to habitat exploitation, permitting niche expansion even at high latitudes

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(Graham *et al.*, 2004). Among all large size Scombrids (Collette *et al.*, 1983), the temperate and cold climate species are represented by the group of the "Bluefin" tunas (Yamanaka *et al.,* 1963). In addition to the *Thunnus thynnus* (commercially called Atlantic Northern Bluefin tuna) (Linnaeus, 1758), two other species, from other parts of the world, show greater affinities: the *Thunnus orientalis* (Temminck *et al.*, 1842), commercially called Pacific Northern Bluefin tuna, and *Thunnus maccoyii* (Castelnau, 1872), called Southern Bluefin tuna. Even in these two species of Bluefin tunas, Hg concentrations vary between inter and intra muscle type. In addition to the protein composition of the muscles, there is another factor that determines a variation in the concentration level and is represented by the lipid content of the tissues, which has a dilution effect on Hg. Therefore, the higher fat content for certain tissues may result in lower concentrations of Hg (De Almeida Rodrigues *et al.*, 2019). Lower Hg level is found in the anterior part of the abdomen muscles respect to tail, as was observed in *Thunnus orientalis*, the Pacific Northern Bluefin tuna (Ando *et al.*, 2008), or in the caudal peduncle muscle than in the rest of the body in the same Pacific Northern Bluefin tuna (Kumar, 2017). Similarly, other studies have also found a significant decrease of total Hg in farmed *Thunnus maccoyii* (Southern Bluefin tuna) with high lipid content (Balshaw *et al.*, 2008). For humans, the tuna consuming represents the most important contribution to Hg ingestion (Bradley *et al.*, 2017) and it is extremely important to properly monitor contamination levels through appropriate sampling procedures.

Finaly, the evidence of uneven distribution of Hg in various muscles, performed on a suitable sample of *Thunnus thynnus* caught in a static trap in the Mediterranean area, will allow to identify the representative muscular part of the Hg levels of the whole carcass that could be sampled for Hg routine food controls.

Materials and Methods

The study started with an "a priori" sample size calculation. Assuming an average difference of the distributions of total mercury (HgTot = inorganic and organic mercury) of ± 0.3 mg/kg than the maximum level of 1.0 mg/kg (European Commission, 2006) and a standard deviation of 0.4 mg/kg, with a type "alpha" error of 0.05 and a power of 0.80 (type "beta" error of 0.20) with a two-tailed test, the calculated sample size was at least 14 tuna (equally divided between males and females). This allowed designing a data analysis and regression model to correlate the Hg-Tot values observed in the tuna samples and some collected parameters (such as gender, weight, and length of the tuna). Considered the lower number of female tuna in the lot (with 6 specimens instead of 7), the sample size was prudently increased to a total of 15 tuna (9 males and 6 females), which constitute a sublot obtained from the primary lots n. 61 e 62, register with BCD (Bluefin-tuna Catch Document) IT-18-900577, caught on 28/06/2018 by static tuna trap of Isola Piana, alongside the coast of Carloforte, in south-western Sardinia. The two days following the catch of the mentioned sublot, muscle portions of about 100 g were taken from the left side of each specimen in 7 specific sites of the carcass. One of these was dark muscle alongside the fishbone, the remaining portions were taken in the white muscle from three points (director A, dorsal) along with the upper loin (composed of epaxial muscles) and from three points along the full-thickness lower loin (director B, ventral) (composed of all hypaxial muscles), therefore including in the anteromedial region also the belly flap (Figure 1). The white muscle sampling points generally overlaps to the tuna (sites) regions that, in Japanese food culture, now widespread all over the world, correspond to meat parts: "se-kami", "senaka", se-shimo" (A-1, A-2, and A-3 respectively) and "hara-kami", "hara-naka", "hara-shimo" (B-1, B-2, and B-3 respectively). Selection of these specific sampling sites was intended to follow protocols applied in similar research on other species of large size Scombrids (Ando *et al.*, 2008; Balshaw *et al.*, 2008; Bosch *et al.*, 2016; Ross *et al.*, 2015; Vieira *et al.*, 2017). Also according to the restrictions legislative of Official Sampling (European Commission, 2007), which specify that the sampling "may be applied provided that it is sufficiently representative", and also that it does not involve "unacceptable commercial consequences". Therefore, sampling points A-1 and A-2 are located respectively at the anterior and posterior edge of the entire cut, locally called "schienale". This sampling was performed without damaging to the meat. The same attention was applied for points B-1 and B-2 locally called "bodano" and "ventresca" in Japanese belly flap or "o-toro", and points A-3 and B-3 located respectively in the anterior and posterior edge of the "codella nera" (or black tail) and of the "codella bianca" (or white tail). All 105 (15x7) muscle samples were immediately packaged, labeled in single bags, and transferred at a controlled temperature to the Istituto Zooprofilattivo Sperimentale della Sardegna laboratory. All samples were stored at temperatures below -20°C until analysis. HgTot concentrations were determined by an inductively coupled plasma mass spectrometry (ICP-MS), under compliance with US EPA 6020B methods for the instrumental analysis and with US EPA 3052 for the sample treatment. The analytical procedure involved the mineralization of the sample (1g) in a glass vessel with 5 mL of ultrapure nitric acid, 70% (J.T. Baker, Phillipsburg, USA), carried out in a Discover SP-D microwave digestion system (CEM Corp., USA) under monitored temperature and pressure. The mineralization process started with a heating-up phase at a temperature of 200°C and continued at this temperature for the other 5 minutes. At the end of the process, the resulting solutions were diluted to 50 mL with ultrapure water MILLI-Q® and before the instrumental analysis, they were diluted again to 1:20 mL. The instrumental analysis was per-

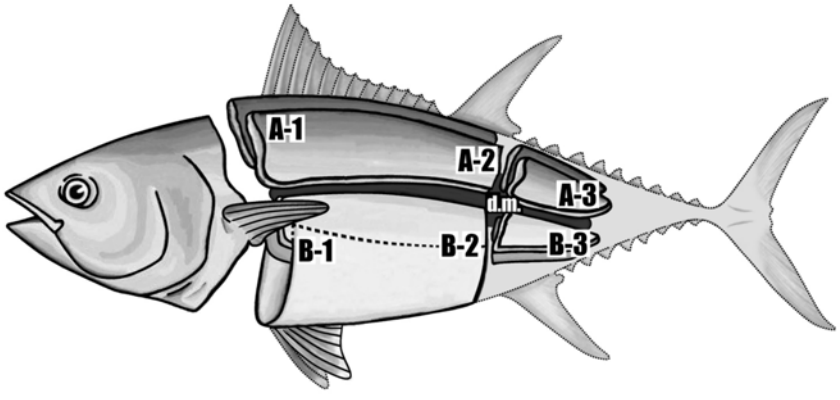

Figure 1. Scheme of the sampling points selected for the analysis of Hg in bluefin tuna caught by traditional static tuna traps in the Mediterranean. With "A" is indicated the upper loin points (epaxial muscles), with "B" the full-thickness lower loin points (all hypaxial muscles, including belly flap) and with "d.m." the dark muscle.

formed with an inductively coupled plasma mass spectrometer ICP-MS/MS (Agilent 8800 Triple Quad) consisting of a collision cell and two quadrupole mass analyzers. In comparison to a single quadrupole ICP-MS system, the triple quadrupole system significantly increases the accuracy of mass separation. HgTot concentrations in the reading solutions were determined by the interpolation of the reading signal with multi-level calibration curves, using ^{202}Hg as the quantification isotope and ^{209}Bi as the internal standard of the compensation signal. The analytical procedure has a LOQ value of 0.005 mg/kg for HgTot. For the Quality Control (QC) and the verification of contaminations and recoveries, the aforementioned system requires the blank process test and the analysis of the certified reference materials: Fish muscle ERM-BB422 (0.601 ± 0.030 mg/kg) and Tuna BCR-463 (2.85 ± 0.16 mg/kg). The testing method used is accredited according to UNI EN ISO 17025/2017.

Results

The HgTot results showed a normal distribution and a homogeneity of variances (Shapiro-Wilk's test and Barlett test respectively). Therefore, the parametric statistical method has been used to evaluate the data. The highest mean concentrations were found in the dark muscle, with statistically significant differences (p=0.0001) from weighted averages concentrations of the white muscle. The averages were calculated for the relative weight of the muscle substrand (Table 1). These averages were expressed as the values of a composite representative of all the white muscle of each carcass. Regarding the weighted averages of HgTot distribution in the different carcasses, statistically positive correlations were found for the size of tuna, weight (p=0.0069), and the length (p=0.0125). Contrary no differences were established for sex (p=0.0679) and nutritional status (p=0.1128) evaluated with "Fulton's condition factor (K)". This factor linked, to the trophic synchronization of the reproductive phase, was in fact relatively homogeneous with an average ratio of 1.638 ± 0.096. Even the white muscle shows significant differences, proceeding longitudinally for both loins, and in particular for the lower loin ($p \leq 0.0001$) and between the two loins (p=0.0004) specifically, the muscle samples taken in their anterior sites the sampling point A-1 for the upper loin and the B-1 for the lower loin, which is more fat due to the presence of the belly flap or "ventresca" (Figure 2). Finally, the values of the different white muscle sampling points were compared with the weighted average HgTot concentration. Significant differences were found in five of the six sampling points although with different significance levels. Point A-1, the anterior part of the upper loin or "schienale", ("se-kami") has a concentration level representative of the average HgTot content of each individual carcass. So the analysis of this muscular part would give results comparable to those obtained from the analysis, more laborious and with greater commercial consequences, of a hypothetical composite sample formed by multiple muscle portions of the carcass, which should also take into account the relative weight of each muscle portion on the total weight of the carcass.

Discussion

The results obtained in the present study are comparable with data reported by other authors for different species of tuna (Ando *et al.*, 2008; Balshaw *et al.*, 2008; Bosch *et al.*, 2016; Ross *et al.*, 2015), showing an uneven distribution of HgTot in the *Thunnus thynnus* muscles. This evidence could affect results obtained for routine analysis of HgTot control. Muscle part sampled may not be representative of the levels of the whole specimen and this could be a critical condition when HgTot concentrations are around the maximum value of 1.0 mg/kg (European Commission, 2006). As suggested by other authors, the HgTot variation observed between dark and white muscle may be due to differences in muscle fiber development and composition (Vieira

Table 1. Mercury levels (mg/kg fresh weight) for carcass and sampling locations in a lot of bluefin tuna caught in the south-western coast of Sardinia in the 2018 fishing season.

COD No	Sex	W.W. (kg)	S.L. (cm)	Fulton's factor (K)	Dark muscle	White muscle						Weighted averages
						A-1	B-1	A-2	B-2	A-3	B-3	
876	F	320.3	265	1.721	1.251	1.315	0.831	1.289	0.908	1.221	1.131	1.111
831	M	315.4	262	1.754	1.537	1.064	0.736	1.468	1.106	1.235	1.179	1.120
812	F	312.3	260	1.777	1.531	1.008	0.808	1.245	1.167	1.132	1.176	1.081
874	F	258.5	258	1.505	0.649	0.565	0.408	0.652	0.632	0.628	0.628	0.579
867	F	253.3	250	1.621	0.793	0.633	0.465	0.736	0.656	0.797	0.776	0.655
875	M	251.6	248	1.649	0.884	0.578	0.518	0.914	0.739	0.807	0.754	0.707
881	F	241.2	242	1.702	1.337	1.052	0.807	0.906	1.029	0.934	0.941	0.947
816	F	224.4	240	1.624	1.438	0.960	0.627	1.214	1.333	1.093	1.092	1.047
821	M	217.5	238	1.613	0.563	0.434	0.321	0.594	0.496	0.569	0.558	0.486
864	M	212.6	233	1.681	0.458	0.416	0.195	0.508	0.521	0.459	0.439	0.420
827	M	211.5	229	1.761	0.782	0.652	0.366	0.696	0.659	0.642	0.652	0.604
897	M	175.4	225	1.540	1.216	0.673	0.752	1.060	0.985	0.991	0.774	0.870
802	M	166.7	223	1.503	0.938	0.859	0.438	0.909	0.959	0.853	0.880	0.807
826	M	140.2	205	1.628	0.486	0.262	0.338	0.479	0.421	0.420	0.411	0.383
818	M	126.6	204	1.491	0.542	0.453	0.380	0.510	0.522	0.452	0.474	0.467
				Average concentration =	0.960	0.728	0.533	0.879	0.809	0.816	0.791	
				Standard deviation =	± 0.392	± 0.299	± 0.210	± 0.318	± 0.280	± 0.281	± 0.268	

COD = numeric code for carcass applied by the Port Authority for fishing; Sex (F = female, M = male); W.W. = whole weight; S.L. = standard (or "fork") length; whole weight and standard length were used to determine the "Fulton's condition factor (K)" of each tuna, using the following formula: $K = (WW / SL^3) \times 10^5$.

Figure 2. Initial processing step of bluefin tuna at Tonnare Sulcitane's establishment in South-West of Sardinia. With "A-1" is indicated, in the upper loin, the representative sampling point of the average Hg contents of the white muscle as a whole.

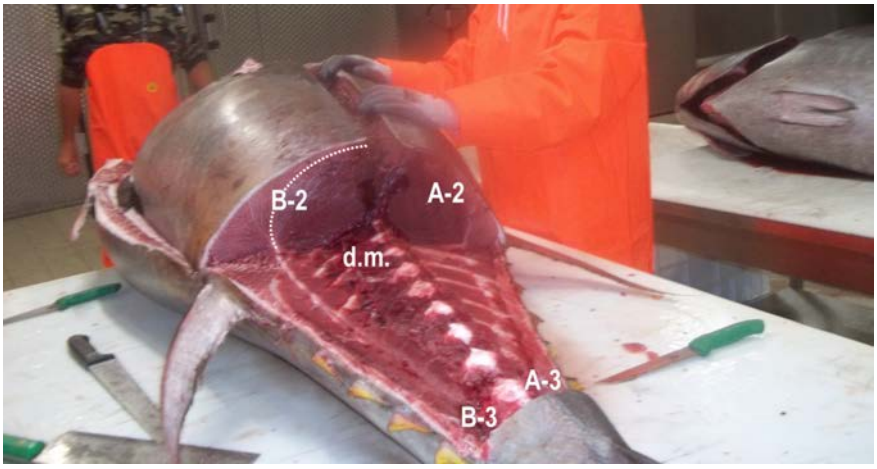

Figure 3. Intermediate processing step of bluefin tuna at Tonnare Sulcitane's establishment after removal of tails. With "A" is indicated the upper loin points, with "B" the full-thickness lower loin points including the backside of the belly flap and with "d.m." the dark muscle.

et al., 2017). Furthermore, the areas that accumulate higher levels of lipid show a lower HgTot concentration than lean tissues (Ross *et al.*, 2015). Therefore, lipid content appears to have a dilution effect on HgTot already associated with fish tissues (Balshaw *et al.*, 2008), and also, there is a longitudinal variation into muscle part in addition to a variation between muscle parts. Structural and functional features of the different muscle types, especially dark and white muscle, as well as the different lipids content of some tissues over others, resulting in a cross-carcass variation of HgTot concentration of tuna muscular tissue. This has clear implications for a compliant assessment of batches. The choice of the tissue sample to be analyzed becomes, therefore, a very critical point in the sampling procedures (Balshaw *et al.*, 2008). Concerning HgTot quantification in fish samples for purposes of public health, although tuna white muscle has generally been indicated as the representative tissue for the edible part of the fish (Bosch *et al.*, 2016), according to other authors, for better monitoring of HgTot levels in the edible muscle, also the dark muscle should be taken in account and analyzed as the "worst-case scenario" (Vieira *et al.*, 2017). Most authors believe that, in order to obtain reliable results, the ideal action when evaluating HgTot is to collect specific white muscle samples (Bosh *et al.*, 2016; De Almeida Rodrigues *et al.*, 2019). As also reported in this work, the most representative sampling points can be found in the anterior part of the upper loin (or "chu-toro" in Japanese) and, in the upper area, between the first dorsal fin and the cranial extremity ("se-kami" in Japanese) of the tissue cut locally called "schienale" and identified with "A-1" in Figures 2 and 3.

Conclusions

The study has shown that, as for other species of large size Scombrids with tropical and temperate-cold marine habitats, also for the Atlantic Northern Bluefin tuna (*Thunnus thynnus*) caught in the Mediterranean, the distribution of HgTot is uneven in the various muscular parts. This evidence may have legal implications, as well as in risk assessment in the scientific field. Although analysis of white meat may give a most accurate measurement of representative fish mercury levels, however producing a composite sample of large size tuna could also be expensive and time-consuming. The results of this study, in agreement with other authors (Balshaw *et al.*, 2008), indicate that, also in *Thunnus thynnus*, the anterior portion of the upper loin is representative of the mercurial content average of whole fish white muscular tissues. Moreover, the indications of sampling for this specific site of Tuna fish, consistent with the indications given in the Commission Regulation (EC) 333/2007 laying down in points B.2.2. and B.2.3. of "Sampling plans" paragraph of sampling and analysis for the control of the levels of contaminants in foodstuffs, would provide useful guidance even for the sampling of very large fish, such as tuna.

References

Altringham JD, Block BA, 1997. Why do tuna maintain elevated slow muscle temperatures? Power output of muscle isolated from endothermic and ectothermic fish. J Exp Biol 200:2617-27.

Altringham JD, Shadwick RE, 2001. Swimming and muscle function. In: Block BA, Stevens ED (eds) Tuna: physiology, ecology, and evolution. Chapter 8. Academic Press, London, pp 314-44.

Ando M, Seoka M, Nakatani M, Tsujisawa T, Katayama Y, Nakao M, Tsukamasa Y, Kawasaki KI, 2008.Trial for quality control in mercury contents by using tailmuscle of full-cycle cultured bluefin tuna (Thunnus orientalis). J Food Prot 71:595-601.

Annibaldi A, Truzzi C, Carnevali O, Pignalosa P, Api M, Scarponi G, Illuminati S, 2019. Determination of Hg in farmed and wild atlantic bluefin tuna (Thunnus thynnus) muscle. Molecules 24:1273.

Balshaw S, Edwards JW, Daughtry BJ, Ross KE, 2007. Mercury in seafood: mechanisms of accumulation and consequences for consumer health. Rev Environ health 22:91-113.

Balshaw S, Edwards JW, Ross KE, Daughtry BJ, 2008. Mercury distribution in the muscular tissue of farmed southern bluefin tuna (Thunnus maccoyii) is inversely related to the lipid content of tissues. Food Chem 111:616-21.

Bosch AC, O'Neill B, Sigge GO, Kerwath SE, Hoffman LC, 2016. Mercury accumulation in yellowfin tuna (Thunnus

albacares) with regards to muscle type, muscle position and fish size. Food Chem 190:351-6.

Bradley MA, Barst BD, Basu N, 2017. A Review of Mercury Bioavailability in Humans and Fish. Int J Env Res Publ Health 14:169.

Cammilleri G, Vazzana M, Arizza V, Giunta F, Vella A, Lo Dico G, Giaccone V, Giofrè SV, Giangrosso G, Cicero N, Ferrantelli V, 2018. Mercury in fish products: what's the best for consumers between bluefin tuna and yellowfin tuna? Nat Prod Res 32:457-62.

Castelnau FL, 1872. Scombridae (Thunnus maccoyii). Contribution to the ichthyology of Australia. In: Proceedings of the Zoological and Acclimatisation Society of Victoria.Volume 1. F.A. Masterman, General Printer, Melbourne, pp 104-7.

Collette BB, Nauen CE (eds) 1983. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO Species Catalogue No 125, Volume 2. Published by arrangement whit the FAO of the ONU, Rome, pp 1-137.

De Almeida Rodrigues P, Gomes Ferrari R, Neves dos Santos L, Adam Conte C, 2019. Mercury in aquatic fauna contamination: a systematic review on its dynamics and potential health risks. J Environ Sci 84:205-18.

European Commission, 2006. Setting maximum levels for certain contaminants in foodstuffs. Consolidated version of the Regulation (EC) No 1881/2006 of 19 December 2006. Official Journal of the EU, L 364:5-24.

European Commission, 2007. Laying down the methods of sampling and analysis for the control of the levels of trace elements and processing contaminants in foodstuffs. Consolidated version of the Regulation (EC) No 333/2007 of 28 March 2007. Official Journal of the EU, L 88:29-38.

European Food Safety Authority (EFSA) Panel on contaminants in the food chain (CONTAM), 2012. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA J 10:2985.

Graham JB, Dickson KA, 2004. Tuna comparative physiology (Commentary). J Exp Biol 207:4015-24.

Harris HH, Pickering IJ, George GN, 2003. The Chemical Form of Mercury in Fish. Science 301:1203.

Houssard P, Point D, Tremblay-Boyer L, Allain V, Pethybridge H, Masbou J, Ferriss BE, Baya PA, Lagane C, Menkes CE, Letourneur Y, Lorrain A, 2019. A model of mercury distribution in tuna from the western and central Pacific Ocean: influence of physiology, ecology and environmental factors. Environ Sci Technol 53:1422-31.

Itano K, Sasaki S. 1983. Binding of mercury compounds to protein components of fish muscle. B Jpn Soc Sci Fish 49: 1849-1853 (original language: Japanese - translated (1984) by "Canadian Translation of Fisheries and Aquatic Sciences No. 5104 (http://www.dfo-mpo.gc.ca/Library/89091.pdf).

Katz SL, 2002. Design of heterothermic muscle in fish (Review). J Exp Biol 205:2251-66.

Kumar G, 2017. Mercury concentrations in fresh and canned tuna: a review. Rev Fish Sci Aquac 26:1-10.

Lavoie RA, Jardine TD, Chumchal MM, Kidd KA, Campbell LM, 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ Sci Technol 47:13385-94.

Linnaeus C, 1758. Pisces thoracici (Thunnus thynnus). In: Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I, editio decima, reformata. Impensis direct. Laurentii Salvi, Stockholm, pp 260-302.

Olson RJ, Young JW, Menard F, Potier M, Allain V, Goñi N, Logan JM, Galván-Magaña F, 2016. Bioenergetics, trophic ecology, and niche separation of tunas. In: Curry BE (eds). Advances in marine biology. Chapter 4. Elsevier Ldt, Orlando, pp 199-344.

Piras PL, Assaretti A, Fiori G, Sanna A, Chessa G, 2019. A case study on the development of representative sampling procedure to determine mercury levels in a lot of tuna caught by static trap. Ital J Food Safe 8:213-7.

Ross K, Edwards J, 2015. Spatial variation in the mercury concentration of muscle myomeres in steaks of farmed southern bluefin tuna. Foods 4:254-62.

Shadwick RE, Schiller LL, Fudge DS, 2013. Physiology of swimming and migration in tunas. In: Palstra AP, Planas JV (eds) Swimming physiology of fish. Chapter 3. Springer-Verlag, Berlin Heidelberg, pp 45-78.

Temminck CJ, Schlegel H, 1842. Les thons (Thunnus orientalis). In: Fauna Japonica sive Descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batava imperium tenent. Regis auspiciis edita. Apud A. Arnz et socios, Leida, pp 94-99.

Vieira HC, Bordalo MD, Morgado F, Soares AMVM, Abreu SN, 2017. Mercury content in the white and dark muscle of skipjack tuna (Katsuwonus pelamis) along the canning process: implications to the consumers. J Food Compos Anal 56:67-72.

Yamanaka H, Tiews K and Robins JP, 1963. Synopsis of biological data on kuromaguro (pacific bluefin tuna), atlantic bluefin tuna and southern bluefin tunas. In: FAO Fisheries Biology Synopsis Nos. 49, 56, 60, SAST - Tuna, Rome, pp 180-217, 422-81, 562-87.