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Mercury accumulation in Yellowfin tuna (*Thunnus albacares*) with regards to muscle type, muscle position and fish size

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ABSTRACT

The concentrations and relationships between individual mercury species and total mercury were investigated in different muscle parts and sizes of Yellowfin tuna (*Thunnus albacares*). Fourteen Yellowfin tuna caught in the South Atlantic off the coast of South Africa had an average total Hg (tHg) concentration of 0.77 mg/kg wet weight. No differences were detected ($p > 0.05$) in tHg, MethylHg (MeHg) or inorganic Hg (iHg) accumulation among the four white muscle portions across the carcass, but both tHg and iHg were found in higher concentrations ($p < 0.001$) in dark muscle than white muscle. Positive linear correlations with fish weight were found for both tHg ($r = 0.79$, $p < 0.001$) and MeHg ($r = 0.75$, $p < 0.001$) concentrations. A prediction model was formulated to calculate toxic MeHg concentrations from measured tHg concentrations and fish weight (cMeHg = 0.073 + 1.365·tHg − 0.008·w). As sampling sites and subsampling methods could affect toxicity measurements, we provide recommendations for sampling guidelines.

1. Introduction

Fish meat is widely consumed and considered a main source of nutrition in many coastal communities. It contributes to a healthy diet by providing high-value amino acids and nutrients (vitamins and minerals) and is an excellent source of essential omega-3 fatty acids associated with many health benefits (Domingo, Bocio, Falcó, & Llobet, 2006). Although highly nutritious, high consumption of some fish meat can have significant adverse effects on human health due to the bioaccumulation of heavy metals in fish muscles from the surrounding aquatic environment (Castro-González & Méndez-Armenta, 2008; Järup, 2003).

The accumulation of heavy metals in fish can occur due to metals being naturally present in the aquatic environment, but can also be exacerbated by anthropogenic activities such as industrial activity and pollution (Järup, 2003). However, not all metals are hazardous as some are essential elements in biological systems, only becoming toxic when present at high concentrations (Munos-Olivas & Camara, 2001; Schroeder & Darrow, 1972). Amongst the metals that accumulated in fish and seafood, mercury (Hg) is one of the most abundant toxic metals (Carvalho, Santiago, & Nunes, 2005; Chen et al., 2012). The total Hg (tHg) content of fish can consist of a combination of several Hg species (MethylHg, EthylHg and inorganic Hg) (Morel, Kraepiel, & Amyot, 1998). The toxicity of these individual Hg species differs; the organic mercury species (MethylHg and EthylHg) are considered toxic and inorganic Hg (iHg) is considered non-toxic as it is not as easily absorbed into living organisms compared to the organic forms and is very slow to cross the blood–brain barrier and therefore does not display toxic effects in fish or humans (Guynup & Safina, 2012). MethylHg (MeHg) is considered the most toxic form and it is also the most abundant Hg species (75–100% of tHg) in fish meat (Burger & Gochfeld, 2004). Measuring the various Hg species can therefore improve the determination of true fish toxicity and subsequently inform regulatory bodies on fish safety.

Current FAO legislation (FAO, 2003) has stipulated a maximum tHg limit of 0.5 mg/kg for fish and seafood with the exception of predatory fish (shark, tuna and swordfish) which has a limit of 1.0 mg/kg. Regulations by the South African Department of Health specify these same limits (0.5 and 1.0 mg/kg) as for MeHg (DOH, 2004) as the main toxic component of tHg. Mercury levels are monitored according to Commission Regulation (EC) No. 333/2007 as enforced by the National Regulator for Compulsory Specifications (NRCS). As it is normally assumed that almost 100% of tHg is present as MeHg, commercial fish samples are only tested for tHg and not specifically for MeHg. The actual levels of toxic Hg in commercial fish remain unknown. Routine monitoring of MeHg concentrations in addition to the current tHg analysis procedure would require Hg speciation and thus additional analyses

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with associated equipment and costs. An accurate model to predict MeHg content from tHg measurements would therefore greatly benefit the fishing industry.

The Commission Regulation (EC) No. 333/2007 describes sampling for routine Hg analysis. However, this regulation lacks detail as to which fish sizes and carcass sites need to be sampled. In large pelagic fish species such as tuna, chemical composition of the various muscles and anatomical sections can vary (Balshaw, Edwards, Ross, & Daughtry, 2008), as these fish have two very distinct muscle types (dark and white muscle), which have different functions (dark = slow, continuous movement; white = fast, sudden movement) and compositions (Te Kronnié, 2000). Mercury is accumulated in the protein fraction of the muscle as it binds to thiol groups (Harris, Pickering, & George, 2003; Nakao, Seoka, Tsukamasa, Kawasaki, & Ando, 2007). The presence of such different muscle types with varying protein compositions can therefore result in variation in heavy metal accumulation and concentration across the fish carcass. Balshaw et al. (2008) found such variation in Hg levels between different commercial cuts of farmed Southern Bluefin tuna (*Thunnus maccoyii*). Sampling and measuring fish muscle at various carcass positions can therefore shed light on the extent of intra fish variability in heavy metal concentrations and can aid method standardisation for fish subsampling.

A positive relationship between fish size/age and tHg and MeHg concentrations has been identified for numerous fish species investigated (Andersen & Depledge, 1997; Campbell, Balirwa, Dixon, & Hecky, 2010; Kraepiel, Keller, Chin, Malcolm, & Morel, 2003; Storelli, Giacominelli-Stuffler, & Marcotrigiano, 2002a; Van den Broek & Tracey, 1981; Walker, 1976). Within a single fish population, Hg concentrations can vary widely (Bosch, 2012) from well below the maximum allowable limit in smaller sized fish to levels substantially above the limit in larger, older fish. Detailed, species-specific research is needed to determine the fish weight above which Hg limits are likely to be exceeded, as this limit might depend on metabolism and growth rates specific to each fish species or sub population. This threshold weight could be used to introduce weight specific catch limits to avoid wastage of fish likely to be considered not suitable for consumption and advise more accurate subsampling for routine analyses avoiding biased results from misrepresented fish sizes.

Yellowfin tuna is a commercially important fish with a large size range and is widely consumed due to its high quality meat. Several studies have reported on the total Hg and Hg species content in Yellowfin tuna (Ferriss & Essington, 2011; Kraepiel et al., 2003; Menasveta & Siriyong, 1977; Ruelas-Inzunza, Patiño-Mejía, Soto-Jiménez, Barba-Quintero, & Spanopoulos-Hernández, 2011; Schmidt et al., 2015; Teffer, Staudinger, Taylor, & Juanes, 2014), however limited knowledge still exists on the extent of individual mercury species and the relationships and variations between the accumulation of these individual species and total mercury in different muscle parts and different sizes of Yellowfin tuna. Therefore, the overarching aim of this study was to investigate the accumulation of total mercury and individual mercury species (methylmercury, ethylmercury, inorganic mercury) in South African Yellowfin tuna (*Thunnus albacares*) meat and determine variation caused by carcass position, muscle type and fish size. A subsample was used to test for correlations between total Hg measurements and Hg speciation results that could be used in prediction models.

2. Materials and methods

2.1. Sampling

Fourteen Yellowfin tuna fish were caught off the Atlantic coast of South Africa (S34°29′ E17°54′ and S34°35′ E17°58′) and ranged in size from 29.0 to 50.8 kg. Six muscle subsamples per fish were used for chemical analysis. Ceramic knives were used for cutting meat samples to minimise sample contamination. Three samples were taken anteriorly in the dorsal (A), mid (B) and ventral (C) axial muscles, located at the start of the first dorsal fin, and three samples were taken at the dorsal (D), mid (E) and ventral (F) axial muscles at the start of the second dorsal fin (Fig. 1). The middle samples (B and E) consisted of dark muscle and the dorsal and ventral samples (A, D, C and F) consisted of white muscle. All meat samples were homogenised prior to further analysis.

2.2. Analyses

2.2.1. Total mercury – ICP-MS

Total Hg was measured by means of inductively coupled plasma mass spectrometry (ICP-MS). Approximately 0.3 g of the homogenised meat sample and standard (certified reference material: BCR®-463) were used for sample digestion. This was done in 2 mL HCl and 8 mL HNO_3 (Merck Suprapur® acids) in a Mars 240/50 microwave digester (produced by CEM) at 160 °C and 800 psi for 20 min after which the solution was diluted to 50 mL with deionised water in a sample bottle cleaned with 5% HNO_3. The digested samples were then analysed on an Agilent 7700 ICP-MS, with Hg measured in no-gas mode, under robust conditions and online dilution with argon gas provided by the unique HMI function of the instrument. The instrument was tuned to optimise sensitivity and minimise oxides to <1%. It was subsequently calibrated using the NIST-traceable standards, with quality control checks performed to verify accuracy of results. A rinse programme was set up to ensure efficient wash-out of Hg in the expected concentration range of the samples. Samples with unexpectedly high concentrations are diluted and re-analysed, as well as samples that followed the initial high concentration sample. Results are given as mg/kg meat sample with the detection limit of tHg at 0.003 mg/kg.

2.2.2. Mercury speciation – HPLC–ICP-MS

2.2.2.1. Instrumentation, standards and reagents. An Agilent 7700 ICP-MS connected to an Agilent 1100 HPLC was used to measure inorganic, methyl- and ethylmercury in prepared samples. The system specifications are given in Table 1. The MassHunter workstation software was used for the setup and control of the coupled HPLC–ICP-MS system. The instrument was tuned to optimise sensitivity and minimise oxides to <1%, with Hg analysed in no-gas mode. The Hg species were separated in a mobile phase of 98% L-cysteine solution (0.1% w/v L-cysteine + 0.1% w/v

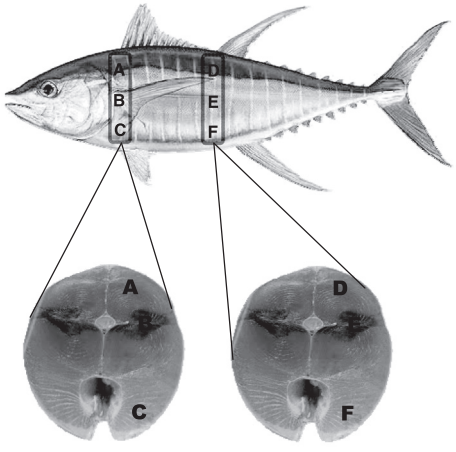

Fig. 1. Tranverse section of a tuna carcass indicating the positions of the white (A, C, D and F) and dark (B and E) muscle. Letters A–F indicate sampling locations.

L-cysteine·HCl·H_2O) + 2% methanol at 1 mL/min. Mercury (II) chloride (ACS reagent, ⩾99.5%, Sigma–Aldrich), methylmercury (II) chloride (PESTANAL®, analytical standard, Sigma–Aldrich) and ethyl mercuric chloride (Supelco analytical standard, Sigma–Aldrich) were used to prepare stock solutions of 2,000,000 μg/L iHg, 1,000,000 μg/L MeHg and 40,000 μg/L EtHg respectively. Calibration standards for the individual species (iHg, MeHg and EtHg) prepared by appropriate dilution of stock solutions in 0.1% w/v L-cysteine hydrochloride monohydrate (L-cysteine·HCl·H_2O) solution to concentrations of 1–20 μg/L were run at the beginning of each analytical batch, with control standards every 8–10 samples. Detection limits for individual species on the ICP-MS were 0.030 μg/L, 0.030 μg/L and 0.050 μg/L for iHg, MeHg and EtHg, respectively. Two certified reference materials (CRMs) (BCR®-463 and ERM®-CE464) from the Institute for Reference Materials and Measurements (IRMM) in Belgium were included in every sample batch for accuracy evaluation. Deionised water was used for all solutions and standards. All glassware used was soaked in 15% HNO_3 for 24 h and rinsed with deionised water before every use to avoid sample contamination.

2.2.2.2. Sample preparation and Hg speciation. Using the mercury extraction process for extraction of iHg, MeHg and EtHg components based on the method described by Hight and Cheng (2006), 0.5 g of homogenised tissue was extracted with L-cysteine·HCl·H_2O solution in a water-bath at 60 °C for 2 h. The extract was filtered through a syringe filter (0.2 μm with a 0.8 μm prefilter) and one to 2 mL of the filtrate collected in a glass auto-sampler vial and kept in the dark, to be analysed on the same day as soon as possible after extraction. The same procedure was used to prepare and extract the lyophilised CRMs with certified values for total Hg and MeHg. Per sample, 20 μL was injected manually into the HPLC–ICP-MS on-line system and the injector rinsed with mobile phase solution in-between every injection. No carry-over was detected for any of the Hg species as monitored between single Hg species standards analysed. Samples were reanalysed if individual MeHg measurements for CRMs were more than 10% from the certified values. Average MeHg levels for BCR®-463 (2.97 ± 0.322) and ERM®-CE464 (5.45 ± 0.356) were within specification according to certified values for MeHg (2.83 ± 0.16 mg Hg/kg for BCR®-463 and 5.12 ± 0.17 mg Hg/kg for ERM®-CE464). The total of the Hg species concentrations determined by speciation had an average recovery of 104% when compared to the total Hg concentrations measured by the ICP-MS method for total metals.

2.3. Statistics

STATISTICA 12.5 was used for data analysis. Preliminary tests (normality) were conducted and true outliers were removed ($n = 1$) prior to analysis. All data conformed to the necessary assumptions. A mixed model repeated measures ANOVA was conducted to determine the variation of Hg concentrations between various fish carcass sites. To determine relationships between tHg, and Hg species and fish weight (before evisceration), Spearman correlations were reported in order to compensate for outliers and a simple regression analysis was conducted. A multiple regression analysis was done to investigate a prediction equation for MeHg concentrations. Results were reported at a 95% confidence level.

Table 1
HPLC–ICP-MS instrument parameters.

Agilent 7700 ICP-MS instrument parameters:	
RF power	1550 W
Sampling depth	8 mm
Carrier gas flow	1.06 L/min
Make-up gas flow	0.18 L/min
Agilent 1100 HPLC parameters:	
Column	ZORBAX Eclipse XDB C-18; 2.1 mm id × 50 mm, 5 μm
Flow rate	1 mL/min
Injection volume	20 μL
Mobile phase	2% methanol + 98% (0.1% w/v L-cysteine + 0.1% w/v L-cysteine·HCl·H_2O)

3. Results

Overall, tHg values ranged from 0.45 to 1.52 mg/kg wet weight with an average concentration of 0.77 mg/kg where the average was calculated from six anatomical sites of 14 tuna ($n = 84$) and 28.6% of the samples analysed were above the maximum allowable limit (1.0 mg/kg). MeHg values ranged from 0.23 to 1.24 mg/kg and iHg was present at much lower values (0.003–0.41 mg/kg). EtHg values were all below the detection limit (0.005 mg/kg) of the analytical method and were therefore considered insignificant and are not analysed or discussed further.

3.1. Cross-carcass Hg (tHg, iHg and MeHg) variation

Both tHg and iHg concentrations varied between sampling sites, where sites B and E (dark muscle sites) had higher ($p < 0.001$) concentrations compared to the rest of the carcass sites (white muscle) (Tables 2 and 3) with one exception (tHg in site B was statistically similar ($p > 0.05$) to site D). In addition, variability within the dark muscle sites was observed where site E had consistently higher concentrations than site B for both iHg ($p < 0.05$) and tHg ($p < 0.001$).

Limited inter-carcass variation in MeHg concentrations was apparent where the only difference ($p < 0.05$) observed was between the dark muscle portions (site E > site B). Therefore MeHg concentration did not vary significantly ($p > 0.05$) between the muscle types (white vs dark).

Overall it was noted that no variation ($p > 0.05$) in iHg, MeHg or tHg was found within the white muscle portions (A, C, D and F).

3.2. Regression analyses

3.2.1. Relationship between fish size and tHg, MeHg and iHg concentrations

Strong positive linear correlations were found between the average tHg concentration of each fish (six carcass sites) and fish weight ($n = 14$) ($r = 0.79$, $p < 0.001$) (Fig. 2) and similar results were found between the average MeHg concentrations per fish (six carcass sites) and fish weight ($n = 14$) ($r = 0.75$, $p < 0.001$). However, iHg was not significantly correlated with weight ($n = 14$) ($r = 0.08$, $p > 0.05$). From these regressions, Yellowfin tuna above 70 kg fresh weight are likely to exceed the tHg maximum limit (Fig. 2).

3.2.2. Relationship between MeHg and tHg concentration

Methylmercury had a strong positive linear correlation with tHg ($r = 0.77$, $p < 0.001$) when all 6 portions from all of the 14 sampled fish were included ($n = 84$). A simple regression analysis showed that when tHg measurements are used to predict the MeHg content in fish, the results have a root mean square error of calibration (RMSEC) of 0.133 mg/kg, which is more than 10% error of the maximum allowable limit of 1 mg/kg. This large RMSEC could be caused by the slight variation of both MeHg and iHg concentrations within the dark muscle (Table 2) and the iHg variation between dark and white muscle of the tuna (Table 3), which can all cause variation within the tHg measurements. Fish weight could also affect the MeHg to tHg relationship, as MeHg

Table 2
Average concentration (±standard deviation) of iHg, MeHg and tHg (mg/kg wet weight) for each carcass sampling site: A–F (n = 14 tuna). Differing superscript letters indicate significant (p < 0.05) differences between sampling sites for each Hg species.

	A	B	C	D	E	F
iHg	$0.06^{c} \pm 0.04$	$0.13^{b} \pm 0.09$	$0.07^{c} \pm 0.04$	$0.07^{c} \pm 0.05$	$0.17^{a} \pm 0.09$	$0.06^{c} \pm 0.04$
MeHg	$0.64^{ab} \pm 0.19$	$0.64^{b} \pm 0.23$	$0.65^{ab} \pm 0.21$	$0.66^{ab} \pm 0.23$	$0.67^{a} \pm 0.23$	$0.66^{ab} \pm 0.20$
tHg	$0.73^{c} \pm 0.21$	$0.84^{b} \pm 0.25$	$0.72^{c} \pm 0.23$	$0.73^{bc} \pm 0.22$	$0.88^{a} \pm 0.30$	$0.73^{c} \pm 0.22$

Table 3
Summary and comparison of average iHg, MeHg and tHg (mg/kg wet weight) concentrations (±standard deviation) in the dark (data from 2 sampling sites per carcass combined) and white muscle (data from 4 sampling sites per carcass combined) of Yellowfin tuna (n = 14 tuna).

	Dark muscle	White muscle	p-Value
iHg	0.159 ± 0.075	0.065 ± 0.035	<0.001
MeHg	0.659 ± 0.235	0.654 ± 0.209	>0.05
tHg	0.873 ± 0.286	0.726 ± 0.216	<0.001

is increasingly accumulated with increasing fish weight whereas iHg was shown to be independent of fish weight and the MeHg proportion of tHg would therefore increase with fish weight. Therefore to minimise the effect of muscle type and to incorporate fish weight, the regression was reanalysed using only data from white muscle portions (average per fish) and including both tHg and fish weight as variables in a multiple regression analyses. This resulted in the following prediction model: cMeHg = 0.073 + 1.365·ctHg − 0.008·w (Fig. 3) where ctHg is the measured total mercury concentration (mg/kg), w is fish weight (kg) and cMeHg is the predicted/calculated concentration for MeHg. A relatively low RMSEC of 0.06 mg/kg (r = 0.95) indicates that this is a more accurate prediction of the true MeHg values.

4. Discussion

The average tHg value (0.77 mg/kg) of the subsample is below the maximum allowable limit of 1.00 mg/kg (DOH, 2004; FAO, 2003). However, four fish had an average tHg concentration exceeding this limit. Therefore, almost 29% of the tuna fish sampled would be considered unsafe for human consumption. Due to bioaccumulation of Hg up the food chain, higher trophic level fish often have tHg levels close to or exceeding the maximum limit

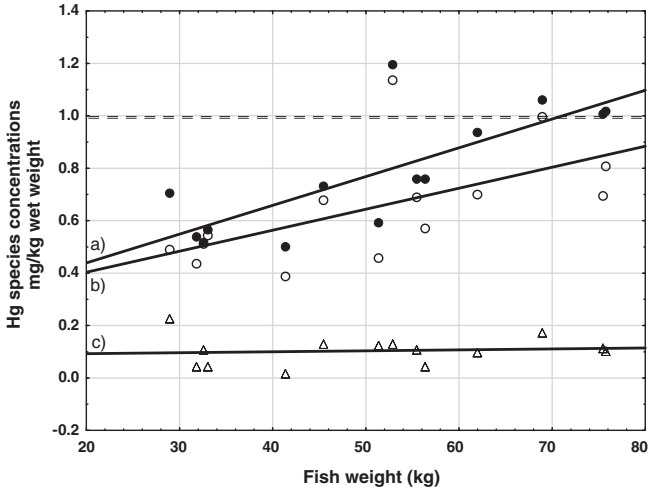

Fig. 2. Correlations between fish weight w (n = 14) and average concentrations of (a) tHg = 0.22 + 0.01 w (●), (b) MeHg = 0.24 + 0.01 w (○) and (c) iHg = 0.09 + 0.0004 w (△); where individual Hg concentrations are given as mg/kg wet weight and the fish weight (w) is in kg. The horizontal dotted line indicates the maximum allowable limit of tHg in tuna meat.

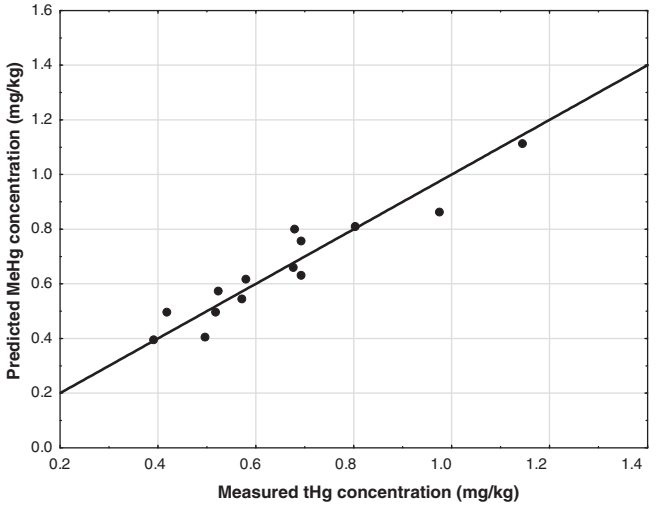

Fig. 3. Scatterplot of predicted MeHg against measured tHg concentrations (mg/kg wet weight). Regression equation: cMeHg = 0.073 + 1.365·ctHg − 0.008·w (r = 0.95).

(Peterson, Klawe, & Sharp, 1973; Storelli, Giacominelli-Stuffler, & Marcotrigiano, 2002b). Total Hg however includes both toxic and non-toxic species and is therefore not necessarily representative of meat toxicity.

Methylmercury is the most toxic and most abundant Hg species (Clarkson, Vyas, & Ballatori, 2007) often assumed to constitute 100% of the tHg present (Andersen & Depledge, 1997; Campbell et al., 2010; Spry & Wiener, 1991; Storelli et al., 2002a; Walker, 1976). The maximum allowable limit for MeHg (DOH, 2004) is the same as for tHg (1.00 mg/kg) (FAO, 2003). However, if we consider the measured MeHg values with regards to the maximum limit, we find that only 14% of the tuna fish measured would be considered unsuitable for human consumption (compared to the 29% when tHg is used). Current research on sampling and measuring protocol with regards to specific mercury species (Schmidt, Bizzi, Duarte, Dressler, & Flores, 2013) and compliance to maximum allowable limits can therefore add to current knowledge and specifications in order to acquire more accurate measurements and reports of the toxicity of fish meat (Branch, 2001; Van Dael, 2001). Therefore, improved understanding and knowledge regarding how MeHg accumulates and the MeHg:tHg ratios in fish meat could potentially reduce unnecessary wastage of fish due to the inaccuracy of toxic classification.

4.1. Cross-carcass Hg variation

The inter and intra muscle type (dark and white) variability in Hg accumulation in Yellowfin tuna suggests that potential biases can exist when subsampling fish for measuring toxicity as iHg concentrations are higher in dark muscle than in white muscle whereas MeHg is equally accumulated in both white and dark muscle. Sampling from dark muscle will therefore result in higher tHg readings and Hg toxicity of the fish carcass could therefore be overestimated. Systematic differences in Hg among different

muscle types need to be taken into account in the sampling protocol in order to obtain representative and accurate monitoring of Hg toxicity in fish.

Other studies (Ando et al., 2008; Balshaw et al., 2008; Lares, Huerta-Diaz, Marinone, & Valdez-Marquez, 2012) have also found inter and intra muscle type variation in Hg concentrations in some tuna species (*Thunnus orientalis* and *T. maccoyii*). Lares et al. (2012) found higher Hg concentrations in the caudal peduncle muscle tissue (CPMT) than in the rest of the body regions as was similarly found in the present study with higher tHg concentrations in the posterior sample (site E) of the dark axial muscle. Both Ando et al. (2008) and Lares et al. (2012) found lower Hg concentrations in the front of the abdomen (white muscle) compared to the rest of the white muscle regions in the fish body. This variation in Hg concentration could be caused by a dilution effect of the higher fat content of this portion of the carcass (Balshaw et al., 2008). No significant differences were, however, found within the white muscle between different body regions in Yellowfin tuna in the current study.

Apart from the possible effect of lipid content of muscle, the Hg variation observed within the dark muscle and between dark and white muscle may be due to differences in muscle function and therefore differing muscle fibre development and composition (Shadwick, Katz, Korsmeyer, Knower, & Covell, 1999). Te Kronnié (2000) found that in zebrafish (*Danio rerio*) larvae, the white muscles used for fast movement were the first to develop with relatively late maturation of the lateral layer of dark (slow) muscle. Stickland (1983) also found differences in the rates of muscle cell growth and increase between dark and white muscle in rainbow trout (*Salmo gairdneri*). Te Kronnié (2000) also found that muscle activity had an effect on the rate of muscle fibre development. Larger migratory fish such as tuna are known for continuous strong swimming driven by the dark muscle with virtually all of the thrust produced at the tail blade (Shadwick et al., 1999). This higher activity in the caudal region could possibly explain a higher rate of dark muscle fibre development in this region of the fish. As Hg is continuously accumulated in fish by binding to protein sites (Harris et al., 2003; Menasveta & Siriyong, 1977; Nakao et al., 2007), it could be expected that Hg accumulation is affected by the rates and regions of muscle development. This relationship between Hg accumulation and muscle development, however, needs further investigation in order to prove such an assumption.

Previous studies have concluded that CPMT of tuna is an appropriate region for subsampling for routine toxicity measurement as it would represent the highest Hg concentration within the carcass (Ando et al., 2008; Lares et al., 2012). This conclusion is however based on investigations of tHg concentrations and not individual Hg species. From the Hg speciation results in the current study, it is apparent that the higher tHg concentration in the caudal dark muscle compared to that in the white muscle of the rest of the carcass would be due to higher non-toxic iHg concentrations while toxic dark muscle MeHg concentrations are in fact not different from concentrations in white muscle regions. Therefore sampling from the white muscle regions for tHg measurements would render more representative results of the true Hg toxicity of the entire edible muscle portion. Previous studies found that sampling from the front abdominal white muscle in certain tuna species (*T. orientalis*) could result in under-representation of the Hg content in the rest of the carcass (Ando et al., 2008; Lares et al., 2012). It would therefore be suggested to sample from any of the other white muscle portions, even though this is not supported by results from the current study.

4.2. Relationship between Hg and fish size

The differences in accumulation patterns of individual Hg components (iHg and MeHg) could be explained by their pathways of absorption and accumulation in the fish body. Both iHg and MeHg is readily absorbed by fish from their diet and the surrounding environment, but the majority of iHg is rapidly eliminated from the fish body whereas MeHg is largely absorbed into fish tissue where it binds to thiol groups and is continually accumulated (Spry & Wiener, 1991). Toxic Hg levels therefore increase with increasing fish age and therefore fish size. This finding is supported by Andersen and Depledge (1997) on edible crab muscle, where a positive correlation between MeHg concentration and carapace length was found whereas iHg concentrations were low and independent of crab size.

A positive correlation between tHg and fish size has been found in numerous fish and marine species from various trophic levels, but especially in top predator species including Yellowfin tuna, Bigeye tuna and several shark species (Andersen & Depledge, 1997; Campbell et al., 2010; Kraepiel et al., 2003; Menasveta & Siriyong, 1977; Storelli et al., 2002a; Van den Broek & Tracey, 1981; Walker, 1976). Fish size could therefore be one of many factors which could give an indication towards estimated Hg levels in individual fish as larger individuals would be more likely to contain Hg levels close to or exceeding the maximum limit (1.0 mg/kg). Results from the current study show that 70 kg is the weight limit above which Yellowfin tuna are likely to contain tHg levels exceeding the regulatory limit (Fig. 2) and avoiding catches of fish above this size would reduce unnecessary wastage of having to discard fish not suitable for consumption.

4.3. Relationship between tHg and MeHg

No prediction model for MeHg has previously been formulated that we are currently aware of. A prediction model as formulated in this study can allow for an accurate prediction of the true toxic Hg levels in tuna meat without additional speciation techniques which would require additional equipment and funds. In addition to tHg values, which are routinely measured by the fishing industry, fish weight is the only other information needed to predict MeHg levels.

As this study only includes 14 tuna, the model presented here should be validated with larger sample sizes. The approach presented here should also be investigated for other fish species. The accumulation of individual and total Hg species in fish muscle, and therefore the correlation between them, could vary between fish species, as muscle type and metabolism vary between species and these factors play a role in Hg accumulation (Walker, 1976).

5. Conclusion

The cross-carcass analysis of Hg species (methylmercury, ethylmercury and inorganic mercury) and total mercury (tHg) in Yellowfin tuna showed that toxic methylmercury (MeHg) concentrations vary only within dark muscle but concentrations do not vary significantly between white and dark muscle, neither does it vary within the white muscle across the carcass. Routine tHg analyses for measuring the toxicity levels of Hg in fish meat can therefore be sampled from any white meat portion for a representative result of Hg toxicity per fish. Sampling from dark meat could result in higher tHg levels caused by higher levels of non-toxic inorganic mercury (iHg), giving a false indication of the Hg toxicity of the flesh. For representative sampling from a batch of fish, samples should be measured from fish of all represented size categories as MeHg concentrations were found to increase with increasing fish size and concentrations of toxic Hg could therefore be higher in larger fish. Due to this increasing MeHg accumulation with increasing fish size, catches of Yellowfin tuna above 70 kg should be avoided for consumption as these fish have higher risks of containing toxic levels of Hg. The low RMSEC values for the prediction

of MeHg based on tHg and fish weight indicates that with further research, MeHg concentrations could be accurately calculated from tHg measurements without extra costs of additional analytical methods for MeHg measurements.

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