



Mercury distribution in the muscular tissue of farmed southern bluefin tuna (*Thunnus maccoyii*) is inversely related to the lipid content of tissues

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ABSTRACT

The lipid content and total mercury concentration were measured in whole tissue composites of all edible tissues of farmed southern bluefin tuna (*Thunnus maccoyii*) and each of the marketed tissue cuts of these fish (akami, chu-toro, o-toro). Despite differences in fish size, condition factor and culture time, the mercury concentrations of tissue cuts and composite samples were found to decrease with increasing lipid content at a consistent rate of $-0.00476 \text{ Hg (mg/kg)/\% lipid}$ within each fish. Consequently, lipid accumulation appears to have a dilution effect on mercury already associated with fish tissues. The increased affinity of lipid for certain tissue cuts (o-toro) over that of others (e.g. akami), results in cross carcass variation in the mercury concentration of fish muscular tissue with clear implications for mercury advisory statements – the tissue sample collected for analysis is critical.

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1. Introduction

Mercury is a naturally occurring, neurotoxic metal that has the potential to accumulate to toxic levels in the biological tissues of humans and animals alike (Food Standards Australia New Zealand (FSANZ), 2004). The primary environmental source of human mercury exposure is seafood. Seafood constitutes up to an estimated 95% of total dietary mercury intake for most populations (e.g. FSANZ, 2004; Nakagawa, Yumita, & Hiromoto, 1997; UK Food Standards Agency (UK FSA), 2003; United Nations Environment Program (UNEP), 2002, US Environmental Protection Agency (US EPA), 2001). Consequently, it is desirable to regulate seafood consumption to minimize the risk of mercury accumulating to toxic levels in consumer populations.

Current international safety guidelines, established by the Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Committee on Food Additives and Contaminants (JEFCA), recommend a maximum tolerable weekly intake level (TWI) for mercury of $1.6 \mu\text{g/kg}$ (body weight) for women of childbearing age and $3.3 \mu\text{g/kg}$ (body weight) for children and the general population (JEFCA, 2003). The numbers of serves of seafood that can be safely consumed while maintaining total dietary intake below these maximum thresholds are determined nation-

ally, based on contaminant levels in marketed seafood, consumer group serving size and average body weight of consumer group populations (e.g. FSANZ, 2004). In order to aid in the accuracy of such recommendations, total mercury contamination of marketed seafood is regulated. Maximum allowable levels differ between countries, but are typically set at around 0.5 mg/kg fresh weight for the majority of fishes (e.g. FSANZ, 2004). However, those species that are recognized as naturally accumulating elevated levels of mercury – large, long-lived, piscivorous fishes, such as sharks, marlin and tuna – are often exempt from this generic maximum allowable level. For such fishes it is typical to see maximum allowable limits of (and never exceeding) 1 mg/kg fresh weight (e.g. FSANZ, 2004).

Considering the concern about the effects of mercury on human health and the thresholds that dictate the suitability of product for local human consumption and export to global markets, continued evaluation of mercury levels in seafood is essential. However, despite a wealth of literature on the mercury content of fishes, an internationally recognized protocol for sample collection is lacking.

The muscular tissue is the primary reservoir for bioavailable mercury in fish (Suzuki, Miyama, & Toyama, 1973), and is also the portion most frequently consumed. Consequently, mercury concentrations in fish muscle have important implications for consumer health and risk assessment, and are the basis for government seafood consumption advisories (e.g. Canadian Food Inspection Agency (CFIA), 2002, FSANZ, 2004, Japanese Ministry of Health, Labour & Welfare (JMHLW), 2003, UK FSA, 2003, US Food and Drug Administration (US FDA), 2001). However, it is often

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impractical to test whole fillets for mercury content, particularly when considering fish species of a large size and of economic significance – such as tuna. For such species, it is typical to sample just a small subsection of tissue, the location of which may vary according to practical and economic convenience (e.g. Adams, 2004; Hellou, Fancey, & Payne, 1992; Kojadinovic, Potier, Le Corre, Cosson, & Bustamante, 2006; Menasveta & Siriyong, 1977; Storelli, Giacomini-Stuffer & Marcotrigiano, 2002; UK FSA, 2003). Moreover, this practice is fuelled by an assumption that mercury is evenly distributed throughout all muscular tissues, despite there being very few published studies on the cross carcass distribution of mercury in the muscular tissues of fishes (Freeman & Horne, 1793; Kim, 1995; Nakao, Seoka, Tsukamasa, Kawasaki, & Ando, 2007; Redmayne, Kim, Closs, & Hunter, 2000; Working Group on Mercury in Fish (WGMF), 1979).

Within the muscular tissue of fish, mercury is believed to bind directly to thiol group complexes, such as cysteine, and as such will be predominantly found in association with the protein fraction of tissues (Harris, Pickering, & George, 2003; Nakao et al., 2007). Itano and Sasaki (1983) report that 71% to 89% of mercury in the muscular tissue of bluefin tuna is found in association with myofibrillar and sarcoplasmic protein fractions. Moreover, comparison of the mercury content of marine mammal lean muscular tissue with that of blubber consistently shows lower mercury concentrations in blubber (Wagemann, Trebacz, Bolia, & Lockhart, 1998). Consequently it would appear that, in order for mercury to be evenly distributed in the muscular tissues of fishes, protein binding-sites must also be evenly distributed. While this could be the case for some fishes, large pelagic species such as tuna are identified as having distinct tissue groups within muscular tissues. Muscular tissue may be categorized as “white” edible tissues, or “dark” muscle that is infrequently consumed (Working Group on Mercury in Fish (WGMF), 1979). In addition to white and dark muscle groups, the white edible muscular tissue of tuna species is frequently marketed as three distinct tissue cuts (akami, chu-toro and o-toro) identifiable by location, muscular structure, lipid content and colour (Nakamura, Ando, Seoka, Kawasaki, & Tsukamasa, 2005). Examination of the proximate composition (protein, moisture, lipid, ash) of tissues in tunas indicates that there are significant differences between these tissue cuts, not only in terms of the proportion of lipids, but also protein (Nakamura, Ando, Seoka, Kawasaki, & Tsukamasa, 2007; Nakamura, Handa et al., 2005). The o-toro, for example, is noted to have an elevated proportion of lipid but reduced protein, water and ash in comparison to all other tissues (Nakamura et al., 2007). Consequently, there is potential for variation in the mercury concentration in tissues. Potential differences in the mercury concentrations between tissues may be particularly apparent within farmed tuna, as the farming process greatly increases the lipid content of tissues and causes increased differentiation between tissue cuts when compared with wild-caught fish of similar size (Aguado-Gimenez & Garcia-Garcia, 2005; Nakamura, Ando et al., 2005).

Here we report on the mercury concentration of tissue composites of all edible tissues of farmed southern bluefin tuna, *Thunnus maccoyii* and each of the edible tissue cuts of these fish (akami, chu-toro, o-toro), in order to determine whether differences in the compositions of these tissues affect their mercury concentration.

2. Materials and methods

2.1. Materials

Specimens were obtained from commercially stocked and operated experimental farm pontoons. The operational procedure con-

sisted of the purse-seine capture of wild southern bluefin tuna (SBT) in the Great Australian Bight in March, 2005. Over a period of weeks, SBT were towed to the coastal waters of Port Lincoln, where they were transferred into sea pontoons and fattened on a mixture of Australian and imported baitfish species until harvest. Five specimens were harvested in August, 2005 after a typical commercial-length fattening period of 18 week's culture, and one additional SBT was harvested in August, 2006, after an experimentally extended culture period of an additional 12 months. SBT were caught and processed according to standard commercial harvesting techniques, and were received, eviscerated and bled as is normal for an export-bound product (Hayward, Aiken, & Nowak, 2007).

In the laboratory, the fork length (cm) and weight (kg) of specimens were recorded. The head and tail were removed, and the remainder of the carcass was split into left and right halves, each of which was separated into 6 sections, labelled 1–6 (Fig. 1). Each half carcass was processed separately, either for compilation of a composite sample of all white muscular tissues on one side of a fish or for compilation of tissue cut (akami chu-toro and o-toro) samples.

Carcasses were halved by slicing all flesh away from the spinal bone and major vertebral bones; any flesh that did not come away cleanly was later scraped off and placed with its corresponding section. The large portion of flesh that remained inside the dorsal head was removed and included as part of section 1. The skin, bones and dark meat were removed from each section and discarded. The remaining tissue was the white (edible) muscle portion of the fish.

For compilation of white muscular tissue composite samples, sections 1–6 were first weighed and homogenised in a stainless steel Hobart™ food processor. Composite samples were composed of a proportionately prepared mixture of sub-samples taken from each of the 6 section homogenates. Weights of homogenate sub-samples used from each section were determined by calculation of each section's percentage of total edible weight. Sub-samples were combined and again homogenised to ensure thorough mixing.

For compilation of tissue cut composite samples, sections 1–6 were further divided into akami, chu-toro and o-toro tissue cuts, which are identifiable by location, muscle structure and colour (Fig. 2). Tissue cut composites were prepared by combining all akami tissues from sections 1–6, combining all chu-toro tissues from sections 1–6, and combining all o-toro tissues, which are found in sections 4 and 5 only. The total weights of all tissue cut composites were recorded and tissues were homogenized.

All samples were stored in polyethylene bags at –80 °C and sent frozen to an external accredited laboratory (AgriQuality, New Zealand) for analysis. Mercury analysis was by means of wet digestion (Aristar nitric acid and Aristar hydrofluoric acid) with quantification by inductively coupled plasma mass spectroscopy (Perkin-El-

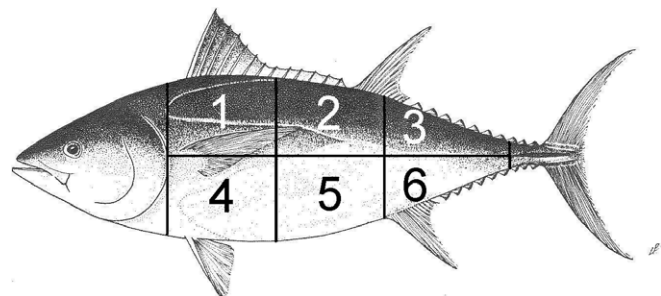


Fig. 1. Schematic diagram of SBT, identifying each of the six cuts (1–6) used to produce the whole tissue composite and the tissue group composites (Source: Collette & Naven, 1983).

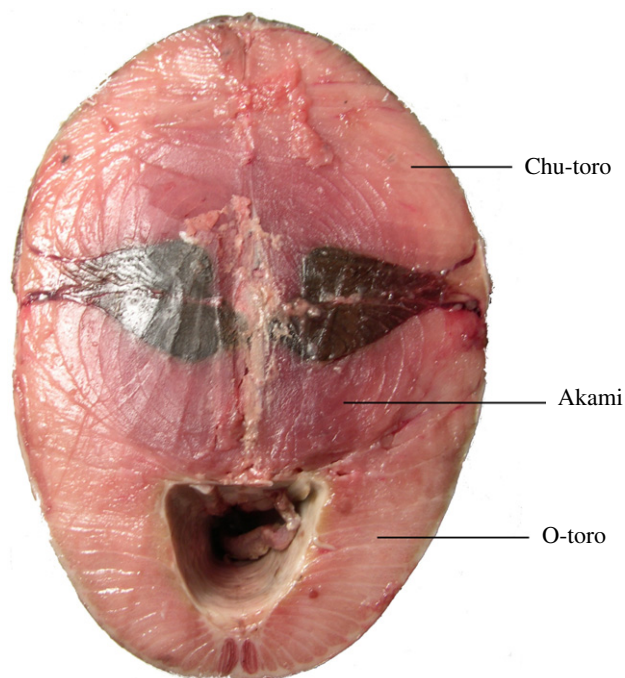


Fig. 2. Cross section of a farmed southern bluefin tuna, indicating each of the tissue cuts (akami, chu-toto and o-toro).

mer Elan 9000). Limit of reporting was 0.01 mg/kg fresh weight. Total lipid determination was by Soxhlet extraction using diethyl ether. Lipid content was reported as a percentage of tissue fresh weight.

2.2. Data analysis

Whole weight was estimated by assuming that the gills and internal organs, which were removed at harvest, were equivalent to 12% of the total body weight (unpublished data). Whole weight estimates and length were used to determine the condition factor of each fish, using the following formula, in which W_w is whole weight (kg) and FL is the fork length (m):

$$CF = \left(\frac{W_w}{FL^3} \right)$$

Statistical analyses (linear regression and analysis of variance) were performed using the R statistical package, version 2.4.0 (R Development Core Team 2006) with a significance value of $P < 0.05$.

3. Results

The harvest information and physical characteristics of experimental SBT are presented in Table 1. Fork length ranged from 0.88 to 1.22 m, whole weight ranged from 16.21 to 41.61 kg and condition factor of fish ranged from 22.91 to 27.04 kg/m³.

Table 1
Physical characteristics of experimental SBT at harvest

Fish	Harvest date	Fork length (m)	Whole weight (kg)	Condition (kg/m ³)
A	August 2005	1.13	37.01	25.65
B	August 2005	1.05	28.06	24.23
C	August 2005	0.90	19.71	27.04
D	August 2005	0.88	16.21	23.78
E	August 2005	0.95	21.67	25.27
F	August 2006	1.22	41.61	22.91

The percent lipid content and fresh weight mercury concentration of SBT tissues are presented in Fig. 3. The lipid content of tissues varied according to tissue cut. The mean percent lipid content of akami, chu-toro and o-toro were $5 \pm 2\%$ ($n = 6$), $20 \pm 5\%$ ($n = 6$) and $33 \pm 5\%$ ($n = 6$), respectively. The whole white tissue composite was found to have a lipid mean of $17 \pm 3\%$ ($n = 6$), which was significantly different from both the akami ($p = 0.00036$) and o-toro ($p = 0.00001$) tissues.

Analysis of mercury concentrations across tissue cuts revealed that the fresh weight mercury concentration decreased with increasing lipid content of tissues. The tissue cut with the lowest lipid content, the akami (lipid content range = 3.5–8.1%), was consistently found to have the highest mercury concentration, while the tissue cut with the highest lipid content, the o-toro (lipid

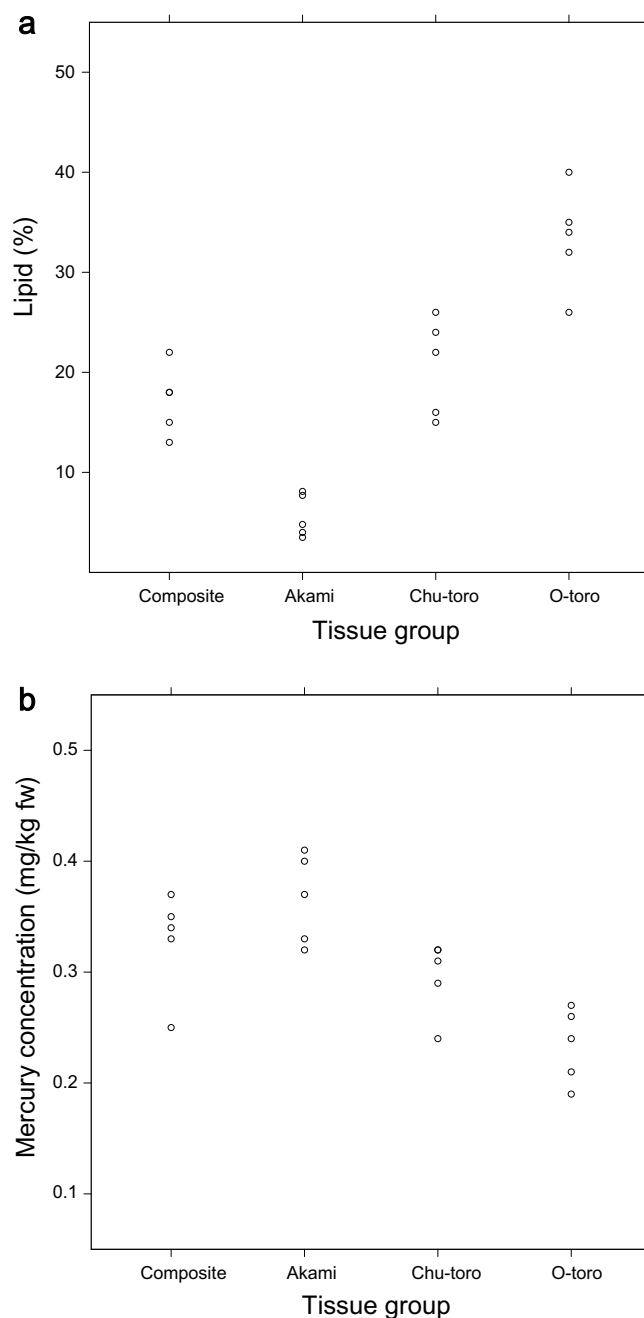


Fig. 3. Percent lipid content (a) and fresh weight mercury concentration (b) of SBT tissue cuts: composite, akami, chu-toro and o-toro.

range = 26–40%) was consistently found to have the lowest mercury concentration (Fig. 3). Mean fresh weight mercury concentrations for akami, chu-toro and o-toro were 0.36 ± 0.02 mg/kg ($n = 6$), 0.28 ± 0.05 mg/kg ($n = 6$) and 0.23 ± 0.05 mg/kg ($n = 6$), respectively. The whole white tissue composite was found to have a mean fresh weight mercury concentration of 0.32 ± 0.03 mg/kg ($n = 6$), which was significantly different from the o-toro tissue only ($p < 0.002$).

Regression analysis showed the slope of the negative linear relationship between lipid content and mercury concentration of tissues was consistent for all experimental fish ($p = 0.72$). However, there were statistically significant differences existing in the intercept of each fish ($p = 0$). In Fig. 4, the mercury concentration of tissues is plotted against lipid content for each individual fish. The common slope of the linear regression fit (least squares fit) to the lipid–mercury relationship is estimated to be -0.00476 (Hg (mg/kg)/% lipid). Identification of a common slope for each fish, despite statistically different intercepts, is indicative that the effect of lipid on the mercury concentration within farmed SBT appears to be consistent, irrespective of differences in fish length, weight, condition and culture time.

4. Discussion

The mercury content of tuna and, consequently, the quantity of tissues that can be safely consumed have previously been shown to vary according to species (Storelli et al., 2002), size (Peterson,

Klawe, & Sharp, 1973), geographic location (Bernhard & Renzoni, 1977), culture time (Nakao et al., 2007) and condition factor of the fish (unpublished data). Consequently, given the variation in fish culture time, condition factor and size observed in the current study, it is not surprising that a small degree of variation in the overall mercury content of fish was observed (indicated by significantly different intercepts in Fig. 4).

However, the observation that, within each individual fish, mercury content is not evenly distributed but is inversely related to the lipid content of tissues, is novel.

It may be expected that the mercury concentration of a tissue will decrease as the protein fraction decreases due to reduced binding sites. However, Nakamura, Handa et al. (2005) report that, in cultured bluefin tuna, *Thunnus orientalis*, the combined total amount of water and lipid in tissues is typically fixed at 80%. The water content of tissues decreases as lipid content increases. Consequently, changes in lipid content of tissues do not affect the proportion of proteins except in cases of extremely high lipid accumulation, as observed in the o-toro of cultured fish. In such cases the relationship between water and lipid is thought to fail, resulting in a proportionate decrease in protein as lipid increases (Nakamura, Handa et al., 2005; Nakao et al., 2007). Furthermore, it is suggested that this reduction in the protein fraction of tissues results in a decrease in mercury concentration (Nakao et al., 2007). However, in order for the o-toro to have a lower mercury concentration due to a reduced protein fraction, all protein binding sites would need to be exhausted. Tissues, such as the chu-toro and aka-

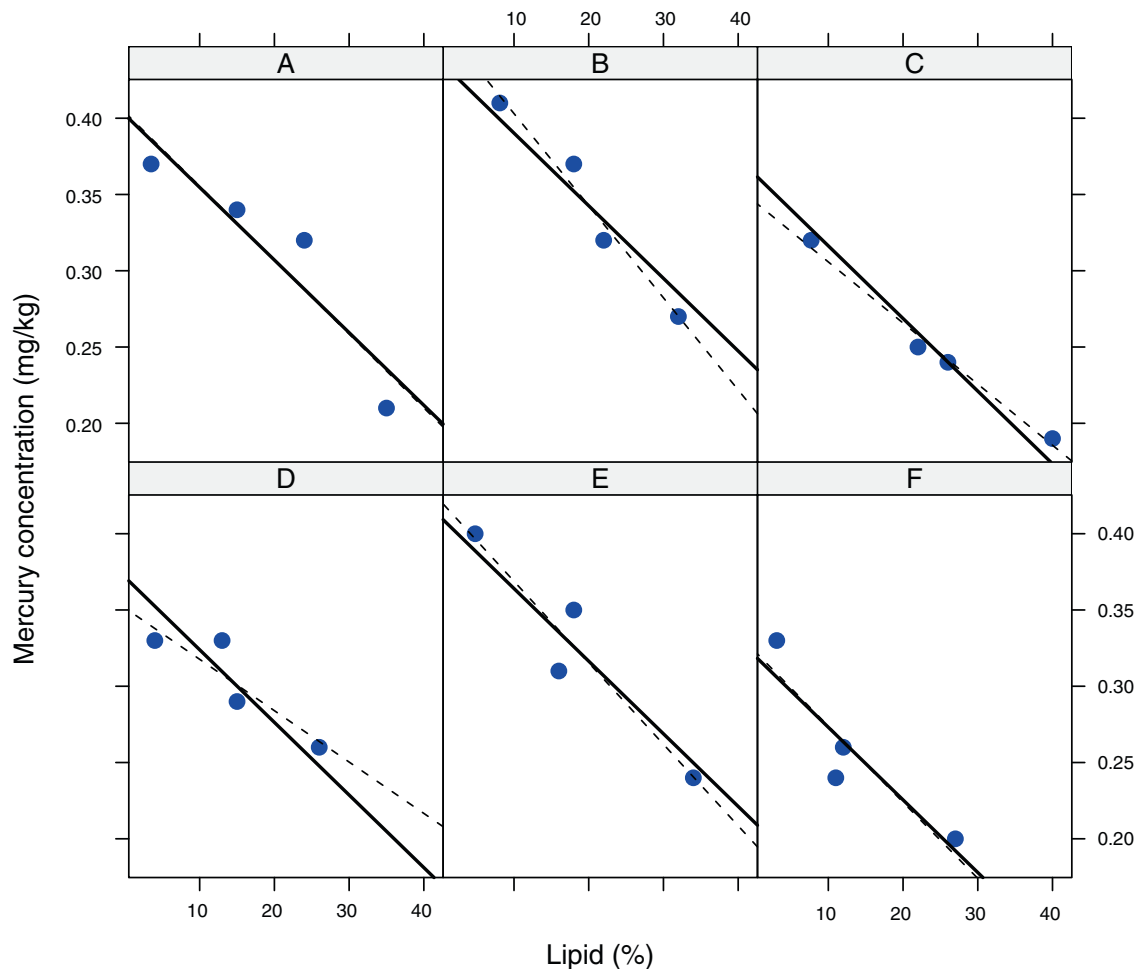


Fig. 4. Mercury concentration in the composite, akami, chu-toro and o-toro samples for five SBT harvested after 18 weeks of culture and a single SBT harvested after an additional 12 months in culture (fish F, bottom right panel). The dashed lines are the individual least-squares regression fit for each individual fish. The solid lines are the common slope for all fish, found to be -0.00476 Hg (mg/kg)/% lipid.

mi, in which the lipid content does not increase to levels high enough to reduce the protein fraction of tissues, might be expected to have equivalent mercury contents, regardless of differences in their lipid contents. Results of the current study indicate this is not the case.

Tuna are recognised as naturally accumulating elevated levels of mercury and are frequently reported with fresh weight tissue concentrations exceeding 1 mg/kg (Adams, 2004; Bernhard & Renzoni, 1977; Storelli et al., 2002). Given that the mercury levels reported in the current study were all below 0.50 mg/kg, it is unlikely that all protein binding sites were exhausted. All tissues were found to have a consistent negative linear relationship between lipid content and mercury concentration, regardless of their lipid content. Consequently, the mercury concentration of tissues appears to decrease as lipid content increases, regardless of the availability of protein binding sites in tissues.

Lipid accumulation appears to have a dilution effect on mercury already associated with fish tissues. The preferred affinity of lipid for certain tissues over others will result in cross-carcass variation in the mercury concentration of fish muscular tissue with clear implications for mercury advisory statements – the tissue sample collected for analysis is critical. Mercury concentrations reported from SBT in the current study (typical in size and culture conditions of the majority of farmed SBT) suggest that under current Food Standards Australia New Zealand (FSANZ) recommendations (see <<http://www.foodstandards.gov.au>>), differences in the mercury concentration between tissue cuts result in an allowance of two additional weekly serves of o-toro and one additional weekly serve of chu-toro in comparison to akami, while maintaining total dietary mercury intake below recommended levels for children, women of child bearing age and adults.

Additionally, the observed cross-carcass variation in mercury content in the white muscular tissues of tunas indicates a level of uncertainty in comparing results between studies that ambiguously describe tissue samples based on location (e.g. dorsal muscle, caudal muscle, abdominal muscle, axial muscle). Reference to locations such as abdominal muscle could include any or all of the three tissue cuts described in this study (see Fig. 1). In order to improve the comparability of future studies, and reduce the risk of inaccuracies in health advisory statements, it is recommendable that future work identifies muscle samples by tissue cut, location, and lipid level. Moreover there is potential for longitudinal variation within a tissue cut in addition to variation between tissue cuts. Such detailed examination is beyond the scope of this particular study, but will be addressed in future papers.

Sampling strategies should be considered in light of their intended purpose. When considering the mercury levels of all frequently consumed tissues on tuna carcasses, preparing white tissue composites will produce the most accurate and robust estimate of whole fish white muscle mercury levels. However the large size of tuna species makes producing composite samples time consuming and costly. Results of the current study indicate that, in the absence of tissue composites, sub samples of the chu-toro will most closely represent the lipid and mercury characteristics of whole fish white muscular tissues. Moreover, as the slope of the linear relationship between the lipid and mercury content of tissues was consistent between all SBT specimens, if the mercury concentration in one tissue is known, predictions can be made as to the mercury concentration of all other edible tissues, provided that the lipid content is known. Validation of these findings will be made as data becomes available.

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