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DNA barcodes reveal species-specific mercury levels in tuna sushi that pose a health risk to consumers

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Excessive ingestion of mercury—a health hazard associated with consuming predatory fishes—damages neurological, sensory-motor and cardiovascular functioning. The mercury levels found in Bigeye Tuna (Thunnus obesus) and bluefin tuna species (Thunnus maccocyii, Thunnus orientalis, and Thunnus thynnus), exceed or approach levels permissible by Canada, the European Union, Japan, the US, and the World Health Organization. We used DNA barcodes to identify tuna sushi samples analysed for mercury and demonstrate that the ability to identify cryptic samples in the marketplace allows regulatory agencies to more accurately measure the risk faced by fish consumers and enact policies that better safeguard their health.

Keywords: mercury; Thunnus; sushi; DNA barcoding; seafood labelling; epidemiology

1. INTRODUCTION
Accurate identification of commercial fish species has many public health and legal applications. DNA barcodes (Hebert et al. 2003)—short nucleotide sequences used to identify species—can serve as an important tool allowing regulatory agencies to recognize ambiguous food items that are fraudulent or hazardous (Wong & Hanner 2008; Yancy et al. 2008). For tuna, DNA barcodes have been used to document market substitution, and in the case of Atlantic Bluefin Tuna (Thunnus thynnus), meet the requirement that species protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) be identifiable in trade (Lowenstein et al. 2009; IUCN & TRAFFIC 2010). We demonstrate one of the first applications of DNA barcoding in a human health context (Cohen et al. 2009) by using mitochondrial DNA to identify tuna sushi to the species level concomitant with mercury testing.

Mercury methylated by microorganisms bioaccumulates, reaching high concentrations in predatory fishes such as tuna (Morel et al. 1998). Excessive mercury consumption is implicated in neurodevelopmental defects including mental retardation, cerebral palsy, deafness, blindness and disarthritis, and adult neuro- and cardiovascular toxicity (National Resource Council 2000). Many countries have established mercury action levels above which fish may not be sold, and have also issued advisories notifying consumers of fishes high in mercury (table 1).

Owing to relaxed international labelling requirements set by the United Nations Food and Agriculture Organization (FAO), species descriptions are often inaccurate, disputed by nations or missing (Jacquet & Pauly 2008). Many countries have ambiguous or no species-specific labelling requirements such as the US where the approved market name for all members of Thunnus in addition to Frigate Tuna (Auxis thazard), Kawakawa (Euthynnus affinis), Skipjack Tuna (Katsuwonus pelamis) and Slender Tuna (Allothunnus fallai) is ‘tuna’ (FDA 2008). Tuna sushi, or maguro in Japanese, is made from five species sometimes specified in restaurants as bluefin tuna (T. maccocyii, T. orientalis, or T. thynnus), Bigeye Tuna/ahi (T. obesus) or Yellowfin Tuna/ahi (T. albacares; Lowenstein et al. 2009). Because of overlap in appearance and taste (Catacari 2005), molecular identification is one of the most precise methods for identifying tuna in the marketplace (Lowenstein et al. 2009; Viñas & Tudela 2009). In the context of mercury analysis, DNA barcodes enabled us to determine which species warrant inclusion in consumer advisories or trade restrictions, and whether the data used by health agencies reflect accurately the mercury threat faced by consumers.

2. MATERIAL AND METHODS
We tested the mercury content of 100 tuna sushi samples from 54 restaurants and 15 supermarkets collected from October 2007 to December 2009 in New York, New Jersey, and Colorado. The New York Times collected 20 samples, and we collected the rest. We identified them using nucleotide characters and BLASTN against NCBI GenBank (see the electronic supplementary material, table S1) using the cytochrome c oxidase subunit I (cox1) gene sequence, following the methodology detailed in Lowenstein et al. (2009). Because the three closely related species of bluefin are often not differentiated in global trade (Catarci 2005) we pooled these data into one category for the mercury analysis. We further categorized samples according to whether they were sold as lean red tuna (ahami in Japanese) or fatty tuna (toro) because mercury and lipid concentrations are inversely proportional in tuna (Balshaw et al. 2008a).

To measure total mercury, a 2 g (wet weight) subsample of fish tissue was digested in trace metal grade nitric acid (Fisher Chemical) in a microwave (CEM, MDX 2000), using a digestion protocol of three stages of 10 min each under 50, 100 and 150 pounds per square inch (3.5, 7 and 10.6 kg cm−2) at 80 per cent power. Digested samples were subsequently diluted to 25 ml with deionized water. All laboratory equipment and containers were washed in 10 per cent HNO3 solution and rinsed with deionized water prior to each use.
Table 1. Mercury advisory levels set by regulatory agencies. (The weekly maximum level recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization for women of childbearing age is equivalent to 0.2 μg kg⁻¹ body weight per day.)

<table>
<thead>
<tr>
<th>agency</th>
<th>mercury advisory level</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Commission</td>
<td>1.0 ppm</td>
<td>European Commission (2008)</td>
</tr>
<tr>
<td>Food and Agriculture Organization</td>
<td>1.6 μg kg⁻¹ body weight per week</td>
<td>Codex Alimentarius Commission (1995)</td>
</tr>
<tr>
<td>Health Canada</td>
<td>1.0 ppm</td>
<td>Health Canada (2007)</td>
</tr>
<tr>
<td>Japanese Ministry of Health</td>
<td>0.4 ppm</td>
<td>Yamashita (2005)</td>
</tr>
<tr>
<td>US Environmental Protection Agency</td>
<td>0.1 μg kg⁻¹ body weight per day</td>
<td>EPA (1997)</td>
</tr>
<tr>
<td>US Food and Drug Administration</td>
<td>1.0 ppm</td>
<td>FDA (2000)</td>
</tr>
<tr>
<td>World Health Organization</td>
<td>1.6 μg kg⁻¹ body weight per week</td>
<td>Codex Alimentarius Commission (1995)</td>
</tr>
</tbody>
</table>

Table 2. Total mercury (Hg) content in tuna sushi samples. (Data from samples identified as one of the three species of bluefin (T. maccoyii, n = 7; T. orientalis, n = 4; T. thynnus, n = 18), were pooled into a single category. Akami is the Japanese word for lean red tuna, and toro for fatty tuna. Total mercury (ppm) varied significantly across sample categories (one-way ANOVA: F₄,₉₅ = 11.81, p < 0.0001). Categories assigned as 'a' were significantly different from those assigned 'b' (Tukey’s multiple comparison test). The mean dose was calculated for the default weight of a 60 kg adult woman (WHO 1972; Health Canada 2007) consuming a single order.)

<table>
<thead>
<tr>
<th>sample category</th>
<th>total Hg (ppm)</th>
<th>mean</th>
<th>median</th>
<th>s.d.</th>
<th>min</th>
<th>max</th>
<th>assignment</th>
<th>mean dose of total Hg (μg kg⁻¹ body weight per day)</th>
<th>sample mass (g; mean ± s.e.)</th>
<th>sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bigeye Tuna akami</td>
<td>0.871</td>
<td>0.794</td>
<td>0.393</td>
<td>0.336</td>
<td>1.716</td>
<td>a</td>
<td>0.344</td>
<td>22.48 ± 2.843</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Bigeye Tuna toro akami</td>
<td>0.989</td>
<td>0.685</td>
<td>0.716</td>
<td>0.365</td>
<td>2.254</td>
<td>a</td>
<td>0.351</td>
<td>20.82 ± 2.941</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>bluefin tuna akami</td>
<td>1.043</td>
<td>1.028</td>
<td>0.478</td>
<td>0.368</td>
<td>1.916</td>
<td>a</td>
<td>0.180</td>
<td>12.09 ± 2.046</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>bluefin tuna toro akami</td>
<td>0.385</td>
<td>0.307</td>
<td>0.244</td>
<td>0.166</td>
<td>1.027</td>
<td>b</td>
<td>0.123</td>
<td>21.18 ± 2.428</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Yellowfin Tuna akami</td>
<td>0.474</td>
<td>0.435</td>
<td>0.294</td>
<td>0.095</td>
<td>1.377</td>
<td>b</td>
<td>0.164</td>
<td>18.34 ± 2.823</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

Mercury was analysed by cold vapour technique using a Perkin Elmer FIMS-100 mercury analyser, with an instrument detection level of 0.004 μg g⁻¹ and a method detection level of 0.010 μg g⁻¹. All samples were tested twice, and 15 samples with the highest mercury levels were tested three times. For 98 of the samples results from all runs were within 5 per cent, and two samples within 10 per cent. As a control, we used the National Institute of Standards and Technology dogfish muscle trace metal reference material (DORM-2) alongside the samples and our results were always within the total mercury certificate range (4.38–4.90 ppm). For sushi-grade tuna, approximately 97 per cent of total mercury is methylmercury (Hight & Cheng 2006). The mercury analysis was carried out at Rutgers University and the genetic identification was carried out at the American Museum of Natural History with no prior knowledge of sample identity or mercury concentration.

Statistical analyses were performed on GraphPad Prism v. 5.00b for Mac OSX (www.graphpad.com). Before using parametric tests, we performed a log(x + 1) transformation on all mercury data used in this study and assessed conformation to a normal distribution using the D’Agostino–Pearson omnibus test (α = 0.05). We assessed homogeneity of variances for the one-way ANOVA using Bartlett’s test, as well as for comparisons of two groups using an F-test (α = 0.05).

3. RESULTS

Mercury concentrations varied significantly across sample categories (one-way ANOVA: F₄,₉₅ = 11.81, p < 0.0001; table 2). The mercury levels in bluefin akami and all Bigeye Tuna samples were significantly higher compared with bluefin toro and Yellowfin Tuna akami. The mean mercury concentrations of all samples exceed the concentration permitted by Japan (Yamashita et al. 2005), and the maximum daily consumption considered safe by the US Environmental Protection Agency (EPA 1997). Mean mercury levels for bluefin akami exceed those permitted by the US Food and Drug Administration (2000), Health Canada (2007) and the European Commission (2008). On average, one order of Bigeye Tuna sushi—the species used most often for sushi (Catarci 2005)—exceeds the safe maximum daily dose recommended by Health Canada (2007) and the safe limit established by the World Health Organization and FAO for women of childbearing age (Codex Alimentarius Commission 1995).

As documented previously for Southern Bluefin Tuna (Balshaw et al. 2008a), we found significantly less mercury in bluefin toro than in akami (t = 5.109, p < 0.0001), but no significant difference for Bigeye Tuna (t = 0.363, p = 0.717). We found no significant difference in bluefin mercury levels comparing data from a study (Storrelli et al. 2002) with greater sampling (n = 161, mean = 1.18 ppm, s.d. = 0.85) to our bluefin akami results (Mann–Whitney U-test, p = 0.59). The total mercury levels we found in Yellowfin Tuna sushi was significantly higher (Mann–Whitney U-test, p = 0.0236) than in samples obtained by the FDA (2004), as was the case for Bigeye Tuna (t-test with Welch’s correction, t = 2.549, p = 0.0162; figure 1). Finally, we found that the concentration of total mercury was also higher in our samples sold in restaurants compared with supermarkets (t = 3.249, p = 0.0018; figure 1).
Our results demonstrate the use of DNA barcodes to enable regulatory agencies to identify unknown and potentially hazardous samples. A multi-locus genetic species identification method was recently proposed for tuna (Viñas & Tudela 2009), and while we agree that multi-locus approaches perform better in cases of introgressive hybridization, this discussion does not have a negative impact on our findings presented here. Mercury concentrations in tuna are positively correlated with body size (Storelli et al. 2002; Yamashita et al. 2005), and larger individuals are more likely to be sushi-grade and valued the highest (Catarci 2005). The finding that the mercury levels in Bigeye Tuna akami and toro were not significantly different may be owing to the fact that premium Bigeye toro cuts on average have half the fat content of bluefin (Shimamoto et al. 2003; Balshaw et al. 2008a) and because larger fish typically have more belly fat and are preferentially selected for toro. Furthermore, whereas thousands of tons of bluefin per year are fattened in farms prior to export (Catarci 2005), which can also reduce mercury (Balshaw et al. 2008b), the vast majority of Bigeye Tuna are harvested directly from the wild. Because the mercury concentrations found in our sushi were significantly higher than levels documented by the Food and Drug Administration (FDA) (figure 1), this could reflect that our samples came from larger fish (the FDA lacks bluefin data). We found significantly lower mercury levels in supermarket sushi (figure 1) because samples were dominated (77%) by Yellowfin Tuna, which comprised a minority of restaurant samples (22%; \( \chi^2 = 18.14, p < 10^{-4} \)) and was found to be the species with the lowest mercury concentration (table 2). By allowing for the direct measurement of samples collected from the marketplace, DNA barcoding has the potential of revealing mercury measurements more reflective of the threat faced by consumers allowing for the enactment of policy that better safeguards consumer health.

Our results suggest health agencies should consider adding Bigeye and bluefin tuna to mercury advisories. For instance, the mercury levels in these species are within the bounds of fish the FDA and EPA advise pregnant or nursing women and children to avoid entirely (EPA & FDA 2004), and thus these tunas should be included in the advisory. Consumers could make more informed health decisions if the FDA, and regulatory agencies in other nations, enforced market-specific names for species high in mercury.

4. DISCUSSION AND CONCLUSIONS

We thank the New York Times for samples, and the Alfred P. Sloan Foundation, the Richard Lounsbery Foundation, and Columbia University for funds. Mercury analyses were partially funded by NIEHS Center Grant P30ES005022. We are grateful for the comments and constructive criticisms from two anonymous reviewers that helped to improve the manuscript.

The potential of revealing mercury measurements more reflective of the threat faced by consumers...


EPA & FDA 2004 What you need to know about mercury in fish and shellfish: advice for women who might become pregnant, women who are pregnant, nursing mothers, young children. EPA-823-R-04-005. See http://www.fda.gov/Food/FoodSafety/Seafood/index.cfm?


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