

Presence of Metals and Metalloids in Wild Salmon Compared to Farmed Salmon

Studied with Hard X-ray Fluorescence Microscopy



Theresa Quain¹, Sam Boroumand¹, Abigail Kargol¹, Thomas Matysik¹, Julian Martinez¹, Andrew Molina¹, Rachel Smith¹, Apoorva Sooranahalli¹, Amanda Thate¹, Eric Wynne¹, Qiaoling Jin², and Sophie Gleber³
¹Science Department, Downers Grove High School South, Downers Grove, IL 60516 ²Department of Physics and Astronomy, Northwestern University, Evanston, IL 60208 ³X-ray Science Division, Argonne National Laboratory, Argonne, IL 60439

ABSTRACT

The presence of metals in fish is an issue of food safety due to persistence in the body as well as their tendency to bioaccumulate. Consumers rely increasingly on farmed fish due to pressure on fisheries. The quality of farmed fish compared to wild fish is therefore of public health interest. The purpose of this research study is to determine if there is a difference in the amount of inorganic contaminants in wild versus farmed salmon. Previous studies have found that in multiple species the concentrations of metals present in wild-caught fish differ from the metal concentrations in farmed fish. Concentrations vary from species to species as well as between tissues in individual fish (Eneji et al., 2011). Whether the concentrations reach harmful levels varies with the species. Previous comparisons of wild and farmed salmon have found that the farmed salmon had significantly higher levels of arsenic while wild salmon had significantly higher concentrations of cobalt, copper, and cadmium, though none of the metals were present in high enough quantities to harm a person (Foran et al., 2004). In this study, samples of wild salmon and farmed salmon were purchased from the same grocery store, which claims that both products meet the highest quality standards; thus this study compares the best-case farmed salmon with the best-case wild salmon. Both samples were chemically fixed followed by sucrose embedding and sectioning at -80°C. Hard x-ray fluorescence microprobe at 2-ID-E was used to map the metal concentrations and distributions with high spatial resolution on these thin sections. Methods of sample preparation and results of the x-ray fluorescence microscopy will be presented. Results showed that Ni and Cr were significantly higher in farmed salmon than in wild salmon. No significant differences in concentrations of other metals were found.

PROCEDURE

Muscle cubes (2 mm x 2mm x 2 mm) were excised from fresh farm raised salmon and wild salmon samples. They were fixed by immersion in phosphate buffer saline containing 2% glutaraldehyde and 2% paraformaldehyde for overnight at 4 °C. After being washed four times in PBS buffer, muscle cubes were infiltrated with 2.3 M sucrose for overnight at 4°C. The sucrose embedded specimens were subsequently trimmed to a block size of 0.5 mm x 0.5 mm x 0.5 mm, placed on a clean specimen pin and frozen in liquid nitrogen. The specimen pin was then inserted into the sample holder in the precooled cryochamber (-80°C) of cryo-ultramicrotome Leica UC7/FC7. Trimming and sectioning were carried out with a dry glass knife at -80°C. Both longitudinal and transverse sections around 800 nm thick were generated, transferred out from the cryochamber with a drop of 2.3 M sucrose and deposited onto silicon nitride windows. After rinsing off sucrose with distilled water, sections were set to dry in the air and micrographed (Figure 1 and 2).

The samples were mounted magnetically to the sample stage at a 15 degree angle to the beamline. XRF mapping of the sample was conducted using x-ray fluorescence microprobe at incident beam energies of 10 keV and 12 keV. A focused x-ray beam of roughly 0.6 µm in diameter was used for the raster scanning the sample. The beam was focused using a zone plate objective lens. Compositional maps of the samples were produced using MAPS software. First, a rough scan of the sample was conducted to find areas of potential high metal concentration. Then, detailed scans of those areas were conducted at high spatial resolution and longer exposure times for highest elemental sensitivity.

RESULTS

Initial results were performed at 10 keV, additional scans at higher energy were then performed to also detect elements like arsenic. Figure 3 shows a comparison of the two samples when run at 12 keV. The black line represents the absolute counts of elements in farmed-raised salmon, while the green line represents the same values in wild salmon. This graph suggests that the absolute counts of two elements, Ni and Cr, were significantly higher in farmed salmon than in wild salmon. Other elements show similar concentrations in the two samples. Due to higher focused flux, the black line (scan 100) is overall slightly higher than the green line (scan 101).

Figure 4 shows elemental maps taken at 10 keV of wild (Fig. 4 left) and farm-raised (Fig. 4 right) salmon. The maps show data quantified using an XRF standard. The Ni signal is about double in the farm-raised salmon. The Cr signal appears to be in the same range for these scan regions.

REFERENCES

Foran, J., Hites, R., Carpenter, D., Hamilton, C., Mathews-Amos, A. & Schwager, S. (2004). *Environmental Toxicology and Chemistry*, 23 (9), pp 2108-10.
Eneji, I. S., Sha' Ato, R., & Annune, P. A. (2011). *Pak. J. Anal. Environ. Chem.*, 12(1 & 2).
http://www.nmfs.noaa.gov/aquaculture/faqs/faq_feeds.html#1what
http://www.wholefoodsmarket.com/sites/default/files/media/Global/Core%20Value/WholeFoodsMarketQS_Farmed-finish-shrimp_Jan1-2014.pdf

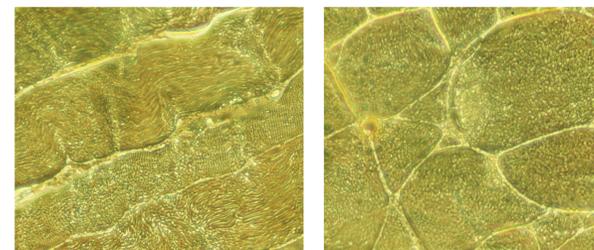


Figure 1: Longitudinal and transverse sections obtained by cryo-ultramicrotome.

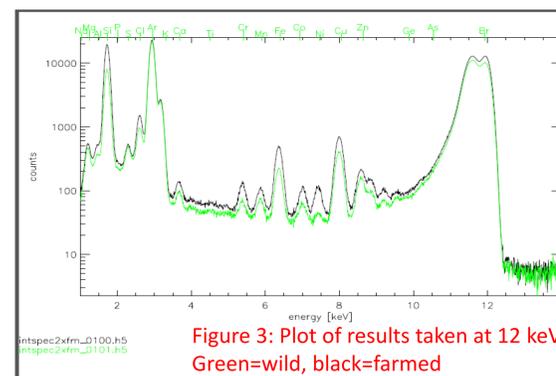


Figure 3: Plot of results taken at 12 keV. Green=wild, black=farmed

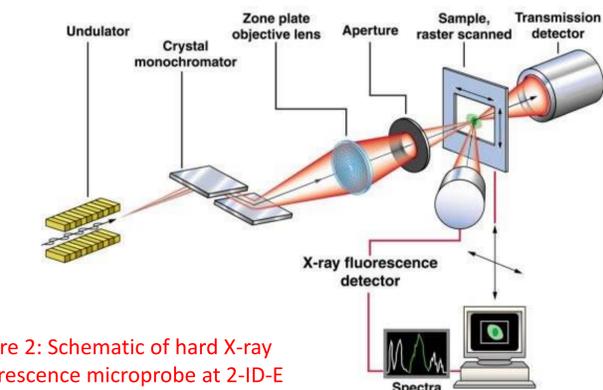


Figure 2: Schematic of hard X-ray fluorescence microprobe at 2-ID-E

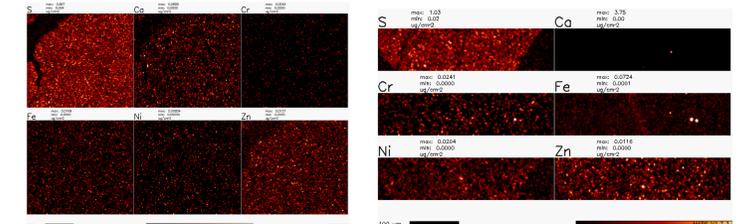


Figure 4: Medium resolution elemental maps of wild (left) and farm-raised (right) salmon. 1 µm pixel size, 15 ms dwell time per pixel.

CONCLUSIONS

Concentrations of all metals were found to be low. However, the farmed sample was found to contain higher concentrations of Ni and Cr compared to the wild sample. Further study is needed to confirm these results and to ascertain the form of chromium that was detected, as some oxidation states of chromium are toxic to humans. The origin of these contaminants may be the pelagic fish meal fed to the fish, feed additives, or some environmental source surrounding the fish farm. Additional research is needed to determine this source. No significant difference was found in concentrations of other metals between the wild and farmed fish. To our knowledge, this is the first study to use hard X-ray fluorescence microscopy on fish flesh. The high fat content of salmon may have contributed to the very low signal detected for metals in general. Repeating the experiment with thicker samples (>1000 nm) will increase detection limits. More samples need to be investigated for statistically relevant conclusions. In addition, other tissues should be studied, as previous research has shown that the flesh of fish shows lower concentrations of metals than other tissues.

ACKNOWLEDGMENTS

This research was made possible through the Exemplary Student Research Program, supported by Argonne National Laboratory's Educational Programs (CEPA), APS User Office, and Downers Grove South teacher T. Quain. Use of the Advanced Photon Source, an Office of Science User Facility operated for U.S. Department of Energy (DOE) by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.