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Caudal Fin Mercury as a Non-Lethal Predictor of Fish-Muscle Mercury

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Environmental Context. *Surveys to assess the body burden of mercury in fish to support research or contamination advisory programs typically involve capturing and killing fish and analyzing muscle tissue for mercury. Lethal sampling may not be feasible in protected waters or in studies involving threatened or endangered species. We analyzed tail fin samples of two fish species for total mercury and compared results with muscle-tissue mercury and concluded that fin-Hg can be used as a predictor of muscle-Hg. This approach enables catch and release studies for mercury in fish.*

Abstract. The caudal (tail) fins from 17 walleye (*Sander vitreus*) and 12 northern pike (*Esox lucius*) from three northern Arizona lakes (Long Lake, Soldier Lake, and Upper Lake Mary) were analyzed for total-Hg by combustion–atomic absorption spectrometry. Results indicate that the fin contains measurable Hg that correlates with muscle-Hg concentrations. As the body burden of Hg increased, the concentration in the fin increased relative to the muscle. Mercury concentrations also increased with fish length and weight, although the relationship was lake- and species-dependent. Fish from Soldier Lake had the most efficient uptake of Hg, likely due to the trophic structure of the lake or the condition of the fish, but possibly due to an acute source of Hg. Overall, this study demonstrates that caudal fin clippings can be used as a non-lethal predictor of muscle-Hg concentrations, which can reduce the number of fish killed in routine monitoring programs.

Keywords. AAS — biological monitoring (animals) — mercury — toxicology

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Numerous state and federal programs monitor mercury (Hg) in fish in their respective waters mainly to keep the fishing public informed of the concentrations in fish skeletal muscle and of the potential risks associated with consumption of the fillet. These programs typically collect fish by a variety of methods, measure them for length and weight, and then kill them for analysis of the muscle tissue. Individually, these programs generally remove a small percentage of fish from their water bodies but collectively the number of fish is large. Introducing non-lethal sampling into routine monitoring programs is desirable because the fish can be released back into the wild. This is particularly important when threatened or endangered species are captured.[1] Moreover, in vivo sampling makes possible re-examination of the same fish on multiple occasions, allowing study of temporal trends on individual fish, if the fish is marked for identification and caught again.

Both muscle plugs, obtained from a biopsy punch, and blood have been explored as a non-lethal measure of Hg concentration and exposure in fish.[2] Muscle plugs are advantageous because they sample the tissue (fillet) that is consumed through recreational fishing. However, the impact on the fish has not been documented, and there is concern about the potential for infection at the site of the biopsy. Blood can also be sampled without killing the fish, but it requires more skill and effort to obtain samples and minimize trauma to the fish. Also, blood-Hg concentrations may fluctuate depending on recent meals.[2] Another possibility for sampling live fish for Hg, which to our knowledge has not been scrutinized, is the caudal (tail) fin. Fins are routinely tagged for various purposes, including identification and tracking of fish. Fin clippings have also been used for stable isotope analysis in trophic-position studies which require release of live fish.[3,4] Removal of a small section of the caudal fin, which is often frayed or damaged to some extent, is not expected to impact the fish.

The purpose of this study is to determine whether the caudal fins (henceforth only fin) of walleye (*Sander vitreus*) and northern pike (*Esox lucius*) contain measurable Hg and, if so, the extent to which it correlates with muscle-Hg. We also examine the bioaccumulation of Hg by fish weight and trophic position. Finally, we evaluate the performance of a commercially available Hg-analyzer based on combustion–atomic absorption spectrometry (AAS) for the rapid and direct analysis of fish fins.

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Experimental

The Arizona Department of Environmental Quality (ADEQ) routinely collects fish from lakes and reservoirs as part of the statewide Hg advisory program. Fish for this study were collected by Arizona Game and Fish Department personnel using gill netting or electroshocking. Fish were transported to the ADEQ preparation laboratory and frozen until sub-sampling. Muscle tissue was sub-sampled using a modified biopsy punch method,[5] whereby the skin and scales were removed from an area bordered by the lateral line, dorsal fin, gill, and tailfin using a stainless steel fillet knife. A 6-mm Fray stainless steel biopsy punch was then used to collect tissue sub-samples, which were then refrozen until analyzed at the Arizona Department of Health Services using a cold vapor Hg analyzer (Perkin–Elmer model 5100) according to Method BLS152 (US EPA 600/4-81-055).[6]

Our study focussed on fish collected from three northern-Arizona reservoirs, Upper Lake Mary (35°4′ N, 111°32′ W), Soldier Lake (34°47′ N, 111°14′ W), and Long Lake (35°0′ N, 111°21′ W). We were permitted to sample caudal fins and muscle from the ADEQ archive of fish collected from these lakes, and to compare our Hg results for fin tissue with the Hg values reported by ADEQ for muscle tissue. We sampled the fish at the ADEQ preparation laboratory and refroze tissue sub-samples until analysis at the Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas.

Fins were thawed and three strips (~2 cm long and ~0.25 cm wide) were cut away using stainless-steel scissors. Samples were analyzed using a commercially available Hg analyzer, of which the AMA-254 (Leco Corp.) and the DMA-80 (Milestone Inc.) are two technically similar models. These instruments integrate sample combustion, preconcentration of Hg by amalgamation, and AAS. Since no sample pretreatment is needed, the technique is fast (<5 min per sample), eliminates reagent waste, and lowers the potential for contamination. Several groups have published details on combustion–AAS application to fish.[5,7,8] Fin segments, weighing between 50 and 300 mg wet weight (ww), were introduced into the instrument's quartz combustion tube where the sample was dried and combusted. Elemental mercury (Hg^0) and other decomposition products are carried to a tube containing gold-coated sand where Hg^0 forms an amalgam while other gaseous components are flushed out of the system. The Hg trap is then rapidly heated to ~700°C, and Hg^0 vapor is carried in a pulse through a spectrophotometer. Instrumental operating times for the drying, combustion, and waiting (post-combustion flushing) periods were 45, 120, and 45 s, respectively. To report fin-Hg relative to dry weight (dw), we determined moisture content on separate fin sub-samples by measuring weight change after heating the sub-samples for 2 h at 100°C.

We also sampled muscle tissue using the same biopsy punch method described above for nitrogen stable-isotope analysis. Samples were refrozen until analysis at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. Samples were dried at 70°C overnight, then crushed to a powder using a mortar and pestle. The dry powder was then weighed into tin boats and combusted in a elemental analyzer (Carlo Erba model 2100), then introduced into a gas isotope ratio mass spectrometer (Thermo-Finnigan Delta^{plus} Advantage) in continuous-flow mode. Nitrogen stable-isotope data are presented in per mille units relative to air.

Standard quality assurance measures were employed throughout the study. The AMA was calibrated using a fish-muscle certified reference material (DORM-2). Calibration checks using standard reference materials, including oyster tissue (NIST 1566a), were performed at the beginning and end of each set of sample analyses (typically 10). Recoveries averaged 97% of the certified values and ranged from 88 to 105%. Blanks (an empty sample boat) were analyzed periodically to confirm that Hg was not being carried over between samples. Moisture content was determined on a separate portion of the samples by drying in a convection oven at 110°C for two hours. Stable-isotope data were normalized using two internationally accepted isotope standards (IAEA N1 and N2). The working standard was peach leaves (NIST SRM 1547).

External precision on these standards was ±0.20‰ or better for $\delta^{15}N$.

Results and Discussion

Mercury concentrations for the direct analysis of the fin clippings ranged from 10 ng g^{-1} to 410 ng g^{-1} ww (Table 1). These concentrations are ≥25× the instrumental detection limit for a 50-mg specimen. Precision for Hg in fin tissue averaged 9% relative standard deviation (RSD) for triplicate measurements (range 2–20%), which is similar to that found for other fish tissues by the same method in a separate study.[2] Moisture content of the fins was variable as some were partially dried out during storage. However, most samples had percent moisture values from the low- to mid-60s. Because of apparent desiccation of some samples during cold storage, we chose to compute correlations between muscle- and fin-Hg data based on the dry weight of the fins.

Although methylmercury (in the unbound state) has appreciable volatility, in fish samples it is stable with time and against temperature cycling.[9] Nevertheless, the drying process should be standardized with respect to temperature (typically biological materials are dried between 80 and 120°C or lypholized) and should include performance criteria such as no additional weight loss.

While we strove to sub-sample the fin in a uniform and consistent manner, we did assess the variability in Hg content within the fin by analyzing different areas of select fins. The fin tends to become thicker as it nears the main body of the fish, and there was concern that the muscle mass in the sample would correspondingly increase resulting in higher Hg concentrations. However, we found no apparent trend for Hg in this axial direction, or in the vertical (top, middle, and bottom). If the fin muscle mass increases toward the body, perhaps it is proportional to the increase in bone, skin, cartilage, and other material making up the fin, or the variability in the results is simply too large to observe these differences. Nevertheless, we recommend using a sub-sampling scheme that is consistent among fish.

We found a positive correlation between muscle-Hg [ng g^{-1}, ww] and fin-Hg [ng g^{-1}, dw] for northern pike and walleye (Fig. 1), although the relative uptake appears to be influenced by environmental factors. Fig. 1 indicates that walleye from Soldier Lake have different Hg uptake characteristics than the rest of the population. We calculated Fulton's condition factor,[10] K, for all fish (Table 1). Soldier Lake walleye had significantly ($\alpha = 0.05$, $P = 0.001$) poorer condition (mean = 0.88) than the pooled population of walleye from Long Lake and Upper Lake Mary (mean = 1.07). Northern pike from Long Lake and Upper Lake Mary (there was only one observation of northern pike from Soldier Lake) did not have significantly different condition ($\alpha = 0.05$, $P = 0.2$), although their conditions were uniformly low (mean = 0.64). As a result, we calculated regression equations for northern pike as a single population, but separated the walleye from Soldier Lake from the remaining walleye (Fig. 1a).

Walleye from Soldier Lake had the lowest body weights, but the highest Hg burdens among the walleye collected. Plots of muscle-Hg versus both fish weight (Fig. 1b) and muscle-$\delta^{15}N$ (Fig. 1c), a measure of trophic position,[11,12]

Table 1. Sample information, Hg concentrations, and trophic position data for northern Arizona fish
Fin-Hg data were obtained by combustion-AAS. Muscle-Hg was measured by wet digestion and cold vapor-AAS (except for fishes 7, 30, and 48, which were obtained by combustion-AAS).

Fish ID	Lake	Taxa	Length [mm]	Weight [g]	Fin-Hg [ng g^{-1}, ww]	SD ($n = 3$)	Fin water [%]	Fin-Hg [ng g^{-1}, dw]	Muscle-Hg [ng g^{-1}, ww]	δ^{15}N Muscle [‰]	*K*-factor
7	Long	N. Pike	432	580	16	1	67	47	147	11.43	0.72
8	Long	N. Pike	770	3300	25	1	62	66	380	11.77	0.72
15	Long	N. Pike	600	1790	29	2	61	73	350	11.05	0.83
30	Long	N. Pike	370	320	15	3	60	38	127	11.36	0.63
41	Long	N. Pike	346	270	16	3	70	52	250	11.29	0.65
48	Long	N. Pike	348	220	10	1	53	22	165	10.95	0.52
16	Long	Walleye	537	1890	65	5	52	137	1200	12.62	1.22
24	Long	Walleye	447	1020	12	1	64	34	390	11.55	1.14
31	Long	Walleye	583	1720	31	6	55	70	620	11.90	0.87
49	Long	Walleye	540	1930	26	3	52	53	630	11.66	1.23
50	Long	Walleye	632	2820	36	2	59	86	870	12.10	1.12
12	Soldier	N. Pike	370	273	65	1	65	185	710	10.92	0.54
17	Soldier	Walleye	400	547	267	6	58	629	2300	11.39	0.85
20	Soldier	Walleye	375	460	155	30	62	403	1500	11.31	0.87
22	Soldier	Walleye	406	610	410	46	34	617	1800	11.23	0.91
23	Soldier	Walleye	360	438	84	5	61	214	1100	11.45	0.94
29	Soldier	Walleye	464	756	271	49	58	645	2700	11.84	0.76
33	Soldier	Walleye	322	308	70	5	60	174	1200	11.64	0.92
35	Soldier	Walleye	343	365	99	14	65	281	1500	11.36	0.90
40	Soldier	Walleye	425	623	178	23	66	523	1700	11.48	0.81
42	Soldier	Walleye	348	391	78	7	64	215	1200	11.28	0.93
26	Mary	N. Pike	417	509	74	2	23	96	290	10.99	0.70
28	Mary	N. Pike	812	3510	171	23	25	230	710	12.43	0.66
46	Mary	N. Pike	606	1308	99	8	52	206	1100	12.38	0.59
52	Mary	N. Pike	641	1330	92	5	19	113	570	11.73	0.50
62	Mary	N. Pike	634	1415	66	7	21	84	590	12.09	0.56
51	Mary	Walleye	613	2123	168	13	17	201	1600	12.87	0.92
53	Mary	Walleye	632	2653	145	6	36	208	1100	12.59	1.05
60	Mary	Walleye	672	3140	76	11	29	107	1100	10.63	1.03

demonstrate a generally positive correlation. Fig. 1b shows that higher muscle-Hg concentrations are generally associated with heavier fish and Fig. 1c shows that the same fish occupying a higher trophic position also carried a heavier Hg burden. Both trends are consistent with bioaccumulation as the primary source of Hg in the fish and indicate that the food-web structures in Long Lake and Upper Lake Mary are likely to be similar. In Figs 1b and 1c, Soldier Lake walleye demonstrate a far steeper slope in muscle-Hg versus both fish weight and δ^{15}N. Although this is consistent with bioaccumulation, these high relative concentrations of Hg may also be due to either a point source of Hg or a greater efficiency in Hg methylation in Soldier Lake. Whereas it is beyond the scope of this study to determine the cause of the relatively high concentrations of Hg in Soldier Lake fish, we do note that biological availability of Hg in aquatic ecosystems is sensitive to processes including redox and bacterial processes at the sediment–water interface,[13] and pH, dissolved organic carbon, and alkalinity in the water column.[14] A more thorough investigation of both Hg cycling and food web dynamics is necessary to resolve these interactions.

The data and regression lines in Fig. 1a show increased fin-Hg relative to muscle-Hg at higher Hg concentrations (e.g. a lower slope for Soldier Lake walleye and a flattening of the logarithmic curve fit to all the data). While the dynamics of Hg in fish are not completely understood, it is clear that concentrations in the blood, liver, brain, and other tissues decrease after exposure, and that skeletal muscle is the recipient of this redistribution.[15] However, at relatively high body burdens the concentration of Hg in other tissues increases relative to the muscle.[2] Thus, if fin, or any other tissue, is going to be used to predict muscle-Hg concentrations, this change in distribution should be incorporated into the model.

In summary, we have observed a positive correlation between fin-Hg and muscle-Hg in two species of fish in northern Arizona. Our preliminary study suggests that both trophic position and physiological condition of fish influence the uptake of fin-Hg relative to muscle-Hg. With regard to analytical procedure, although our statistical analyses were based on dry weight of fin tissue, analysis of fin wet weight may save time and effort. The combustion–AAS instrument is an affordable and accurate technique for rapidly measuring total-Hg concentrations in fish fin. Productivity can be as high as high as 15 samples per hour making it useful for laboratories that analyze large numbers of fish for Hg. With regard to fin analysis as a non-lethal predictor of muscle-Hg, a case could be made for a predictive model and fin measurements could replace some muscle measurements in monitoring programs. For those fish that are still killed for muscle data, analysis of the fins could be used to evaluate and/or add data to the model, possibly refining the relationship. This level of modelling will likely require consideration of factors related to fish physiology, such as fish condition or other metrics. Since the fin-Hg and muscle-Hg relationship

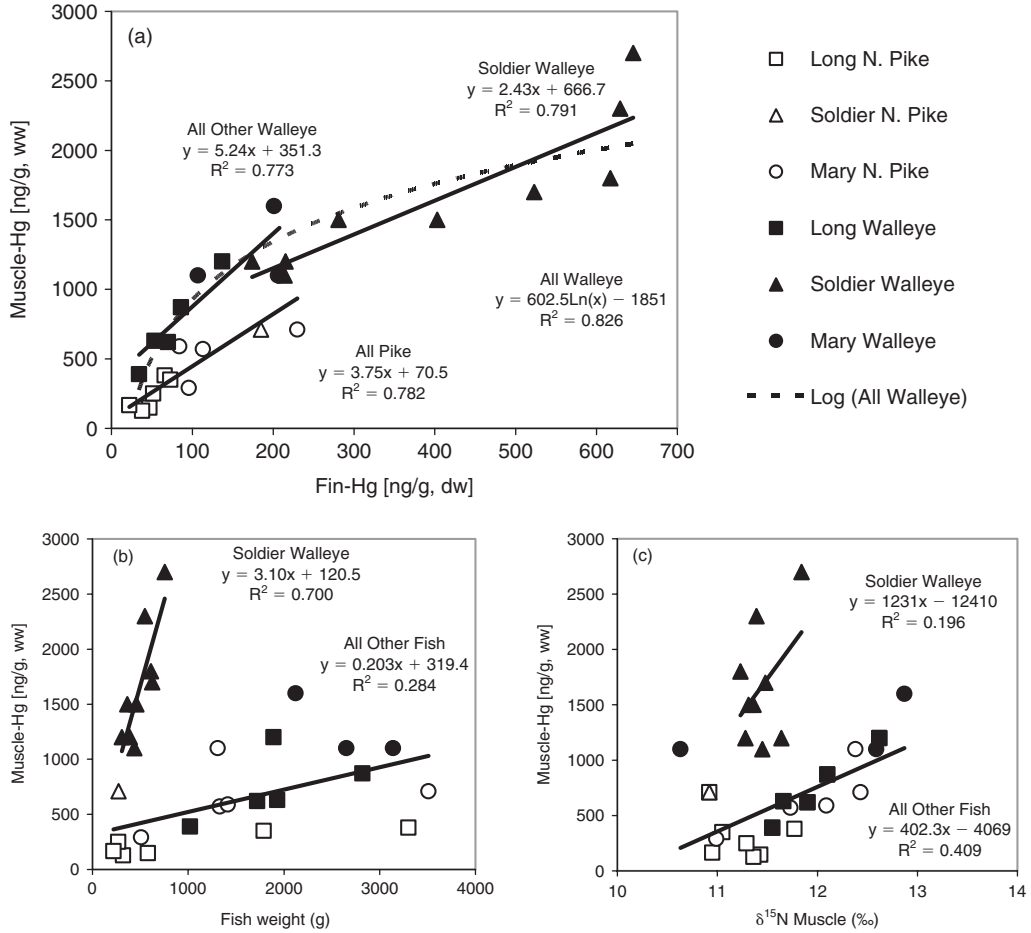

Fig. 1. Relationship between fish muscle-Hg and (a) fin-Hg, (b) fish weight, and (c) δ^{15}N muscle, showing species by lake.

is likely species-dependent (namely, depends on how a given species distributes, retains, and copes with Hg), it would be interesting to evaluate whether correlations obtained for a species from one lake would be valid for other water bodies. If the model is robust, it may be transferable and used by others.

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References

[1] B. H. Waddell, T. W. May, *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 321. doi:10.1007/BF00213109

[2] J. V. Cizdziel, T. A. Hinners, C. Cross, J. Pollard, *J. Environ. Monit.* **2003**, *5*, 802. doi:10.1039/B307641P

[3] J. C. Finlay, S. Khandwala, M. E. Power, *Ecology* **2002**, *83*, 1845.

[4] J. P. Shannon, D. W. Blinn, G. A. Haden, E. P. Benenati, K. P. Wilson, *Isotopes Environ. Health Stud.* **2001**, *37*, 179.

[5] J. V. Cizdziel, T. A. Hinners, E. M. Heithmar, *Water Air Soil Pollut.* **2002**, *135*, 355. doi:10.1023/A:1014798012212

[6] S. Rector, *Methylmercury in Fish Tissue from Arizona Lakes and Reservoirs* **2004** (Arizona Department of Environmental Quality: Phoenix, AZ).

[7] N. Salvato, C. Pirola, *Microchim. Acta* **1996**, *123*, 63.

[8] C. T. Costley, K. F. Mossop, R. J. Dean, L. M. Garden, J. Marshall, J. Carroll, *Anal. Chim. Acta* **2000**, *405*, 179. doi:10.1016/S0003-2670(99)00742-4

[9] M. Horvat, A. R. Byrne, *Analyst* **1992**, *117*, 665. doi:10.1039/AN9921700665

[10] T. Bolger, P. L. Connolly, *J. Fish Biol.* **1989**, *34*, 171.

[11] B. J. Peterson, B. Fry, *Annu. Rev. Ecol. Syst.* **1987**, *18*, 293. doi:10.1146/ANNUREV.ES.18.110187.001453

[12] K. Latjha, R. H. Michener, *Stable Isotopes in Ecology and Environmental Science* **1994** (Blackwell: New York, NY).

[13] J. K. King, J. E. Kostka, M. E. Frischer, F. M. Saunders, R. A. Jahnke, *Environ. Sci. Technol.* **2001**, *35*, 2491. doi:10.1021/ES001813Q

[14] B. A. Monson, P. L. Brezonic, *Environ. Toxicol. Chem.* **1999**, *18*, 560. doi:10.1897/1551-5028(1999)018<0560:IOFAHA>2.3.CO;2

[15] J. G. Wiener, D. J. Spry, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (Eds W. N. Beyer, G. H. Heinz, A. W. Redmon) **1996** (Lewis: Boca Raton, FL).