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Evaluation of Nonlethal Methods for the Analysis of Mercury in Fish Tissue

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Abstract.—Thousands of fish are sacrificed each year to determine potential human exposure to mercury (Hg) from fish consumption. In this paper, we use lake whitefish *Coregonus clupeaformis* and northern pike *Esox lucius* to demonstrate that accurate and reliable measures of fish muscle Hg concentrations can be determined from small samples (<100 mg) harvested with biopsy tools. Reliability of results primarily depends upon analytical methodology and tissue sample weight. Mercury concentrations estimated by use of cold-vapor atomic absorption spectrophotometry (CVAAS) on small composite tissue samples harvested with a Tru-Cut (TC) biopsy needle (mean sample wet weight = 47 mg) were less precise than estimates from tissue samples harvested with a dermal punch (DP; mean sample wet weight = 126 mg). Precision differences presumably occurred because TC samples weighed less than the prescribed minimum weight (>100 mg) for CVAAS. There was no difference in precision of Hg concentrations among tissue extraction methods when biopsy samples were analyzed via cold-vapor atomic fluorescence spectrophotometry (CVAFS). Mean tissue Hg concentrations obtained with the biopsy techniques and CVAAS or CVAFS were similar to benchmark concentrations in fillet samples (within 6%), even for TC–CVAAS. A field study of the effects of the DP biopsy method on survival of northern pike showed that tissue harvesting did not reduce survival. Our results clearly demonstrate that analysis of Hg content in muscle harvested with biopsy tools provides Hg measures comparable in accuracy to traditional, whole-fish methods but without causing mortality.

Fish consumption is the main pathway of mercury (Hg) exposure in humans. In Ontario in 2003–2004, 98% of fish consumption advisories from inland lakes were due to Hg (OME 2003). In the USA in 2002, there were 2,140 fish consumption advisories for Hg across 45 states, with 28 states issuing statewide advisories for freshwater fish consumption (USEPA 2003). Sampling of fish to determine tissue Hg concentration is a routine part of many studies and traditionally requires that fish be killed to acquire sufficient tissue volume for analysis. Implementation of monitoring programs for freshwater fish may necessarily require the sacrifice of large numbers of fish each year. With regulatory agencies becoming increasingly reluctant to permit destructive sampling, particularly for rare or endangered fish species, there is a clear need for wide-scale application of nonlethal techniques that reliably measure Hg concentrations in fish muscle.

In comparison with traditional methods, nonlethal sampling has several distinct advantages related to management issues. For example, larger sample sizes can be gathered to generate more accurate relationships between fish size and Hg concentration (Baker 2001a). Regulatory authorities may permit sampling of fish where none was permitted before (Osmundson et al. 2000; Baker et al. 2002). Accumulation or depuration of contaminants by individual fish can be monitored over time through successive biopsies (Hamilton et al. 2002). Rare or threatened species can be sampled

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without causing mortality (Osmundson et al. 2000; Baker 2001b; Baker et al. 2002; Hamilton et al. 2002).

Nonlethal tissue biopsy techniques have been used in animal and human studies for more than 30 years, although they have been infrequently applied to fish. Recent technological advances, however, have resulted in relatively smaller tissue requirements for a variety of analyses, including Hg (Moy and Dredge 1979; Tyus et al. 1999) and selenium (Waddell and May 1995; Osmundson et al. 2000; Hamilton et al. 2002). Some examples of nonlethal sampling of fish include gill, muscle, and liver biopsies for genetic characterization (Whitmore et al. 1992), electrophoretic evaluation (Crawford et al. 1977; McAndrew 1981; Mair 1989; Van Meter 1995), contaminants monitoring (Moy and Dredge 1979; Waddell and May 1995; Hamilton et al. 2002), and physiological measures (McCormick 1993; Martinellit-Liedtke et al. 1999). Uthe (1971) used a 14-gauge biopsy needle to extract small (40-mg) muscle samples. Uthe found that Hg concentrations of biopsy samples did not differ from Hg concentrations in whole muscle (although no data were provided) and reported ''excellent'' survival of biopsied fish held in aquaria. Lockhart et al. (1972) subsequently applied Uthe's (1971) technique in a field study to monitor changes in Hg concentrations in live fish over 2 years, but did not study survival of biopsied fish. Tyus et al. (1999) extracted liver (surgical incision) and muscle tissue by use of a dermal punch (DP) from rainbow trout *Oncorhynchus mykiss*, razorback suckers *Xyrauchen texanus*, and bonytail *Gila elegans*, and found similar survival and growth rates relative to control groups in a hatchery environment. Osmundson et al. (2000) used 5-mm DPs to determine selenium concentrations in Colorado pikeminnow *Ptychocheilus lucius* and razorback suckers in 1994 and 1995. Some of the fish sampled in 1994 were recaptured in 1995, providing evidence that fish survived the procedure, although quantifying survival was not part of the study design. Hamilton et al. (2002) used 4- or 5-mm DPs in 1996 and 1997 to repeatedly harvest tissue from razorback suckers held in ponds to monitor changes in selenium concentration over time in the same group of fish.

Nearly all studies that have employed nonlethal biopsy techniques to harvest muscle tissue have generally shown low mortality and no long-term, sublethal effects on fish (Lockhart et al. 1972; Crawford et al. 1977; Moy and Dredge 1979; Leitner and Isely 1994). Most of the referenced studies have been conducted under laboratory conditions (e.g., Crawford et al. 1977; McAndrew 1981; Tyus et al. 1999), however, and few studies have examined survival of fish under field conditions or compared the relative survival of biopsied and non-biopsied fish. Despite the success of laboratory studies, nonlethal techniques have not been widely applied in monitoring programs, perhaps because of lack of awareness, logistics, cost, or lack of certainty by researchers that Hg concentrations measured in biopsy samples accurately represents Hg concentrations in large (>5 g) tissues samples.

In this study, we compared total Hg concentrations in muscle biopsy samples harvested with needles and DPs from lake whitefish *Coregonus clupeaformis* and northern pike *Esox lucius* against the traditional, destructive, fillet-style technique, which served as our benchmark concentration. Multiple biopsy samples harvested with both tools from the same fish were analyzed by cold-vapor atomic absorption spectrophotometry (CVAAS) and cold-vapor atomic fluorescence spectrophotometry (CVAFS) to determine possible differences related to analytical procedure. Cold-vapor atomic absorption spectrophotometry is a widely available and inexpensive procedure that is most commonly used to determine total Hg in tissues weighing at least 200 mg. Cold-vapor atomic fluorescence spectrophotometry is not widely available and is more expensive, but has lower detection limits and is better suited to analyzing Hg in small tissue quantities. Neutron activation has been used to determine selenium in tissue plugs (Waddell and May 1995; Hamilton et al. 2002), but this technique requires a nuclear reactor, is not widely commercially available, and has rarely been used for routine Hg testing.

In this study, we addressed several questions. (1) Do biopsy samples provide accurate estimates of fish Hg concentration? (2) Are Hg concentrations determined from tissue samples harvested with biopsy needles versus DPs comparable to one another and to benchmark values? (3) Does precision of Hg concentration in biopsy sample replicates vary depending on analytical method (i.e., CVAAS or CVAFS)? In addition, we examined survival of biopsied fish relative to non-biopsied fish in a 1-year study of wild northern pike populations at the Experimental Lakes Area (ELA), Ontario, to determine whether survival was reduced by tissue extraction procedures.

Methods

Tissue extraction procedures.—Ten northern pike and five lake whitefish were captured from the Churchill River, Manitoba (58.7°N, 94.2°W), in early June 2000 and were sacrificed and shipped fresh on ice to Vancouver, British Columbia. Fish were weighed (nearest 25 g) and measured (fork length [FL], mm) prior to processing. Muscle biopsy samples were harvested from multiple locations on the left dorsal musculature of each fish by use of a Tru-Cut (TC) biopsy needle and a 4-mm DP. The DP is designed to collect approximately 60 mg of subcutaneous muscle tissue and epidermis, whereas the TC biopsy needle is designed to collect approximately 25 mg of muscle about 1 cm below the skin. Our experience has shown that both tools are effective at harvesting tissue from fish larger than 180 mm FL.

The TC biopsy needle is a 14-gauge, 4-cm-long, double-barreled device with a 2-cm cannula for containing harvested muscle. To collect a tissue sample, the TC needle was inserted forward at an oblique angle beneath a scale into the dorsal musculature. The outer barrel, which possesses a sharp leading edge, was then extended over the inner needle to cut and capture a small tissue plug within the cannula. The needle was withdrawn and opened, and the tissue sample was removed with tweezers. An experienced person required only about 10 s to harvest a single tissue sample with the TC needle.

The DP consists of a sharp, hollow, 4-mm-diameter, stainless steel punch attached to a plastic holder. To collect a tissue sample, several scales were removed with the tip, and the DP was placed against the exposed epidermis. A downward twisting action was applied to allow the punch to penetrate several millimeters into the tissue. The DP was rotated parallel to the fish and twisted to sever and capture a small piece of muscle and epidermis in the distal end of the punch. The tissue plug was mouth blown onto a clean glass slide, and the epidermis was removed with a sharp scalpel. Approximately 30–45 s were required to collect a tissue sample with the DP.

The DP collected a larger sample of tissue per application than the TC needle, but was more time consuming, required the removal of epidermal tissue and several scales, and left a small wound that had to be sealed with a tissue adhesive. To increase sample weight, individual tissue samples for both the TC and DP biopsy techniques were comprised of a composite of two plugs extracted from immediately adjacent locations. After collection, all biopsy samples were weighed ($\pm$0.001 g), placed in 7-mL, labeled plastic vials, and frozen immediately on dry ice. All frozen tissue samples were sent to the Department of Fisheries and Oceans Freshwater Institute (FWI), where they were freeze-dried before analysis.

Study design.—The TC needle and DP biopsy tools were used to harvest an equal number of tissue samples from both species for Hg analysis. Five biopsy samples were harvested with both the DP and TC biopsy tools from each of the five lake whitefish (i.e., 5 replicates $\times$ 2 tools $\times$ 5 fish = 50 tissue samples). Three biopsy samples were harvested with both the DP and TC tools from each of the 10 northern pike (i.e., 3 replicates $\times$ 2 tools $\times$ 10 fish = 60 tissue samples). This first set of samples was placed on dry ice and frozen. Next, a second complete set of samples was harvested from the same fish and frozen on dry ice. In all, 220 tissue samples were collected; the first set (n = 110) was earmarked for CVAAS analysis of Hg, and the second set (n = 110) was earmarked for CVAFS analysis. Once all biopsy tissue samples were collected, four large ''fillet'' (10 g) samples were harvested from each fish. Two fillet samples were analyzed for total Hg as wet tissue (μg/g wet weight [ww]) and two were analyzed after freeze-drying (μg/g dry weight [dw]). All Hg determinations from fillet samples were analyzed by use of the conventional CVAAS method. The mean dry-weight Hg concentration of freeze-dried fillets was used as the benchmark concentration against which Hg concentrations from freeze-dried replicate biopsy samples were compared. Tissue samples were freeze-dried at the FWI and weighed with a Perkin-Elmer Autobalance AD-2Z (nearest 0.001 mg). Moisture content of biopsy samples from individual fish was derived to allow conversion back to wet-weight concentrations before data analysis.

Analysis of mercury concentration.—Total Hg concentration (μg/g) in biopsy tissues were determined by the Flett Research, Winnipeg, Manitoba, and FWI laboratories for CVAFS and CVAAS, respectively. Both laboratories have demonstrated ongoing reliable performance through quarterly participation in the Mercury Quality Assurance Program (MQAP), a proficiency program for Hg analysis in fish, run by the Canadian Food Inspection Agency. To demonstrate analytical control, previously characterized fish tissues from the MQAP were run by both laboratories during all analyses presented in this study.

CVAAS analysis at the Freshwater Institute.—At the FWI, freeze-dried biopsy samples were transferred to 25-mm × 200-mm Folin Woo digestion tubes and were digested in 5 mL of a 3:2 mixture of sulfuric acid and nitric acid at 180°C for a period of 5 h in an aluminum hotblock. Samples were cooled to room temperature prior to the addition of $KHSO_4$ and $KMnO_4$ as oxidants, followed by reduction of excess oxidant by hydroxylamine sulfate. The resulting solution (brought to volume with deionized water) was analyzed with an LDC Analytical, Hg Monitor 3200 (elemental Hg detector) by use of standard CVAAS protocols (modified from Armstrong and Uthe [1971] and Hendzel and Jamieson [1976]). Peak areas were transformed digitally with a Thermo Separation Products SP4400 Integrator.

CVAFS analysis by Flett Research.—Freeze-dried biopsy samples analyzed by Flett Research were transferred to 20-mm × 150-mm, acid-cleaned Pyrex culture tubes and were digested in 10 mL of a 2.5:1 mixture of sulfuric acid and nitric acid at 180°C for a period of 6 h in an aluminum hotblock. The samples were cooled to room temperature, 200 μL of BrCl was added, and the samples were then increased to 25 mL with deionized water. Analysis of digests was performed by CVAFS in a Brooks Rand II Hg fluorometer, according to the U.S. Environmental Protection Agency's Method 1631 protocol (USEPA 2001); a single gold trap was used for analysis. Peak areas were determined with a Spectra-Physics 4200 integrator.

Statistical procedures.—The following statistical procedures were employed to address the various study questions, posed as null hypotheses.

H_0 (1) *Mercury concentrations of freeze-dried tissues do not differ from wet-weight benchmark values after correction for moisture content. Moisture content of biopsy samples, relative to whole muscle from fillets (i.e., benchmark values), does not differ among techniques.* Comparisons of moisture content among biopsy tissue samples and determination of the effect of freeze-drying on Hg concentration in fillet samples were performed with one-way analysis of variance (ANOVA) and paired *t*-tests (Sokal and Rohlf 1981).

H_0 (2) *Precision of replicate Hg concentrations does not vary among the different biopsy techniques and analytical methods.* The precision of TC needle and DP tissue extraction techniques analyzed with both CVAAS and CVAFS was determined by comparisons of coefficient of variation (CV = 100 × SD/mean) data.

H_0 (3) *Mercury concentrations of tissues harvested with the TC needle or DP do not differ relative to benchmark concentrations when analyzed by either CVAAS or CVAFS.* To determine the accuracy of different tissue extraction techniques, it was necessary to know the true Hg concentration for each fish (i.e., the benchmark). These values could not be determined with certainty, and there was no a priori reason for choosing the results of one technique over another. We chose to use Hg concentrations determined by CVAAS of freeze-dried fillet samples as our benchmark. This was because fillets are used by most regulatory agencies, and because most research applications and laboratories use CVAAS to determine Hg concentration. An alternative was to use the grand mean of all techniques as the benchmark; this approach resulted in similar interpretations. Dry-weight tissue Hg concentrations (μg/g dw) acquired with CVAAS and CVAFS of the TC needle and DP replicates were compared by use of one-way analysis of covariance (ANCOVA) with fillet dry-weight Hg concentrations. Each individual fish was the independent covariate. We used the mean Hg concentration of the three or five biopsy replicates for each fish and analytical method as the dependent variables in these analyses. Analyses based on back-calculated wet-weight concentrations (μg/g ww) produced similar results. Tukey's post hoc multiple comparisons were used to distinguish differences among all possible comparisons. Potential biases in Hg determinations made by the different biopsy sampling methods were further examined by conducting paired *t*-tests with fillet Hg concentrations. Data were log-transformed as necessary to reduce heteroscedasticity. All statistical comparisons were undertaken in SYSTAT 8 (SPSS Science, Chicago).

Survival study.—For biopsy methods to gain wide acceptance, mortality from capture, handling, and tissue extraction must be minimal. We used the DP to acquire tissue samples from northern pike in October 2000 in two lakes (L240, L658) within the ELA, northwestern Ontario (49°34′N to 49°47′N, 93°36′W to 93°52′W). Northern pike were captured with short-set gill nets or by angling and were placed in a large holding pen. Before tissue extraction, live fish were anesthetized with MS-222 (tricaine methanesulfonate) to minimize injury from struggling. Once anesthetized, fish were measured for FL (mm) and weight (g), and passive integrated transponder (PIT) tags (Biomark, Boise, Idaho) were inserted into the cheek tissue. Single tissue plugs were harvested with the

DP from about two-thirds of all fish captured. Exposed wounds were closed with Nexaband, a sterile tissue adhesive that forms a solid seal within several seconds after application. Once processing was completed, fish were returned to a freshwater holding tub and allowed to recover prior to release into the lake. The same methods were used to capture and mark fish in the two lakes in May–June 2001 and again in October–November 2001, except that clove oil was used as an anesthetic during these sampling periods. A chi-squared test with Yates' correction was used to compare the probability of recapture of biopsied and non-biopsied fish in spring 2001 (overwinter survival) and fall 2001 (annual survival).

Results and Discussion

Fish Size, Moisture, and Hg Data

The northern pike and lake whitefish used in the comparison study were obtained from an ongoing research program in Churchill, Manitoba, and incorporated fish sizes and Hg concentrations typical of fish Hg monitoring studies. Northern pike ranged from 451 to 673 mm FL (mean = 561 mm) and weighed 600–2,300 g (mean = 1,280 g). Lake whitefish ranged from 362 to 510 mm FL (mean = 423 mm) and weighed 625–2,025 g (mean = 1,220 g). Mean benchmark wet-weight tissue Hg concentrations ranged from 0.174 to 0.459 μg/g in northern pike and from 0.087 to 0.143 μg/g in lake whitefish (Figure 1). As expected, mean wet weights of composite TC (mean ± 1 SD; 46.8 ± 9.1 mg), DP (126.4 ± 32.8 mg), and fillet (220 ± 24.9 mg) tissues differed and influenced the precision of Hg estimates.

Mean moisture content of muscle from TC (78.4%) and DP (78.9%) techniques did not significantly differ from moisture content of fillets (79.0%), and demonstrated that tissue moisture content was not affected by extraction with biopsy tools. Previous biopsy techniques that involved suctioning of tissue with needles (Moy and Dredge 1979; Van Meter 1995) or compaction of tissue with noncutting tools (Uthe 1971; Lockhart et al. 1972; Wang et al. 1994) may cause hemorrhaging or compaction and alter moisture content, thus affecting Hg concentrations. We weighed biopsy samples immediately after harvesting, prior to storage and freeze-drying, to further eliminate the potential confounding effect of moisture loss on Hg concentration when converting from dry-weight to wet-weight Hg concentrations. We recognize that it may not be possible to acquire precise wet weights for biopsy samples in the field or to freeze-dry tissues before analysis. If accurate tissue weights cannot be acquired in the field and if freeze-drying is not possible, then biopsy samples should be weighed and analyzed as quickly as possible after harvesting to minimize the moisture loss that could cause Hg concentrations to be overestimated.

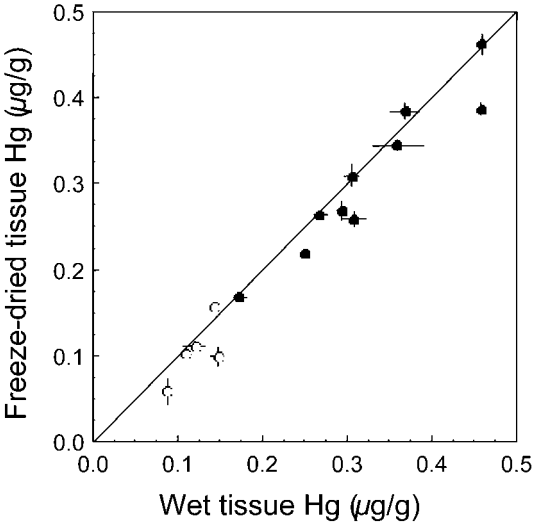

FIGURE 1.—Mean Hg concentration (±SD) of freeze-dried whole-muscle (fillet) tissue versus mean Hg concentration of wet whole-muscle tissue from individual lake whitefish (○) and northern pike (●). A 1:1 relationship is represented by the solid line.

Mean Hg concentrations of freeze-dried (μg/g dw) and wet muscle tissues (μg/g ww) from fillet-style samples were highly correlated ($r^2 = 0.92$, $n = 15$, $P < 0.001$; Figure 1). Although freeze-drying is not known to cause a loss of Hg in tissue (Lasorsa and Allen-Gil 1995; de Boer and Smedes 1997), we found that mean back-calculated Hg concentrations of freeze-dried fillet subsamples were significantly lower (91.4%) than concentrations based on wet weight (paired *t*-test: df = 14, $P = 0.02$). Examination of Figure 1 indicates that the majority of points lie on or just below the 1:1 ratio between dry-weight and wet-weight Hg values. We subsequently undertook a second comparison of Hg concentrations determined from wet and freeze-dried tissue and found no significant difference. Nevertheless, freeze-drying may have caused a small loss of Hg in this study.

Precision of Hg Data Obtained with Biopsy Tools

Mercury concentrations of replicate biopsy samples harvested with the TC needle and analyzed

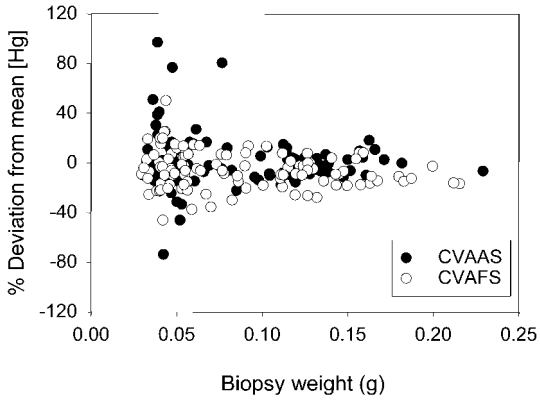

FIGURE 2.—Relationship between tissue biopsy weight (grams wet weight) and percent deviation from mean benchmark Hg concentrations based on cold-vapor atomic absorption spectrophotometry (CVAAS) (●) and cold-vapor atomic fluorescence spectrophotometry (CVAFS) (○) of lake whitefish and northern pike samples.

with CVAAS were significantly more variable, based on CV values, than samples acquired with the DP. On average, the CV for the TC–CVAAS method was significantly higher (CV = 13.7%) than for samples analyzed by the other three combinations of biopsy tool and Hg analysis (mean CV = 9.2%). The greater variability of Hg concentrations from TC–CVAAS was presumably due to the small mean weight of tissue (47 mg), which is lower than the prescribed minimum of 100 mg for CVAAS (Hendzel and Jamieson 1976). Variance in Hg concentration among replicate samples increased substantially when samples weighed less than 80 mg ww and were analyzed with CVAAS (Figure 2). These data indicated that greater precision of Hg concentration was obtained from composite tissue samples weighing over 80 mg, a size more easily achieved by the DP technique. Note that overall precision was lower in our study than is typically seen in quality assurance/quality control (QA/QC) inter-laboratory comparisons. Most QA/QC programs use large, well-homogenized tissue samples, as opposed to the small, discrete samples we collected from different parts of the same fish.

Accuracy of Hg Data Acquired with Biopsy Tools

Mean Hg concentrations of freeze-dried tissue samples harvested with the TC needle or DP and analyzed by CVAFS were statistically indistinguishable from benchmark concentrations (Tables 1, 2; Figure 3A, C; paired *t*-tests). The TC–CVAAS samples (Figure 3B) were slightly but significantly higher than benchmark concentrations (paired *t*-test: df = 14, $P < 0.02$). Samples analyzed with CVAAS (Figure 3D) also had marginally but significantly higher Hg concentrations than replicate samples analyzed with CVAFS (Table 2). On average, mean Hg concentrations estimated by CVAAS were 6% higher than those of replicate tissues analyzed by CVAFS; this is a relatively small difference. Statistically significant among-method or among-laboratory differences are common in QA/QC studies (Wernimont 1985), and this magnitude of difference is not unusual. For example, Waddell and May (1995) found that the CV of selenium concentration in multiple replicates from a 20-g razorback sucker muscle fillet was 6.5%. The CV for selenium in muscle plugs from razorback suckers and common carp *Cyprinus carpio* in the Waddell and May (1995) study was 10% and did not differ significantly from fillet-style samples.

In summary, the CV of replicate samples relative to wet and dry benchmark tissue Hg concentration was of a similar magnitude for DP and TC biopsy tissues analyzed with CVAFS and for DP analyzed with CVAAS. These results indicate that the variation in Hg concentrations of tissues harvested with these techniques was no greater than the variation in Hg concentration of fillet-style tissues requiring destructive sampling. Although

TABLE 1.—Results of analysis of covariance comparison of Hg concentrations in lake whitefish and northern pike obtained by use of different biopsy methods. Benchmark Hg concentrations obtained from fillet samples were used as the independent covariate; SS = sum of squares, MS = mean square.

Source	SS	df	MS	*F*-ratio	*P*
Method	0.01	3	0.01	4.21	0.01
Benchmark	0.73	1	0.73	661.86	<0.01
Error	0.06	55	0.001		

TABLE 2.—Matrix of probabilities for Tukey's post hoc comparison of different biopsy methods for determining Hg concentration in lake whitefish and northern pike. Biopsy tools were Tru-Cut (TC) needle and dermal punch (DP). Analysis methods were cold-vapor atomic absorption spectrophotometry (CVAAS) and cold-vapor atomic fluorescence spectrophotometry (CVAFS).

Method	TC–CVAFS	DP–CVAFS	DP–CVAAS
DP–CVAFS			0.39
TC–CVAFS		0.96	0.70
TC–CVAAS	0.04	0.01	0.38

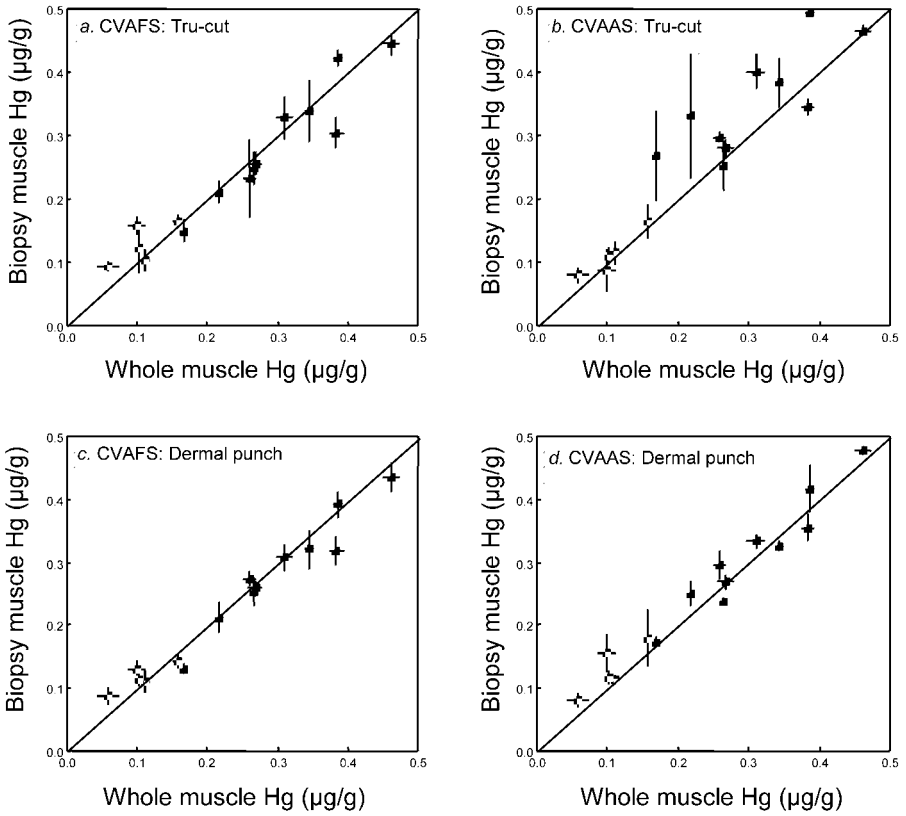

FIGURE 3.—Relationships between mean biopsy Hg concentrations (µg/g wet weight [ww]) and benchmark concentrations (µg/g ww) (± SD) for individual lake whitefish (○) and northern pike (●). Biopsies were collected by dermal punch or Tru-Cut needle, and were analyzed with cold-vapor atomic fluorescence spectrophotometry (CVAFS) or cold-vapor atomic absorption spectrophotometry (CVAAS). A 1:1 relationship is represented by the solid line.

there were statistical differences in Hg concentration between biopsy samples and fillet-style samples, the magnitude of these differences was quite small and within the range one would expect in multiple-sample, multiple-laboratory comparisons. Therefore, we believe that the use of nonlethal biopsy techniques for either CVAAS or CVAFS analysis of Hg content will provide accurate and reliable data comparable to the traditional method (i.e., fillet-style samples analyzed by CVAAS). We caution that precision of CVAAS is unacceptably low if wet tissue weight is less than 80 mg (Figure 2). As a result, when CVAAS is used, we recommend sampling by DP because of the larger mass of tissue harvested with this tool.

Field Survival of Biopsied Fish

Biopsy tissue extraction procedures are of little benefit if there is significant mortality of fish. Although numerous studies have demonstrated minimal mortality or sublethal effects on fish as a result of handling and muscle tissue extraction under laboratory conditions (e.g., Van Meter 1995; Tyus et al. 1999), no study has specifically examined survival under natural conditions. Recently, muscle plugs from razorback suckers and Colorado pikeminnow were acquired under field conditions to determine muscle selenium concentration by use of neutron activation (Waddell and May 1995; Osmundson et al. 2000; Hamilton et al. 2002). Although the effect of muscle plug harvesting on infection and survival was not an objective of these studies, Waddell and May (1995) indicated that recaptured fish did not appear to demonstrate adverse effects. Razorback suckers that were held in ponds for up to 1 year after the biopsy procedure also appeared to be unharmed by tissue harvesting (Hamilton et al. 2002).

In fall 2000 at the ELA, we biopsied 15 of 19

TABLE 3.—Mark and recapture data from the Experimental Lakes Area, Ontario, used to estimate overwinter and annual survival of biopsied and nonbiopsied northern pike. Single dermal punches were used on biopsied fish.

Lake	Biopsy group	Number marked in fall 2000	Number recaptured in	
			Spring 2001	Fall 2001
L240	Biopsied	15	6	4
	Nonbiopsied	4	1	0
L658	Biopsied	48	22	16
	Nonbiopsied	32	10	10

northern pike captured from L240 and 48 of 80 northern pike from L658. During capture events in spring and fall 2001, the recapture ratio of biopsied to nonbiopsied fish was similar to the ratio at which fish were originally biopsied (Table 3). In other words, there was no difference in the recapture probabilities of biopsied versus non-biopsied fish between fall 2000 and the subsequent spring (L240: $\chi^2 = 0.00$, $P = 0.98$; L658: $\chi^2 = 1.20$, $P = 0.28$) or after 1 year (L240: $\chi^2 = 0.22$, $P = 0.64$; L658: $\chi^2 = 0.00$, $P = 0.96$) in either lake. All biopsy wounds appeared free of infection and showed complete regrowth of skin and scales over the wound after 1 year. These data are the first, to our knowledge, to quantitatively demonstrate that fish handling and biopsy techniques do not affect fish survival in the wild. Although Osmundson et al. (2000) conducted repeat biopsy sampling of the same PIT-tagged fish in 1994 and 1995, they did not examine survival of biopsied fish per se. Hamilton et al. (2002) repeatedly sampled razorback suckers with dermal plugs to monitor changes in tissue selenium concentration. A few individual fish died or escaped from holding ponds during the study, but no adverse effects were ascribed to the biopsy procedure.

Summary

Results of our study demonstrated that small tissue quantities collected with nonlethal biopsy tools provided accurate and precise estimates of Hg concentration in fish muscle relative to benchmark values from the traditional, fillet-style method that requires sacrifice of fish. Reliability of results depended upon the biopsy tool, analytical methodology (i.e., CVAAS versus CVAFS), and tissue sample weight, and possibly upon the loss of Hg from freeze-drying.

A field study at the ELA with the DP method demonstrated that tissue biopsy sampling did not reduce survival of recaptured northern pike after 1 year and that this method allows for monitoring of Hg concentration from the same fish over time.

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