A comparison of fish tissue mercury concentrations from homogenized fillet and nonlethal biopsy plugs

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ABSTRACT

The use of biopsy plugs to sample fish muscle tissue for mercury analyses is a viable alternative to lethal sampling; however, the practice has yet to be widely implemented in routine monitoring due to concerns about variability of mercury concentrations in fish muscle tissues. Here we examine distribution of mercury in fillets of four fish species (Walleye, Northern Pike, Smallmouth Bass and Lake Trout), suitability of left/right side of fillet for biopsy sampling, and appropriateness of re-using a biopsy punch. The results showed that average mercury concentrations in left and right fillets of fish are similar. Mercury concentrations in biopsy plug samples, taken from the anterior dorsal area of the fish fillet, were statistically equivalent to the mercury concentrations in homogenized fillets. There was no discernible cross contamination between samples when a biopsy punch was reused after washing in hot soapy water, and as such, biopsy punches can be recycled during sampling to reduce the sampling cost. If a tissue mass collected from a specific site on the fillet is insufficient, then we suggest sampling corresponding locations on the other fillet rather than sampling two adjacent sites on one fillet to obtain more tissue. The results presented here can improve the accuracy of fillet biopsy plug sampling, minimize fish mortality for mercury monitoring, and reduce labor and material costs in monitoring programs.

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Introduction

Mercury typically accumulates in biota in the form of methylmercury, which is readily absorbed from the gastrointestinal tract and distributed to tissues, where it tightly binds to proteins (Health Canada, 2008). This behavior allows bioaccumulation and biomagnification of methylmercury in the food web, resulting in its higher concentrations and presence as the dominant form of mercury in top predatory species including fish and humans (Health Canada, 2008; Environment Canada, 2013a). Methylmercury also readily crosses the blood–brain barrier to affect proper functioning of the nervous system including sensory impairment (Harada, 1995; Clarkson et al., 2003; Mergler et al., 2007). In cases of extremely high exposure, methylmercury can be deadly (Harada, 1995; Health Canada, 2008).

As fish consumption is the foremost mercury exposure pathway for humans, safe dosages have been derived from toxicological studies (e.g., 3.3 μg/kg bw per week for the general population and 1.6 μg/kg bw per week for the sensitive

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population of women of child bearing age and children; Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives). The sampling and analyses of fish samples for mercury content is considered important for public health and safety (Harada, 1995; Health Canada, 2008; UNEP/WHO, 2008; UNEP, 2013). Based on monitoring data, advice on safe consumption of fish (both locally caught and commercially purchased) has been issued by a variety of agencies (Environment Canada, 2013b; OMOECC, 2015; USEPA, 2015). Various governments have also set regulations on mercury content for retail and international sale of fish products (CODEX, 2009; Health Canada, 2011).

Monitoring of mercury concentrations in fish, especially for issuing advice on safe consumption, is typically conducted using measurements on homogenized fillets (with skin intact or removed) to provide an accurate measure of the average concentrations of mercury in the most edible portion (Monson et al., 2011). This method is an extensive process in which a number of fish are caught, sacrificed, filleted, transported to a laboratory, stored frozen, thawed and cut into small pieces that are then ground into a homogenized tissue to be subsampled for a mercury analysis (Bhavsar et al., 2010). From an ethical perspective, the most concerning aspect of this effort is fish mortality to provide a very small sample of muscle tissue required for mercury analysis.

A number of studies have recommended the use of a biopsy punch to obtain small tissue samples directly from a live fish as an alternative to the homogenized fillet method for mercury monitoring (Uthe, 1971; Bishop and Neary, 1975; Cizdziel et al., 2002; Baker et al., 2004; Schmitt and Brumbaugh, 2007; Ackerson et al., 2014). In comparison with the traditional method, the biopsy plug method is beneficial in many ways as it: (1) eliminates the need to sacrifice the specimen, (2) reduces processing time and labor cost, and (3) allows for the sampling of fish that would otherwise be prohibited due to conservation restrictions. However, most previous studies on reliability of fish tissue biopsy plug mercury measurements have been limited by types of fish considered, sample size, number of biopsy plugs taken from a fillet, coverage of fillet area, fillet from which side of a fish considered, or method of the assessment (e.g., compared to fillet or whole fish; from the same or other fish). Although 40+ years have passed since the initial studies indicating that a biopsy plug mercury measurement is a feasible and viable alternative to lethal, homogenized fillet mercury monitoring, the practice has yet to be widely implemented in routine monitoring (Schmitt and Brumbaugh, 2007; Chalmers et al., 2011; Monson et al., 2011).

In this study, we investigate distribution of mercury in fillets of four fish species, and present an area on fish fillet to collect a biopsy plug that would provide the most accurate representation of the corresponding average fillet mercury concentration. We also examine whether a biopsy plug sample from the right or left side of a fish would be more appropriate. Finally, we assess if re-use of a biopsy punch, which could save resources and thereby reduce sampling cost, would be suitable. The results presented here can improve the accuracy of biopsy plug sampling, minimize fish mortality for mercury monitoring, and reduce labor and material costs in monitoring programs. We hope this outcome will lead to aid in implementing Article 19 of the Minamata Convention on Mercury requiring the parties to develop and improve geographically representative monitoring of mercury in environmental media (UNEP, 2013).

1. Materials and methods

1.1. Sample collection

Four fish species namely Walleye (Sander vitreus), Northern Pike (Esox lucius), Smallmouth Bass (Micropterus dolomieu) and Lake Trout (Salvelinus namaycush) were selected for this study due to the following reasons: (1) widely available at many waterbodies in Ontario, Canada, (2) generally higher in mercury concentrations due to biomagnification from consumption of other fish, and/or (3) preferred by humans/wildlife for consumption (Scott and Crossman, 1973; Awad, 2006; Bhavsar et al., 2011). Samples were collected in 2015 by electrofishing or gillnetting from publicly accessible sites at the Niagara River (43°6′17″N, 79°3′34″W), Montreal River (47°56′16″N, 80°39′10″W), Spanish River (46°15′18″N, 81°43′3″W), Long Lake (46°22′20″N, 81°4′54″W) and Anstruther Lake (44°4′44″N, 78°12′48″W), in the Province of Ontario, Canada. Fish samples were collected as a part of regular fish contaminant monitoring being conducted in the Province of Ontario for 45 years, and ranged in sizes (Walleye: 42.8–54.3 cm, 730–1650 g; Northern Pike: 52.4–90.2 cm, 930–5330 g; Smallmouth Bass: 34–47 cm, 600–1400 g; Lake Trout: 47.5–72.8 cm, 820–3750 g). The sample collection was approved by Ontario Ministry of Natural Resources Aquatic Research and Development Section Animal Care Committee, and did not involve endangered species or locations. After collection, fish were euthanized by blow to the head, weighed and measured for total length and kept on ice during transportation to the Ontario Ministry of the Environment, Conservation and Parks (OMOEC) laboratory in Toronto. The samples were filleted (skin removed) and kept frozen at −20°C until ready for fillet homogenization and biopsy plug collection.

1.2. Fillet and biopsy plug sampling

The study was performed in four steps to conduct an in-depth comparison of two sampling methods: fillet homogenization and fillet biopsy plug sampling. Two skinless fillets were collected from each fish sample using a pre-cleaned stainless steel filleting knife. Fillets chosen for homogenization were sliced into manageable sizes and homogenized using the BÜCHI B-400 Mixer (Switzerland), Robot Coupe Blixer® 2 (USA), or Robot Coupe R15 Vertical Cutter (USA) depending on size of a fillet. The 0.2–0.4 g of homogenized tissue from each fillet was then subsampled in sterile 2 mL cryogenic vials and refrozen at −20°C until lab analysis.

Biopsy plug sampling was conducted in the laboratory on thawed fillets as opposed to in the field on live fish because of collection of multiple plugs from various parts of a fillet and the involvement of the homogenization method which requires a whole fillet from a dead fish. Biopsy samples were collected using Integra™ Milite® (USA) 6 and 8 mm diameter stainless steel disposable biopsy punches attached to plastic holders. These punches obtained approximately 0.2–0.8 g of subcutaneous wet weight fillet tissue per insertion of about...
1–2 cm deep. The method is described by Baker et al. (2004) and briefly summarized here. Once a fillet was thawed, it was placed onto a glass cutting board that was washed with hot soapy water between each sample. The sterile biopsy punch was vertically inserted into the fillet with slight pressure and a twisting motion to sever the muscle tissue to simulate the sampling of a live specimen. The tissue sample was retrieved and blown through a hole in the top of the hollow punch using a rubber squeeze bulb into a sterile 2 mL cryogenic vial. The biopsy plug samples were then weighed (correcting for weight of the empty vials) to ensure retrieval of at least 0.2 g of tissue and kept frozen at −20°C until total mercury analysis.

1.3. Left and right fillet comparison

Left and right side fillets were homogenized separately (Fig. 1a) to allow for a comparison of mercury concentrations between the two sides. This was a precautionary step to confirm that fillets from either side of the sample would yield the same mercury concentration in order to proceed with the study. Two fillets were acquired from each of three samples of the four species, and were homogenized following the procedure as described above. Each homogenized fillet was split into five samples to capture analytical variability in the measurements. Fish fillets with differing mercury concentrations were selected to examine if the left and right side fillets could be considered equivalent in total mercury concentrations (Smallmouth Bass: 0.1–0.3 μg/g; Lake Trout: 0.2–0.4 μg/g; Walleye: 0.4–0.6 μg/g; Northern Pike: 0.3–1.0 μg/g; Fig. 2). The first part involved a total of 120 mercury analyses.

1.4. Homogenized fillet and biopsy plug comparison

Left fillets were subjected to the homogenization treatment and right fillets to the biopsy sampling (Fig. 1b) to determine whether the biopsy plug samples could accurately represent the average tissue concentration from a homogenized fillet. Two fillets were acquired from five samples of each of the four fish species. The left fillet from each fish was homogenized following the previously described process, and then was split into five subsamples that were individually homogenized using the Virtis® 6301 Model Homogenizer. Each re-homogenized tissue was subsampled into three portions and frozen in sterile 2 mL cryogenic vials at −20°C until total mercury analysis (Fig. 1b). In total, there were 15 samples per fillet, and 75 samples per species (Fig. 1b). The additional homogenization of a part of a homogenized fillet before further splitting was aimed at preparing a truly homogenized sample to investigate analytical variability. The right fillet was sampled using the biopsy method in patterns of 8–20 plugs (Appendix A Fig. S1), with 2 fish of each species sampled 8 times and the other 3 fish sampled 20 times (depending on the size of the fillet) to provide higher resolution of spatial variability of mercury within a fillet.

1.5. Left and right biopsy plug sample comparison

Left and right side fillets were both sampled three times using the biopsy punch method from a narrow area established during the second part of the study, as well as literature to examine potential differences in mercury concentrations in

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**Fig. 1** - Sampling schematics for the four parts of the study. a: left and right fillet comparison, b: homogenized fillet and biopsy plug comparison, c: left and right biopsy plug sample comparison, and d: re-use of biopsy punch ((A) single use of a punch, (B) reuse of a washed punch to sample a clean fish after sampling a mercury contaminated fish).
biopsy plugs from this area on two fillets of a fish (Fig. 1c). Two fillets were acquired from five samples of the four species, and underwent biopsy punch sampling following the described procedure. The selected area for the plug collection was the anterior dorsal musculature in the top front-middle part of the fillet where the tissue was thickest (Appendix A Fig. S1).

1.6. Re-use of biopsy punch

We investigated whether disposable biopsy punches could be re-used after cleaning for the collection of multiple samples in the case of mercury testing (Fig. 1d). Traditionally, the instrument would be disposed of after one use to prevent cross-contamination (Schmitt and Brumbaugh, 2007). However, mercury is typically tightly bound to muscle tissue and does not easily transfer through physical contact. The re-use of punches could prove to be a cost-efficient option, and also reduces plastic waste produced during sampling. To test this, a fillet from a typically high mercury concentrated species (Walleye) was sampled in tandem with a laboratory raised Rainbow Trout (Oncorhynchus mykiss) fillet containing very little mercury to observe whether any cross-contamination of mercury occurred between the tissue samples. A baseline concentration for the Rainbow Trout tissue was established using three fish by first homogenizing the left fillets, splitting each into five subsamples, and then biopsy sampling the right fillets, with a new sterile punch being used for each of the five plugs collected from a fillet. To test the re-usability, three fish from both the Walleye and Rainbow Trout species were filleted and biopsy sampled on their right fillets by alternating the single punch between species from one fillet to the other, with the punch being washed with soap and hot tap water between each sample. A new punch was used for each set of fillets from the two species.

1.7. Analytical methods

The samples were analyzed for total mercury content using acid digestion and cold vapor flameless atomic absorption spectroscopy as per the OMOE method HGBIO-E3057 (OMOECC, 2016). The method is described by Bhavsar et al. (2010) and briefly summarized here. Thawed 0.2–0.4 g of wet weight fish tissue was transferred into Folin–Wu digestion tubes using a spatula, and 5 mL of a 4:1 (V/V) sulfuric:nitric acid mixture were added to each tube. The tubes were placed in aluminum blocks on hot plates and samples were digested overnight at a temperature of 215–235°C. The samples were cooled and brought up to 25 mL with pure water. The solutions were transferred to a cold vapor flameless atomic absorption spectroscopy system by a Gilson Minipuls peristaltic pump (Mandel Scientific, Canada) using a Gilson Anacol SC30 automated sampler (Mandel Scientific, Canada). The mercury was then reduced by a stannous chloride solution to its elemental form. An air stream carries the mercury vapor through a gas–liquid separator (LaSalle Scientific, Canada) and an impinger containing sulfuric acid into a flow-through elemental mercury detector (Milton Roy, USA) with a light source set at 253.7 nm wavelength. The data was collected using a Labtronics DP1000 (Labtronics, Canada) data acquisition system.

Two blanks and four mercury calibration standards, made from a stock solution traceable to the National Institute of Standards and Technology (NIST), were processed with every batch of 50–60 samples. The calibration was checked with a NIST traceable solution (NIST991304), and the correlation
coefficient of the relationship was between 0.990 and 1.00. The calibration standards were re-analyzed at the end of the run to ensure that the sensitivity did not change >10% during the run. The digestion efficiency was checked against two in-house reference materials, which were composite samples of previously analyzed fish fillet samples with mercury concentrations representing low and high values within the desired concentration range. Within-run precision based on duplicates of a sample and one in-house reference material was <10% relative standard deviation (RSD). Between-run method precision measured using the two in-house reference materials was <9% RSD. The RSD for a NIST standard (1 mg/L Hg) was 2.95% and for DORM-2 (3.54 μg/g) was 8.4%. Between-run instrument precision measured using an undigested standard of 0.30 mg/L was at 2.63% RSD. Recoveries monitored by spiking both a sample and reference material were on-average about 98.5%. This method participates in two international round robin comparison studies namely Quality Assurance of Information for Marine Environmental Monitoring In Europe (QUASIMEME) and Northern Contaminants Program, and has performed well.

1.8. Statistical analysis

Statistical tests were performed at each step of the study to compare mercury concentrations in accordance with the goals of the study. The statistical tools utilized included unpaired and 2 one-sided t-tests, as well as one-way analysis of variance. While the unpaired t-test was a valuable tool for comparing means, it could only determine difference in means, with no output on their equivalency. The 2 one-sided t-tests (Schuirmann, 1987; Phillips, 1990) allow for equivalence testing between two means which was more suitable in the context of this study.

The first step compared mercury concentrations in homogenized samples between left and right fillets. The second step compared mercury concentrations between subsamples of homogenized left fillet and biopsy plugs from the right fillet, as well as averages from the homogenized samples and concentrations from biopsy plugs in the defined optimal sampling regions. The third step involved a comparison between mercury concentrations in ‘optimal site’ biopsy plugs on the right fillet, and concentrations from corresponding biopsy plugs on the left fillet. The final step compared mercury concentrations in biopsy plugs extracted from Rainbow Trout fillets using single-use sterile biopsy punches and plugs extracted from Rainbow Trout fillets using a re-used punch that sampled a Rainbow Trout and a Walleye fillet in tandem. Unpaired t-tests were performed under a significance level of \( p < 0.05 \). One-sided t-tests were performed with an equivalence region of 5% of the homogenized average (approximately 0.01–0.02 μg/g). Two one-sided t-tests (TOST) were also performed to develop an understanding of the equivalence of compared data at each step of the procedure (i.e., left/right side homogenized fillets, biopsy plug vs. fillet average, etc). Comparisons were performed with an equivalence region of 5% between the selected data. Statistical analysis was performed in Microsoft™ Excel 2010.

2. Results

2.1. Left and right fillet comparison

Overall, mercury concentrations in left and right fillets of the four species were not statistically different (differed by only 0–0.02 μg/g or up to 6%; Fig. 2 and Appendix A Fig. S2). Walleye had the smallest concentration difference between the fillets (0–0.01 μg/g or 1%–2%). Smallmouth Bass and Lake Trout left and right fillets had slight differences in mercury concentrations (0–0.01 μg/g or 2%–5%, and 0.01 μg/g or 0%–4%, respectively). Northern Pike left and right fillets had the largest, but still very modest difference (0.01–0.02 μg/g or 2%–6%). These results confirmed that the use of fillet from one side of a fish for a homogenized fillet measurement and from the other side of the fish for biopsy sampling to compare with the homogenized fillet measurement is appropriate.

2.2. Homogenized fillet and biopsy plug comparison

The mercury concentrations in the homogenized samples were generally within 3%–5% of the corresponding fillet averages for all four species (Fig. 3a and Appendix A Fig. S4a). These differences are similar to the analytical variability reported for such a measurement (Appendix A Fig. S3a) (OMOE, 2006). The mercury concentrations in the biopsy plugs taken from the right fillet were compared to the average mercury concentration from the 15 subsamples of the corresponding homogenized left fillet. Mercury concentrations in the biopsy plug samples from individual fish showed a similar pattern as observed in the homogenized fillet samples (Appendix A Fig. S3c). Although differences in mercury concentrations between biopsy plug samples and the corresponding fillet averages were typically within 10%, the differences were greater than 20% in some cases and most of the biopsy plug measurements were lower (Fig. 3b and Appendix A Fig. S4b). Walleye and Northern Pike samples showed the least deviation with the majority of the concentrations within 7% of the averages, and maximum differences of 18% and 25%, respectively. Smallmouth Bass and Lake Trout showed slightly more deviation with the majority of the concentrations within 11% of the averages, but with maximum differences of 25% and 23%, respectively (Fig. 3b and Appendix A Fig. S4b). Differences were lower in all species when only biopsy plug mercury concentrations from samples taken in ‘optimal sites’ along the anterior dorsal were compared to the corresponding fillet data. Almost all samples from these sites were within 5% of the corresponding fillet averages, with maximum difference of 13% (Appendix A Fig. S4c–d). Overall, the differences in mercury concentrations between the right fillet biopsy plugs from the optimal sampling area and the left fillet homogenized subsamples were not statistically significant.

Biopsy plug samples provided spatial patterns of mercury within fillets, indicating ideal representative sampling sites located along the anterior dorsal musculature of the fillets of all four species (Fig. 4). Differences between biopsy plug concentrations and homogenized averages were consistently lower in this area than in any other location along the fillet
For Smallmouth Bass, almost all biopsy plug samples from the anterior dorsal tissue were in very good agreement (within 5%) with the corresponding fillet averages. This was also the case for the Walleye, Northern Pike and Lake Trout biopsy plugs from the anterior dorsal area (5%), but these species also displayed concurrence to the corresponding whole fillet averages in biopsy plugs from the posterior dorsal and anterior ventral areas (10%).

2.3. Left and right biopsy plug comparison

Mercury concentrations in anterior dorsal muscle biopsy plugs from individual fish fillets were directly compared to the biopsy plug group averages, as well as corresponding biopsy plug concentrations from the opposite fillet. Mercury concentrations in left and right biopsy plugs from all four species were generally within <5% of the corresponding biopsy plug group averages, with maximum differences of 11% (Fig. 5 and Appendix A Fig. S5a). Left plugs typically had differences within 5% of the averages with a maximum of 8%, while right plugs had similar variation of 5% with a marginally higher maximum of 11%. Walleye and Lake Trout biopsy plugs showed the least variation with mercury concentrations within 5% of the averages, and mean standard deviations of <3%. Smallmouth Bass and Northern Pike biopsy plugs showed slightly more variation with concentrations within 8% and 11% of the averages, and mean standard deviations of 3%-4%. In terms of punch location, samples taken from the front and middle of the anterior dorsal in both side fillets displayed little variability (6%) from the fillet averages. Some rear biopsy plugs displayed slightly higher but still reasonable variability (11%), but most were lower than this. When comparing corresponding biopsy plug concentrations between the left and right fillets, all biopsy plugs displayed correlation with the corresponding opposite fillet biopsy plug (Appendix A Fig. S5b–d). Middle and rear biopsy plug differences between the fillets (12%-13%) showed slightly

**Fig. 3** – Boxplots of percent differences in mercury concentrations (μg/g ww) in (a) 15 subsamples of homogenized left fillets from each of five samples of the four fish species compared to the corresponding group homogenized averages, and (b) groups of 40 and 76 tissue biopsy plugs taken from five right fillets of the four species compared to the corresponding homogenized left fillet averages. The box indicates the first and third quartile values, the line within the box indicates median, the whiskers indicate the highest and lowest values not classified as statistical outlier values more than 1.5 times away from the interquartile range.

**Fig. 4** – Average percent differences in tissue biopsy plug mercury concentrations compared to the corresponding average homogenized fillet concentrations in five samples of each of the four fish species. The black filled circle indicates a difference within 5%, the gray filled circle indicates a difference greater than 5%, the black open circle indicates an optimal tissue biopsy plug sampling area, and a dotted circle indicates a larger acceptable sampling area.
less deviation than front biopsy plugs (15%) but were still similar.

2.4. Re-use of biopsy punch

Both homogenized fillet and biopsy plug sampling using new sterile punches on the other fillet of three laboratory raised Rainbow Trout had low and not statistically different mercury concentrations (0-0.01 μg/g difference between the two fillets; Appendix A Fig. S6). Re-use of a biopsy punch after conducting biopsy sampling on field collected, more mercury contaminated Walleye also resulted in low and not statistically different mercury concentrations for fillets of laboratory raised Rainbow Trout (Appendix A Fig. S6). The maximum impact of the re-used biopsy punch was an increase of 0.01 μg/g of mercury, which is much lower than the variability described above (Appendix A Fig. S6).

3. Discussion

Tissue mercury concentrations from fillet biopsy plugs were almost always within 5%-10% of the corresponding homogenized fillet average concentrations (Fig. 4 and Appendix A Fig. S4b). These findings are in agreement with previous reports (Baker et al., 2004; Peterson et al., 2005; Bank et al., 2007; Schmitt and Brumbaugh, 2007; Ackerson et al., 2014). The differences between the biopsy plugs and corresponding homogenized fillets were insignificant both statistically (p > 0.05) and practically. Biopsy plug samples resulting in greater mismatch compared to average fillet mercury concentrations can be avoided by taking biopsy plugs from anterior dorsal muscle as discussed below.

3.1. Spatial distribution of fillet mercury

The biopsy plug measurements showed a relatively more even distribution of mercury in the fillets of Walleye, Lake Trout, and Northern Pike, while the fillets of Smallmouth Bass showed the anterior dorsal area to be a better representative of the average fillet mercury concentrations. The findings highlight some species-specific dependence in fillet mercury distribution. We did not explore differences in mercury concentration between interior and exterior fillet tissues. Minimal differences have been reported for Northern Pike, Walleye, and Lake Trout species, while some bottom feeding species such as Channel Catfish and Carp may have higher (10%-50%) concentrations in interior muscle tissue (1975).

Bishop and Neary (1975) previously concluded that biopsy plug sampling was not a viable alternative to the homogenized fillet method due to greater (double) variance in mercury concentrations for biopsy plugs taken from various sections of a fillet. Mercury can display variability in its distribution within a fillet largely due to the variation in proportion of muscle tissue relative to connective tissue (mycommata), with high proportions of muscle tissue containing more protein generally exhibiting greater mercury concentrations (Bishop and Neary, 1975; Health Canada, 2008). Since Bishop and Neary (1975) studied tissue biopsy plugs from all parts of a fillet with varying muscle tissue to connective tissue proportions, a high variability in mercury concentrations was likely. As the anterior dorsal area contains the greatest fillet mass as well as muscle tissue relative to mycommata, biopsy plug sampling from this area, rather than sampling along the whole length of the fillet, results in statistically not different mercury concentrations compared

![Fig. 5 - Percent differences in mercury concentrations (μg/g ww) in three tissue biopsy plug samples taken from the front, middle, and rear sections of the anterior dorsal region of five left (◊) and right (×) fillets of the four fish species compared to the corresponding biopsy plug group averages for each side.](image-url)
to the fillet average. Biopsy plugs sampled from areas of low muscle tissue mass and relatively more connective tissues, such as the anterior ventral and tail region, resulted in higher deviations (25%) from the average fillet concentrations and should be avoided when sampling.

Other studies (Bishop and Neary, 1975; Cizdziel et al., 2003) have also described the anterior dorsal area as the best representation of fillet mercury concentration. The consistency of mercury concentrations in the anterior dorsal biopsy plugs within and between fillets found in this study suggests that any part of the anterior dorsal muscle would result in a reliable representation of the fillet average and serve well as a location for biopsy sampling.

### 3.2. Biopsy method implementation

Although the biopsy procedure for nonlethal mercury sampling has been described since the early 1970s, the method has yet to be widely adopted in routine monitoring. In field and laboratory settings, implementation of a nonlethal biopsy punch method can provide multiple benefits, including lower sample processing time, labor and equipment costs, freezer space requirements, and fish mortality. Biopsy sampling presents a portable, inexpensive alternative to large and in many cases costly homogenizing machines. Further cost saving is feasible through re-use of punches as described in this study.

Large biopsy tools such as the 6 and 8 mm punches used in this study served as adequate tools for extracting sufficient tissue mass (0.2–0.8 g wet weight), but may be too invasive in a nonlethal application. Baker et al. (2004) and Tyus et al. (1999) collected biopsy plugs using 4 mm punches and reported no reduction in survival post sampling. Ackerson et al. (2014) used 5 mm biopsy punches to collect biopsy plugs from 31 living Smallmouth Bass and reported high (mean 97%) survival rate. Similarly, other studies have reported high survival rates of fish post biopsy sampling (Utke, 1971; Moy and Dredge, 1979; Leitner and Isely, 1994). These studies have established that a nonlethal biopsy method could promote conservation by limiting fish mortality for mercury monitoring.

From our experience with the method, a 5–6 mm punch is an optimal size as it will likely provide adequate tissue for repeat analysis if required and also serve as nonlethal sampling. If adequate tissue cannot be collected with a single biopsy plug using a 5–6 mm punch (Peterson et al., 2005), we suggest sampling corresponding locations on the left and right fillets of the fish rather than sampling two adjacent sites on one fillet as suggested by Baker et al. (2004). The left and right fillets are sufficiently similar in their mercury distribution within the optimal sampling area that one biopsy plug sample from each side can collectively provide a representative mercury concentration and potentially increase the chances of fish survival compared to two side-by-side biopsy plugs from one side.

An alternative nonlethal method to biopsy punch sampling would be the use of a biopsy needle. Although both biopsy punch and needle are expected to result in similar mercury measurements, punch sampling could be considered a preferred method due to three major reasons: (1) ease of sample collection, (2) greater precision due to greater tissue mass collected, and (3) ease of cleaning and reuse (Baker et al., 2004; Schmitt and Brumbaugh, 2007).

As this study only explored a fish tissue biopsy method for mercury monitoring, we cannot contend that these findings extend to other chemical or biological measurements. The biopsy method has been used for monitoring selenium, stable isotope, fish coloration as well as genetic analyses (Leitner and Isely, 1994; Waddell and May, 1995; Osmundson et al., 2000; Lepak et al., 2006). Many persistent organic pollutants (POPs) are lipophilic and have tendency for greater accumulation in fatty tissues of a fish (Gewurtz et al., 2011). Since distribution of lipid in a fish fillet is typically more variable than myocommata especially between anterior and posterior areas (Bishop and Neary, 1975; Palmeri et al., 2007), concentrations of POPs in a fish fillet may be more variable than mercury and biopsy punch method may be less appropriate for the monitoring of lipophilic compounds.

Fish fillets and tissue biopsy plugs considered in this study were skinless. It is possible that tissue biopsy plugs collected in field include skin, even though scraping of a few scales before collecting a biopsy plug is recommended and skin can be easily cut and removed once a biopsy plug is collected (Peterson et al., 2005; Schmitt and Brumbaugh, 2007). However, inclusion of skin in a biopsy plug sample should not have any major impact on adopting findings of this study because either similar or marginally lower mercury concentrations can be expected if skin is kept intact (Zhang et al., 2013).

In summary, for more than 40 years studies have indicated that a biopsy plug mercury measurement is a feasible and viable alternative to lethal, homogenized fillet mercury monitoring; however, the practice has yet to be widely implemented in routine monitoring due to concerns about variability in mercury concentrations in fish muscle tissue. We hope that this study will increase the adoption of nonlethal biopsy sampling for the analyses of mercury in fish tissue, and that this may be a template for other studies that may similarly examine other contaminants of concern.

### Conflict of interest

The authors declare no conflicts of interest.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.12.004.

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