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Recommended Citation
Drouillard, Ken G.; Gandhi, Nilima; Bhavsar, Satyendra P.; Gewurtz, Sarah B.; Arhonditsis, George B.; and Petro, Steve. (2016). Is it appropriate to composite fish samples for mercury trend monitoring and consumption advisories?. Environmental Science and Technology.
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Is it appropriate to composite fish samples for mercury trend monitoring and consumption advisories?

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Abstract

Monitoring mercury levels in fish can be costly because variation by space, time, and fish type/size needs to be captured. Here, we explored if compositing fish samples to decrease analytical costs would reduce the effectiveness of the monitoring objectives. Six compositing methods were evaluated by applying them to an existing extensive dataset and examining their performance in reproducing the fish consumption advisories and temporal trends. The methods resulted in varying amount (average 34-72%) of reductions in samples, but all (except one) reproduced advisories very well (96-97% of the advisories did not change or were one category more restrictive compared to analysis of individual samples). Similarly, the methods performed reasonably well in recreating temporal trends, especially when longer-term and frequent measurements were considered. The results indicate that compositing samples within 5 cm fish size bins or retaining the largest/smallest individuals and compositing in-between samples in batches of 5 with decreasing fish size would be the best approaches. Based on the literature, the findings from this study are applicable to fillet, muscle plug and whole fish mercury monitoring studies. The compositing methods may also be suitable for monitoring Persistent Organic Pollutants (POPs) in fish. Overall, compositing fish samples for mercury monitoring could result in a substantial savings (approximately 60% of the analytical cost) and should be considered in fish mercury monitoring, especially in long-term programs or when study cost is a concern.

Keywords: Mercury Hg; compositing/pooling; fish; monitoring; advisories; sensitive population
Graphical Abstract

Compositing fish, ok?

Highlights

- We test if compositing fish to decrease costs biases Hg monitoring
- Six compositing methods were assessed using an extensive dataset from Ontario
- Five methods reproduced advisories very well (96-97% same or more stringent)
- Methods performed well in recreating temporal trends, especially for longer-terms
- Methods resulted in average 34-72% reductions in samples and should be considered
1. Introduction

Mercury is a contaminant of global concern (UNEP, 2013a). Virtually every fish in North America, and possibly worldwide, contains mercury (Stahl et al., 2009; Depew et al., 2013; Evers et al., 2013). Consumption of fish is generally a dominant route of human exposure to mercury (UNEP/WHO, 2008). Mercury is responsible for the most number of restrictive fish consumption advisories, at least in North America (e.g., USEPA, 2013a, b; OMOECC, 2015).

Due to spatial variation in fish mercury levels, location-specific advisories are typically provided (e.g., USEPA, 2013a; OMOECC, 2015). Since mercury levels vary by fish species and size (Gewurtz et al., 2011b), monitoring efforts to issue fish consumption advisories and track long-term changes require collection and analysis of a variety of fish spanning their natural size range (USEPA, 2013b). As a result, the total number of annual samples required to adequately monitor fish mercury levels for numerous locations can range from hundreds to tens of thousands.

Due to analytical costs, most contaminant studies limit sample size by reducing the fish species monitored, replication of samples, sampling frequency and/or study period; however, these options are generally not suitable for agencies that rely on the data for long-term trend monitoring and issuing of fish consumption advisories aimed at protecting human health (Gewurtz et al., 2011a). Further, Article 19 of the recently formulated Minamata Convention on Mercury requires parties to develop and improve geographically representative mercury monitoring in environmental media, including fish (UNEP, 2013b). In less than a decade, monitoring data will be called upon to assist in the implementation and evaluation of the convention, which emphasizes the importance of improving monitoring efforts to optimize both the quality of the programs as well as costs.
To decrease program costs, combining multiple temporally or spatially discrete samples, widely known as composites, has been suggested as an effective alternative to chemical analysis on individual samples (USEPA, 2002; Gewurtz et al., 2011a). In addition to substantially reducing analytical cost, the data collected through compositing samples can provide wider temporal and spatial coverage without increasing the sample count. The analysis of data may give more representative estimates of mean concentrations than can the same number of discrete sampling, albeit at the cost of variability in the observations (USEPA, 2002).

There are several potential approaches to compositing fish contaminant monitoring samples that incorporate different dimensions of the study, such as time (within/across years), location, fish species, and fish size. The optimal compositing approach would be one that reduces the total number of samples for analysis without compromising the objectives of the monitoring program. In addition, the composite method chosen should follow assumptions that correspond to the statistical analysis that is ultimately applied to the data. Several studies have used compositing as a part of their designs for both organic and inorganic contaminants in all media including biota (Rajagopal and Williams, 1989; Turle and Collins, 1992; Blomqvist, 2001; Braune and Noble, 2009; Gewurtz et al., 2011a). However, to our knowledge, a comprehensive study investigating the effectiveness of various compositing approaches for monitoring mercury in fish is lacking in the literature, especially for programs designed to generate fish consumption advice, where variability and the presence of outliers can affect overall risk (Gewurtz et al., 2011a).

In this study, we evaluate six methods of compositing fish samples by examining their performance if they would have been utilized instead of collecting >220,000 individual mercury measurements for >3000 locations by the Province of Ontario, Canada over nearly 50 years. The
Effectiveness of the composite methods was evaluated by comparing the fish consumption advisories and temporal trends from individual measurements (current sampling design) with estimated composite values, calculated by averaging the individual measurements included in each composite. The findings of the study determine whether a compositing method can effectively minimize costs for regular, long term, large scale monitoring programs and set advisories for fish consumption.

2. Methods

2.1 Compositing Methods

Fish mercury levels vary by species and size, and can change seasonally as well as over time under the influence of a variety of internal and external factors, such as bioenergetics and ambient water chemistry (Bhavsar et al., 2010; Azim et al., 2011; Gewurtz et al., 2011a; Stern et al., 2012; Greenfield et al., 2013). As such, we opted to group species-specific samples collected during the same sampling event within the composites.

There is a well-known relationship between mercury concentrations and fish size that is typically described by the power-series regression (Gewurtz et al., 2011b). As such, similar sized samples could be considered for creating a composite sample. However, the resultant fish size range (i.e., maximum-minimum fish lengths) would likely be less than the regular, individual measurements. This could result in trimming of a regression at the extreme ends, and thereby loss of advisories for certain fish sizes. Alternatively, if one or two of the largest and/or smallest individuals are retained with all other samples being composited, then the fish size
range could be captured, and a power series regression between fish length and compositing mercury concentrations might be improved.

Compositing of 3, 5, 7, 10 or more samples have been used in many studies (Hites et al., 2004; Carlson and Swackhamer, 2006; French et al., 2011; Pantazopoulos et al., 2013). Since a collection of about 20 fish samples per species and sampling event over a possible maximum size range is generally considered a preferred method for mercury monitoring (e.g., Gewurtz et al., 2011a), compositing more than 5 samples (i.e., having less than four composites), may not be sufficient for characterizing the fish size/mercury relationships. Alternatively, compositing samples within a narrow size range (e.g., 35-40 cm, 40-45 cm and so on) regardless of the number of samples within that size range may be appropriate as the impact on the fish size/mercury relationship would likely be minimal.

Based on the above notes, we considered six compositing methods: (1) composite samples in batches of five in the order of decreasing fish size (Figure 1a,b), (2) retain individual samples for the largest and smallest fish and composite samples in between in batches of five in order of decreasing fish size (Figure 1a,c), (3) retain the two largest and smallest individual samples and composite the samples in between in batches of five in order of decreasing fish size (Figure 1a,d), (4) retain the largest and smallest individual samples and composite the samples in between in batches of three in order of decreasing fish size (Figure 1a,e), (5) retain the two largest and smallest individual samples and composite the samples in between in batches of three in the order of decreasing fish size (Figure 1a,f), and (6) composite samples within a 5 cm size range (Figure 1a,g).

2.2 Data Source
The above described compositing methods were evaluated by simulating composite data from the individual fish measurements, assuming that the same mass of each fish is added to the composite. For this purpose, we used an extensive and consistent fish mercury dataset comprising 223,318 individual, widely varying measurements for skinless, boneless dorsal fillets of >10 cm fish of 66 fish species (Table S1) collected by the Ontario Ministry of the Environment and Climate Change (OMOECC), Canada in partnership with the Ontario Ministry of Natural Resources and Forestry and other agencies over nearly 50 years (1967-2014) from >3000 locations in the Province of Ontario, Canada, that spans 41º to 56º N and 74º to 95º W (Figure S1). The samples were analysed for total mercury using acid digestion and cold vapor flameless atomic absorption spectroscopy as described in detail by Bhavsar et al. (2010). The dataset contained 16,900 species/location/year combinations for 6,440 sampling events (location/year) and varied widely (1 to 274) in the number of individual samples for a species in a sampling event (species/location/year) (Figure S2).

2.3 Statistical analysis

The performance of each composite method in comparison to the regular, individual measurements was evaluated based on its accuracy in reproducing the fish consumption advisories as well as the direction and magnitude of the long-term temporal trends. As illustrated in Figure S3, a power series regression was conducted for each of 16,900 species/location/year-specific sampling events using the regular, individual measurements as well as the composite values calculated using the six methods considered in this study. Using these total 118,300 power series regressions (i.e., 16,900 x 7), fish mercury levels were calculated at 5 cm intervals for the available size range in each species-specific sampling event (Figure S3). These mercury concentrations were used in calculating fish consumption advisories using the benchmarks for
the general population and sensitive population (children and women of child-bearing age),
which is the standard method used by the Province of Ontario, Canada (Table S2, Figure S3).
Advisories for each 5 cm interval calculated using the six composite methods were compared
with those from the regular, individual measurements (Table S4), and classified into three
categories: 1) same, 2) more restrictive, and 3) less restrictive.

For a comparison of temporal trend analyses from the regular and composite methods,
rates of changes in fish mercury levels (µg/g decade) were calculated using the slope of the
linear relationship between year and mercury concentration standardised to a fish length. Since
the purpose is to compare rates from the regular and composite methods, appropriateness of a
linear regression is essentially a moot point (Azim et al., 2011). Since a temporal trend analysis
is typically conducted on a suitable indicator species with good monitoring data, four species,
namely Lake Trout (*Salvelinus namaycush*), Walleye (*Sander vitreus*), Northern Pike (*Esox
Lucius*) and Smallmouth Bass (*Micropterus dolomieu*), were considered. Mercury
concentrations standardized to 50 cm fish size were used. The standardization was conducted
using a power series regression \( y = a x^b \), where \( y \) is concentration in µg/g, \( x \) is fish length in cm,
and \( a \) and \( b \) are regression coefficients. The number of temporal trend rate estimates was
maximized by considering every combination of the start and end years as illustrated in Figure
S4. In total, 83,664 rates of fish mercury changes were calculated. All statistical analyses were
conducted in either Excel 2010 or R-3.2.0 for Windows™ (R Core Development Team, 2015).

3. **Results**

3.1 **Reductions in samples**
The composite method 1 resulted in the highest (average/median 72/78%) reduction in number of samples to be analysed for mercury (Figure 2). The composite methods 2 and 3 required retention of one and two extreme sized individual samples, respectively. As such, the reductions in number of samples were less (method 2: 54/64%; method 3: 40/50%; Figure 2). The methods 4 and 5 required compositing samples in the batches of 3, compared to 5 for the methods 2 and 3. As a result, reductions in the number of samples by implementing the methods 4 and 5 were less (method 4: 45/53%; method 5: 34/42%; Figure 2). Although the composite method 6 resulted in more variable (0-98%) reductions in the samples because of its dependence on number of samples in 5 cm fish size bins, overall reductions were similar to the method 2 (55/60%; Figures 2, S5).

3.2 Performance in reproducing advisories

Seven sets of fish consumption advisories (regular plus six composite methods) were calculated for each sampling event (species/location/year) as illustrated in Figure S3, and compared as shown in Table S3. The resultant fish size ranges (minimum to maximum length) for the composite method 1 were lower than from the regular, individual measurements for many sampling events. In addition, method 1 produced one composite for each of 3,681 sampling events with ≤5 samples (Figure S2), resulting in no power series regression for an advisory calculation. Therefore, about 35% of the advisories from method 1 were missing (Figure 3, Table S3).

The advisories were calculated using power series regressions on fish size vs mercury concentrations for each sampling event (location/year/species). The statistical significance of the regressions was evaluated on the basis of their p-values. Since the composites were aimed at
reducing the sample size, which is generally positively related to a p-value of a regression, it was not surprising to observe lower statistical significance for regressions from a composite method that produced a greater reduction in sample sizes (Figures 2, S6).

Overall, advisories for the general population from the methods 2 to 6 were largely (85-91%) similar to those from the regular, individual measurements (Figure 3, Table S4a). About 6-11% of the advisories were more restrictive, mostly by only one advisory category (Figure 3, Table S4a). Only 3-4% of the advisories were less restrictive, again mostly by only one advisory category (Figure 3, Table S4a). The results for the sensitive population advisories were even better (similar: 88-93%; more restrictive 5-9%; less restrictive 2-3%; Figure 3, Table S4b).

The increasingly fewer reductions in the number of samples from the composite methods 2 to 5 only marginally improved reproduction of the advisories (Figure 3). The performance of the method 6 was similar to the method 4 and overall second best among the methods (Figure 3, Table S4). Based on the reductions in the number of samples and performances in reproducing the advisories, we focus further analysis and the following discussions on results for the general population using the methods 2 and 6.

Next we examined if there was a pattern in the underestimation of mercury concentrations and thereby less restrictive advisories from the composite methods that could be linked to sample size, species, fish size class, and/or level of mercury. As shown in Tables S5-S8, individually these four factors had minimal impact on the performance of the composite methods 2 and 6. The only exception was that increasing fish size worsened the performance of method 2, with relatively more cases of less restrictive advisories for large size categories within individual species (Table S9). Nevertheless, there were only 3-4 combinations of species/size
for which the total number of advisories were >100 and >10% of the advisories were less restrictive (Table S9). Similarly, there was no fish species-specific mercury concentration that substantially affected the performance of the composite methods 2 and 6 (Table S10).

### 3.3 Performance in reproducing temporal trends

In this assessment, we examined if the nature of the mercury versus time slopes from the composite methods corresponded with the regular method. The composite methods resulted in the same temporal trends as observed for the individual samples in most (90-94%) cases (Figure S7). The performances of the composite methods improved from 90-94% to 94-96% when cases with a minimum time span of 15 years and 5 sampling years were considered, and to 95-97% when cases with a minimum time span of 15 years and 10 sampling years were considered (Figure S7).

For a majority (72-82%) of the cases, the rates of changes in fish mercury levels from the composite methods were within a factor of two of the corresponding rates from the regular method (Figure S8). Approximately 81-88% of the rates were within a factor of three (Figure S8). When cases with a minimum time span of 15 years and 5 sampling years were considered, the percentages of cases improved to 81-88% for within a factor of two and 88-92% for within a factor of three (Figure S8). The corresponding results for cases with a minimum time span of 15 years and 10 sampling years were better at 83-90% and 89-93%, respectively (Figure S8).

The performance of the composite methods in reproducing the rates of changes was also evaluated for each of the four selected fish species. All composite methods provided the same temporal trends for a majority (83-95%) of the cases for all species (Figure S9). When cases with a minimum time span of 15 years and 10 sampling years were considered, the percentages
of cases improved to 97-100% for Lake Trout, Northern Pike and Walleye, and 86-90% for Smallmouth Bass (Figure S9). Likewise, performances of all methods in reproducing the rates within a factor of two were comparatively similar for all species (Figure 4). When a more robust dataset (cases with a minimum time span of 15 years and 10 sampling years) was considered, all methods resulted in rates that were within a factor of three in 97-100% of the cases for Lake Trout, Northern Pike and Walleye (Figure 4). The performance of the composite samples in reproducing the rates of change for Smallmouth Bass was less (86-90%) compared to the other three species (Figure 4), indicating that Smallmouth Bass is the least preferred species for trend monitoring when a composite method is utilised.

As expected, the composite methods that resulted in fewer reductions in the number of fish mercury measurements provided better estimates of the rates of changes in the fish mercury levels (Figures 2 and 4). Although reductions (55/60%) in number of measurements from method 6 were comparable to method 2 (54/64%), method 6 provided better estimates of the rates of change (Figure 2 and 4). Furthermore, the performance of method 6 was comparable to the method 3, which consisted of relatively more mercury measurements (Figures 2 and 4). The differences in the performance of the methods in reproducing the rates were minimal when cases with a minimum time span of 15 years and 10 sampling years were considered (Figure 4).

4. Discussion

Composite sampling combines environmental samples or subsamples to form a new sample on which chemical or biological analyses are performed. Compared to evaluating individuals, composite sampling is beneficial as it decreases analytical cost by analyzing fewer
samples and reduces/simplifies the sample handling process (USEPA, 2002). Composite sampling is recommended when laboratory costs are substantially greater than field sampling costs (USEPA, 2002). The collection of a few more fish samples at a particular location may not substantially increase the field cost. However, the analytical savings associated with composite sampling in long-term fish mercury monitoring and for issuance of fish consumption advisories can be substantial, especially over time. For example, the approximately 60% reductions in sample analyses in the OMOECC dataset used in this study would have resulted in approximately 134,000 fewer fish mercury analyses over the 47 year period, which sums to about $5,400,000 (or $114,000 per year) at an average rate of $40 per sample. Similarly, about $1,000,000 could be saved for the dataset compiled by USGS from data collected by US states (Hearn et al., 2006). Further, the composite sampling would have resulted in substantial saving in other operational costs due to reduced number of samples to handle. Although the extent of cost saving would depend on nature of the program (e.g., how many individual samples of which fish species and sizes are presently analysed for mercury) and analytical cost, which has been declining with advances in the analytical technology, the results presented in this study show that savings can be achieved without any major impact on the quality of the advisories or temporal trend assessments.

There are, however, some potential disadvantages of the composite sampling approach. For example, composite sampling can result in a loss of information on extreme contamination levels and variability. Although this is true in many cases, a composite method retaining one or two largest and smallest individual samples as suggested in this study can potentially capture extreme fish mercury levels due to the strong relationship of fish size and mercury concentration. Although method 6 considered in this study may not preserve individual samples, a power series
relationship between fish length and mercury indicates that compositing within a 5 cm fish size bin would likely be able to provide values closer to the extreme levels. This could be a result of the pattern in fish mercury levels, where even though there is a strong relationship between fish length and mercury levels, it is not necessary that the biggest fish has the highest concentration and the smallest fish has the lowest concentration likely due to differences in mercury levels in spatially integrated fish samples. Compositing reduces sample size, and as such decreases statistical power; however, statistical formulas can be used to derive composite size that results in a sufficient power (Rohlf et al., 1996). The composite methods examined in this study also resulted in some loss of statistical significance (Figure S6). Nevertheless, the methods performed reasonably well in reproducing the advisories and temporal trends (Figures 3, 4, S7).

If contaminants other than mercury are also of interest, further evaluation of the compositing methods may be necessary. For North America, other major contaminants of concern include persistent organic pollutants (POPs) for which compositing is often performed (Hites et al., 2004; Gewurtz et al., 2011a) for studies focused on the health of fish themselves and not on the generation of fish consumption advice. Gewurtz et al. (2011a) found compositing fish samples appropriate for temporal trend monitoring of polychlorinated biphenyls (PCBs) based on a limited evaluation of Lake Ontario lake trout measurements from different Canadian and U.S. monitoring programs. However, their evaluation did not consider the impact of compositing on the ability to detect outliers. It should be noted that the relationship between fish length and POPs, such as PCBs, is much weaker than is typically observed for mercury (e.g., Gewurtz et al., 2011b). As such, compositing fish samples based on size categories (e.g., method 6 in this study) may be less effective in capturing outliers for POPs. However, many agencies use the "75% rule" (i.e., the length of the smallest fish in a composite should be at least 75% of the
length of the largest fish) for compositing fish samples for POP monitoring (e.g., Stahl et al., 2009). The method considered in this study will composite samples within a 5 cm size range (Figure 1a,g) and follow the 75% rule (except for fish smaller than 15 cm, which are generally not considered sport fish anyway). Similarly, the method 2 (and probably the other methods considered) will also create composites (Figure 1a,c) that has a high potential to follow the 75% rule (Tables S11-S12), depending on the extent of sample collection by a program. As such, the compositing methods and findings of this study may also be suitable for monitoring POPs in fish.

A reliable temporal trend analysis depends on within-year samples and duration of monitoring (Sokal and Rohlf, 1995). Based on an exploratory analysis performed on data collected by some Great Lakes biomonitoring programs and a comparison with the literature, it was concluded that >10 years of monitoring with 10-15 samples per year is optimal to achieve 80% statistical power, which is typically considered adequate (Gewurtz et al., 2011a). This is largely due to diminished sensitivity of a temporal trend analysis to start and end points when a reasonable length of monitoring data is available (Gewurtz et al., 2011a). In this study, the correspondence between the results from the regular and composite methods improved when a longer time span and increased number of sampling years were considered (Figures 4, S7-S9). As such, compositing samples may not be advisable for a short term assessment; however, the accuracy of the regular method based on individual samples may also be poor.

In this study, we utilized skinless, boneless fillet mercury measurements to evaluate the compositing methods. However, some monitoring programs use muscle plug or whole fish measurements to track environmental conditions. Since fish fillet, muscle plug and whole fish mercury measurements can be linked to one another (Baker et al., 2004; Peterson et al., 2005), findings from this study should be applicable to muscle plug and whole fish mercury monitoring.
studies as well. The Ontario’s fish contaminant monitoring is conducted exclusively in temperate environments and thus the results from this study have broad applicability to other monitoring programs in temperate latitudes. Although the in-depth analyses conducted on an extensive dataset indicate that the findings should be applicable to tropical environment as well, further work to verify these results in tropical environment may be warranted.

In summary, we explored the suitability of six composite methods for fish mercury monitoring using an extensive dataset. The methods resulted in varying amount of reductions in number of samples to be analyzed. In general, all compositing methods performed well for both advisories on consumption of fish and temporal trend monitoring. The methods resulting in lower reductions in sample count performed marginally better. Overall, compositing samples would have resulted in a substantial cost savings for OMOECC (approximately $5.4 M over 47 years assuming 60% sample reduction), and should be considered in fish mercury monitoring especially in long-term extensive monitoring programs or when study cost is a concern.

Acknowledgements

We thank Ontario Ministry of the Environment and Climate Change, Canada, for the long-term fish mercury dataset.

Supplementary Material

Additional 12 tables and 9 figures. This material is available free of charge via the Internet at ??????

Notes

The authors declare no competing financial interest.
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http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance.
Figure 1: Illustration of six compositing methods considered in the study.

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Individual measurements are shown in grey
Same coloured individuals belong to the same composite

For this example sampling event
Figure 2: Overall reduction (%) in number of samples per sampling event (location/year/species) analyzed in each of the six composite methods compared to the regular method of analyzing all individual fish samples for mercury.
Figure 3: Comparison on fish consumption advisories for mercury for the general and sensitive populations using composite methods compared to the current OMOECC method of analyzing individual fish samples.
Figure 4: Comparison of rates of change in fish mercury levels of the six composite methods with those from the current OMOECC method of analyzing individual fish samples for mercury. The results have been presented as percentage of the total number of rate estimates within 2 and 3 times the corresponding rates from the current OMOECC method.