

Variation in Organochlorine Bioaccumulation by a Predatory Fish; Gender, Geography, and Data Analysis Methods

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Σ PCB and *p,p'*-DDE levels within and among walleye (*Stizostedion vitreum*) populations were examined to determine how the method of data analysis could influence the interpretation of (i) gender differences and (ii) geographic variation. In the lower Great Lakes (Huron, Erie, and Ontario) whole-body burdens of both contaminants tended to increase with body mass at a faster rate in males than in females. Thus, males generally had higher burdens than females at large body sizes but not at small body sizes. This result was not strongly influenced by the method of expressing contaminant level (burden, wet mass concentration, or lipid mass concentration) but was influenced by the choice of covariate (body mass, body length, or age) in some cases. Mean Σ PCB and *p,p'*-DDE concentrations of walleye muscle declined along a gradient from the lower Great Lakes to the Northwest Territories. Analyses using means adjusted for age yielded a stronger contrast between Great Lakes and non-Great Lakes populations than analyses using means adjusted for body length. The gender composition of fish samples and the type and level of covariate used in statistical analyses should be considered in studies of spatiotemporal variation in organochlorine bioaccumulation in fish.

Introduction

Organochlorines (OCs) are a large group of persistent organic pollutants of agricultural and industrial origin that are widespread in ecosystems of the northern hemisphere (1, 2). Many OCs are toxic and resistant to biodegradation, biomagnify readily in aquatic food webs (3), and are believed to be poorly metabolized by fish (4). Thus, in some freshwater ecosystems, such as the Laurentian Great Lakes of North America, predatory fish may contain high concentrations of OCs. Most OCs, such as polychlorinated biphenyls (PCBs), were banned for use in North America in the late 1970s; this was followed by declining OC levels in Great Lakes biota from the late 1970s to the early 1990s (5, 6). However, since the early 1990s PCB levels have stopped declining in many

components of the Great Lakes ecosystem (7). Consequently, OCs in the environment are still a human health issue and consumption advisories remain in place for many Great Lakes fishes (6). Further research to define the patterns of variation in OC concentrations within and among fish populations is essential to refining these consumption guidelines and to understanding the distribution, dynamics and fate of these contaminants.

One impediment to our understanding of OC dynamics in wild fish populations is the lack of standardization in methods of analyses. Because contaminant levels can vary widely among individuals, the sample size, gender composition, and age and body size composition of the fish sample are the first considerations. Following collection, investigators must decide if contaminant levels are to be measured on whole bodies or only on certain body parts, such as edible muscle tissue. Laboratory analyses may follow a variety of techniques, and the results may be reported as body burdens (mass of contaminant per fish) or concentrations in either wet tissue or lipid. Finally, data analyses may involve simple comparisons of means or more elaborate procedures in which contaminant levels are adjusted to particular ages and/or body sizes of fish. This wide variety of approaches often makes comparisons between current and previous research quite difficult. However, few studies have explored how the methods of analyses may influence the interpretation of spatiotemporal OC data.

Our primary objective in this study was to explore how methods of data analyses may be influencing our interpretations of OC variation in fish. We chose to examine this variation at two scales: (i) with respect to gender (within-population variability) and (ii) with respect to geography (interpopulation variability). Recent studies of individual fish populations have indicated that bioaccumulation rates may differ substantially between genders (8, 9), but it is unknown if this is a general phenomenon in fish or if this result is influenced by the methods of analyses. Furthermore, because growth (size at age) characteristics can vary substantially among populations, it is possible that interpretations based on body size adjustment may differ greatly from those based on age adjustment. Thus, covariate selection may be influencing the interpretation of results.

Methods

Field Sampling and Fish Processing. Our target species for this research was walleye (*Stizostedion vitreum*), a freshwater percid. Relatively little research has been carried out on spatial variation in OC concentrations of this species despite its high economic value and importance in many commercial and recreational fisheries. The native range of walleye extends from the southern United States to the MacKenzie River delta in northern Canada. Growth characteristics and longevity vary substantially over this range, not simply with respect to temperature but also with respect to waterbody trophic status and changes in population density brought about by exploitation. Individuals may attain ages >25 years in very slow-growing populations but seldom exceed 10 years of age in very fast-growing populations. Walleye are piscivorous and a top predator in the aquatic ecosystems they inhabit. They are also iteroparous, with spawning taking place in early spring each year. Maturity appears to be linked to body size and, thus, age of maturity is related to growth rate. Males generally reach maturity first at smaller body sizes and younger ages than females. Walleye may mature as early as 2 or 3 years of age in fast-growing populations and as late as 10+ years of age in very slow-growing populations. Several

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works provide more detailed biological information on this species (10, 11).

We obtained our walleye contaminant data from several concurrent research projects (12–14) carried out during the 1990s. Lower Great Lakes' walleye populations (Huron, Erie, and Ontario) were sampled over many years, whereas other populations were generally sampled in one or two years during this period. Most sampling was conducted in mid-summer (July–August). Fish were captured using gill nets, trap nets, or angling gear and immediately sacrificed and placed on wet ice. Fish were processed either in the field or in the laboratory; all flesh used in contaminant analyses was frozen until analyses could be performed. For all fish, gender was determined by internal inspection of gonads. Fish for which the gender could not be determined were eliminated from our analyses. For fish collected from Lakes Huron, Erie, and Ontario, length was measured from the tip of the snout to the end of the folded caudal fin (total length, TL, ± 1 mm), and scales were removed from the dorsal, midbody region for age determinations. For fish from all other populations, length was measured from the tip of the snout to the inner notch of the caudal fin (fork length, FL, ± 1 mm), and opercles were removed as the aging structures. Total lengths were converted to fork lengths by the empirical relationship $FL = -13.6 + 0.972(TL)$ (T. A. Johnston, unpublished data), and fork lengths were used in all subsequent analyses. Fresh total (round) body mass (± 1 g) was determined for all sampled fish. Ages of fish were determined by counting annular rings on the aging structures. Opercles were soaked in hot water to remove all flesh, allowed to air-dry, and then viewed under a dissecting microscope at $6\times$ power using reflected light. Acetate impressions of scales were viewed on a microfiche reader at $20\text{--}40\times$ power using transmitted light. Scales were used for age determination only in relatively fast-growing populations where old fish were uncommon. There was good agreement between scale and opercle ages in these populations.

The tissue taken for contaminant analyses differed among walleye populations sampled in the earlier studies. For fish processed in the laboratory [Slave River, Leland (in part), and Lakes Huron, Erie, and Ontario] contaminant analyses were performed on subsamples of the whole-body homogenate. For fish processed in the field, muscle samples (~ 50 g) were taken from alongside the first dorsal fin and above the lateral line. Skin was left attached to the muscle for the fish of most of these populations [Liard River, Leland (in part), Musclow, Orange, Linge, Trout, Sidney, and Lake Superior] but was removed for the fish of one population (Manitoba). For several fish collected from Lake Ontario in 2000 we homogenized both muscle samples and the whole body in order to compare contaminant concentrations and develop tissue conversion factors.

Organochlorine Analyses. Methods of OC analyses differed slightly among the various study lakes. However, all samples from a given population were analyzed in the same manner. We restricted our analyses to the sum of PCBs (Σ PCB) and *p,p'*-DDE because they were measured in all study populations and because they are generally found at higher concentrations than other OCs in fish. Whole fish and muscle samples were homogenized either fresh in food grade equipment (steel meat grinder or glass and steel food processor) or cryogenically by grinding with dry ice. All homogenizing equipment was washed and hexane- and acetone-rinsed between samples. Preweighed subsamples of tissue were homogenized further by grinding with anhydrous Na_2SO_4 and added recovery standards. Extraction was performed using either dichloromethane (DCM) in a glass column or a DCM/hexane mixture in a Soxhlet apparatus. Bulk lipids were removed by automated gel permeation chromatography (GPC). Percentage of lipid in

the homogenate subsamples was estimated gravimetrically. The PCBs and *p,p'*-DDE were separated from other OCs by consecutive elutions through deactivated silica gel columns.

Concentrations of OCs in lipid extracts were determined by high-resolution gas chromatography electron-capture detection (GC-ECD). Sample analyses can be divided into two groups based on methodologies employed for Σ PCB determinations, hereafter referred to as the Aroclor and congener summation techniques. Samples from Liard River, Slave River, Leland Lake, and Lakes Huron, Erie, and Ontario were analyzed following modifications of the Aroclor technique (15, 16), whereas samples from all other populations were analyzed following the congener summation technique (13, 14). Σ PCB determinations from these two approaches yield comparable results, but the Aroclor method tends to provide slightly lower estimates (5).

Samples subjected to the Aroclor technique were analyzed using a GC with dual ECDs; an RTX-5 60 \times 0.25 mm \times 0.25 mm column was used with an RTX-1701 60 \times 0.25 mm \times 0.25 mm as the confirmation column. Samples were processed in batches of ~ 15 including OC spikes, duplicates, blanks, and reference materials. Recoveries of internally spiked standards averaged $> 90\%$. For Lakes Huron, Erie, and Ontario OCs were quantified against a three-point calibration curve; final results were corrected for recoveries and blanks (when necessary). Total PCBs were quantified using a standard of Aroclor 1254. For samples from Liard River, Slave River, and Leland Lake OCs were quantified against an eight-point calibration curve and PCBs were quantified using a standard containing a 1:1:1 mixture of Aroclors 1242, 1254, and 1260.

For the congener summation technique, lipid-free eluates were analyzed on a Varian 3600 GC-ECD equipped with a 60 \times 0.25 mm DB-5 column (J&W Scientific, Folsom, CA) with H_2 carrier gas. External standards were run after every six samples. Ninety individual PCB congeners were quantified using congener mixtures obtained from the National Research Council (NRC, Halifax, NS, Canada) and Ultra Scientific (Hope, RI). Σ PCB was the sum of all quantified congeners. Recovery of the internal standards PCB 30 and OCN ranged from 80 to 100%, and concentrations were not corrected for recovery efficiency.

Data Analyses. Levels of OCs in walleye were calculated in three ways: as total body burden (milligrams per fish), as wet mass concentration (nanograms of OC per gram of wet mass), and as lipid concentration (nanograms of OC per gram of lipid). All of our statistical analyses were conducted following standard SAS procedures (e.g., GLM, NLIN, etc.) (17). Both dependent and independent variables were \log_e -transformed as necessary to linearize relationships and/or normalize residuals. Standard body sizes and ages of walleye for estimating mean contaminant levels were determined by comparing growth characteristics of the various populations. Prior to interpopulation analyses, we modeled fork length as a function of age for each population and gender by nonlinear least-squares using the von Bertalanffy growth model (18). Predicted mean lengths at age were estimated from the fitted models. Standard body sizes and ages used for comparisons were selected as the region of greatest overlap in the growth trajectories among the populations. In all OC analyses, predicted means and standard errors of OC levels were estimated at standard covariate levels from fitted ANCOVA models (LSMEANS option, GLM procedure) (17).

Gender analyses were restricted to the three lower Great Lakes' populations (Huron, Erie, and Ontario), where male and female sample sizes were largest. We also restricted our analyses on these populations to pooled data from the years 1994–1996, inclusive, as temporal analyses indicated that OC levels were most stable over this time period. Gender differences were initially assessed by ANCOVA using whole-

TABLE 1. Characteristics (Mean \pm SD) of Walleye Analyzed for Organochlorines^a

population ^b	year(s) sampled	females			males		
		n	age (years)	FL (mm)	n	age (years)	FL (mm)
1) Liard River, NWT	1994				14	8.6 \pm 3.2	440 \pm 69.0
2) Slave River, NWT	1990–1993	3	7.7 \pm 0.58	460 \pm 17.3	28	6.2 \pm 1.5	438 \pm 41.0
3) Leland, AB	1990–1993	6	9.0 \pm 0.63	543 \pm 27.5	9	8.7 \pm 1.0	505 \pm 31.7
4) Manitoba, MB	1996	10	10 \pm 5.4	608 \pm 115			
5) Musclow, ON	1990–1992	7	8.6 \pm 0.79	437 \pm 40.7	11	9.6 \pm 2.1	413 \pm 35.8
6) Orange, ON	1990–1992	7	11 \pm 3.4	453 \pm 30.1	10	15 \pm 3.5	446 \pm 12.8
7) Linge, ON	1990–1992	9	15 \pm 2.2	461 \pm 19.7	9	12 \pm 3.0	414 \pm 35.6
8) Trout, ON	1990–1992	9	10 \pm 3.0	486 \pm 34.0	9	12 \pm 2.7	467 \pm 27.3
9) Sidney, ON	1990–1992	9	5.7 \pm 1.6	429 \pm 37.0	9	9.0 \pm 2.1	403 \pm 36.3
10) Superior, ON	1997	9	8.0 \pm 3.8	519 \pm 41.5	3	7.0 \pm 2.6	466 \pm 15.0
11) Huron, ON	1994–1996	24	6.8 \pm 2.0	537 \pm 80.8	51	5.5 \pm 2.2	480 \pm 92.3
12) Erie, ON	1994–1996	51	5.2 \pm 1.4	546 \pm 64.6	36	3.8 \pm 1.2	452 \pm 72.1
13) Ontario, ON	1994–1996	12	6.3 \pm 2.0	510 \pm 117	66	3.7 \pm 1.4	396 \pm 85.4

^a Populations refer to lakes unless specified otherwise and are ordered from northwest to southeast. Sampling locations for the larger waterbodies were the south basin of Lake Manitoba, the Thunder Bay region of Lake Superior, northern Georgian Bay in Lake Huron, the western basin of Lake Erie, and the Bay of Quinte in Lake Ontario. ^b NWT, Northwest Territories; AB, Alberta; MB, Manitoba; ON, Ontario.

body burden as the dependent variable and body mass as the covariate of adjustment. These results were compared with those of subsequent analyses where wet mass and lipid mass concentrations were used as the dependent variable, and fork length and age were used as covariates.

For interpopulation analyses we used muscle concentrations as this was the tissue most commonly analyzed for populations outside of the Great Lakes. Using a sample of Lake Ontario fish, we determined the ratios of OC concentrations in muscle (both with and without skin) to whole-body concentrations and used these to convert all whole-body values to muscle values for the interpopulation analyses. To assess geographic variation, we estimated least-squares means and SEs of OC concentrations for each population using an ANCOVA model that initially included lake and gender as discrete variables, fish size or age as the covariate and all respective interaction terms. Nonsignificant terms (partial *F* test, *P* < 0.05) were sequentially removed by backward elimination prior to estimation. This approach produced mean estimates that were intermediate between male and female concentrations. However, because samples from some populations did not include both genders (Liard River and Lake Manitoba; Table 1), we eliminated the gender \times lake interaction term from all fitted models in order to estimate population means. This is equivalent to assuming that the effect of gender on OC concentration is similar in all lakes.

Results

Gender Differences. Scatter plots indicated a divergence in OC burdens between genders with increasing body mass in lower Great Lakes populations (Figure 1). At small body sizes, burdens appeared to be similar between genders, but at larger body sizes males tended to have higher burdens than females (Figure 1). Statistical analyses confirmed this pattern; the slope of Σ PCB burden versus body mass was significantly higher for males than for females in each of Lakes Huron, Erie, and Ontario (ANCOVA, heterogeneity of slopes, *P* < 0.05). Similarly, slopes of *p,p'*-DDE versus body mass were higher for males than for females in all three populations, and this difference was significant in Lakes Erie and Ontario (ANCOVA, heterogeneity of slopes, *P* < 0.05). Subsequent comparisons between genders were made at standard body sizes of 1000 and 2500 g (comparisons of least-squares means, Tukey–Kramer adjustment). Differences in mean burdens between genders were not consistent at 1000 g (Figures 2 and 3). However, at a body size of 2500 g differences were more pronounced, with males having significantly higher

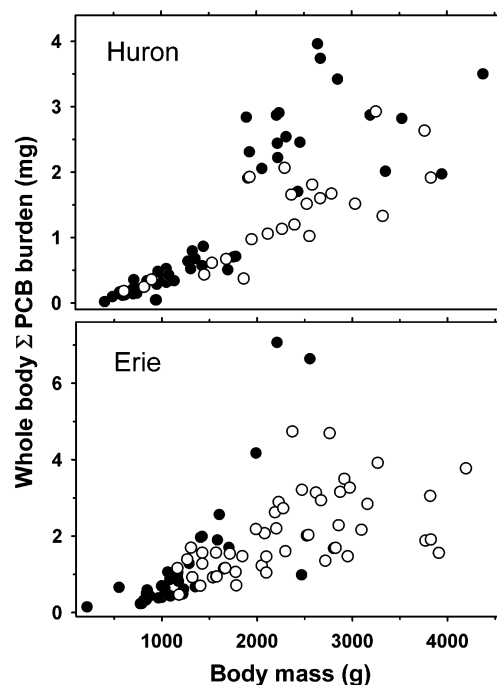


FIGURE 1. Scatter plots of whole-body Σ PCB burdens versus total body mass for male (solid circles) and female (open circles) walleye of Lakes Huron (top) and Erie (bottom).

burdens than females for both Σ PCB and *p,p'*-DDE in all three lower Great Lakes populations (Figures 2 and 3).

We repeated this analysis using wet mass and lipid mass OC concentrations rather than body burdens. The outcome of our analysis did not change markedly when OC levels were expressed as wet mass concentrations (nanograms of OC per gram of wet tissue). Slopes of the wet mass OC concentration versus body mass relationships were generally higher in males than females, and males had significantly higher mean concentrations than females, particularly at larger body sizes. Expressing OC levels as lipid mass concentrations (nanograms of OC per gram of lipid) reduced the interaction between gender effects and body mass covariation; slopes of the lipid mass OC concentration versus body mass relationships did not differ significantly between genders (ANCOVA, heterogeneity of slopes, *P* > 0.05) except in Lake Erie (male slope > female slope). However, the general pattern was for male mean concentrations to be significantly higher than female mean concentrations as observed in the

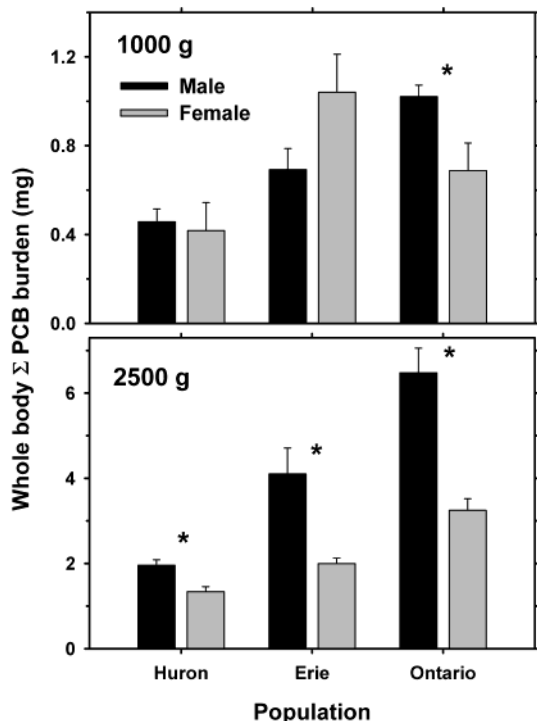


FIGURE 2. Whole-body Σ PCB burdens of male (black bars) and female (gray bars) walleye from Lakes Huron, Erie, and Ontario. Bars represent least-squares mean estimates (+1 SE) following adjustment to standard body masses of 1000 g (top) and 2500 g (bottom). Asterisks indicate significant differences between genders ($P < 0.05$). Scales of axes differ between plots.

burden analysis. Patterns in body lipid content were not consistent among these three populations. Body lipid content was positively related to body mass in Lake Ontario but not in Lake Huron or Erie. At a standard mass of 1700 g, mean lipid contents (percent of wet mass) were 8.9 and 10.1% for males and females, respectively, in Lake Huron, 12.3 and 13.5% for males and females, respectively, in Lake Erie, and 11.3 and 10.0% for males and females, respectively, in Lake Ontario.

Finally, we repeated our initial gender analysis using body burden as the dependent variable but replacing body mass with either \log_e fork length or \log_e age as the covariate. Comparisons of means were made at standard FLs of 400 and 600 mm and at standard ages of 4 and 7 years. Changing covariates altered the outcomes of the gender tests for Lake Ontario walleye but not for Lake Huron or Erie walleye. For Lake Ontario walleye, both Σ PCB and p,p' -DDE burdens increased with age at a faster rate in females than in males, contrary to previous results when body mass was used as the covariate. Furthermore, mean burdens at age or fork length were not significantly higher in males than in females in Lake Ontario. Changing the covariate of analysis appears to have affected results in Lake Ontario more than in the other populations because of gender-based differences in growth among these populations. Growth rates, as represented by the slopes of fork length (or body mass) versus age were significantly higher for females than males in Lake Ontario walleye (ANCOVA, heterogeneity of slopes, $P < 0.05$) but did not differ between genders in the other two populations. Interestingly, the effect of changing covariates in our gender-based analyses was also influenced by the choice of dependent variable. When OC levels were expressed as wet mass concentrations, changing the covariate from body mass to fork length or age did not drastically alter the outcomes of the gender analyses; males tended to have higher OC

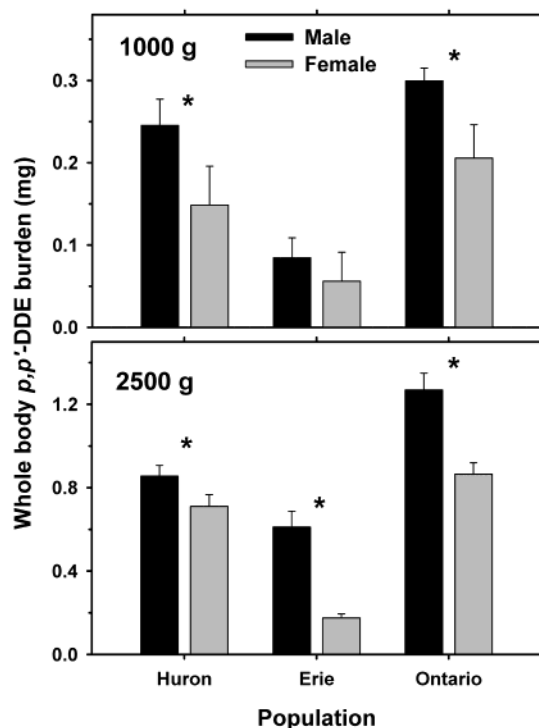


FIGURE 3. Whole-body p,p' -DDE burdens of male (black bars) and female (gray bars) walleye from Lakes Huron, Erie, and Ontario. Bars represent least-squares mean estimates (+1 SE) following adjustment to standard body masses of 1000 g (top) and 2500 g (bottom). Asterisks indicate significant differences between genders ($P < 0.05$). Scales of axes differ between plots.

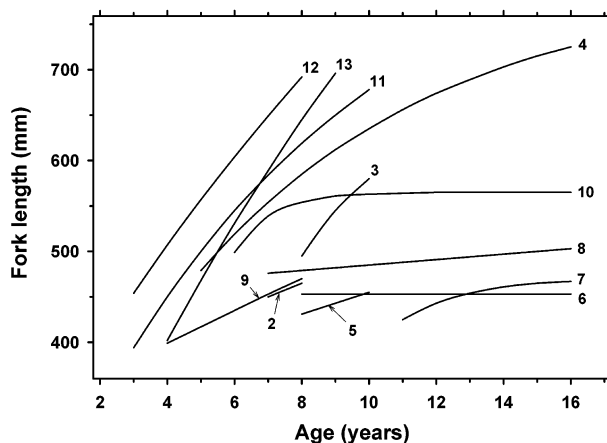


FIGURE 4. Growth characteristics of female walleye from populations sampled for this study. Numbers correspond to populations listed in Table 1 (no females were captured from population 1, Liard River). Predicted mean fork length at age over the range of female ages sampled in each population was estimated from a fitted von Bertalanffy growth model. When the model could not be fitted (population 6, Orange Lake), mean fish size was plotted.

concentrations than females, particularly at greater FLs and ages.

Geographic Variation. Walleye OC data were obtained from a total of 13 Canadian populations from the Liard River in the northwest ($60^{\circ} 30' N$, $123^{\circ} 30' W$) to Lake Ontario in the southeast ($43^{\circ} 30' N$, $78^{\circ} 00' W$) (Table 1). Mean sizes and ages varied greatly among the sampled populations (Table 1) as did growth patterns (Figure 4). The fastest growing walleye were those of the lower Great Lakes, particularly Lake Erie, whereas the slowest growing walleye were those of lightly exploited populations in northwestern Ontario, particularly

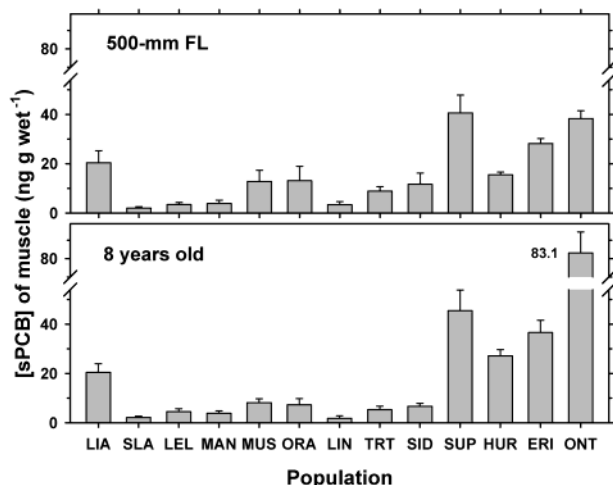


FIGURE 5. Whole-body Σ PCB concentrations of walleye from the 13 populations listed in Table 1. Populations are roughly ordered from northwest to southeast (left to right) and labeled with the first three letters of their name as listed in Table 1. Bars represent least-squares mean estimates (+1 SE) following adjustment for gender and for size (top) or age (bottom) effects.

Orange, Linge, and Trout Lakes (Figure 4). Females grew more rapidly; on average, females were 7.2% longer than males at 8 years of age. Comparison of growth trajectories revealed that no particular combination of fish age and size could reasonably exist in all populations. We selected a size and age of 500 mm FL and 8 years, respectively, as our standards of comparison in subsequent analyses as these appeared to represent the region of overlap for most population samples. Mean ratios ($n=4$, Lake Ontario walleye) of muscle to whole body OC concentrations used as conversion factors were 0.059 for Σ PCB and 0.036 for p,p' -DDE for wet mass concentrations and 0.67 for Σ PCB and 0.41 for p,p' -DDE for lipid mass concentrations.

In all geographic analyses, there were significant differences in OC concentrations among populations. Patterns of geographic variation differed between Σ PCB and p,p' -DDE concentrations. Mean muscle Σ PCB concentrations were highest in the Great Lakes ($>20 \text{ ng g}^{-1}$) and lower in more northerly lakes ($<10 \text{ ng g}^{-1}$) (Figure 5); the one exception to this trend was the Liard River population, which exhibited a relatively high mean Σ PCB concentration despite its northern location. Mean p,p' -DDE concentrations were extremely low for the four most northwesterly populations sampled ($<3 \text{ ng g}^{-1}$) (Figure 6). For the remaining lakes, mean p,p' -DDE concentrations of 500 mm walleye were generally in the range of 8–15 ng g^{-1} with a few exceptions (Linge and Erie) (Figure 6). For both Σ PCB and p,p' -DDE concentrations, standardization to age 8 years rather than 500 mm FL increased the mean concentration of Great Lakes populations relative to northwestern Ontario populations (Musclow, Orange, Linge, Trout, and Sidney) and accentuated the contrast among geographic regions (Figures 5 and 6). For example, in comparisons of mean Σ PCB concentrations, Lake Huron walleye differed significantly from four of nine populations outside the Great Lakes at 500 mm but differed from eight of nine of these populations at 8 years of age ($P < 0.05$, Tukey–Kramer adjustment). Similarly, in comparisons of mean p,p' -DDE concentrations, Lake Huron walleye differed significantly from five of nine populations outside the Great Lakes at 500 mm but differed from nine of nine of these populations at 8 years of age.

We repeated the geographic analysis using lipid-adjusted muscle OC concentrations (nanograms of OC per gram of lipid) instead of wet mass concentrations. Muscle lipid concentrations differed significantly among these popula-

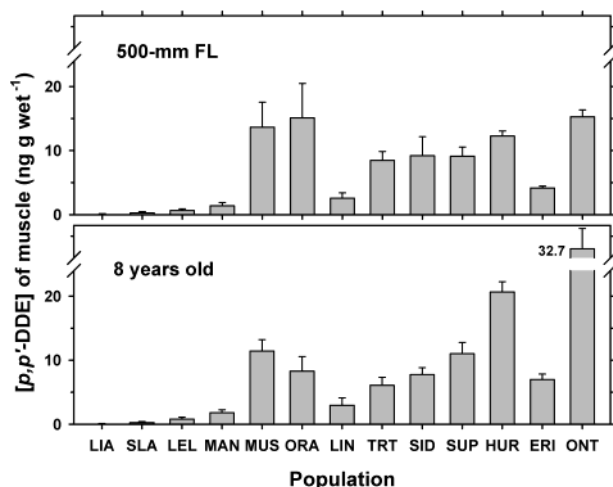


FIGURE 6. Whole-body p,p' -DDE concentrations of walleye from the 13 populations listed in Table 1. Populations are roughly ordered from northwest to southeast (left to right) and labeled with the first three letters of their name as listed in Table 1. Bars represent least-squares mean estimates (+1 SE) following adjustment for gender and for size (top) or age (bottom) effects.

tions, but this variation exhibited no discernible geographic pattern. Analyses of lipid-adjusted OC concentrations yielded trends that were nearly identical to those of analyses based on wet mass OC concentrations.

Discussion

Interpretations of spatial and temporal trends in contaminant concentrations of freshwater fish may be influenced by a variety of factors. Investigators must decide upon the tissue to analyze (e.g., muscle or whole fish), the analytical methods of contaminant analysis, the means of expressing concentrations, and the methods of data analysis. All of these decisions may influence the final interpretation of results, but the magnitude of their influence has rarely been explored. In this study we examined variation in OC concentrations at two spatial scales and tried to determine how the methods of data analysis may influence our interpretation of results. To achieve this, we brought together data from a variety of studies employing different methods in sample preparation and contaminant analyses, particularly for Σ PCBs. However, this should influence only variability in interpopulation comparisons. All fish within populations were analyzed in a similar manner and thus, intrapopulation analyses, such as for gender effects, would not be affected.

We observed significant differences in bioaccumulation patterns of both Σ PCB and p,p' -DDE between male and female walleye in three Great Lakes populations. To our knowledge, this is the first study to report gender-based differences in OC bioaccumulation in multiple populations. This suggests that the phenomenon of gender differences may be more widespread than was previously thought, at least in walleye. In general, the magnitude of the gender differences increased with increasing body size and age, and OC levels were lower in females than in males. Previous studies may not have detected such differences because they failed to account for size or age covariance in their analyses or their samples included too few large and/or old fish. Our analyses clearly demonstrated the need to account for size/age covariation and that the level of the covariate selected for comparison can affect the magnitude and statistical significance of the observed gender differences.

Changing the method of expressing the dependent variable had little effect on the outcome of our gender comparison analyses. Results based on analyses of wet mass

concentrations were virtually identical to those based on body burdens. Using lipid mass concentrations altered the body size covariance effect, but the general pattern of higher concentrations in males than in females remained the same. The effects of switching from body burdens to wet mass or lipid mass concentrations may vary with relative differences in body composition between genders. Both water and lipid content of fishes vary seasonally, and often inversely to one another (19). Gender differences in whole-body lipid content were not consistent among our three study populations. However, these differences may be more pronounced under certain conditions. It is currently unknown how the lipid content of males relative to females varies within walleye populations with respect to body size/age or season or how it varies among walleye populations with respect to growth conditions. These may be factors to consider when OC analyses are based on lipid mass concentrations.

The choice of covariate influenced the outcome of our gender analyses for one population. Using fork length or age instead of body mass as the covariate reduced the mean predicted burdens of males relative to females in the Lake Ontario population. This effect was not evident when OC levels were expressed as wet mass concentrations instead of burdens. The effect of changing covariate may have been related to gender-based differences in growth (length at age and/or mass at length) that were stronger in Lake Ontario walleye than in the Lake Huron or Erie walleye. Regardless of the mechanisms, this result demonstrates that the standard used for comparison of the genders can influence the interpretation of results in some populations.

We had expected that using age as the covariate rather than some index of size (length or mass) would increase female OC concentrations relative to male concentrations and reduce the magnitude of the observed gender effect. This was based on the assumption that females grow more rapidly than males (11) and, thus, comparison of genders at a standard age would be a comparison of larger females with smaller males. In contrast, comparison of genders at a standard size would be a comparison of younger females with older males. Contaminant concentration is influenced by both the age and size of fish; thus, for a given age of fish larger individuals tend to have higher concentrations, and for a given size of fish older individuals tend to have higher concentrations. Consequently, comparative analyses using a standard age will tend to increase the mean predicted concentrations of faster-growing groups (i.e., greater size at age) relative to slower-growing groups, whereas using a standard length will have the opposite effect. We expected that this effect would apply within (e.g., between genders) as well as among populations. Although we did observe higher growth rates in females than in males on average across all 13 populations, the difference was less pronounced in the lower Great Lakes populations that we analyzed for gender differences. We suggest that further studies on gender-based differences within populations should compare analyses based on all covariates, particularly if growth rates differ substantially between genders.

Previous studies have noted lower concentrations of OCs in females relative to males in individual populations of northern pike (8) and walleye (9). Females also have lower OC concentrations than males in other taxa such as some migratory birds (20) and marine mammals (21). These differences may arise for a variety of ecological and physiological reasons. Females may have lower OC concentrations because they lose disproportionately more lipid and hence lipophilic OCs through egg production (fish and birds) or lactation (mammals). In iteroparous fish such as walleye the mature ovaries may represent 20% or more of the body mass and body burden of OCs. Thus, spawning represents a considerable annual reduction in body burden that is not

experienced by the males. The proportion of body burden lost during spawning may be even greater in other species (8). Whether or not this leads to long-term reductions in female relative to male OC levels may depend on the body weight lost during spawning as well as the nature of other gender-based differences such as diet, growth rates, or habitat use. For example, higher PCB concentrations in male walleye of Saginaw Bay (Lake Huron) were attributed to habitat selection; males of this population spent more time in OC-contaminated areas than females (9). The magnitude of the gender difference observed in the Saginaw Bay walleye appeared to be much greater than what we observed in our study populations. This suggests that the combination of factors leading to gender differences may vary among populations.

Geographic patterns in walleye OC concentrations generally followed our expectations based on historical distribution of these contaminants. Concentrations were usually highest in Great Lakes populations and declined moving northwest, but the pattern differed between Σ PCB and p,p' -DDE. The observed variation among populations may include some methodological variability (primarily Σ PCB, see Methods), some temporal variability because populations were not all sampled in the same year, and some gender variability because we assumed the effects of gender on OC concentrations were similar among populations (no gender \times population interaction). Although we feel that the additional variability thus introduced was small, we have limited our interpretation to the most distinct trends.

For Σ PCB in the Great Lakes, concentrations were highest in Lake Ontario and Lake Superior. Lake trout OC concentrations in the Great Lakes decline going downstream from Lake Superior to Lake Ontario (7, 22). Outside the Great Lakes, Σ PCB concentrations were uniformly lower with the exception of elevated levels in the Liard River population. The higher than expected mean predicted PCB levels of Liard River walleye may be an artifact of the sampling program as only males were collected from this population (Table 1). Concentrations of OCs, including PCBs, in burbot (*Lota lota*) from outside the Great Lakes decline from south to north (23). Concentrations of p,p' -DDE were very low in the four most northwesterly walleye populations examined but did not differ greatly among the remaining populations. As a metabolite of pesticide residues, p,p' -DDE may be less prevalent in far northern regions where DDT was not historically applied for forestry or agriculture. Among the Great Lakes, p,p' -DDE concentrations were markedly lower in Lake Erie than in the remaining populations, a pattern observed in OC concentrations of other species (22). This has been attributed to the higher productivity and more rapid turnover of the Erie system. However, it is not clear why the pattern of p,p' -DDE concentrations differs so markedly from the pattern of Σ PCB concentrations for walleye of the lower Great Lakes.

For both Σ PCB and p,p' -DDE concentrations the geographic pattern observed was influenced by the choice of covariate. The contrast between Great Lakes and other populations (particularly northwestern Ontario lakes: Musclove, Orange, Linge, Trout, and Sidney) was much greater when age was used as the covariate than when length was used as the covariate. We believe this phenomenon reflects differences in growth rates among populations and is analogous to the effect of differential growth rates on gender differences discussed earlier. Lower Great Lakes populations exhibited much faster growth rates than the northwestern Ontario populations. As a result, comparisons at 500 mm FL were of older non-Great Lakes fish versus younger Great Lakes fish, whereas comparisons at 8 years old were of smaller non-Great Lakes fish versus larger Great Lakes fish. Variability in growth rates was much greater among the 13 populations

we examined than between genders within any of these populations and, thus, the effect of changing covariates appears to be much greater in the geographic analyses than in the gender analyses. Our analysis was not meant to indicate which of age or size standardization is more appropriate. Furthermore, because the age and size composition of sampled fish varied considerably among populations, we were unable to test the effect of changing covariate levels on geographic comparisons. However, in comparisons of groups of fish with widely different growth characteristics we recommend presenting the results of both age- and size-adjusted analyses. This allows a clearer assessment of the robustness of the interpretation.

We have demonstrated that walleye exhibit considerable variation in OC concentrations across their native Canadian range. More importantly, we have shown that the interpretation of these trends can be influenced by the gender composition of the sample and the statistical methods applied. These are important considerations for future sampling programs, particularly those covering populations with widely varying growth characteristics. Furthermore, the demonstration of gender differences in OC concentrations of multiple populations is of interest from two perspectives. First, it is ecologically intriguing as it suggests that male versus female ecologies could vary more substantially than was previously thought. Second, it is of interest from a human health perspective because it provides an additional criterion for assessing consumption risk. Future research should attempt to understand the mechanisms that lead to gender-specific differences in contaminant concentrations and, in so doing, determine which populations of fish would be most likely to exhibit these differences. This would be beneficial to refining consumption guidelines, particularly for those harvesters targeting larger, mature fish.

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