J. Great Lakes Res. 24(4):917–928
Internat. Assoc. Great Lakes Res., 1998

Maternal Transfer of Organochlorines to Eggs of Walleye (*Stizostedion vitreum*) in Lake Manitoba and Western Lake Superior

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ABSTRACT. *Gravid walleye were sampled from Lake Manitoba and western Lake Superior (St. Louis River) to measure the concentrations of organochlorine contaminants (OCs) in eggs and muscle and to assess the influence of maternal age and size on the transfer of OCs from mother to egg. Concentrations of most OCs in Lake Superior walleye eggs were 1 to 3 orders of magnitude greater than in Lake Manitoba eggs. Toxaphene (mean concentration (wet weight) ± 1 SE, 1580 ± 462 ng/g) and polychlorinated biphenyls (PCBs) (240 ± 24 ng/g) were the predominant OCs in Lake Superior walleye eggs, whereas DDT and metabolites (eggs 16 ± 1.5 ng/g, muscle 2.1 ± 0.36 ng/g) and PCBs (eggs 9.2 ± 0.83 ng/g, muscle 2.0 ± 2.4 ng/g) were the most common OCs in Lake Manitoba walleye eggs and female muscle. Egg size (dry mass) and the concentration of most OCs in Lake Manitoba walleye eggs were positively correlated with female length and age. This relationship was strongest for more hydrophobic OCs (e.g., PCBs) but was not significant for less hydrophobic OCs (e.g., hexachlorocyclohexanes (HCHs)). Neither egg size nor egg OC concentration of Lake Superior walleye were significantly correlated with female length or age. There was no relationship between OC concentrations in muscle tissue and female length or age of Lake Manitoba walleye. OC concentrations in Lake Manitoba walleye eggs were not correlated with concentrations in the muscle tissue of the mothers, suggesting that OCs in walleye eggs are derived from various tissues. A positive relationship between the egg:muscle ratio of PCB concentrations and the egg:muscle ratio of lipid in freshwater fish suggests that the maternal transfer of PCBs in freshwater fish is related to the relative amounts of lipid in the eggs and mother. The transfer of hydrophobic OCs from mother to eggs in freshwater fish appears to vary within and among fish species and with the hydrophobicity of the OC.*

INDEX WORDS: *Pollutants, contaminant dynamics, reproduction, fish size, lipid.*

INTRODUCTION

Persistent organochlorine pollutants (OCs) are found throughout the world and have been implicated in reduced reproductive success of both fish (Giesy *et al.* 1986, Walker *et al.* 1991) and fish-eating birds (Weseloh *et al.* 1983). Hydrophobic OCs are transferred from mother to eggs in fish (Guiney *et al.* 1979), and levels in eggs have been found to be positively correlated with muscle concentrations of the mother in chinook salmon (*Oncorhynchus tshawytscha*) and lake trout (*Salvelinus namaycush*) (Miller 1993) and the fraction of the mother's total body lipid contained in the eggs for five species of freshwater fish (Niimi 1983). High burdens of OCs, such as polychlorinated biphenyls (PCBs), in fish eggs have been correlated with higher egg mortality (Monod 1985) and reduced juvenile growth (Black *et al.* 1988).

The walleye is the most economically important and most intensively managed freshwater fish species in central Canada and the United States. As a piscivore, walleye also tend to accumulate higher burdens of environmental contaminants, such as OCs, than species feeding at lower trophic levels. Because most egg production is derived from so-

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matic tissue (Wiegand 1996) and because walleye produce eggs with a very high lipid content (Moodie *et al.* 1989), the resultant embryos and larvae will presumably have OC concentrations equal to or greater than those found in the female. A limited number of studies have determined OC levels in adult walleye muscle (Niimi *et al.* 1994, De Vault *et al.* 1996, Morrison 1996, Giesy *et al.* 1997, Paterson *et al.* 1998), but none has determined OC concentrations in walleye eggs.

The objective in this study was to examine and compare the maternal transfer of OCs in walleye from Lakes Manitoba and Superior. OC concentrations in fish have been shown to increase with fish age and size (Larsson *et al.* 1992, Miller 1994) and thus, it was hypothesized that older, larger females would produce eggs with higher OC concentrations than younger, smaller females.

MATERIALS AND METHODS

Field Collection

Gravid walleye were captured from western Lake Superior by electrofishing in the St. Louis River and from the south basin of Lake Manitoba by setting trap nets in Swan Creek (Fig. 1). Most females in both stocks were in non-spawning condition (eggs not free-flowing) when captured and were placed in holding pens for one to several days until they attained spawning condition (eggs free-flowing). Egg samples were collected from the Lake Superior walleye on 3 May (n = 1.0 fish) and from Lake Manitoba walleye on 10 May (n = 18 fish) and 14 May (n = 2 fish) 1996. Females were selected to cover the entire size range of fish in the spawning populations. Each fish was measured (fork length, ± 1.0 mm) and the second and third dorsal spines were removed. From each female, a sample (~ 25 mL) of eggs was collected from the posterior third of the expelled egg volume (i.e., first third expelled) as the fish was stripped. Ten of the Lake Manitoba females sampled for eggs on 10 May were killed and muscle tissues samples (~ 50 g) were taken from below the first dorsal fin and above the lateral line. Egg and muscle samples were placed in Whirl-Pak bags and transported on ice or freezer packs to the laboratory.

Egg Dry Mass and Female Age Determination

Mean individual egg dry mass for each female was determined by drying two subsamples of 30 eggs each for 24 h at 60°C. Muscle samples and the remaining eggs were transferred to hexane-rinsed aluminum foil and hexane-rinsed glass vials, re-

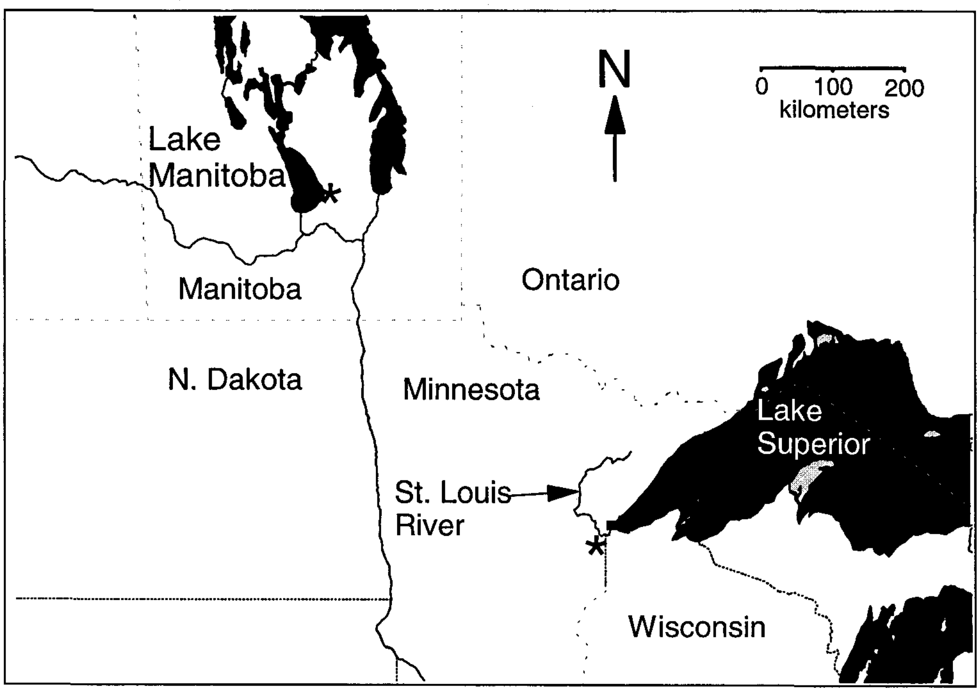

FIG. 1. Location of sampling sites (*).

spectively, and stored at –4°C until organochlorine analyses were conducted.

Walleye ages were estimated by counting annuli on dorsal spine sections using a modification of the methods outlined by Chilton and Beamish (1982). Dorsal spines were allowed to air dry, set in epoxy, then sectioned with a low speed saw. Three sections from each fish were mounted in clear epoxy on a glass slide and viewed under a compound microscope with a dark field condenser at 100 × magnification.

Extraction and Organochlorine Analysis

Eggs (~10 g) were crushed in a beaker using a glass pestle and approximately 50 g of Na_2SO_4. An additional 200 g of Na_2SO_4 was added to the sample and mixed until a free-flowing mixture was obtained. Muscle tissue samples (skinless) were homogenized with dry ice in a blender, and 10 g was then added to 250 g of Na_2SO_4 and mixed until a free-flowing mixture was obtained. PCB 30 and octachloronapthalene (OCN) were added to all samples as a recovery standard (recoveries > 80%, concentrations were not corrected for recovery efficiencies). Egg and muscle samples were then Soxhlet extracted with 350 mL of DCM:hexane (1:1) for 4 h. For every five samples a blank (250 g Na_2SO_4) was also extracted. A fraction of the extract was used to determine lipids gravimetrically. Lipids were removed from the sample by gel permeation chromatography (GPC). The GPC columns (inner diameter, 29.5 mm; length, 400 mm, reservoir, 500 mL) were packed with 60 g (dry weight) of 200- to 400-mesh Bio-Beeds® S-X3 beads (Bio-Rad Laboratories, Hercules, CA, USA). The column was eluted with 300 mL of DCM:hexane (1:1), the first 125 mL contained lipids and was discarded. The lipid-free eluate, containing the OCs, was evaporated to 1 mL and applied to a Florisil column (8 g, 1.2% deactivated). The OCs were recovered by consecutive elution with 35 mL hexane (Fraction 1 (F1)), 38 mL of 85% hexane: 15% DCM (F2), and 52 mL of 50% hexane: 50% DCM (F3). F1 contained 90% of the chlorobenzenes and mirex, 75% of trans-nonachlor, 50% of the *o,p*-DDE and *o,p*-DDT, 15% of the *p,p*-DDT, 95% of the *p,p*-DDE, ~33% of the toxaphene, and 100% of the PCBs. F2 contained 5% of *p,p*-DDE, 10% of the chlorobenzenes and mirex, 25% of trans-nonachlor, 50% *o,p*-DDT and *o,p*-DDE, 90% of *p,p*-DDT, and 100% of (-hexachlorocyclohexane (HCH), oxychlordane, trans-chlordane, cis-chlordane, *o,p*-DDD, *p,p*-DDD and cis-nonachlor. Fraction 3 (F3) contained 100% of endosulfan I and II, dieldrin, and endrin. All fractions were roto-evaporated, transferred to 2,2,4-trimethyl pentane, and were evaporated to approximately 100 μL. Aldrin was added as a volume corrector.

Samples (100 μL) were analyzed on a Varian 3600 gas chromatograph (GC) equipped with a 60 m x 0.25 mm DB-5 column (J & W Scientific) and a ^{63}Ni-electron capture detector (ECD). The carrier gas was H_2 and N_2 was used as the make-up gas for the ECD. External standards were run after every six samples. Three randomly chosen egg and muscle samples from Lake Manitoba were also analyzed by GC-MS using a Hewlett Packard 5971 MSD in SIM mode to confirm PCB concentrations.

Data Analysis

OCs which were not detectable were assigned a concentration of 0 for calculation of means and standard errors. OC concentrations used in regression analysis were on a wet weight basis. Regression analysis was only performed on individual OCs which had measurable concentrations in all samples. All statistical analyses were performed using Systat for Windows (SYSTAT, Evanston, IL).

RESULTS

The mean age and fork length of walleye sampled from Lake Manitoba were not significantly different from those of walleye sampled from Lake Superior (*t*-test, p = 0.50) (Table 1). Eggs from Lake Superior walleye had significantly greater dry mass and lipid content than eggs from Lake Manitoba walleye (*t*-test, p < 0.01) (Table 1). Egg dry mass was positively correlated with female length and age in Lake Manitoba walleye but not in Lake Superior walleye (Fig. 2). These egg size vs female age/size relationships held for both lakes when a larger data set was used in a single year (n = 40 females) and even among different years (1994 to 1998) in the Lake Manitoba population (Johnston 1997, and T.A. Johnston *unpublished data*).

Lipid content of eggs was negatively correlated with female length in Lake Superior walleye (linear regression; F = 20.9; df = 1,8; p = 0.02; r^2 = 0.72) but not in Lake Manitoba walleye (linear regression; F = 0.20; df = 1,18; p = 0.66; r^2 = 0.01). There was no correlation between muscle lipid content and fork length in female walleye from Lake Mani-

TABLE 1. Characteristics of gravid female walleye sampled from Lakes Manitoba (n = 20) and Superior (n = 10) for organochlorine analyses.

	mean ± 1 SE	range
Fork length (mm)		
Superior	585 ± 26.5	462–684
Manitoba	616 ± 21.5	452–740
Age (years)		
Superior	11.7 ± 1.15	7–17
Manitoba	10.7 ± 0.990	5–18
Egg lipid (% wet weight)		
Superior	10 ± 0.25	9.1–12
Manitoba	8.8 ± 0.34	4.4–10
Muscle lipid (% wet weight)		
Superior	–	–
Manitoba	0.93 ± 0.12	0.35–1.3
Egg dry mass (mg)[a]		
Superior	1.2 ± 0.023	1.0 –1.3
Manitoba	0.87 ± 0.027	0.66–1.1

[a]The mean water contents (n = 5) of the Lake Superior and Lake Manitoba walleye eggs were 67 and 68%, respectively.

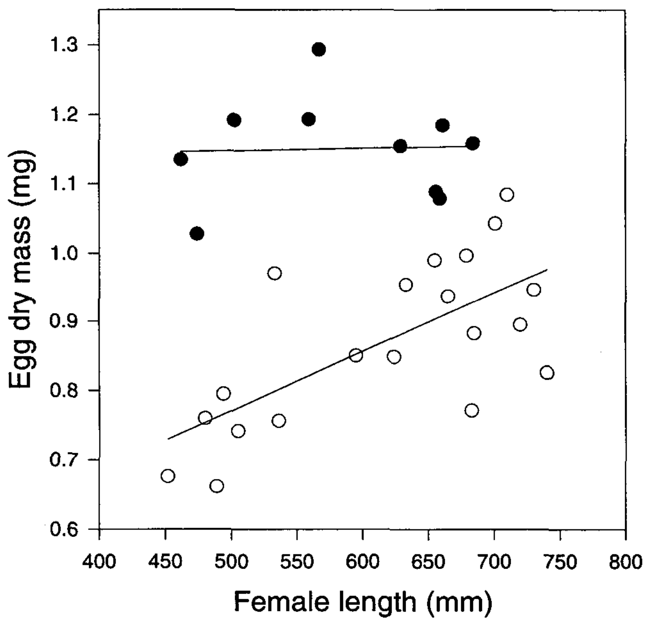

FIG. 2. Relationships between mean individual egg dry mass and female fork length for walleye collected from Lakes Manitoba (○, r^2 = 0.47, p < 0.001, n = 20) and Superior (●, r^2 = 0.002, p = 0.91, n= 10).

toba (linear regression; F = 0.67; df = 1,8; p = 0.44; r^2 = 0.08).

OC concentrations in Lake Superior walleye eggs were much higher than in Lake Manitoba walleye eggs and muscle tissue, and relative concentrations of individual OCs varied between these lakes (Table 2, Fig. 3). Toxaphene, ΣPCB (sum of 85 PCB congeners), ΣDDT (sum of *o,p*-DDT, *p,p*-DDT, *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, *p,p*-DDD), ΣCHL (sum of oxychlordane, trans-chlordane, cis-chlordane, trans-chlordane, cis-nonachlor and heptachloro-epoxide), ΣHCH (sum of αHCH, βHCH and γHCH) and ΣCBz (sum of 1,2,3-trichlorobenzene (triCBz), pentachlorobenzene (PCBz) and hexachlorobenzene (HCBz)) concentrations in Lake Superior walleye eggs were 350, 26, 4, 21, 2, and 5 times greater than in Lake Manitoba walleye eggs, respectively. Dieldrin and endrin concentrations were also much higher in the Lake Superior eggs (Table 2). OC concentrations in eggs were greater than in muscle tissue from Lake Manitoba female walleye, and this trend was fairly consistent among the OC groups.

Egg concentrations of ΣPCB, ΣDDT, ΣCHL, ΣCBz, and a large number of individual compounds were positively correlated with female length in Lake Manitoba but not in Lake Superior (Fig. 4, Table 3). Similar relationships for both lakes were also observed using female age instead of length. Concentrations of organochlorines in Lake Manitoba female walleye muscle were not significantly correlated with length or age (Table 3). These relationships appeared to be quadratic rather than linear, although only the ΣDDT-age quadratic relationship was statistically significant (quadratic regression; F = 11.0; df = 2, 16; p = 0.001; r^2 = 0.58).

Concentrations of OCs in Lake Manitoba walleye eggs were not significantly correlated with female muscle concentrations.

DISCUSSION

OC Concentrations

There are no published data on OC concentrations in walleye, or walleye eggs, of Lake Manitoba. Lipid corrected PCB concentrations (ng/g lipid) in the Lake Manitoba walleye muscle are approximately half of those reported for walleye muscle (skin on) collected at a number of smaller lakes in Northwestern Ontario which are in the same general geographical area (Paterson *et al.* 1998). The higher concentrations of OCs in the Northwestern

TABLE 2. Organochlorine concentrations (mean ± 1 SE, ng/g wet weight) in walleye eggs from Lakes Superior and Manitoba and female walleye muscle from Lake Manitoba. Organochlorines that were below detection limits were given a value of 0 for mean and standard error calculations.

Organochlorine	Lake Superior eggs (n = 10)	Lake Manitoba eggs (n = 19)[a]	Lake Manitoba female muscle (n = 10)
ΣPCB[b]	240 ± 24	9.2 ± 0.83	2.0 ± 0.24
ΣDDT	61 ± 8.8	16 ± 1.5	2.1 ± 0.36
o,p'-DDE	0.40 ± 0.15	0.06 ± 0.01	0.03 ± 0.02
p,p'-DDE	49 ± 6.0	8.5 ± 0.80	1.0 ± 0.22
o,p'-DDD	0.94 ± 0.34	0.22 ± 0.12	0.04 ± 0.01
p,p'-DDD	7.1 ± 1.5	6.4 ± 0.69	0.88 ± 0.19
o,p'-DDT	4.1 ± 1.8	0.02 ± 0.01	0.02 ± 0.01
p,p'-DDT	< 0.02	0.24 ± 0.06	0.06 ± 0.03
ΣCHL	77 ± 15	3.7 ± 0.36	0.59 ± 0.07
oxychlordane	7.5 ± 1.4	0.30 ± 0.03	0.11 ± 0.02
trans-chlordane	4.3 ± 1.1	0.42 ± 0.04	0.05 ± 0.01
cis-chlordane	14 ± 3.5	0.92 ± 0.10	0.14 ± 0.02
trans-nonachlor	28 ± 4.9	1.1 ± 0.12	0.16 ± 0.02
cis-nonachlor	19 ± 4.3	0.51 ± 0.06	0.08 ± 0.01
heptachloro-epoxide	4.4 ± 0.40	0.39 ± 0.03	0.05 ± 0.01
ΣHCH	3.2 ± 0.76	1.3 ± 0.12	0.20 ± 0.02
αHCH	2.3 ± 0.59	0.61 ± 0.07	0.09 ± 0.01
βHCH	0.16 ± 0.04	< 0.02	< 0.02
γHCH	0.74 ± 0.14	0.64 ± 0.06	0.11 ± 0.01
ΣCBz	2.6 ± 0.27	0.53 ± 0.03	0.09 ± 0.01
1,2,3,4-triCBz	0.05 ± 0.00	< 0.02	< 0.02
PCBz	0.21 ± 0.01	0.07 ± 0.01	0.02 ± 0.01
HCBz	2.3 ± 0.26	0.44 ± 0.02	0.06 ± 0.01
toxaphene	1580 ± 460	5.6 ± 0.75	1.6 ± 0.30
dieldrin	18 ± 2.3	1.2 ± 0.08	0.17 ± 0.03
endrin	1.7 ± 0.26	< 0.02	< 0.02
endosulfan I	0.10 ± 0.02	0.12 ± 0.01	0.02 ± 0.00
endosulfan II	0.04 ± 0.01	< 0.02	0.01 ± 0.01

[a]One Lake Manitoba walleye egg Florisil fraction 2 was lost and therefore only 19 samples were available for all organochlorines except PCBs.
[b]ΣPCB is the summation of PCB congeners: 4/10, 6, 8/5, 19, 18, 17, 24/27, 16/32, 26, 25, 31, 28, 33, 22, 45, 46, 52, 49, 47, 48, 44, 42, 41/71, 64, 40, 74, 70/76, 95/66, 56/60, 91, 84/89, 101, 99, 83, 97, 87, 136, 110/82, 151, 144/135, 149, 118, 134, 114, 131, 146, 153, 132, 105, 141, 130/176, 179, 137, 138/163, 158, 178/129, 175, 187, 183, 128, 185, 174, 177, 171, 156, 201/197, 174/197, 180, 193, 191, 170, 190, 198, 199, 196/203, 189, 208, 195, 207, 194, 205, 206 and 209.

Ontario walleye are probably due to a combination of the more oligotrophic nature of the Northwestern Ontario lakes and lower lipid contents of the Lake Manitoba samples. Higher trophic level fish from less productive lakes have been found to have greater OC burdens than similar fish from more productive lakes due to a combination of factors including slower growth rates and dilution of OCs at the bottom of the food chain (Larsson *et al.* 1992). The Lake Manitoba muscle samples may have had lower lipid contents because of the removal of skin, and this probably caused less common OC compounds to fall below detection limits. The hydrophobic nature of OCs results in partitioning of

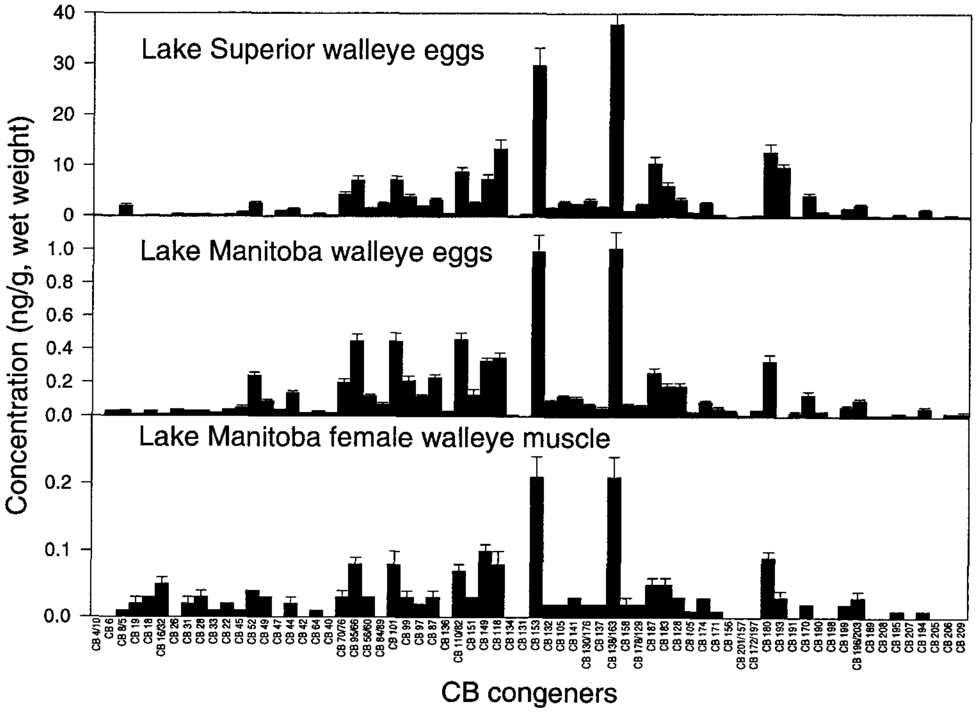

FIG. 3. Concentrations (mean ± 1 SE) of CB congeners in walleye eggs from Lake Superior (n = 10) and Lake Manitoba (n = 20) and female walleye muscle from Lake Manitoba (n = 10). OCs that were below detection limits were given a value of 0 for mean and standard error calculations.

OCs into the lipids of fish. Removing a fatty portion of a sample, such as the skin, would lower the OC burden in the sample resulting in concentrations which may not be detectable for less common OCs. The Northwestern Ontario walleye samples were analyzed using skin-on muscle samples (Paterson *et al.* 1998). Other factors could explain greater OC concentrations in walleye from the Northwestern Ontario lakes, such as greater food chain length (Rasmussen *et al.* 1990) or greater atmospheric input of OCs, although no data are available on Lake Manitoba to evaluate these factors.

Concentrations of OCs in the Lake Superior walleye eggs were similar, or slightly lower, than those reported in eggs of other Lake Superior fish. Mac *et al.* (1993) measured ΣPCB concentrations of 321 and 254 ng/g (wet weight) in lake trout eggs collected in 1987 and 1988, respectively. Miller and Amrhein (1995) found ΣPCB concentrations of 450 ng/g in siscowet eggs, a fatty, deep water subspecies of lake trout found in Lake Superior. Further, Miller (1994) observed a ΣPCB concentration of 300 ng/g in eggs of Lake Superior lake trout, which was an order of magnitude less than those observed in eggs of Lake Michigan lake trout. There are no reported data on toxaphene levels in Lake Superior fish eggs, however the high levels that we found were not unexpected. Although Lake Superior is considered the least contaminated of the Great Lakes, it has some of the highest toxaphene concentrations in lake trout (Glassmeyer *et al.* 1997, De Vault *et al.* 1996). Toxaphene has also been found to be the dominant OC in fish collected from Siskiwit Lake, on Isle Royale, in Lake Superior (Swackhamer and Hites 1988) and in lake trout from Lake Superior (De Vault *et al.* 1996).

Large differences in OC concentrations of walleye eggs, particularly toxaphene, between Lakes Superior and Manitoba were observed despite the close proximity of these lakes. These results were not unexpected because the Great Lakes have a history of being highly contaminated due to large surface areas, long water residence times, proximity to point sources, and long food chains (Jeremiason *et*

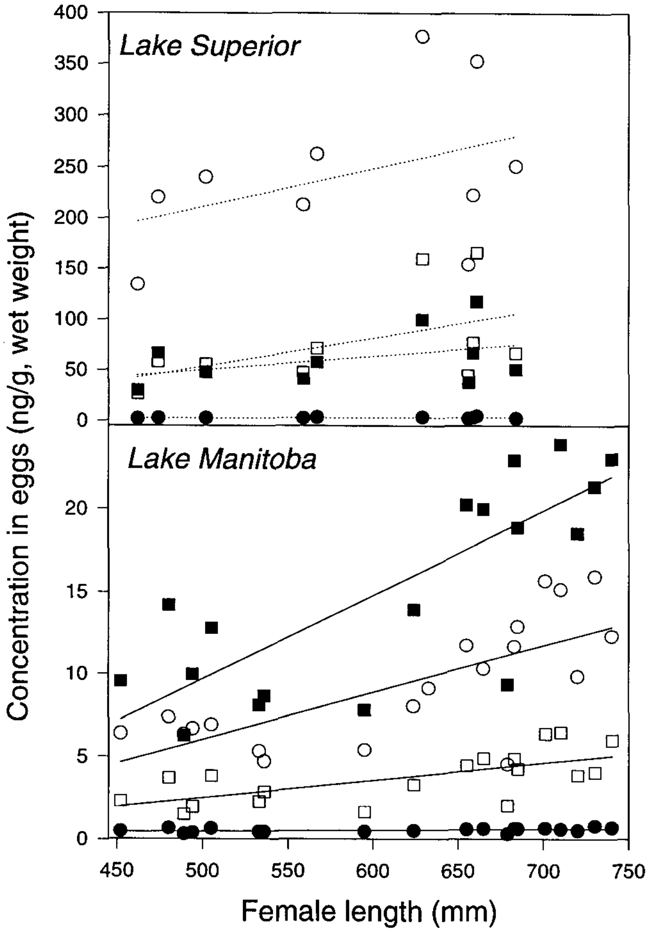

FIG. 4. Linear relationships between organochlorine concentrations in eggs and female fork length for walleye of Lakes Superior (n = 10) and Manitoba (n = 20 for ΣPCBs; n = 19 for all other OCs) (■ - ΣPCB, □ - ΣCHL, ○ - ΣDDT, and ● - ΣCBz). Significant non- significant relationships are represented by solid and dashed lines, respectively. r^2 values for groups and individual chemicals are found in Table 3.

al. 1994). For example, Paterson *et al.* (1998) found that ΣPCB concentrations in lake trout collected from a number of smaller lakes in Northwestern Ontario were nearly 20 times less than those of Lake Superior lake trout. Although atmospheric delivery is probably the predominant route of OCs into these lakes, a number of factors are probably contributing to differences in OC concentrations. Lake Manitoba has no large or obvious point sources for OCs besides agricultural runoff, which probably does not contribute large amounts of OCs compared with atmospheric deposition. Lake Superior has a number of point sources, in particular the St. Louis River, a US EPA superfund site, where the walleye eggs were collected. As well, the Lake Superior walleye may be feeding at a higher trophic level, due to a longer food chain, than the Lake Manitoba walleye, resulting in greater bioaccumulation and biomagnification (Rasmussen *et al.* 1990). Higher proportions of more highly chlorinated CB congeners in the Lake Superior walleye eggs suggest greater bioaccumulation and partially support the hypothesis of a longer food chain, but could also be due to local point sources. Lastly, Lake Manitoba is a more eutrophic system than Lake Superior, and greater productivity in lakes has been associated with reduced bioaccumulation of OCs in higher trophic level fish (Larsson *et al.* 1992).

Maternal Transfer of OCs in Fish

The transfer of hydrophobic OCs from mother to eggs in freshwater fish appears to vary within and between fish species and with the hydrophobicity of the OC, but further research is needed to clarify these mechanisms.

Concentrations of hydrophobic OCs, including ΣPCBs, PCB congeners, ΣDDT, ΣCHL, and ΣCBz, in Lake Manitoba walleye eggs were positively correlated with the age and size of the mother, but this relationship was not observed for Lake Superior walleye eggs. An explanation for the lack of this relationship in the Lake Superior samples is not apparent from these data. It may be due to the mixing of walleye stocks from different areas of Lake Superior. If OC concentrations varied among these walleye stocks then relationships from the Lake Superior samples may be confounded. It should be noted that only 10 egg samples from Lake Superior were analyzed and the size and age range was smaller than for the Lake Manitoba walleye. As well, there were no data on Lake Superior female muscle OC concentrations, which may have provided insight into their concentrations in the eggs.

The maternal transfer of hydrophobic OCs in freshwater fish species appears to be related to the relative amounts of lipid in the eggs and somatic tissue of the mother (Fig. 5). Niimi (1983) also concluded that the percent lipid in fish and the total lipid deposited in eggs significantly influenced maternal transfer of hydrophobic OCs. However, although the egg:mother ratio of ΣPCB concentration was found to be positively correlated with the

TABLE 3. ***r^2 values from regression of organochlorine concentrations in eggs and muscle against female length for Lake Superior and Lake Manitoba walleye (- represents organochlorines which were non-detectable in one or more samples and therefore no regression analysis was performed; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).***

Organochlorine	Lake Superior walleye eggs (n = 10)	Lake Manitoba walleye eggs (n = 19)	Lake Manitoba female walleye muscle (n = 10)
ΣPCB	0.18	0.53***	0.00
CB 8/5	0.01	0.55***	0.03
CB 28	0.16	0.02	0.28
CB 52	0.11	0.38**	0.02
B 95/66	0.12	0.58***	0.02
CB 101	0.13	0.53***	0.11
CB 149	0.04	0.32***	0.06
CB 118	0.36	0.45**	0.02
CB 153	0.21	0.55***	0.04
CB 138	0.18	0.55***	0.00
CB 187	0.29	0.44**	0.07
CB 180	0.23	0.55***	0.01
CB 196/203	0.29	0.46***	0.02
CB 206	0.31	0.40**	—
CB 209	0.01	0.14	—
mono/di	0.12	0.20*	0.10
tri	0.02	0.29*	0.22
tetra	0.11	0.53***	0.08
penta	0.20	0.58***	0.02
hexa	0.16	0.52***	0.00
hepta	0.16	0.46***	0.00
octa	0.32	0.49***	0.00
nona	0.26	0.48***	—
ΣDDT	0.18	0.61***	0.31
o,p'-DDE	0.30	—	—
p,p'-DDE	0.09	0.61***	0.31
o,p'-DDD	0.23	—	0.02
p,p'-DDD	0.22	0.53***	0.28
o,p'-DDT	0.25	—	—
p,p'-DDT	—	—	—
ΣCHLORDANE	0.26	0.45**	0.05
oxychlordane	0.25	0.30*	0.00
trans-chlordane	0.32	0.29*	0.10
cis-chlordane	0.30	0.38**	0.00
trans-nonachlor	0.14	0.59***	0.19
cis-nonachlor	0.28	0.55***	0.06
heptachloro-epoxide	0.17	0.04	0.00
ΣHCH	0.08	0.04	0.06
αHCH	0.09	0.07	0.13
βHCH	0.07	—	—
γHCH	0.04	0.02	0.01
ΣCBz	0.23	0.24*	0.30
1,2,3,4-triCBz	0.24	0.14	—
PCBz	0.03	0.03	—
HCBz	0.25	0.34**	—
toxaphene	0.38	0.02	0.26
dieldrin	0.12	0.02	—
endrin	0.18	—	—
endosulfan I	0.01	—	—
endosulfan II	—	—	—

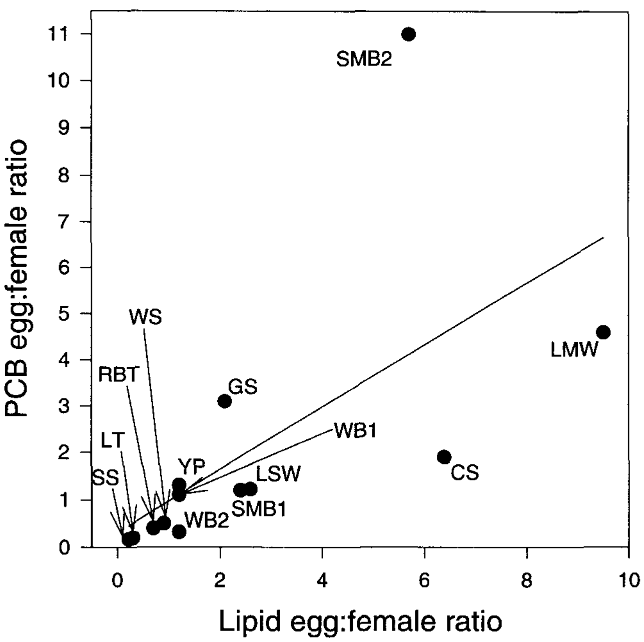

FIG. 5. ***Relationship between the egg:female ratios of ΣPCB concentration (wet weight) and lipid content for various fish species (PCB egg:female ratio = 0.3 + 0.7 [lipid egg:female ratio],*** $r^2 = 0.40$, $p = 0.02$***). Lipid and PCB concentrations for SMB1 (smallmouth bass), YP (yellow perch), WB1 (white bass), RBT (rainbow trout) and WS (white sucker) were determined for eggs and whole fish (Niimi 1983). Lipid and PCB concentrations for CS (chinook salmon) and LT (lake trout) are from Miller (1993); SS (siscowet) from Miller (1995); and GS (gizzard shad), SMB2 (smallmouth bass) and WB2 (white bass) are from Koslowski et al. 1994); and were determined for eggs and muscle tissue only. ΣPCB concentrations and lipid content in LSW (Lake Superior walleye) were determined in fish collected and analyzed in 1991 (Wisconsin Department of Natural Resources, unpublished data) and represent skin on fillets. Data for LMW (Lake Manitoba walleye) eggs and muscle and LSW eggs are from this work.***

egg:mother ratio of lipid content in a number of fish species (Fig. 5), it only explained 40% of the observed variation, and thus, other factors are likely influencing this process. These factors could include artifacts of sampling design, such as the tissues used to determine lipid and PCBs in the adult fish and/or the species of fish, or physiological factors such as high and variable lipid contents. This work, and the work of Miller (1993, 1994) and Koslowski *et al.* (1994), used muscle fillets to determine PCBs and lipids. The material used for egg production may originate from a number of tissues, and therefore muscle may not play the most important role in the transfer of lipids and PCBs to the eggs. Miller (1993) concluded that egg lipid levels did not influence the maternal transfer of OCs in chinook salmon which have high and variable lipid contents. Lipids from other tissues of these fish may have influenced the maternal transfer of OCs.

Interestingly, lipid content of the walleye eggs and muscle tissue did not vary with maternal age or fork length, and therefore lipid does not appear to explain the OC-female age and size relationships in the Lake Manitoba walleye. The lack of a relationship between lipid levels in eggs and maternal age/size was expected because the energy content of the eggs (J/g), derived mainly from neutral lipids, did not vary with maternal age and size in Lake Manitoba walleye (Johnston 1997). Lipid content of the Lake Superior walleye eggs decreased with maternal age and length, but the rate of change was minor and lipid corrected concentrations (ng/g fat) did not cause a significant change in the egg OC vs female OC relationship.

The maternal transfer of OCs also appears to be related to their hydrophobicity. The relationship between egg OC concentrations and maternal age/size in the Lake Manitoba walleye that was observed for PCBs, DDT and chlordane compounds, was not observed for the less hydrophobic ΣHCH. This suggests that a chemical's hydrophobicity is an important parameter in the maternal transfer of OCs in fish.

There was no significant relationship between OC concentrations in eggs and muscle of Lake Manitoba walleye, in contrast to significant relationships observed in lake trout (*Salvelinus namaycush namaycush*), siscowet (*Salvelinus namaycush siscowet*) and chinook salmon (*Oncorhynchus tshawytscha*) of the Great Lakes (Miller 1994, Miller 1993, Miller and Amrhein 1995). However, muscle lipid content may not be indicative of lipid content for the whole fish. Older, larger walleye appeared to have larger stores of visceral fat, although this trend was not quantified. Therefore, it may not be possible to predict OC concentrations in walleye eggs based on concentrations in maternal muscle tissue. The lack of a correlation between OC concentrations in egg and female muscle also suggests that a large percentage of the OC burden in walleye eggs may be derived from tissues other than the dorsal muscle, such as visceral fat stores. OC concentrations in eggs of walleye may be more strongly correlated with whole body OC concentrations.

Mechanisms of Maternal Transfer of OC

Developing fish eggs likely accumulate OCs via uptake of lipids or through diffusion from blood into the ovaries. Lipids are known to be delivered to eggs by vitellogenin, a precursor protein produced in the liver, but lipids are also synthesized within the egg yolk in the early stages of vitellogenisis (Wiegand 1996). Fish vitellogenin is a large molecule which is high in lipids (~20%), although a majority are polar lipids (Mommsen and Walsh 1988), and OCs could partition onto or into this large molecule and be transported into the eggs. A second vehicle for OC movement into eggs would be diffusion of OCs from the blood into the high lipid tissue of the ovary, although this vehicle of movement would become less important with increasing hydrophobicity of the OC.

There were similar proportions of OCs, and in particular CB congeners, in the eggs and muscle tissue of the Lake Manitoba walleye, suggesting that OCs in walleye eggs were derived from somatic tissue of the mother and not directly from her diet. The relative abundance of PCB congeners tends to shift to a greater portion of more highly chlorinated congeners towards the top of aquatic food chains (Oliver and Nimbi 1988). If egg lipids were derived directly from the diet there would likely have been a greater proportion of lower chlorinated PCB congeners because these compounds are less hydrophobic than higher chlorinated PCB congeners. As well, walleye eggs grow over the winter and are spawned in early spring. Energy intake for walleye in the winter is low, and therefore, lipids and OCs in walleye eggs are probably derived from somatic tissue.

Implications of Maternal Transfer of OCs

Concentrations of most OCs in Lake Manitoba and Lake Superior walleye eggs do not appear to be in the range which could cause toxic effects. ΣPCB concentrations in Lake Manitoba and Lake Superior walleye eggs are well below levels identified by Niimi (1996) to cause toxic effects in fish. Hatching success and fry survival were high (> 94.3%) in Lake Superior lake trout despite ΣPCB concentrations in eggs of 254 and 321 ng/g (Mac *et al.* 1993). Broyles and Noveck (1979) reported that the threshold PCB concentration for mortality in lake trout eggs was 3.6 to 7.6 μg/g. Threshold DDT concentrations for mortality in lake trout eggs range from 1.54 to 4.75 μ/g (Burdick *et al.* 1964, Macek 1968). All of these concentrations are greater than those measured in Lake Superior walleye eggs and orders of magnitude greater than those of Lake Manitoba walleye eggs. High toxaphene concentrations in the Lake Superior walleye eggs may pose a problem, however there is no appropriate data with which to evaluate these concentrations.

If the hypothesis that the lipid ratio between mother and eggs in fish controls the maternal transfer of OCs is correct, certain fish populations, or fish within a population, which produce eggs of high lipid content may be more vulnerable to the effects of hydrophobic OCs due to greater OC burdens in their eggs.

Larger fish eggs may have a greater survivorship than smaller eggs due to physiological and ecological advantages (Roff 1992). For walleye, Moodie *et al.* (1989) found that the smallest eggs from Crean Lake, Saskatchewan, had deficiencies in the (n-3) series of polyunsaturated fatty acids, and higher rates of body deformities and mortalities. However, this trend was not observed in a second population of walleye from Lake of the Prairies, Manitoba. Larger, older walleye from Lake Manitoba produced eggs of greater size and OC concentration, than smaller, younger females. As discussed above, the OC concentrations in the Lake Manitoba walleye eggs do not appear to pose a threat to the health of this population. However, the increase in OC burden of eggs with maternal age and length has implications for walleye populations, as well as other fish species, from more polluted ecosystems.

There is evidence that the earliest life stages of fish are the most susceptible to the effects of contaminants (Walker *et al.* 1991). On a rate basis, the ΣPCB exposure dose in larvae was calculated to be approximately 44 times greater than that in adult walleye of Lake Manitoba (Table 4). Therefore, eggs and newly hatched larvae may be the life stage of walleye at the greatest risk to toxicity associated with hydrophobic OCs. It seems likely that the signs of stress from OC contamination in a walleye population would be observed first in early life history stages.

TABLE 4. Estimated ΣPCB exposure rates of Lake Manitoba walleye adult female and swim-up larvae (first 8 days after hatching).

	egg	adult
feeding rate[a]	1/8 oil drop/d	24.5 g/d
exposure concentration (ng/g)[b]	0.0092	2.0
exposure rate (ng/g/d)[c]	0.44	0.010

[a]Assuming a water temperature of 15°C and no exogenous feeding, a newly hatched walleye larva will consume it's oil droplet in approximately 8 days (personal observation). Averaged over a year, an adult walleye will consume approximately 1% of it's body weight per day (Swenson and Smith 1973). The average mass of the Lake Manitoba female walleye was 2.45 kg.

[b]ΣPCB exposure concentration for the egg is the mean ΣPCB concentration in the Lake Manitoba walleye eggs (n = 20). ΣPCB exposure concentration for the adult is the amount assimilated per day from prey. Concentration of ΣPCBs in the prey of walleye has been found to be approximately equal to the walleye muscle concentrations (wet weight) (Paterson *et al.* 1998). We assumed that 50% of the ΣPCB would be assimilated by the adult walleye (Fisk *et al.* 1998).

[c] Exposure rate = [exposure concentration (ng/g) / feeding rate (1/8 oil droplet or g/d)] / egg or adult mass (g).

ACKNOWLEDGMENTS

The authors would like to thank Derek Muir for providing analytical supplies, as well as thoughtful insight on experimental design and an earlier draft of this manuscript. We would also like to thank Michael A. Miller and Dave DeVault for constructive reviews of the original manuscript. Eggs and fish samples were collected with the cooperation of the Manitoba Department of Natural Resources and the Minnesota Department of Natural Resources. Susanna Wiens and Rob Currie assisted in the field. Rick Wastle determined walleye ages. Bruno Rosenberg determined toxaphene concentrations. We thank the Freshwater Institute, Department of Fisheries and Oceans for use of laboratory space. This research was supported by grants from Fish Futures Manitoba, Manitoba Hydro, and the Habitat Action Plan of the Department of Fisheries and Oceans. Ph.D. funding for Aaron Fisk was provided by a grant from the Natrural Science and Engineering Research Council of Canada to Derek C. G. Muir.

REFERENCES

Black, D. E., Phelps, D. K., and Lapan, R. P. 1988. The effect of inherited contamination on egg and larval winter flounder, *Pseudopleuronectes americanus. Mar. Environ. Res.* 25:45–62.

Broyles, R. H., and Noveck, M. I.. 1979. Uptake and distribution of 2,4,5,2′,4′,5′-hexachlorobiphenyl in fry of lake trout and chinook salmon and its effects on viability. *Toxc. Appl. Pharm.* 50:299–308.

Burdick, G. E., Harris, E.J., Dean, H. J., Walker, T. M., Skea, J., and Colby, D. 1964. The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* 93:127–136.

Chilton, D.E., and Beamish, R. J. 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. *Can. Spec. Publ. Fish. Aquat. Sci.* 60.

De Vault, D. S., Hesselberg, R., Rodgers, P. W., and Feist, T. J.. 1996. Contaminant trends in lake trout and walleye from the Laurentian great lakes. *J. Great Lakes Res.* 22:884–895.

Fisk, A. T., Norstrom, R. J., Cymbalisty, C. D., and Muir, D. C. G.. 1997. Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with K_{ow}. *Environ. Toxicol. Chem.* 17:951–961.

Giesy, J. P., Newsted, J., and Garling, D. L. 1986. Relationships between chlorinated hydrocarbon concentrations and rearing mortality of chinook salmon (*Oncorhynchus tshawytscha*) eggs from Lake Michigan. *J. Great Lakes Res.* 12:82–98.

______, Jude, D. J., Tillitt, E., Gale, R. W., Meadows, J. C., Zajieck, J. L., Peterman, P. H., Verbrugge, D. A., Sanderson, J. T., Schwartz, T. R., and Tuchman, M. L. 1997. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in fishes from Saginaw Bay, Michigan. *Environ. Toxicol. Chem.* 16:713–724.

Glassmeyer, S. T., De Vault, D. S., Myers, T. R., and Hites, R. A. 1997. Toxaphene in Great Lakes fish: A temporal, spatial, and trophic study. *Environ. Sci. Technol.* 31:84–88.

Guiney, P. D., Melancon Jr., M. J., Lech, J.J., and Peterson, R. E.. 1979. Effects of egg and sperm maturation and spawning on the distribution and elimination of a polychlorinated biphenyl in rainbow trout (*Salmo gairdneri*). *Toxicol. Appl. Pharmacol.* 47:261–272.

Jeremiason, J. D., Hornbuckle, K. C., and Eisenreich, S. J. 1994. PCBs in Lake Superior, 1978–1992: Decreases in water concentrations reflect loss by volatilization. *Environ. Sci. Technol.* 28:903–914.

Johnston, T. A. 1997. Within-population variability in egg characteristics of walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersonii*). *Can. J. Fish. Aquat. Sci.* 54: 1006–1014.

Koslowski, S.E., Metcalfe, C.D., Lazar, R., and Haffner, G.D. 1994. The distribution of 42 PCBs, including three coplanar congeners, in the food web of the western basin of Lake Erie. *J. Great Lakes Res.* 20:260–270.

Larsson, P., Collvin L., Okla, L., and Meyer, G. 1992. Lake productivity and water chemistry as governors of the uptake of persistent pollutants in fish. *Environ. Sci. Technol.* 26:346–353.

Mac, M. J., Schwartz, T.R., Edsall, C. C., and Frank, A. M.. 1993. Polychlorinated biphenyls in Great Lakes lake trout and their eggs: Relations to survival and congener composition 1979–1988. *J. Great Lakes Res.* 19:752–765.

Macek, K. J. 1968. Reproduction in brook trout (*Salvelinus fontinalis*), fed sublethal concentrations of DDT. *J. Fish. Res. Board Can.* 25:1787–1796.

Miller, M. A. 1993. Maternal transfer of organochlorine compounds in salmonines to their eggs. *Can. J. Fish. Aquat. Sci.* 50:1405–1413.

______. 1994. Organochlorine concentrate dynamics in Lake Michigan chinook salmon *(Oncorhynchus tshawytscha). Arch. Environ. Contam. Toxicol.* 27:367–374.

______, and Amrhein, J. F. 1995. Maternal transfer of organochlorine compounds in Lake Superior siscowet (*Salvelinus namaycush siscowet*) to their eggs. *Bull. Environ. Contam. Toxicol.* 55:96–103.

Mommsen, P., and Walsh, P. J. 1988. Vitellogenesis and oocyte assembly. In *Fish Physiology. Vol. XI The physiology of developing fish. Part A Eggs and larvae,* eds. W. S. Hoar and D.J. Randall, pp. 348–395. San Diego, CA: Academic Press.

Monod, G. 1985. Egg mortality of Lake Geneva charr (*Salvelinus alpinus* L.) contaminated by PCB and DDT derivatives. *Bull. Environ. Contam. Toxicol.* 35:531–536.

Moodie, G. E. E., Loadman, N. L., Wiegand, M. D., and Mathias, J. A.. 1989. Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizostedion vitreum*). *Can. J. Fish. Aquat. Sci.* 46: 516–521.

Morrison, H. A. 1996. The effects of zebra mussels (*Dreissena polymorpha*) on the distribution and dynamics of polychlorinated biphenyls in the western Lake Erie food web. Ph.D. thesis. University of Windsor, Windsor, ON.

Niimi, A. J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. *Can. J. Fish. Aquat. Sci.* 40:306–312.

______. 1996. PCBs in aquatic organisms. In *Environmental Contaminants in Wildlife: Interpreting tissue concentrations,* eds. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwodd, pp. 117–152. Boca Raton, FL: CRC Lewis Publishers.

______, Metcalfe, C. D., and Huestis, S. Y. 1994. Chlorinated diphenyl ethers in Great Lakes fish and their environmental implication. *Environ. Toxicol. Chem.* 13:1133–1138.

Oliver, B. G., and Niimi, A. J. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario Ecosystem. *Environ. Sci. Technol.* 22: 388–397.

Paterson, M. J., Muir, D. C. G., Rosenberg, B., Fee, E. J., Anema, C., and Franzin, W. 1998. Does lake size affect concentrations of atmospherically-derived PCBs in water, sediment, zooplankton, and fish?. *Can. J. Fish. Aquat. Sci.* (in press).

Rasmussen, J. B., Rowan, D. J., Lean, D. R. S., and Carey, J. H. 1990. Food chain structure in Ontario lakes determines PCB levels in Lake Trout (*Salvelinus namaycush*) and other pelagic fish. *Can. J. Fish. Aquat. Sci.* 47:2030–2038.

Roff, D. A. 1992. *The evolution of life histories: Theory and Analysis.* New York, NY: Chapman and Hill.

Swackhamer, D. L., and Hites, R. A. 1988. Occurrence and bioaccumulation of organochlorine compounds in fishes from Siskiwit Lake, Isle Royale, Lake Superior. *Environ. Sci. Technol.* 22:543–548.

Swenson, W. A., and Smith Jr., L. L. 1973. Gastric digestion, food consumption, feeding periodicity, and food conversion efficiency in walleye (*Stizostedion vitreum vitreum*). *J. Fish. Res. Board Can.* 30:1327–1336.

Walker, M. K., Spitsbergen, J. M., Olson, J. R., and Peterson, R. E. 1991. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* 48:875–883.

Weseloh, D. V., Teeple, S. M., and Gilbertson, M. 1983. Double-crested cormorants of the Great Lakes: egg-laying parameters, reproductive failure, and contaminant residues in eggs, Lake Huron 1972–1973. *Can. J. Zool.* 61:427–436.

Wiegand, M. D. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. *Rev. Fish Biol. Fish.* 6:259–286.

Submitted: 17 December 1997
Accepted: 6 September 1998
Editorial handling: Arthur J. Niimi