Intrapopulation Variation in Egg Lipid and Fatty Acid Composition and Embryo Viability in a Naturally Spawning Walleye Population from an Inland Reservoir

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Abstract.—The objective of the study was to evaluate the variation in embryo viability within a population of walleye *Sander vitreus* from an inland reservoir throughout the spawning season. Egg size, egg lipid content, and fatty acid composition were used as criteria to evaluate egg quality. Additionally, we sought to verify whether any particular size-class of females produces superior-quality eggs or whether the time of spawning (early, middle, or late) has an effect on egg quality. Seventy-seven ovulating walleye females (total length, 465–885 mm) were captured in Salt Fork Reservoir, Ohio, throughout the spawning season. Although egg diameter after water hardening varied among females (1.85–2.38 mm), egg size did not correlate with female length ($P > 0.05$). Average egg lipid content was $12.0 \pm 1.3\%$ (mean $\pm$ SD) of wet weight and was unrelated to egg diameter ($P > 0.05$). Neutral and phospholipid classes in eggs comprised $77.5 \pm 4.7\%$ and $22.5 \pm 4.7\%$ of total lipids, respectively. Egg diameter was not significantly related to any of the specific fatty acids from neutral or phospholipid fractions ($P > 0.05$). Moreover, egg fatty acid compositions from both neutral lipids and phospholipids did not change during the spawning season. High survival of embryos ($90.0 \pm 8.7\%$) from females across the observed size range was recorded regardless of the spawning period. We concluded that the quality of walleye eggs was consistently high and thus that the timing of gamete collection would not compromise hatchery programs.

The walleye *Sander vitreus* is a highly valued game and food species in the north-central region of the United States and in central Canada. Public hatcheries annually produce over 1×10^9 walleye fingerlings for stocking into lakes and rivers to enhance natural resources (Fenton et al. 1996; Summerfelt 1996). Although many aspects of walleye culture have been significantly improved (for a review, see Summerfelt 1996), the majority of walleye juveniles used for stocking are currently produced from eggs and sperm collected from mature, wild fish (Summerfelt 1996; Mauk and Brown 2001). Therefore, the quality of eggs collected from natural populations may be an initial bottleneck in the production of stocking material.

A combination of physical (e.g., water discharge from the river, turbidity, and temperature) and eco-

logical (e.g., prey density and predation) factors may have profound impacts on the year-class strength of walleyes (Mion et al. 1998). All of these factors influence the age structure and size distribution of walleye populations, and consequently might affect the quantity and quality of eggs spawned every year. Egg quality is usually reflected in egg size and composition. Johnston and Leggett (2002) reported that within walleye populations, egg size increases with maternal size and age. These authors postulated that larger females produce larger eggs because (1) they breed earlier and consequently their offspring are exposed to less favorable growth conditions (e.g., cooler temperatures and lower food resources), and (2) they can acquire better spawning sites. Larger walleye eggs are usually characterized by larger yolks and oil globules, and therefore possess higher nutritional reserves than smaller eggs. Whether this variation reflects differences among females in the spawning population is unknown (Moodie et al. 1989).

Egg size, as well as egg composition (especially lipids and fatty acids), can have a significant impact on the early life history of fish. Walleye egg size varies among populations with respect to both latitude and water body productivity (Johnston and Leggett 2002). The smallest walleye eggs contained low levels of n-3 polyunsaturated fatty acids

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[2] In this fatty acid notation, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

(PUFAs), and larvae from these eggs survived for only 10 d after hatching (Moodie et al. 1989). The eicosapentaenoic acid (EPA; 20:5[n-3][2]) and docosahexaenoic acid (DHA; 22:6[n-3]) contents of neutral lipids in walleye eggs have been correlated positively with embryo survival (Czesny and Dabrowski 1998). A negative correlation was also observed between survival and the ratio of EPA to arachidonic acid (AA; 20:4[n-6]) in whole-body phospholipids of juvenile walleyes (Czesny et al. 1999).

In our previous work (Czesny and Dabrowski 1998), walleye eggs from three distinct populations were compared in terms of lipid mass and fatty acid composition. We found that egg lipid content and fatty acid composition differed significantly among populations. However, within individual populations, egg composition on a year-to-year basis was relatively constant. In a recent study, Wiegand et al. (2004) reported that the lipid mass composition of eggs was very consistent among 10 walleye populations. In contrast, the fatty acid composition of the egg lipids differed significantly among walleye populations.

In the present study, we quantified the degree of intrapopulation variation in walleye female size, egg lipid composition, and fatty acid composition and examined correlations between these traits and embryo survival or timing of spawning. Our objectives were to determine whether any particular size-class of females produces superior-quality eggs or whether egg quality changes as the spawning season progresses. Such information could benefit fishery management operations by indicating the specific ''target'' females that could be used to optimize fingerling production for stocking programs.

Methods

Ovulating females ($n = 77$) were captured in trap nets over 15 consecutive days in 1997 (March 21–April 5) in Salt Fork Reservoir, Ohio, during the annual gamete collection performed by the staff of the Ohio Division of Wildlife's Senecaville State Fish Hatchery. Walleyes have been stocked in Salt Fork Reservoir annually since 1968 and originated from a Lake Erie river-spawning population. The population in the reservoir is not self-sustaining (J. Navarro, Ohio Division of Wildlife, personal communication). The spawning season was arbitrarily divided into three periods: early spawning (March 21–24), middle spawning (March 25–31), and late spawning (April 1–5). Each period included fish from several days pooled

together ($n = 18$ early-spawning, 41 middle-spawning, and 18 late-spawning females).

At each sampling date, fish were measured and stripped of eggs. A sample of eggs (5 g) was immediately frozen in liquid nitrogen and then stored at $-83°C$ prior to lipid and fatty acid analysis. Eggs from each female were fertilized in duplicate with an excess of fresh, pooled sperm (~0.1 mL) obtained from three to four males. All females collected on a given sampling date were fertilized with the same pooled sperm. At 1 min after fertilization, eggs were washed with a solution of tannic acid (400 mg of tannic acid/L of water) and were stirred continuously for 3 min to remove adhesiveness. Samples of eggs (200–400) were placed into separate polyethylene bags and transported in a cooler above ice at 5–6°C to the Ohio State University aquaculture laboratory, Columbus. Egg size was determined by measuring the diameter of 30 randomly selected eggs from each female. The measurement was taken a few hours after fertilization to allow for complete egg hydration and hardening. Eggs were then placed in separate polyvinyl chloride baskets with screen bottoms and were incubated in California-type hatching trays (Flex-a-Lite Consolidated, Inc., Tacoma, Washington) supplied with recirculating, UV-sterilized water at 11–14°C. From day 2 of incubation until just prior to hatching, formaldehyde treatments were applied daily at a concentration of 50–100 mg of formaldehyde/L of water to prevent development of fungi. The survival percentage was determined at the pigmented eyed embryo stage. Latif et al. (1999) reported that 80% of walleye egg mortality occurred during 50–100 h postfertilization. Thus, embryo survival to the pigmented eyed stage is usually considered a good indicator for the viability at hatching.

Total lipids were extracted with chloroform–methanol (2:1 [volume : volume]) containing 0.025% of butylated hydroxytoluene as an antioxidant according to the procedure of Folch et al. (1957). The organic solvent was evaporated under a stream of nitrogen, and the lipid content was gravimetrically determined. Neutral lipid (mostly triglycerides) and polar lipid (phospholipids) fractions were extracted from the crude lipids by use of silica September-Pak cartridges (Waters, Division of Millipore Corp., Midford, Massachusetts). Chloroform and methanol were used as solvents to elude neutral and polar lipids, respectively. The organic solvents from both fractions were evaporated under a stream of nitrogen, and the amounts of neutral and polar lipids were determined gravi-

TABLE 1.—Reproductive characteristics (mean ± SD) of female walleyes and their eggs collected throughout the spawning season from Salt Fork Reservoir, Ohio, in 1997. Means within a row that have different letters are significantly different (ANOVA: $P < 0.05$). Spawning seasons are defined as follows: early, March 21–24; middle, March 25–31; and late, April 1–5.

Group and variable	Spawning season		
	Early	Middle	Late
Females			
N	18	41	18
Length (mm)	680.7 ± 79.7 y	617.3 ± 75.7 z	596.8 ± 62.4 z
Eggs			
Diameter (mm)	2.0 ± 1.1 z	2.0 ± 1.1 z	2.0 ± 1.1 z
Total lipid (%)[a]	11.9 ± 1.1 z	12.0 ± 1.5 z	11.9 ± 1.2 z
Neutral lipid (%)[b]	78.2 ± 2.9 z	77.8 ± 5.3 z	76.3 ± 4.8 z
Phospholipids (%)[b]	21.8 ± 2.9 z	22.2 ± 5.3 z	23.7 ± 4.8 z
Embryo survival (%)[c]	89.5 ± 4.4 z	91.4 ± 7.6 z	93.1 ± 3.6 z

[a] Total lipids are expressed as the percentage of wet weight.
[b] Neutral lipids and phospholipids are expressed as a percentage of total lipids.
[c] Embryo survival determined at the pigmented eyed stage.

metrically. Fatty acid methyl esters were prepared from both lipid fractions according to methods described by Metcalfe and Schmitz (1961). Methyl esters were formed by saponification in NaOH (0.5 N) in methanol and subsequent esterification with BF_3 in methanol. The fatty methyl esters were separated by gas chromatography (Varian 3300, Varian, Inc., Walnut Creek, California) as previously described by Czesny and Dabrowski (1998). One run was performed per extract; the amounts were then quantified using nonadecanoic acid (C19:0) as an internal standard.

Results are presented as means and SDs. All statistical tests were performed in the SigmaStat Statistical Software package (SPSS 1997); the significance level α was set at 0.05. Percentage data were arcsine transformed prior to statistical analysis. All data were tested for normality and homogeneity of variance with the Shapiro–Wilk W and Bartlett's test, respectively. For each spawning period, we calculated mean values for all physiological variables and subjected them individually to one-way analysis of variance (ANOVA). When the F-test was significant, we then used Tukey's multiple comparison procedure to compare the means. A nonparametric method (Kruskal–Wallis H-test) was used when assumptions about normality and homogeneity of variance could not be satisfied. The concentrations of each fatty acid were also compared between the neutral and phospholipid fractions by use of the t-test or the Mann–Whitney rank-sum test. Relationships between maternal length and the lipid concentration and diameter of eggs were explored by use of linear

regression, and correlation coefficients were calculated. Finally, multiple linear regression analysis was used to test whether egg fatty acid composition in both neutral lipid and phospholipid fractions could predict embryo survival.

Results

Walleye females sampled from the Salt Fork Reservoir spawning population ranged in total length from 465 to 885 mm. Females collected during the early spawning period were significantly larger than the ones collected during the middle and late spawning periods (Table 1; $F = 6.61$, df = 2, $P = 0.002$). Egg diameter varied among females and ranged from 1.85 to 2.38 mm. Egg diameter did not differ significantly among spawning periods (Table 1; $H = 5.03$, df = 2, $P = 0.08$) and was not correlated with female length (Figure 1; early spawning: $F = 0.45$, df = 17, $P = 0.51$, $r^2 = 0.02$; middle spawning: $F = 0.09$, df = 41, $P = 0.76$, $r^2 = 0.05$; late spawning: $F = 5.29$, df = 17, $P = 0.06$, $r^2 = 0.04$). The concentration of total lipids in walleye eggs did not differ significantly among spawning periods (Table 1; $F = 0.04$, df = 2, $P = 0.96$). Neutral lipids and phospholipids of eggs comprised 77.5 ± 4.7% and 22.5 ± 4.7% of total lipids, respectively. No significant difference was observed in the proportions of neutral lipids and phospholipids throughout the spawning season (Table 1; $H = 1.12$, df = 2, $P = 0.57$). The average egg lipid concentration was 12% ± 1.3% of wet weight and was unrelated to egg diameter (Figure 2; $F = 0.93$, df = 76, $P = 0.34$, $r^2 = 0.01$).
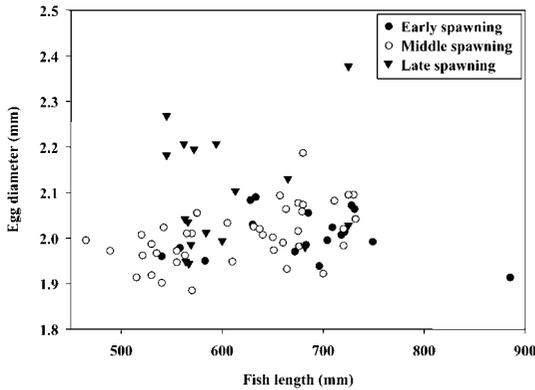
FIGURE 1.—Relationship between maternal total length and diameter of eggs produced by female walleyes ($n = 77$) collected from Salt Fork Reservoir, Ohio, in 1997. Spawning periods are defined as follows: early, March 21–24; middle, March 25–31; and late, April 1–5.
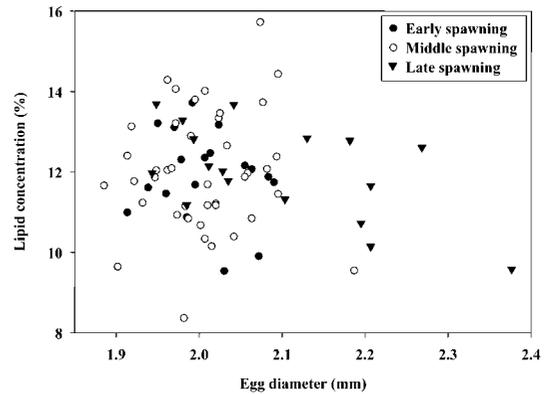

FIGURE 2.—Relationship between the average diameter and lipid concentration of eggs produced by female walleyes collected from Salt Fork Reservoir, Ohio, in 1997. Spawning periods are defined as follows: early, March 21–24; middle, March 25–31; and late, April 1–5.

The fatty acid compositions in neutral lipid and phospholipid fractions of eggs collected throughout the spawning season are shown in Tables 2 and 3. Regardless of the spawning period, both lipid fractions were characterized by high levels of palmitic acid (16:0), palmitoleic acid (16:1, both n-7 and n-9 isomers), vaccenic and oleic acids (18:1[n-7] and 18:1[n-9], respectively), and DHA. The neutral lipid fraction was also characterized by high levels of linolenic acid (18:3[n-3]), whereas the phospholipid fraction contained high levels of AA and EPA. The egg fatty acid composition of both neutral lipids and phospholipids did not change significantly during the spawning season (Tables 2, 3). The phospholipid fraction contained a higher proportion of saturated fatty acids and n-3 PUFAs than the neutral lipids did (saturated: Mann–Whitney test, $T = 3{,}003$, $P < 0.001$; n-3 polyunsaturated: $t = 36.3$, df $= 152$, $P < 0.001$). The higher proportion of n-3 PUFAs in the phospholipid fraction was mainly due to the higher proportion of DHA ($T = 8{,}932$, $P < 0.001$) and EPA ($t = 38.3$, df $= 152$, $P < 0.001$). In contrast, monounsaturated fatty acids were more abundant in the neutral lipid fraction than in the phospholipid fraction (Tables 2, 3; $T = 3{,}003$, $P < 0.001$) due to the higher concentrations of palmitoleic ($T = 3{,}003$, $P < 0.001$) and oleic ($T = 3{,}080$, $P < 0.001$) acids present in the neutral lipid fraction.

Survival of embryos was high regardless of egg or female size and averaged $90 \pm 8.7\%$, indicating that under laboratory incubation conditions the range of fatty acid concentrations was sufficient to ensure a high rate of hatching. Moreover, there was no significant effect of spawning period (early, middle, and late) on embryo survival (Table 1; $F = 1.31$, df $= 2$, $P = 0.28$). No significant relationship was found between embryo viability and any of the fatty acid concentrations from the neutral lipid or phospholipid fraction (neutral lipid: $F = 1.34$, df $= 76$, $P = 0.19$, $r^2 = 0.29$; phospholipid: $F = 0.84$, df $= 76$, $P = 0.66$, $r^2 = 0.27$). Concentrations of the two major PUFAs (EPA, DHA) in both lipid fractions were not correlated with embryo survival at the pigmented eyed stage (Figure 3; DHA in neutral lipid: $F = 0.0004$, df $= 76$, $P = 0.98$, $r^2 = 0.001$; DHA in phospholipid: $F = 0.72$, df $= 76$, $P = 0.40$, $r^2 = 0.007$; EPA in neutral lipid: $F = 0.74$, df $= 76$, $P = 0.11$, $r^2 = 0.002$; EPA in phospholipid: $F = 0.87$, df $= 76$, $P = 0.03$, $r^2 = 0.001$).

Discussion

Both smaller and larger walleye females have the potential to reproduce successfully (Scott and Crossman 1973; Johnston and Leggett 2002), and indeed the individuals captured in the trap nets at Salt Fork Reservoir during the reproductive season in 1997 showed no significant variation in egg viability. As reported previously by Serns (1982), Craig et al. (1995), and Bushong et al. (1996), walleye female size does not correlate with egg size. Our results were also consistent with those reported by Johnston and Leggett (2002), who examined 34 walleye populations and indicated that egg size and maternal size were strongly correlated in populations located at the northern or southern

TABLE 2.—Fatty acid composition (mean $\pm$ SD, expressed as percent of total lipids detected) of neutral lipids in walleye eggs collected from Salt Fork Reservoir, Ohio, in 1997. The concentration of each fatty acid did not differ significantly (ANOVA: $P > 0.05$) throughout the spawning season. See footnote 2 in the text for an explanation of the fatty acid notation; see Table 1 for spawning season definitions.

Fatty acid	Spawning season			
	Early	Middle	Late	Entire
14:0	4.5 $\pm$ 0.9	4.6 $\pm$ 1.0	4.4 $\pm$ 0.4	4.5 $\pm$ 0.9
15:0	0.9 $\pm$ 0.3	1.1 $\pm$ 0.2	1.0 $\pm$ 0.3	1.0 $\pm$ 0.3
16:0	8.3 $\pm$ 0.6	8.0 $\pm$ 0.5	8.1 $\pm$ 0.3	8.1 $\pm$ 0.5
16:1[a]	17.0 $\pm$ 0.7	16.6 $\pm$ 1.4	17.0 $\pm$ 1.3	16.8 $\pm$ 1.3
16:4(n-3)	2.7 $\pm$ 0.3	2.7 $\pm$ 0.3	2.6 $\pm$ 0.2	2.7 $\pm$ 0.3
17:0	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2	0.6 $\pm$ 0.1	0.7 $\pm$ 0.2
18:0	0.5 $\pm$ 0.1	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2
18:1[a]	20.7 $\pm$ 1.2	19.3 $\pm$ 3.1	19.4 $\pm$ 1.0	19.8 $\pm$ 2.5
18:2(n-6)	5.5 $\pm$ 0.3	5.4 $\pm$ 0.5	5.4 $\pm$ 0.4	5.4 $\pm$ 0.4
18:3(n-3)	7.9 $\pm$ 0.8	8.8 $\pm$ 1.2	9.1 $\pm$ 0.8	8.6 $\pm$ 1.1
18:4(n-3)	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2 $\pm$ 0.2	0.3 $\pm$ 0.2
20:1[a]	2.4 $\pm$ 0.3	2.7 $\pm$ 0.5	2.7 $\pm$ 0.3	2.6 $\pm$ 0.4
20:2(n-6)	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
20:3(n-6)	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.2 $\pm$ 0.0	0.3 $\pm$ 0.1
20:4(n-6)	3.2 $\pm$ 0.2	3.2 $\pm$ 0.3	2.9 $\pm$ 0.2	3.1 $\pm$ 0.3
20:4(n-3)	1.1 $\pm$ 0.1	1.2 $\pm$ 0.2	1.2 $\pm$ 0.1	1.2 $\pm$ 0.2
20:5(n-3)	4.5 $\pm$ 0.3	4.6 $\pm$ 0.4	4.7 $\pm$ 0.9	4.6 $\pm$ 0.5
22:4(n-6)	0.8 $\pm$ 0.1	1.0 $\pm$ 0.7	1.1 $\pm$ 0.9	1.0 $\pm$ 0.7
22:5(n-6)	3.2 $\pm$ 1.3	3.3 $\pm$ 1.5	3.2 $\pm$ 0.6	3.3 $\pm$ 1.3
22:5(n-3)	2.1 $\pm$ 0.4	2.0 $\pm$ 0.3	2.1 $\pm$ 0.3	2.1 $\pm$ 0.3
22:6(n-3)	12.2 $\pm$ 0.8	12.4 $\pm$ 1.0	12.5 $\pm$ 0.8	12.3 $\pm$ 0.9
All saturated	14.9 $\pm$ 1.4	15.0 $\pm$ 1.2	14.6 $\pm$ 0.7	14.8 $\pm$ 1.7
All monounsaturated	40.2 $\pm$ 1.7	38.8 $\pm$ 2.9	39.0 $\pm$ 1.6	39.2 $\pm$ 2.3
All polyunsaturated	41.2 $\pm$ 2.0	42.7 $\pm$ 2.7	42.9 $\pm$ 1.7	42.2 $\pm$ 2.5
All n-3	30.8 $\pm$ 1.3	32.1 $\pm$ 2.2	32.0 $\pm$ 1.7	32.3 $\pm$ 2.0
All n-6	13.1 $\pm$ 1.5	13.2 $\pm$ 1.3	13.5 $\pm$ 1.8	13.3 $\pm$ 1.4
All n-3 and n-6	2.4 $\pm$ 0.3	2.5 $\pm$ 0.3	2.4 $\pm$ 0.3	2.4 $\pm$ 0.3

[a] Includes both (n-7) and (n-9) isomers.

limits of walleye native distribution but less significantly correlated in mid-range populations. The examined population in Ohio is in the middle range of walleye geographical distribution.

The average total lipid concentration and the fatty acid compositions in both neutral lipid and phospholipid fractions of walleye eggs were consistent with those found in the same population during three previous years (Czesny and Dabrowski 1998). Moreover, these results were similar to those reported for walleye eggs from a Lake Erie stock (Czesny and Dabrowski 1998) and from Lake Superior (Wiegand et al. 2000).

It has been demonstrated that within a given species, egg size significantly affects survival (Heath et al. 2003), early life history, and growth of larvae (Miller et al. 1988; Bromage 1995). It is commonly accepted that larger eggs have better survival (Heath et al. 2003) and produce larger offspring (Elliott 1984; Thorpe et al. 1984; Hinckley 1990; Johnston 1997). Larvae from bigger eggs have larger mouth gapes (Knutsen and Tilseth 1985), better feeding success (Knutsen and Tilseth

1985; Moodie et al. 1989), faster growth (Wallace and Aasjord 1984), greater resistance to starvation (Marsh 1986), and a lower mortality rate (Elliott 1984; Hutchings 1991). Kamler (1992) suggested that mid-size female rainbow trout *Oncorhynchus mykiss* within a single population often produce the largest eggs, whereas the smallest or oldest females tend to produce smaller eggs (Kamler 1992). High metabolic energy demand of older and larger females prevents them from producing high-quality eggs, whereas the smallest fish in the population are generally known to produce smaller and thus lower quality eggs. If quality (viability) can be correlated with size, it is logical to assume that the mid-size females should produce the highest quality eggs. In our study, however, this relationship was not apparent, suggesting that factors other than egg size alone drive egg viability.

Fatty acid composition largely influences the quality of eggs (Kjorsvik et al. 1990). A deficiency of n-3 fatty acids, particularly EPA and DHA, caused physiological dysfunction of the developing embryo and increased the incidence of early

TABLE 3.—Fatty acid composition (mean ± SD, expressed as percent of total lipids detected) of phospholipids in walleye eggs collected from Salt Fork Reservoir, Ohio, in 1997. The concentration of each fatty acid did not differ significantly (ANOVA: $P > 0.05$) throughout the spawning season. See footnote 2 in the text for an explanation of the fatty acid notation; see Table 1 for spawning season definitions.

| Fatty acid	Spawning season			
	Early	Middle	Late	Entire
14:0	1.4 ± 0.2	1.5 ± 0.3	1.3 ± 0.1	1.4 ± 0.2
15:0	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
16:0	18.1 ± 0.9	17.6 ± 1.2	17.6 ± 1.0	17.7 ± 1.1
16:1[a]	5.3 ± 0.3	5.4 ± 0.5	5.2 ± 0.3	5.3 ± 0.4
16:4(n-3)	1.0 ± 0.2	1.0 ± 0.3	1.1 ± 0.4	1.0 ± 0.3
17:0	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
18:0	5.2 ± 0.4	5.3 ± 0.5	5.3 ± 0.4	5.3 ± 0.5
18:1[a]	10.0 ± 0.8	9.9 ± 0.9	9.8 ± 0.7	9.9 ± 0.8
18:2(n-6)	1.1 ± 1.4	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
18:3(n-3)	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.2
18:4(n-3)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20:1[a]	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
20:2(n-6)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20:3(n-6)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
20:4(n-6)	9.1 ± 1.2	8.7 ± 0.9	8.5 ± 0.7	8.8 ± 1.0
20:4(n-3)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
20:5(n-3)	7.8 ± 0.5	8.0 ± 0.6	7.9 ± 0.5	8.0 ± 0.5
22:4(n-6)	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.2
22:5(n-6)	2.7 ± 0.3	2.9 ± 0.5	3.3 ± 1.4	3.0 ± 0.8
22:5(n-3)	2.2 ± 0.5	2.2 ± 0.8	2.2 ± 0.7	2.2 ± 0.7
22:6(n-3)	29.6 ± 1.1	29.5 ± 1.9	29.8 ± 1.3	29.6 ± 1.6
All saturated	26.5 ± 1.0	26.5 ± 1.7	26.2 ± 0.9	26.4 ± 1.4
All monounsaturated	15.8 ± 1.0	15.9 ± 1.2	15.6 ± 0.9	16.7 ± 1.1
All polyunsaturated	55.8 ± 1.2	55.8 ± 2.3	56.1 ± 1.0	56.8 ± 1.8
All n-3	42.6 ± 1.2	43.0 ± 2.2	43.2 ± 1.6	43.0 ± 1.9
All n-6	14.2 ± 1.6	13.8 ± 1.1	14.0 ± 1.4	13.9 ± 1.2
All n-3 and n-6	3.0 ± 0.4	3.1 ± 0.3	3.1 ± 0.4	3.1 ± 0.3

[a] Includes both (n-7) and (n-9) isomers.
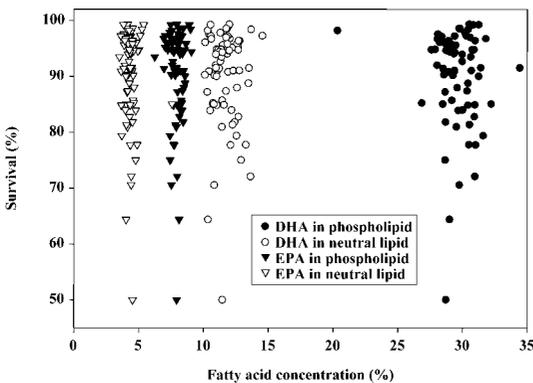
FIGURE 3.—Relationships between the percentages of eicosapentaenoic acid (EPA; 20:5[n-3]) and docosahexaenoic acid (DHA; 22:6[n-3]) in the neutral lipid or phospholipid fraction of walleye egg lipids and embryo survival at the pigmented eyed stage. Eggs were produced by females collected in Salt Fork Reservoir, Ohio, in 1997.

embryonic mortality (Watanabe 1982). Moodie et al. (1989) reported that walleye larvae hatched from small eggs deficient in n-3 PUFAs had higher incidences of body deformities and higher mortality. In the present study, the survival of embryos was generally very high, which suggests that the fatty acid composition of egg lipids satisfied the embryonic nutritional needs. However, as reported by Moodie et al. (1989), the monitoring of embryo survival to 7–12 d posthatch would provide more conclusive results about progeny viability.

In conclusion, the different indicators used in this study, including female size, egg diameter, egg biochemical composition, and embryo survival, suggest that egg and larval quality do not vary during the walleye spawning season. No significant relationship was found between lipid or fatty acid composition of walleye eggs and female size, egg size, and embryo survival. The quality of walleye eggs was consistently high throughout the spawning season and was not affected by female size. Thus, the collection of walleye gametes for hatchery programs can be performed during the

entire spawning season without compromising off-spring survival.

References

Bromage, N. R. 1995. Broodstock management and seed quality: general considerations. Pages 1–25 *in* N. R. Bromage and R. J. Roberts, editors. Broodstock management and egg and larval quality in fish. Blackwell Scientific Publications, Oxford, UK.

Bushong, D. L., I. R. Adelman, and A. S. Csallany. 1996. Vitamin E, fatty acids, and other characteristics of eggs from the fish, walleye (*Stizostedion vitreum vitreum* Mitchill). Recent Research and Development in Nutrition 1:27–32.

Craig, J. F., J. A. Babaluk, S. G. Stevenson, and P. C. Williams. 1995. Variation in growth and reproduction of walleye (*Stizostedion vitreum*) in three Manitoba lakes. Canadian Journal of Zoology 73:367–372.

Czesny, S., and K. Dabrowski. 1998. The effect of fatty acids concentration in wild and domesticated walleye (*Stizostedion vitreum*) eggs on embryos' viability. Aquatic Living Resources 11:371–378.

Czesny, S., S. Kolkovski, K. Dabrowski, and D. Culver. 1999. Growth, survival, and quality of juvenile walleye *Stizostedion vitreum* as influenced by n-3 HUFA enriched *Artemia nauplii*. Aquaculture 178:103–115.

Elliott, J. M. 1984. Numerical changes and population regulation in young migratory trout *Salmo trutta* in a Lake District stream, 1966–83. Journal of Animal Ecology 53:327–350.

Fenton, R., J. A. Mathias, and G. G. E. Moodie. 1996. Recent and future demand for walleye in North America. Fisheries 21(1):6–12.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biology and Chemistry 226:497–509.

Heath, D. D., J. W. Heath, C. A. Bryden, R. M. Johnson, and C. W. Fox. 2003. Rapid evolution of egg size in captive salmon. Science 299:1738–1740.

Hinckley, S. 1990. Variation of egg size of walleye pollock *Theragra chalcogramma* with a preliminary examination of the effect of egg size on larval size. Fishery Bulletin (Washington, D.C.) 88:471–483.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution 45:1162–1168.

Johnston, T. A. 1997. Within-population variability in egg characteristics of walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*). Canadian Journal of Fisheries and Aquatic Sciences 54:1006–1014.

Johnston, T. A., and W. C. Leggett. 2002. Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83:1777–1791.

Kamler, E. 1992. Early life history of fish: an energetic approach. Chapman and Hall, London.

Kjorsvik, E., A. Mangor-Jensen, and I. Homelfjord. 1990. Egg quality in fishes. Advances in Marine Biology 31:71–113.

Knutsen, O. S., and S. Tilseth. 1985. Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Transactions of the American Fisheries Society 114:507–511.

Latif, M. A., R. A. Bodaly, T. A. Johnston, and R. J. P. Fudge. 1999. Critical stage in developing walleye eggs. North American Journal of Aquaculture 61:34–37.

Marsh, E. 1986. Effects of egg size on offspring fitness and maternal fecundity in the orangethroat darter, *Etheostoma spectabile* (Pisces: Percidae). Copeia 1986:18–30.

Mauk, R. J., and M. L. Brown. 2001. Performance of walleye progeny from Missouri river tributaries in South Dakota. North American Journal of Aquaculture 63:167–170.

Miller, T. J., L. B. Crowder, J. A. Rice, and E. A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Canadian Journal of Fisheries and Aquatic Sciences 46:516–521.

Metcalfe, L. D., and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Analytical Chemistry 33:363–364.

Mion, J. B., R. A. Stein, and E. A. Marschall. 1998. River discharge drives survival of larval walleye. Ecological Applications 8:88–103.

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

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Ottawa.

Serns, S. L. 1982. Walleye fecundity, potential egg deposition, and survival from egg to fall young-of-year in Escanaba Lake, Wisconsin, 1979–1981. North American Journal of Fisheries Management 4:388–394.

SPSS. 1997. SigmaStat for Windows, version 2.03. SPSS, Chicago.

Summerfelt, R. C. 1996. Walleye culture manual. Iowa State University, North Central Regional Aquaculture Center Culture Publications Office, NCRAC Culture Series 101, Ames.

Thorpe, J. E., M. S. Miles, and D. S. Keay. 1984. Developmental rate, fecundity, and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture 43:289–305.

Wallace, J. C., and D. Aasjord. 1984. An investigation

of the consequences of egg size for the culture of Artic charr, *Salvelinus alpinus* (L.). Journal of Fish Biology 24:427–435.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology 72B:3–15.

Wiegand, M. D., T. A. Johnston, and J. Brooks. 2000. Egg neutral and polar lipid fatty acid compositions from seven reproductively isolated populations of walleye (*Stizostedion vitreum*). Pages 88–91 *in* B. Norberg, O. S. Kjesbu, G. L. Taranger, E. Andersson, and S. O. Stefansson, editors. Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish. Institute of Marine Research and University of Bergen, Bergen, Norway.

Weigand, M. D., T. A. Johnston, J. M. Martin, and W. C. Leggett. 2004. Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Canadian Journal of Fisheries and Aquatic Sciences 61:110–121.