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The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye *(Stizostedion vitreum)*

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Abstract – Eggs from three distinct populations of walleye *Stizostedion vitreum*, one domesticated (London State Fish Hatchery) and two wild (Lake Erie and Salt Fork Reservoir), were compared in terms of total lipid content and fatty acid profiles (phospholipids and neutral lipids). The concentrations of total lipids in eggs from domesticated broodstock were significantly lower (8.6 ± 1.0 % of wet weight) than those of both wild populations (13.3 ± 0.9 % and 10.9 ± 0.6 % of wet weight for Lake Erie and Salt Fork Reservoir, respectively). The profiles of fatty acids in egg lipids differed significantly among the three investigated populations. Domesticated females fed a formulated diet produced eggs containing significantly higher levels of linoleic acid (18:2n-6), characteristic of plant lipids. However, arachidonic acid (20:4n-6), was at significantly higher levels in eggs of wild walleye stocks. Although eicosapentaenoic acid (20:5n-3) was detected at significantly higher levels in eggs from Lake Erie walleye, docosahexaenoic acid (22:6n-3), the most abundant in polar lipids, was found at similar levels in eggs of all three populations. Survival of walleye embryos was correlated with the concentrations of polyunsaturated long chain fatty acids. Our data suggest that deficiency in n-3 fatty acids might be associated with impaired development of walleye, and thus poor larval and juvenile viability. © Ifremer/Elsevier, Paris

Fish eggs / lipids / fatty acids / *Stizostedion vitreum*

Résumé – Influence de la concentration des œufs en acides gras sur la viabilité des embryons du (sandre) doré jaune sauvage et d'élevage (*Stizostedion vitreum*). Des œufs de trois populations distinctes du poisson *Stizostedion vitreum* ont été comparés en termes de lipides totaux et de profils d'acides gras (phospholipides et lipides neutres) ; une population provenant d'un élevage (écloserie de poissons de London State) et deux populations sauvages (du lac Érié et du réservoir de Salt Fork). Les concentrations en lipides totaux dans les œufs de la souche d'élevage sont significativement plus faibles (8,6 ± 1,0 % du poids humide) que celles des deux populations sauvages (13,3 ± 0,9 % et 10,9 ± 0,6 % du poids humide, respectivement pour le lac Érié et le réservoir de Salt Fork). Les profils d'acides gras dans les œufs diffèrent de façon significative parmi les trois populations étudiées. Les femelles d'élevage sont nourries avec un aliment composé et produisent des œufs contenant des niveaux plus élevés d'acide linoléïque (18:2n-6), caractéristique des lipides de végétaux. Cependant, l'acide arachidonique (20:2n-6) est à des niveaux plus élevés dans les œufs des stocks de dorés jaunes sauvages. Bien que l'acide eicosapenténoïque (20:5n-3) soit détecté à des niveaux plus élevés dans les œufs des dorés jaunes du lac Érié, l'acide docosahexénoïque (22:6n-3), le plus abondant en lipides polaires est trouvé à des niveaux similaires dans les œufs des trois populations. La survie des embryons de dorés jaunes est corrélée avec les concentrations en acides gras polyinsaturés à longues chaînes. Nos données conduisent à penser que le déficit en acides gras n-3 peut être associé avec un arrêt du développement du doré jaune, et donc une faible viabilité des stades larvaires et des juvéniles. © Ifremer/Elsevier, Paris

Œufs / lipides / acides gras / *Stizostedion vitreum*

1. INTRODUCTION

Walleye *Stizostedion vitreum* is being considered as a potential aquaculture species because of the limited number of commercial catches in the Great Lakes region. Therefore, a better understanding of biochemical events in the walleye's early life history would be useful for propagation. The morphological stages of walleye development have been well defined [22]. However, morphological events were not related to metabolic and biochemical changes during ontogeny [17]. The period of yolk absorption and transition from endogenous to exogenous feeding is particularly critical to eleutheroembryo survival and larval recruitment. Moreover, the high mortality experienced among walleye embryos may be related to insufficient nutri-

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tional status of the eggs [24] and consequently, the nutritional history of females during gametogenesis.

In their geographical range, walleye often exhibit high year-to-year variation in larval production [13]. This variation may be in part explained by the variable abiotic conditions during or shortly after spawning. Another source of variation may be size and age structure of the spawning population [12].

The quality of maternal nutrition has a direct influence on egg quality and hence on embryo and larval development throughout the period of endogenous feeding. Lipids are the second most abundant egg constituent after proteins [6] and play a major role as membrane components and a primary source of energy in fish embryos [32]. Unbalanced maternal diets negatively affect fecundity and egg viability, resulting in embryos with yolk reserves deficient in some important nutrients such as essential fatty acids [21].

Fatty acids with n-3 and n-6 configurations are necessary for the normal development of fish. A deficiency of n-3 fatty acids in the rainbow trout *Oncorhynchus mykiss* broodstock diet decreases the number and size of eggs [35], increases the incidence of early embryonic mortality, and causes physiological dysfunction in the developing fish [34]. Reduced hatching success as well as poor larval viability associated with low n-3 polyunsaturated fatty acid (PUFA) levels have been observed in experimental studies in rainbow trout [21]. In contrast, a higher egg production, fertilization and hatching rates were observed in gilthead seabream when broodstock was fed a diet containing 1.8 % (n-3) HUFA compared to fish fed (n-3) HUFA deficient diets [27].

We hypothesized that eggs from walleye broodstocks of various origins (domestic and feral) and life history (migratory and non-migratory) may differ from each other in terms of essential fatty acid concentrations due to differences in dietary lipids. The present study was conducted to determine whether there are differences among cultured walleye stock in London State Fish Hatchery, Ohio (LO), a heavily stocked reservoir population, Salt Fork Reservoir, Ohio, (SF) and the wild population from Lake Erie (LE). We examined the lipid content in eggs and fatty acid composition of two classes, neutral lipids (mostly triglycerides) and polar lipids (mostly phospholipids). Finally, we asked the question whether different levels of fatty acids influence the viability of embryos.

2. MATERIALS AND METHODS

2.1. Egg sampling

Eggs of cultured fish (LO) were sampled from walleye kept for several generations in London State Fish Hatchery, Ohio Division of Wildlife [26], during routine egg collection (spring 1994 and 1995). The LO stock was obtained in 1975, 1976 and 1977 from the Pymaturing and Mosquito Reservoirs. It is believed, however, that the original source of walleye in these reservoirs was Lake Erie. Starting from the F_2 generation of domesticated walleye, fish were fed with floating trout chow (PMI Nutrition International Inc., Brent Wood, MO). Eggs from walleye in Salt Fork Reservoir (SF) were sampled during egg collection by Senecaville State Fish Hatchery personnel in the spring of 1994, 1995 and 1996. The eggs from Lake Erie fish were obtained during their upstream spawning run in the Maumee River in the spring of 1995 and 1996. Total length of each female at all locations was taken prior to their release (± 0.5 cm).

Samples (5 g) of eggs from individual, completely ovulated females were taken during stripping before fertilization. For lipid concentration and fatty acid profiles analysis, eggs were placed in small plastic containers, immediately frozen in liquid nitrogen, and then stored in a bio-freezer at –86 °C prior to biochemical analysis. Additional portions (5 g) of eggs were sampled from each fish and fertilized with sperm obtained from several walleye males. Immediately after fertilization, eggs were treated with a 400-ppm solution of tannic acid for 3 min and stirred continuously in order to prevent egg-to-egg adhesion. Eggs were then washed with water. Fertilized eggs were transported (5–6 °C) to the Ohio State University aquaculture laboratory for incubation. Walleye embryos from each female were incubated separately in order to determine egg lipid profile on survival rate to the pigmented eyed-stage. The eggs from each female were incubated in separate trays (in 3 replicates) until they reached that stage. This occurred 10 to 14 d after fertilization depending on the water temperature in a given year (11 to 15 °C). Eyeing success of the embryos was calculated as the mean percentage of viable eggs from each female.

2.2. Lipid and fatty acid analyses

Total lipids were extracted from homogenized egg samples according to the procedure of Folch et al. [9]. Crude lipid extracts were separated into polar (phospholipids) and neutral (mostly triglycerides) fractions using silica sep-pak cartridges (Waters, Division of Millipore Corp., Milford, MS). The residual lipid (≅ 0.15 g) was applied on a silica filter and the neutral lipids were eluted with 30 mL chloroform. After elution of neutral lipids, the polar fraction was eluted with 30 mL methanol. Solvents from both fractions were then evaporated under nitrogen and the amounts of polar and neutral lipids were determined gravimetrically. Fatty acid methyl esters mixtures were prepared from both fractions of lipids according to Metcalfe and Schmitz [23]. The crude lipid extract was saponified with NaOH in methanol and fatty acid methyl esters were prepared by transmethylation with BF_3 in methanol. Fatty acid methyl esters were obtained on a Varian 3300 Gas Chromatograph equipped with a glass column (custom-made 304.8 cm × 6.35 mm OD × 2 mm

ID), packed with 10 % SP 2330 on 100/120 SUPELCOPORT. The carrier gas was helium with a pressure of 80 psi. The thermal gradient was 175 °C for 26 min, then increased by 2 °C·min^{-1} to 205 °C and held at 205 °C for 30 min. The individual fatty acids were identified by comparing their retention times with the retention times of known fatty acids, available in mixtures of external standards (Nu-Chek-Prep Inc., Elysian, MN). The fatty acids were quantified (% by weight) by comparing their peaks areas with that of the peak of a known amount of an added internal standard, C19:0 (nonadecanoate).

2.3. Statistical analyses

Data are presented as means ± SD. One-way ANOVA, followed by Scheffe's F-test was used for mean comparisons (StatView^{TM} 512+; Brain Power Inc., Calabasas, CA). Percentage data were subjected to arc sine transformation. Linear regressions were performed between the survival of walleye embryos and the concentrations of polyunsaturated long chain fatty acids. Rejection level for all statistical analyses was set at $P = 0.05$.

3. RESULTS

Females from the LO domesticated stock were significantly smaller (mean length 440 ± 39 mm, $n = 8$) than females from either the reservoir stock SF (665 ± 44 mm, $n = 17$) or Lake Erie stock (630 ± 62 mm, $n = 15$). No significant differences were noted between SF and LE stocks.

3.1. Total lipids and fatty acid profiles

Total lipid concentrations in walleye eggs sampled from three different populations, during three consecutive spawning seasons are presented in *figure 1*. The highest levels of total lipids were found in eggs sampled from the Lake Erie walleye population, averaging 13.3 ± 0.9 % ($n = 21$) of wet egg weight. LO walleye population yielded eggs with the lowest total lipid content. For both years, lipids in the eggs comprised 8.6 ± 1.0 % ($n = 13$) of wet weight. Intermediate amounts of lipids were found in walleye eggs from Salt Fork Reservoir. Significant differences in egg lipid content were found among walleye populations ($P = 0.0001$). The amounts of phospholipids were significantly lower in cultured fish eggs (*figure 1*), although the relative amount (percentage of total lipids) was not altered and varied between 18 and 20 % for fish from all examined locations.

Lipid analysis revealed high consistency in fatty acid profiles among populations regardless of the sampling season. Therefore, we have pooled the data from different years together and considered them as three separate populations for comparison. The fatty acids (FA) profiles of egg lipids sampled from London State Fish Hatchery, Salt Fork Reservoir, and Lake Erie, separated into neutral (NL) and polar (PL) fractions, are given in *tables I* and *II*, respectively. The sums of saturated FA (SAFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), n-3, and n-6 include all FA identified.

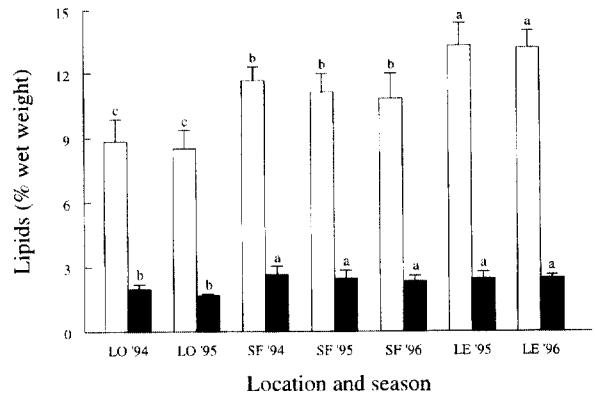

Figure 1. Concentrations of total lipids (white bars) and phospholipids (dark bars) in eggs sampled from ovulated walleye females. London State Fish Hatchery (LO), Salt Fork Reservoir (SF) and Lake Erie (LE). Values across common bars with different letters differ at $P < 0.05$.

Among saturated fatty acids, the most abundant was palmitic acid (16:0) in all three groups. The monoenoic fatty acids were represented by two major compounds, palmitoleic acid (16:1) and oleic acid (18:1). A significantly higher concentration of linoleic acid (18:2n-6) was found in the NL fraction of lipids from eggs of the LO stock than in those from both wild populations. In contrast, linolenic acid (18:3n-3), arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3) (EPA) and docosahexaenoic acid (22:6n-3) (DHA) were significantly higher in NL lipid fraction from wild fish eggs than in eggs from domesticated fish. The differences in the large number of fatty acids from the NL fraction in the three investigated populations resulted in significantly lower concentrations of n-6 fatty acids in wild eggs than in cultured eggs. This fact, together with the opposite tendency in the case of n-3 fatty acid concentrations resulted in n-3/n-6 ratios that were 2- to 3-fold higher in the neutral lipids of wild eggs than in cultured eggs (*figure 2*).

The differences in fatty acid profiles of polar lipids between wild and cultured walleye stocks were not as pronounced as for neutral lipids (*table II*). We detected a similar reciprocal pattern in regard to linoleic (18:2 n-6) and linolenic (18:3n-3) acids; the former was at a significantly higher level in domesticated fish eggs than in wild fish eggs, whereas the latter showed the opposite trend. Furthermore, eggs from wild fish contained significantly higher concentrations of arachidonic (20:4n-6) acid than did eggs from cultured fish. Although EPA (20:5n-3) was detected at significantly higher levels in eggs from Lake Erie, DHA (22:6n-3),

Table I. Fatty acid composition of walleye egg neutral lipids (% of weight of total neutral lipids ± SD). Means across rows with different superscript differ at $P < 0.05$. Means were tested by ANOVA.

Fatty acids	London State Fish Hatchery (1994 and 1995) $n = 12$	Salt Fork Reservoir (1994–1996) $n = 39$	Lake Erie (1995 and 1996) $n = 21$
Saturated			
14:0	1.52 ± 0.18 [c]	2.47 ± 0.37 [a]	2.06 ± 0.19 [b]
16:0	5.76 ± 0.96 [b]	5.53 ± 0.42 [b]	6.52 ± 0.57 [a]
18:0	0.35 ± 0.11 [a]	0.33 ± 0.08 [b]	0.44 ± 0.18 [a]
Monoenoic*			
16:1	11.00 ± 1.21 [b]	11.41 ± 0.95 [b]	15.19 ± 2.00 [a]
18:1	16.58 ± 1.76 [a]	14.39 ± 1.19 [b]	16.92 ± 1.08 [a]
20:1	0.92 ± 0.15 [b]	1.60 ± 0.33 [a]	1.43 ± 0.19 [a]
n-6			
18:2n-6	10.03 ± 1.25 [a]	3.82 ± 0.38 [b]	3.09 ± 0.60 [c]
20:2n-6	0.09 ± 0.04 [b]	0.16 ± 0.06 [a]	0.10 ± 0.05 [b]
20:4n-6	0.50 ± 0.18 [c]	2.22 ± 0.29 [a]	1.52 ± 0.31 [b]
22:4n-6	0.48 ± 0.32 [a]	0.59 ± 0.20 [a]	0.59 ± 0.07 [a]
22:5n-6	1.40 ± 0.90 [b]	2.54 ± 0.71 [a]	1.28 ± 0.35 [b]
n-3			
16:3n-3	0.33 ± 0.04 [a]	0.24 ± 0.04 [b]	0.25 ± 0.06 [b]
16:4n-3	0.71 ± 0.09 [c]	1.85 ± 0.21 [a]	1.51 ± 0.34 [b]
18:3n-3	1.06 ± 0.16 [c]	5.59 ± 0.99 [a]	3.67 ± 0.37 [b]
18:4n-3	0.31 ± 0.04 [a]	0.03 ± 0.08 [b]	0.32 ± 0.05 [a]
20:4n-3	0.45 ± 0.19 [b]	0.82 ± 0.27 [a]	0.83 ± 0.18 [a]
20:5n-3	2.29 ± 0.81 [c]	3.13 ± 0.71 [b]	4.38 ± 0.36 [a]
22:5n-3	1.01 ± 0.31 [c]	1.32 ± 0.17 [b]	1.64 ± 0.17 [a]
22:6n-3	5.79 ± 0.90 [c]	8.01 ± 0.88 [a]	7.00 ± 1.37 [b]
Total saturated	8.19 ± 1.35 [b]	8.45 ± 0.77 [a b]	9.03 ± 0.69 [a]
Total monoenoic	28.50 ± 2.89 [b]	27.40 ± 2.00 [b]	33.54 ± 2.13 [a]
Total polyunsaturated	24.92 ± 3.05 [b]	30.75 ± 3.30 [a]	26.60 ± 2.01 [b]

*Includes both (n-7) and (n-9) isomers.

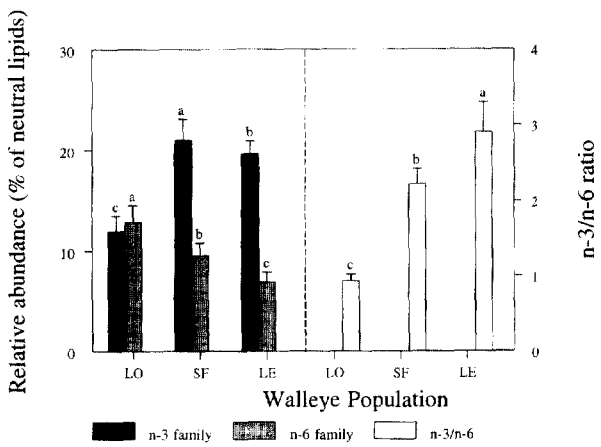

Figure 2. Relative amounts of n-3 and n-6 fatty acids in neutral lipids and n-3/n-6 ratio in eggs sampled from ovulated female walleye from London State Fish Hatchery (LO), Salt Fork Reservoir (SF) and Lake Erie (LE). Values across common bars with different letters differ at $P < 0.05$.

the most abundant in PL, was found at similar levels in eggs from all three walleye populations. The sums of PUFA in the polar fraction of egg lipids from both wild populations were significantly larger than those from domesticated stock.

3.2. Survival of walleye embryos

We found that survival rate varied greatly among individual females. The survival rate of embryos obtained from Salt Fork Reservoir ranged from 33 up to 78 % in 1994 and from 10 to 92 % in 1995. In the case of the London State Fish Hatchery in 1995, the survival rate was similarly variable, with the lowest value at 9.5 % and the highest at 87 %. Four out of six sets of embryos originating from Lake Erie in 1995 had survival rates of 81 % or higher, whereas the lowest was 32 %. The survival of the embryos from Lake Erie in 1996 ranged from 45 to 95 % among the 15 observed sets of eggs.

The correlation between the survival rates of embryos and the concentration of EPA (20:5n-3) and DHA (22:6n-3) in egg neutral lipids were demonstrated for samples from Salt Fork Reservoir in 1994 (*figures 3* and *4*). The correlation coefficients were 0.9 ($n = 5$, $P = 0.01$) and 0.89 ($n = 5$, $P = 0.01$), respectively. Nevertheless, we were not able to confirm these observations in the following years. In 1995, there was no significant correlation between either EPA or DHA and survival of the embryos (*figures 3* and *4*). Furthermore, in 1996, a significant negative correlation between egg DHA content and embryo survival was found (*figure 4*). The concentrations of fatty acids from

Table II. Fatty acid composition of walleye egg polar lipids (% of weight of total polar lipids ± SD). Means across rows with different superscript differ at $P < 0.05$. Means were tested by ANOVA.

Fatty acids	London State Fish Hatchery (1994 and 1995) $n = 8$	Salt Fork Reservoir (1994–1996) $n = 35$	Lake Erie (1995 and 1996) $n = 21$
Saturated			
14:0	0.75 ± 0.10^{b}	0.89 ± 0.13^{a}	0.66 ± 0.08^{b}
16:0	10.88 ± 0.95^{b}	11.57 ± 1.32^{b}	12.41 ± 1.00^{a}
18:0	3.12 ± 0.34^{b}	3.42 ± 0.37^{b}	3.88 ± 0.40^{a}
Monoenoic*			
16:1	3.53 ± 0.61^{a}	3.43 ± 0.46^{a}	3.65 ± 0.33^{a}
18:1	8.41 ± 1.25^{a}	6.71 ± 1.31^{b}	7.77 ± 0.81^{a}
20:1	0.25 ± 0.03^{a}	0.32 ± 0.87^{a}	0.32 ± 0.17^{a}
22:1	0.41 ± 0.10^{b}	0.64 ± 0.15^{a}	0.66 ± 0.10^{a}
n-6			
18:2n-6	1.83 ± 0.61^{a}	0.63 ± 0.48^{b}	0.46 ± 0.09^{b}
20:2n-6	0.16 ± 0.06^{a}	0.26 ± 0.09^{a}	0.24 ± 0.15^{a}
20:4n-6	2.42 ± 1.05^{c}	5.71 ± 0.88^{a}	4.25 ± 0.66^{b}
22:4n-6	0.24 ± 0.06^{b}	0.43 ± 0.12^{a}	0.36 ± 0.11^{a}
22:5n-6	0.51 ± 0.42^{b}	1.93 ± 0.60^{a}	0.80 ± 0.27^{b}
n-3			
18:3n-3	0.21 ± 0.15^{c}	0.71 ± 0.17^{a}	0.49 ± 0.27^{b}
18:4n-3	$0.34 \pm 0.11^{a\,b}$	0.27 ± 0.10^{b}	0.39 ± 0.12^{a}
20:4n-3	0.35 ± 0.11^{a}	0.32 ± 0.07^{a}	0.34 ± 0.09^{a}
20:5n-3	4.78 ± 0.51^{b}	4.96 ± 0.82^{b}	7.46 ± 1.16^{a}
22:5n-3	1.18 ± 0.10^{b}	1.31 ± 0.21^{b}	1.81 ± 0.35^{a}
22:6n-3	18.28 ± 1.50^{a}	19.05 ± 1.95^{a}	18.30 ± 2.04^{a}
Total saturated	14.94 ± 1.35^{b}	$15.92 \pm 1.72^{a\,b}$	16.96 ± 1.29^{a}
Total monoenoic	12.60 ± 1.72^{a}	11.09 ± 1.70^{b}	12.41 ± 0.99^{a}
Total polyunsaturated	30.89 ± 1.92^{b}	35.98 ± 3.61^{a}	35.12 ± 2.89^{a}
Total n-6	5.15 ± 1.10^{b}	8.98 ± 1.20^{a}	6.20 ± 0.88^{b}
Total n-3	25.24 ± 1.84^{b}	26.85 ± 2.89^{b}	28.79 ± 2.61^{a}
n-3/n-6	5.08 ± 1.01^{a}	3.03 ± 0.42^{b}	4.72 ± 0.69^{a}

* Includes both (n-7) and (n-9) isomers.

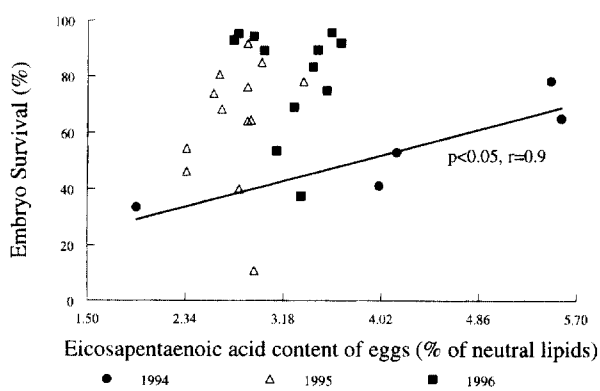

Figure 3. Relationship between survival of Salt Fork Reservoir walleye embryos up to eyed-stage and concentration of eicosapentaenoic acid in the neutral lipids of the eggs. The line represents 1994 data only.

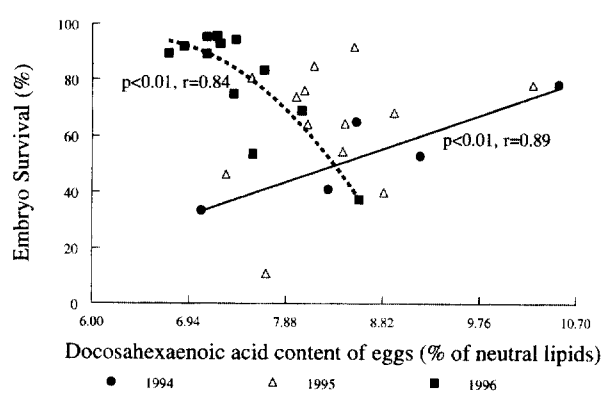

Figure 4. Relationship between survival of Salt Fork Reservoir walleye embryos up to eyed-stage and concentration of docosahexaenoic acid in the neutral lipids of the eggs. The solid line represents 1994 data and the broken line represents 1996 data.

both neutral and polar lipids of walleye eggs, other then EPA and DHA in SF, did not correlate with embryo viability. No correlation was found between total egg lipid content and viability of embryos in any of the walleye population studied.

4. DISCUSSION

The two wild and one domesticated walleye populations evaluated in this study were distinctly different

from each other in terms of egg total lipid content and fatty acid profiles. Notwithstanding the significant differences between ova lipids, we found walleye eggs to be relatively rich in total lipids regardless of broodstock origin. For most freshwater fish species, ripe eggs contain between 2 and 10 % lipids based on wet weight. Kaitaranta and Ackman [15] reported that roach *Rutilus rutilus* and perch *Perca fluviatilis* eggs have less then 5 % lipids, whereas vendace *Coregonus albula* and whitefish *Coregonus lavaretus* eggs were reported to contain about 9.7 and 11.5 % lipids, respectively [7, 14]. Compared to other freshwater fish eggs, walleye ova are among the richest in lipids reaching between 10 and 13 % of wet weight in wild populations. Similar values (10–12 % wet weight) were recently reported for walleye eggs from two Minnesota lakes [4]. However, the large increase of water content in the eggs upon ovulation can be a potential source of variation in total lipid content. Therefore, time of sampling with respect to maturation stage of the ova becomes very important [36].

The amount of lipids in eggs can vary not only between species but also within the same species, depending on the nutritional status of the broodstock. The dietary lipids of the commercial feed of captive broodfish differ from those of the wild fish diet. Research on wild and domesticated striped bass eggs demonstrated significantly higher levels of total lipids in wild fish eggs [10]. In contrast, Sheikh-Eldin et al. [30] reported no differences between the levels of egg total lipids obtained from wild and tank-reared macquarie perch *Macquaria australasica*. However, the perch were fed live brown trout *Salmo trutta* fingerlings rather than a formulated diet and this likely contributed to the lack of differences.

A relationship between total lipid content of eggs and their viability has been observed in freshwater fishes. However, contradictory reports exist with respect to the character of this relationship. High egg lipid content increased larval viability in roach [19] as well as in bream [37]. However, a negative effect was observed by Devauchelle et al. [8] in a number of marine fish species such as turbot *Scophthalmus maximus*, sole *Solea solea* and sea bass *Dicentrarchus labrax*. Kamler et al. [16] observed that higher lipid levels in vendace eggs were indicative of higher rates of egg viability, while Sheikh-Eldin et al. [30] found macquarie perch eggs highly variable in terms of viability regardless of lipid content similarity. In our study, we were not able to demonstrate a definite relationship between total egg lipid content and walleye egg viability. Contradictory data in the literature suggest that there are additional sources of variation that need to be considered.

Fatty acid composition in eggs obtained from wild spawning females of a given species should mirror the proportion of specific fatty acids required to secure embryonic development up to the first feeding of newly hatched larvae. Although preferential accumulation of n-3 PUFA in egg lipids has been reported in fish [32], it has been widely documented that fatty acids profiles of broodstock diets are closely reflected in egg lipids [20, 34]. Broodstock diet greatly influenced the fatty acid composition of total lipids in eggs of gilthead seabream *Sparus aurata* [25]. Earlier fish nutrition studies demonstrated that vegetable oil based diets give relatively poor embryo viability in freshwater fishes [31]. The requirement of n-3 PUFA by fish is generally accepted and has been estimated quantitatively, whereas the degree of n-6 PUFA requirement has not yet been established unequivocally [29].

The phospholipid (polar) fraction of walleye egg lipids was noticeably less affected by the different nutritional status of the walleye broodstock fish. The neutral egg lipids from both wild walleye populations were twice as rich in n-3 fatty acids as those of domesticated fish, whereas polar lipids contained similar proportions of n-3 fatty acids in the eggs of all three populations examined. Similarly, conservative deposition of n-3 PUFA into polar lipids of chinook salmon *Oncorhynchus tshawytscha* eggs, regardless of the dietary regime, was observed by Ashton et al. [1]. Furthermore, our results, like those of Ashton et al. [1], contradict the earlier conclusion by Watanabe [33] that dietary lipids have greater effect on eggs' phospholipids fatty acids than on neutral fatty acids. The aspects of dietary lipid incorporation into yolk polar and neutral lipids has been recently reviewed by Wiegand [36].

The levels of linoleic and linolenic acids, precursors of longer chain PUFAs from both the n-3 and n-6 series, are the major differences between fatty acid profiles of neutral egg lipids from cultured and wild walleye stocks. While the eggs from cultured fish contain 2.5–3 times more linoleic acid than linolenic acid, the eggs from wild fish appear to have more balanced amounts of these essential compounds due to their strict carnivorous diet in Lake Erie [18]. The consequence of low levels of linolenic acid coupled with very high levels of linoleic acid in neutral lipids resulted in a very low n-3/n-6 ratio (1.2) in egg lipids from domesticated stock, whereas eggs from Lake Erie walleye reached a ratio of 3.4. Low levels of n-3 fatty acids and consequently low n-3/n-6 ratios have been reported to cause decreased fecundity [35], impaired embryonic development, reduced hatching success, and poor larval viability in rainbow trout [21]. Santiago and Reyes [28] reported that the total n-3/n-6 fatty acid ratio in the tissues influenced the reproductive performance of Nile tilapia *Oreochromis niloticus*. We did not observe any pronounced negative relationship, although the low n-3/n-6 ratio may have caused lower survival rate in embryos of the domesticated walleye.

The importance of (n-3) HUFA, especially EPA and DHA, has been recognized in studies on larval stages of fish [11]. DHA is thought to play an important role in the proper development and function of the retina in rainbow trout [2]. It has also been shown that herring larvae fed a diet deficient in DHA exhibited impaired

foraging behavior associated with obscured vision at low light intensity [3]. Therefore, it would be advantageous for embryos to possess significant amounts of DHA in yolk reserves to sustain high requirement during development. The DHA deficiency in the lipid pool available to embryos should have a negative effect on their survival.

In walleye at the embryonic stage of development, however, we were not able to detect any conforming relationship between EPA or DHA and embryo performance. The year-to-year inconsistency in the character of the relationship between the concentration of EPA and DHA in egg lipids and viability of walleye embryos in our study may be due to the time of sampling which fell during the spawning season. The duration of walleye spawning in wild stocks in Ohio may extend up to 3 weeks depending on the temperature gradient in a particular reservoir or lake. Therefore, in a single population there are early and late spawners and this segregation is determined by the age of both females and males. We sampled walleye eggs each year only during a spawning peak, one to four days, which should minimize the effect of fish age on the results. Egg lipid content is likely to be influenced by egg size which in turn was found to correlate with size and age of walleye females [5]. Hatching success of walleye eggs was positively correlated with female age [12]. Therefore, one can expect intrapopulation variation in egg quality throughout the entire walleye spawning season. However, this hypothesis requires additional research.

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