Contrasting strategies of ova lipid provisioning in relation to maternal characteristics in three walleye (*Sander vitreus*) populations

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Abstract: We examined how the lipid content and fatty acid composition of walleye (*Sander vitreus*) ova varied with respect to maternal characteristics, particularly indices of body nutrient reserves, within three spawning populations that varied in maternal age, size, and somatic lipid reserves. We also compared the variability in ova lipid composition among three populations with that observed among spawning years within one of these populations. Maternal characteristics had little influence on ova lipid content. In Lake Ontario, percentages of docosahexaenoic acid (22:6(*n*-3), DHA) and arachidonic acid (20:4(*n*-6), AA) increased with maternal length, while those of eicosapentaenoic acid (20:5(*n*-3), EPA) declined. In Lake Nipissing, maternal age had similar effects on AA and EPA, but not DHA. The Lake Winnipeg population did not conform to the trends of the other two populations, and ova from this population had very low levels of AA. We hypothesize that there are similar selection pressures on the Lake Ontario and Lake Nipissing populations with respect to desirable ova fatty acid profiles. Lake Winnipeg walleye may experience different selection pressures or may be unable to conform to the trends observed in the other two lakes.

Résumé : Nous examinons la variation du contenu lipidique et de la composition en acides gras des oeufs de dorés (*Sander vitreus*) en fonction des caractéristiques maternelles, en particulier des indices de réserves nutritives corporelles, chez trois populations reproductives qui diffèrent par l'âge maternel, la taille et les réserves lipidiques somatiques. Nous comparons aussi la variabilité de la composition lipidique des oeufs chez les trois populations avec celle obtenue au cours des années de fraie chez l'une de ces populations. Les caractéristiques maternelles ont peu d'influence sur le contenu lipidique des oeufs. Au lac Ontario, les pourcentages d'acides docosahéxaénoïque (22:6(*n*-3), DHA) et arachidonique (20:4(*n*-6), AA) augmentent en fonction de la longueur de la mère, alors que celui de l'acide eicosapentaénoïque (20:5(*n*-3), EPA) décline. Au lac Nipissing, l'âge de la mère a le même effet sur AA et EPA, mais non sur DHA. La population du lac Winnipeg ne suit pas les tendances des autres populations et les oeufs de cette populations contiennent de très faibles concentrations d'AA. Nous formulons l'hypothèse selon laquelle les pressions de sélection concernant les profils souhaitables d'acides gras dans les oeufs sont semblables dans les populations des lacs Ontario et Nipissing. Les dorés du lac Winnipeg peuvent subir des pressions sélectives différentes ou alors ils sont incapables de suivre les tendances observées dans les deux autres lacs.

[Traduit par la Rédaction]

Introduction

The quantity and quality of nutrient reserves provided to individual offspring will influence their early life growth and survival and hence both offspring and maternal fitness (Lack 1954). Iteroparous organisms, including many teleost fishes, face the necessity of a trade-off between investment in an individual year's reproductive effort and maintenance of the parent, potentially through a period of nutrient deprivation, for subsequent episodes of reproduction (Adams 1999). Indeed, some teleosts are known to forego gonad recrudescence if somatic nutrient reserves are low in the pregametogenic period (Trippel and Harvey 1989; Burton 1994; Rideout et al. 2000). Maternal lipid reserves, in particular, are believed to limit reproductive effort in some species (Henderson et al. 1996; Adams 1999; Marshall et al. 1999). Within a spawning stock, it is expected that individual females will differ in the quantity and composition of their stored nutrient reserves, in their available dietary resources, and in the quantity and quality of their ova nutrients. The

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question arises to what extent variation in the proximate factors of maternal age, size, condition, and nutrient status influences ova composition and quality. The influence of fatty acid (FA) composition on quality of ova has been intensively investigated in aquaculture studies (Wiegand 1996*a*; Sargent et al. 1999); however, much less is known about the situation in wild fish.

Recent research has examined the influence of maternal size and lipid status on reproductive effort in walleye (*Sander vitreus*), an iteroparous, broadcast-spawning fish native to central North America. There is evidence from some walleye populations that females of reproductive size may skip reproduction in years when they lack sufficient body lipid reserves (Henderson et al. 1996). Thus, absolute lipid quantity or the quantity of some particular lipid may be limiting to ova production in walleye.

Quality of ova lipid reserves will depend, in large part, on the availability of certain polyunsaturated fatty acids (PUFA), namely docosahexaenoic acid (22:6(*n*-3), DHA), arachidonic acid (20:4(*n*-6), AA), and eicosapentaenoic acid (20:5(*n*-3), EPA), which can influence offspring survival in teleosts, including walleye (Rainuzzo et al. 1997; Czesny and Dabrowski 1998; Czesny et al. 1999). All are quantitatively significant in structural phospholipids (Bell and Dick 1991; Dey et al. 1993). DHA has specific structural roles in nervous tissue, including retina (Sargent et al. 1993), and DHA deficiencies have been associated with behavioural impairment in fish larvae (Bell et al. 1995; Masuda et al. 1998; Ishizaki et al. 2001). A higher supply of DHA available after the start of feeding would be advantageous to the rapid development of membrane systems (Bell and Dick 1993; Tocher 2003). In addition to structural roles, both EPA and AA serve as precursors for families of chemical messengers collectively known as eicosanoids (Tocher et al. 1991; Knight et al. 1995). AA is the preferred substrate for the two eicosanoid synthesis systems (Tocher et al. 1996), and eicosanoids derived from AA are generally more biologically active than those derived from EPA (Tocher 2003). The importance of adequate levels of AA and of appropriate EPA/AA ratios have been recognized for both freshwater and marine fishes. In particular, elevated levels of AA have been associated with superior resistance to infection, egg quality, growth, and in salmonids, adaptation to seawater (Ackman and Takeuchi 1986; Bell and Sargent 2003). By contrast, pathologies have also been associated with low EPA/AA ratios (Sargent et al. 1995). It is apparent that the optimal ratio of EPA/AA is species-specific and related to life history (Sargent et al. 1999). In addition, palmitic acid (16:0) and oleic acid (18:1(*n*-9), OA) have significant quantitative or qualitative roles in structural phospholipids (Bell and Dick 1991; Dey et al. 1993).

The FA profiles of walleye ova lipids have been shown to vary with maternal size in some populations; ova of larger females have higher proportions of DHA and AA and lower ratios of EPA/AA in their lipids (Wiegand et al. 2004). It is possible that these results may be linked; that is, the FA composition of the ova may depend upon the lipid status of the female. To our knowledge, this has yet to be examined.

We expanded upon this earlier research by more closely examining the relationships between indices of ova quality and maternal characteristics in walleye. We studied three walleye populations that varied in maternal size, age, condition, and lipid status to determine how the maternal influence on ova composition may vary spatially. We sampled one of these populations over multiple years to determine if observed differences in ova composition varied temporally. Our primary hypothesis was that ova composition was related to indices of maternal age, size, and condition, particularly maternal lipid status; we predicted that females with higher lipid reserves should have produced higher quality ova in terms of FA profiles. Furthermore, we predicted that the relationship between ova composition and maternal lipid status would be stronger in populations with relatively lower body lipid contents. Our secondary hypothesis was that ova composition varied among spawning years within a population. This latter hypothesis could only be tested in the Lake Ontario population, where sampling was conducted over multiple years.

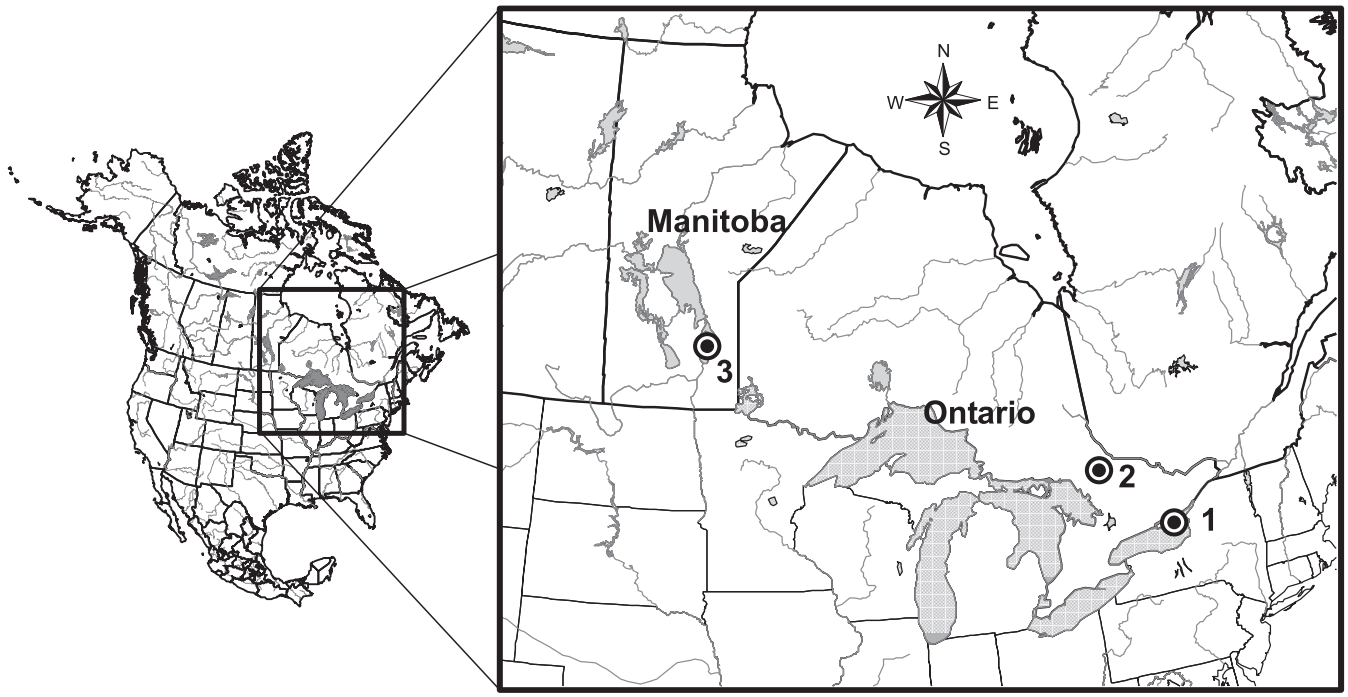
Materials and methods

Field sampling and fish processing

Mature female walleye were captured at the time of spawning over 3 consecutive years in Lake Ontario (44°00′N, 76°40′W) and in single years in Lakes Winnipeg (51°01′N, 96°53′W) and Nipissing (46°12′N, 79°24′W) (Fig. 1). For the Lake Ontario population, fish were captured by dip net from the Napanee River, a spawning tributary of the Bay of Quinte in 2002, and were captured in a trap net set by a shoreline spawning area in the Bay of Quinte in 2003 and 2004. For the Lake Winnipeg population, all females were captured by overnight sets of gill nets at spawning areas along the northwestern shore of the south basin in 2004. For the Lake Nipissing population, all females were captured in a trap net set at the Wasi River inflow to Callander Bay in 2005. Sampling took place from early to late April in Lake Ontario, during early May in Lake Nipissing, and during mid-May in Lake Winnipeg. Females were selected to cover a wide size distribution, and all had free-flowing ova with the exception of several Lake Winnipeg females that had not yet ovulated. The Lake Winnipeg walleye population contains both normal and slow-growing (dwarf) morphotypes of walleye (W. Lysack, Manitoba Conservation, 200 Saulteaux Crescent, Winnipeg, Manitoba R3J 3W3, unpublished data). For the purposes of this study, we restricted our sampling to the normal form by selecting females >400 mm fork length (FL) (Moles 2006). At capture, each female was killed by a blow to the head, tagged with an identification number, and packed on wet ice for transport to the processing site.

Each fish was processed as follows. An ova sample (~150 mL) was stripped (or excised from the ovary midsection in unovulated fish), then divided into subamples that were frozen in small plastic bags at –70 °C for FA analyses and at –20 °C for all other analyses. Females were measured for FL (±10 mm), then weighed without gonads (somatic mass, ±10 g) and without viscera (eviscerated mass, ±10 g). The liver was weighed (±0.1 g), then frozen in a small plastic bag at –20 °C. Sagittal otoliths were removed for age determination. The carcass (without liver or gonads) was then frozen whole at –20 °C. To compare total reproductive effort among the populations, we calculated gonadosomatic index

Fig. 1. Location of Canadian walleye (*Sander vitreus*) populations examined in this study: 1, Lake Ontario (44°00′N, 76°40′W) (Bay of Quinte); 2, Lake Nipissing (46°12′N, 79°24′W); 3, Lake Winnipeg (51°01′N, 96°53′W) (south basin).



(GSI) values from pre-ovulated females that were sampled just prior to spawning (n = 41 for Ontario, n = 41 for Nipissing, n = 61 for Winnipeg; T.A. Johnston, unpublished data).

Laboratory analyses

Ages of the walleye were determined by counting annuli on sagittal otoliths. Otoliths were allowed to air dry and then set in epoxy. Transverse, thin sections (360 ± 30 μm) were cut through the nucleus using a Buehler® Isomet low-speed saw. These sections or acetate replicas of these sections were interpreted following standard procedures (Casselman 1987; Casselman and Gunn 1992) using techniques validated with known-age walleye extant in natural environments for 2 to 8 years. Because fish were sampled at spawning, the outer edge of the otolith section was always counted as an annulus.

All fish tissues underwent further processing prior to total lipid analyses. Female carcasses were thawed, cut coarsely into pieces, and homogenized by passing three times through a commercial stainless steel meat grinder. After thoroughly mixing the homogenate, an aliquot (~25 g) was removed and frozen in a glass vial at –20 °C. Ova subsamples stored at –20 °C, livers, and carcass homogenate subsamples were all freeze-dried for 7 days. Ovum size was estimated as mean dry mass per ovum (±0.01 mg) by weighing two subsamples of 30 freeze-dried ova. All freeze-dried tissues were then reduced to a coarse powder in a ball mill.

Total lipid contents of the freeze-dried ova, liver, and body were determined gravimetrically using a chloroform–methanol extraction procedure modified from earlier studies (Folch et al. 1957; Herbes and Allen 1983). An aliquot of the powdered tissue (~0.20 g) was weighed (±0.0001 g) into a 15 mL glass centrifuge tube. Five millilitres of chloroform–methanol solvent (2:1 by volume) was added to the tissue, and the tube was sealed with a teflon-lined cap. After soaking overnight (>12 h), the mixture was centrifuged (10 min at 1000g) and the supernatant transferred to a second centrifuge tube (hereafter, the supernatant tube) by glass pipette. One millilitre of chloroform–methanol solvent was rinsed through the pipette into the supernatant tube following transfer. The residue in the first centrifuge tube received an additional 3 mL of chloroform–methanol and was vortexed and centrifuged again. The resulting supernatant was added to the supernatant tube followed by another 1 mL of solvent for rinsing the pipette (total supernatant volume ~10 mL). The solvent and lipid mixture was washed by adding 2.5 mL of 0.88% KCl (0.88 g KCl in 100 mL distilled water) solution to the supernatant tube, agitating on a wrist-action shaker (15 min), then centrifuging (10 min at 1000g). After drawing off and discarding the upper aqueous layer, the lower solvent layer was transferred to a preweighed (±0.0001 g) aluminum pan by glass pipette, and the pipette was rinsed twice (1 mL solvent each) into the pan. Pans were placed in a fume hood at room temperature for ~1 h to allow the solvent to evaporate, transferred to a desiccator for ~1 h, then weighed (±0.0001 g) to determine lipid mass. Two extractions were performed for all samples, and a third was carried out if the coefficient of variation for the first two exceeded 10%.

FA profiles of ova total lipids were determined using the subsamples stored at –70 °C. Previous research has demonstrated that FA profiles of walleye ova lipids are more variable in the neutral than the polar lipid fraction (Wiegand et al. 2004). FA composition was determined by flame ionization gas chromatography (FIGC). Procedures for lipid isolation, preparation of FA methyl esters, and analysis by FIGC have been previously described in detail (Wiegand et al. 2004). At least two chromatograms were run for each FA

methyl ester sample. Profiles were developed from the 28 FAs that were identified in the chromatograms, and the data were expressed as percentages of total FAs normalized to 100%.

Statistical analysis

Our dependent variables of interest were indices of ova quality, namely size (mg dry mass), total lipid content (percentage of dry mass), and various measures of lipid FA composition. Our independent variables of interest were spawning year (Lake Ontario only) and various maternal traits, including age (years), FL (mm), somatic mass (g wet), somatic condition, hepatosomatic index (HSI, ratio of liver to somatic mass), somatic lipid content (percentage of dry mass), and liver lipid content (percentage of dry mass). Somatic condition was estimated as the residual from the $\log_e$-somatic mass vs. $\log_e$-FL relationship for the pooled females from all populations. Somatic lipid content was reconstructed from carcass plus liver lipid contents. Ovum size and ova lipid content were also included as independent variables when analyzing ova lipid FA composition as a dependent variable. Some maternal traits were $\log_e$-transformed as required to linearize observed relationships or to stabilize variance. Relative abundances of individual ovum lipid FAs were arcsine square root transformed prior to analyses.

We tested our hypotheses concerning maternal influences on ova composition using linear models. Differences in sampling years among the populations necessitated different model structures, and thus, all statistical analyses were conducted separately for the three populations. All analyses were conducted using SAS statistical packages (SAS Institute Inc. 1999).

For the Lake Ontario population, we constructed analysis of covariance (ANCOVA) models with sampling year as the class variable and the maternal traits as covariates. If sampling year accounted for significant ($P < 0.05$) variation in the dependent variable (one-way analysis of variance, ANOVA), then covariates were added to the model sequentially based on their ability to account for remaining residual variation (partial F test), and each was tested for interaction with sampling year (ANCOVA, heterogeneity of slopes). If sampling year did not account for significant variation in the dependent variable (one-way ANOVA), then sampling year was dropped, and the model was fitted by a multiple regression approach using a stepwise selection of covariates. For the Lake Winnipeg and Lake Nipissing population analyses, sampling year was not included, and models were always fitted by a multiple regression approach using stepwise selection.

Analyses of FA composition were conducted in two steps. First, principal components (PCs) were determined from the 28 FA relative abundances using all female data. These were used to compare overall FA profiles among populations and among sampling years. Second, individual FA contents and FA ratios were examined with respect to maternal traits within populations using the ANCOVA and multiple regression approach described above. All FA relative abundances were arcsine square root transformed for these analyses. Because the relative abundances of individual FAs are correlated with each other, we restricted our analyses to a subset of these, namely, DHA, EPA, AA, EPA/AA, 16:0, OA, palmitoleic acid (16:1(*n*-7), PLA), and α-linolenic acid (18:3(*n*-3)). These particular FAs and ratios were selected because of their prominent structural or functional roles described above or because they contributed greatly to among-female variation in ova FA profiles based on our principal components analysis (PCA).

Results

Maternal characteristics

Female walleye sampled from the three populations varied considerably in age and size distribution, lipid status, and reproductive characteristics. Lake Nipissing females were the youngest and smallest, whereas those from Lake Ontario were the largest and oldest (Table 1). Somatic lipid reserves were lowest in the Lake Nipissing population and highest in the Lake Winnipeg population. However, despite their apparent lipid surplus, Lake Winnipeg females produced the smallest gonads, as represented by GSI, and smallest ova of the three populations. Gonad size was largest in the Lake Ontario females and decreased from south to north among the populations. Lake Nipissing females had the lowest gonad lipid concentration, as well as the lowest somatic lipid concentration. Lake Ontario females had larger livers, as represented by HSI, than females from the other two populations, but liver lipid content did not vary greatly among the populations.

We examined interdependencies in these maternal traits by correlation analysis. As expected, maternal age, length, and mass were all highly correlated with each other in all populations (Table 2). However, correlations among other maternal traits were much less consistent across populations. Somatic lipid content was negatively correlated with maternal age and size in the Lake Ontario and Lake Nipissing populations, but this relationship was weak in the Lake Winnipeg population. Somatic condition was positively related to somatic lipid content in two populations. Liver size (HSI) was positively related to somatic condition and (or) somatic lipid content in all populations. Liver lipid content increased with female age and size and decreased with somatic lipid content in the Lake Ontario population, but these relationships were not seen in the other two populations. One particularly old female from the Lake Ontario stock had unusually low somatic lipid content as well as unusually high liver lipid content and therefore created strong leverage in relationships among these traits. Subsequent analyses were conducted with and without this female, and only those tests that were statistically significant under both conditions were reported.

Ova size and total lipid concentration

For Lake Ontario walleye, the relationship between ova size and maternal FL varied among the sampling years (ANCOVA, heterogeneity of slopes, $F_{[2,56]} = 7.13$, $P = 0.0017$); the relationship was more positive in 2004 than in 2002 and 2003. The full model with sampling year, maternal fork length, and their interaction accounted for 34% of the variance in ova size. Replacing maternal length with somatic mass accounted for slightly more variation ($R^2 = 0.36$), but the interaction with sampling year remained significant. No other maternal traits were able to account for additional vari-

Table 1. Comparison of traits among female walleye (*Sander vitreus*) sampled from Lakes Ontario, Nipissing, and Winnipeg.

	Population		
Trait	Lake Ontario (n = 62)	Lake Nipissing (n = 23)	Lake Winnipeg (n = 24)
Age (years)	11±0.50	6.9±0.39	7.9±0.62
Fork length (mm)	652±9	455±11	552±13
Somatic mass (g wet)	2950±118	1075±79	1992±124
Somatic condition	–0.052±0.011	0.056±0.020	0.067±0.015
Somatic lipid (% dry mass)	24.5±1.1	18.0±3.3	34.5±1.2
HSI (%)	3.24±0.10	2.40±0.32	2.56±0.12
Liver lipid (% dry mass)	13.3±0.62	11.6±1.90	14.7±0.70
GSI (%)	26.7±0.75	18.0±0.69	14.1±0.63
Gonad lipid (% dry mass)	37.6±0.3	33.7±0.8	36.9±0.3
Ova size (mg dry)	0.96±0.022	0.96±0.069	0.81±0.025

Note: All values (mean ± standard error, SE) were calculated from the same ovulated females used in this study, except for gonadosomatic index (GSI) values, which were estimated from pre-ovulated females sampled just prior to spawning (n = 41 for Lake Ontario, n = 41 for Lake Nipissing, n = 61 for Lake Winnipeg; T.A. Johnston, unpublished data). Somatic condition was estimated as the residual from the $\log_e$ somatic mass vs. $\log_e$ fork length regression for the combined data. Estimates of somatic condition, hepatosomatic index (HSI), GSI, ova size, and tissue lipid contents were adjusted to a common somatic mass of 2000 g.

Table 2. Correlation (r) matrices for characteristics of mature female walleye (*Sander vitreus*) sampled from Lakes Ontario, Nipissing, and Winnipeg.

	AGE	FL	SOMA	COND	SOMA-L	HSI
Lake Ontario, 2002–2004 (n = 62)						
FL	0.82 (< 0.001)*	—	—	—	—	—
SOMA	0.78 (< 0.001)*	0.95 (<0.001)*	—	—	—	—
COND	0.04 (0.75)	0.07 (0.59)	0.33 (0.01)*	—	—	—
SOMA-L	–0.55 (< 0.001)*	–0.35 (0.0059)*	–0.22 (0.091)	0.37 (0.0034)*	—	—
HSI	–0.15 (0.24)	0.06 (0.64)	0.08 (0.56)	0.21 (0.11)	0.32 (0.011)*	—
LIVER-L	0.72 (< 0.001)*	0.66 (<0.001)*	0.56 (<0.001)*	–0.19 (0.14)	–0.53 (< 0.001)*	–0.21 (0.095)
Lake Nipissing, 2005 (n = 23)						
FL	0.80 (< 0.001)*	—	—	—	—	—
SOMA	0.75 (< 0.001)*	0.99 (<0.001)*	—	—	—	—
COND	–0.26 (0.23)	–0.10 (0.66)	0.03 (0.88)	—	—	—
SOMA-L	–0.53 (0.009)*	–0.42 (0.043)*	–0.43 (0.042)*	0.25 (0.25)	—	—
HSI	–0.26 (0.23)	–0.09 (0.69)	–0.06 (0.78)	0.47 (0.021)*	0.44 (0.036)*	—
LIVER-L	–0.11 (0.62)	–0.04 (0.88)	–0.03 (0.89)	0.38 (0.076)	–0.40 (0.061)	–0.38 (0.074)
Lake Winnipeg, 2004 (n = 24)						
FL	0.76 (< 0.001)*	—	—	—	—	—
SOMA	0.65 (< 0.001)*	0.97 (<0.001)*	—	—	—	—
COND	–0.47 (0.021)*	–0.04 (0.85)	0.15 (0.47)	—	—	—
SOMA-L	–0.40 (0.050)	–0.10 (0.64)	0.03 (0.89)	0.63 (0.0010)*	—	—
HSI	–0.34 (0.10)	–0.39 (0.057)	–0.28 (0.18)	0.47 (0.021)*	0.28 (0.18)	—
LIVER-L	0.07 (0.76)	0.00 (0.99)	0.00 (0.99)	0.13 (0.56)	0.14 (0.53)	0.51 (0.012)*

Note: Values in parentheses are probabilities (P) of larger absolute values of r. Statistically significant correlations ($P < 0.05$) are indicated with an asterisk (*). Characteristics abbreviations are as follows: AGE, age (years); FL, fork length (mm); SOMA, somatic mass (g wet); COND, somatic condition (residual $\log_e$ wet mass); SOMA-L, somatic lipid concentration (percentage of dry mass); HSI, hepatosomatic index (g liver·g soma^{-1}); LIVER-L, liver lipid concentration (percentage of dry mass).

ance in ova size. For the Lake Nipissing walleye, the strongest univariate predictor of ova size was liver lipid content; ova size declined with increasing liver lipid content (regression analysis, $F_{[1,21]} = 16.0$, $P < 0.001$, $R^2 = 0.43$). The remaining variance in ova size was positively related to $\log_e$ somatic mass (multiple regression analysis, partial $F_{[1,20]} = 6.32$, $P = 0.021$). In Lake Winnipeg walleye, ova size increased with maternal size and age, and the strongest univariate relationship was with $\log_e$ fork length (regression analysis, $F_{[1,22]} = 6.01$, $P = 0.023$, $R^2 = 0.21$). No other maternal traits accounted for additional variance. Thus, among the three populations, ova size was more consistently related to maternal size than to indices of maternal condition or lipid status.

Ova lipid content, expressed as a percentage of dry mass, did not vary among years in Lake Ontario walleye, so data

Table 3. Percentages (unadjusted mean ± standard deviation, SD) of major individual fatty acids, fatty acid families, and fatty acid ratios in ova total lipids of walleye (*Sander vitreus*; *n* = number of females) sampled from Lakes Ontario, Nipissing, and Winnipeg.

	Population and sampling year				
	Lake Ontario			Lake Nipissing	Lake Winnipeg
Fatty acid	2002 (*n* = 25)	2003 (*n* = 19)	2004 (*n* = 18)	2005 (*n* = 23)	2004 (*n* = 24)
16:0	11.4±0.6	8.0±1.2	8.3±1.7	10.2±0.8	10.0±1.1
16:1(*n*-9)	3.4±0.5	2.2±0.4	2.2±0.5	3.1±0.5	2.4±0.4
16:1(*n*-7)	10.7±2.1	7.8±3.3	8.8±3.2	13.5±1.8	13.4±1.2
18:0	1.9±0.2	1.5±0.2	1.7±0.3	1.8±0.4	2.0±0.3
18:1(*n*-9)	18.8±1.2	18.8±1.7	18.1±1.6	14.2±1.8	16.7±1.5
18:1(*n*-7)	3.5±0.8	2.9±0.8	3.5±0.9	4.4±1.0	4.7±0.5
18:2(*n*-6)	4.6±0.9	4.5±0.9	4.5±1.0	5.1±0.6	4.8±0.4
18:3(*n*-3)	4.2±0.5	3.9±0.6	4.9±0.7	5.6±1.6	5.9±0.8
20:4(*n*-6)	4.1±0.6	5.0±0.8	4.7±0.8	4.3±0.6	2.6±0.2
20:5(*n*-3)	6.6±0.9	8.0±0.9	8.6±1.0	7.3±1.5	7.2±0.7
22:5(*n*-6)	1.4±0.3	2.0±0.4	1.6±0.5	1.3±0.3	1.0±0.1
22:5(*n*-3)	2.7±0.3	3.5±0.3	3.2±0.4	2.5±0.4	2.5±0.3
22:6(*n*-3)	17.9±2.3	23.9±3.9	21.2±3.5	17.5±1.4	18.8±2.0
Σ saturated	15.6±0.7	10.9±1.8	11.4±2.4	14.5±0.9	13.8±1.6
Σ monounsaturated	38.4±2.8	33.5±3.9	34.6±3.5	37.3±3.7	39.0±2.1
Σ (*n*-6)	11.5±1.7	13.0±2.0	12.3±2.1	12.2±1.1	9.5±0.7
Σ (*n*-3)	34.6±1.8	42.7±3.8	41.7±3.8	36.0±4.5	37.7±2.7
Ratio EPA/AA	1.6±0.5	1.7±0.6	1.9±0.4	1.7±0.5	2.8±0.3
Ratio DHA/EPA	2.8±0.7	3.1±0.6	2.5±0.5	2.5±0.6	2.6±0.3
Ratio (*n*-3)/(*n*-6)	3.1±0.5	3.4±0.5	3.4±0.5	3.0±0.5	4.0±0.4

Note: Only fatty acids comprising 2% or more of total fatty acids are listed. Other fatty acids found were 14:0, 15:0, 16:1(*n*-5), 17:0, 17:1 (isomer unknown), 18:3(*n*-6), 18:4(*n*-3), 20:0, 20:1(*n*-9), 20:1(*n*-7), 20:2(*n*-6), 20:3(*n*-6), 20:3(*n*-3), 20:4(*n*-3), and 22:4(*n*-6). EPA, eicosapentaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid.

from the three sample years were pooled for further analysis. Ova lipid content increased with HSI (regression analysis, ANOVA, $F_{[1,60]} = 11.6$, $P = 0.0012$, $R^2 = 0.16$). The residuals from this model showed a positive relationship with somatic lipid content but the relationship was not statistically significant (multiple regression analysis, ANOVA, partial $F_{[1,59]} = 3.70$, $P = 0.059$). For Lake Nipissing walleye, somatic condition was the best independent predictor of ova lipid content; ova lipid content decreased with increasing somatic condition (regression analysis, $F_{[1,21]} = 4.60$, $P = 0.044$, $R^2 = 0.18$). The remaining variation in ova lipid content was most strongly related to HSI (positive relationship), but this effect was not statistically significant (regression analysis, partial $F_{[1,20]} = 3.61$, $P = 0.072$). For Lake Winnipeg walleye, HSI was the best independent predictor variable. Ova lipid content increased with HSI, but the relationship was not statistically significant (regression analysis, ANOVA, $F_{[1,22]} = 1.97$, $P = 0.17$, $R^2 = 0.082$). Thus, the most consistent, albeit weak, predictor of ova lipid content among populations appeared to be HSI, whereas relationships between ova lipid content and indices of maternal condition and body lipid status appeared to be both inconsistent and weak.

Fatty acid composition of ova lipids

Mean levels of major ova total lipid FAs exhibited considerable variation among sampling years for the Lake Ontario walleye population (Table 3). Ova sampled in 2002 were relatively poor in major (*n*-3) polyunsaturated fatty acids (PUFA) and comparatively rich in saturated FAs and monounsaturated fatty acids (MUFA). In this respect, the Lake Ontario ova of 2002 were more similar to those of the other two populations than to ova of the Lake Ontario population sampled in 2003 and 2004. Ova from Lake Winnipeg females had FA profiles with lower relative amounts of AA (20:4(*n*-6)) and a higher EPA/AA ratio compared with those of the other two populations.

PCA indicated that most of the variation in ova lipid FA profiles among females was due to variation in 16:0, PLA, OA, 18:3(*n*-3), AA, and DHA. The first three PCs accounted for 65%, 15%, and 8% of the total observed variability. Overall differences in FA profiles among sampling years and populations were assessed by using the first two PCs (PC1 and PC2) as dependent variables in ANCOVA models with maternal age or size as the covariate. In analyses of both PC1 and PC2, there was significant interaction between population and covariate effects when either age or FL were used as the covariate (ANCOVA, heterogeneity of slopes, partial $F_{[2,103]} > 4.1$, $P < 0.020$). When maternal somatic mass was used as the covariate, there was significant interaction between population and mass for analysis of PC2 (ANCOVA, heterogeneity of slopes, partial $F_{[2,103]} = 9.21$, $P < 0.001$) but not for PC1 (ANCOVA, heterogeneity of slopes, partial $F_{[2,103]} = 3.06$, $P = 0.051$). Following removal of the interaction term, PC1 varied significantly with both somatic mass (ANCOVA, partial $F_{[1,105]} = 26.4$, $P < 0.001$)

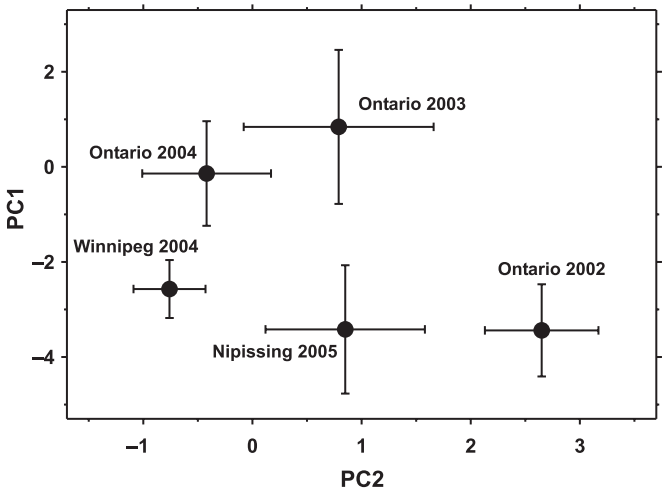
and population (ANCOVA, partial $F_{[1,105]} = 4.02$, $P = 0.021$). A scatter plot of size-adjusted mean PC1 vs. PC2 illustrated that for 550 mm FL females, the variability in ova lipid FA profiles among three sampling years in the Lake Ontario population was as great as the variability among the three populations (Fig. 2). Similar patterns emerged when PCs were adjusted for either maternal somatic mass or age. Subsequent analyses of individual FAs were conducted separately for each population.

Scatter plots of the percentage of particular FAs vs. maternal size or age indicated that such relationships were fairly consistent among sampling years in the Lake Ontario population, but were less consistent among the other spawning populations. For example, the percentage of ova lipid AA increased with maternal length in all sampling years for the Lake Ontario population (Fig. 3). For the single sampling year in the Lake Nipissing population, there was substantial scatter, whereas the ova lipid AA content was much lower and invariant with maternal length in the Lake Winnipeg population (Fig. 3). When AA content was examined with respect to maternal somatic lipid content, there was no clear relationship in the Lake Ontario population, but a modest negative relationship in the Lake Nipissing and Lake Winnipeg populations (Fig. 4). An unexpected result was a difference in the relationship of two MUFAs, OA and PLA, with maternal length. In Lake Ontario, percentages of PLA decreased significantly with increasing maternal length, while those of OA did not change (Fig. 5, Table 4). In Lake Nipissing, percentages of OA increased with maternal age, while those of PLA did not change, and neither FA was related to maternal characteristics in Lake Winnipeg (Table 4).

Interannual variation in individual FA proportions and FA ratios for ova of the Lake Ontario population was assessed by ANCOVA using maternal FL as the covariate. For all FA proportions and FA ratios examined, there was no significant interaction between sampling year and maternal FL (ANCOVA, heterogeneity of slopes, $F_{[2,56]} < 2.3$, $P > 0.10$). Furthermore, there was significant interannual variation in the proportion of ova lipid FAs as DHA, AA, EPA, PLA, 16:0, and 18:3(n-3) (ANCOVA, $F_{[2,59]} > 7.1$, $P < 0.0018$). Of the ova lipid FA variables examined, only the proportion as OA and the EPA/AA ratio did not vary significantly among sampling years. All FA variables for the Lake Ontario population were adjusted for this sampling year effect before assessing maternal effects.

The stepwise multiple regression procedure indicated that indices of maternal size or age were generally the strongest predictors of ova lipid FA composition for all populations and all FA variables examined (Table 4). Maternal FL was the most consistent predictor variable of FA composition in the Lake Ontario population, but age or age and somatic mass were stronger predictors in the other two populations. Liver size and lipid content accounted for additional variance in some ova lipid FA proportions in the Lake Ontario population but were generally poor predictor variables in the other populations. Indices of overall maternal nutrient status, namely, somatic condition and lipid content, did not explain significant variation in ova lipid FA content in the Lake Ontario population, but did account for significant variation in some FA variables, most notably the EPA/AA ratio, in the other two populations. Our results suggest that ova lipid FA composition in these walleye populations is more strongly linked to maternal age and size than to the state of maternal lipid reserves.

Fig. 2. Scatter plot of first versus second principal components (PC1 and PC2, respectively) calculated from the relative abundances of 28 fatty acids found in ova total lipids of walleye (*Sander vitreus*) from two populations sampled in single years (Lakes Nipissing and Winnipeg) and one population sampled in multiple years (Lake Ontario). Symbols are means (± standard error, SE) adjusted to a standard female size of 550 mm fork length.



Discussion

Somatic characteristics

The three populations of walleye differed substantially in their somatic characteristics. Lake Winnipeg fish had the highest condition factors and highest somatic lipid reserves, but the smallest ova and GSI. Lake Nipissing fish were smaller, had the lowest somatic and hepatic lipid reserves, and the lowest ova lipid contents, but had large ova. Lake Ontario fish were older than those of the other two lakes, had the lowest somatic condition, but had high liver lipid content and produced large ova with high levels of lipid.

In both Lake Ontario and Lake Nipissing fish, somatic lipid reserves remaining at the time of spawning were negatively related to female age and FL. In those populations, older, larger fish were thus more depleted after supporting ovarian recrudescence than younger, smaller fish. The liver is the site of assembly of the two major sources of oocyte lipid, vitellogenin and very low density lipoprotein, for which FA can be mobilized from stored reserves (Wiegand 1996*a*). In Lake Ontario walleye, liver lipid content increased with female age and size, suggesting an increase in metabolic activity directed towards vitellogenesis in older, larger females that had not been terminated at the time of spawning. Taken together, these observations suggest, especially for the Lake Ontario population where the range of sample ages was greatest, that the older, larger females have an increased quantitative commitment of energy reserves towards reproduction in a given year and, possibly, a lesser commitment

Fig. 3. Percentages of arachidonic acid (AA, 20:4(*n*-6)) in ova total lipids vs. fork length for mature female walleye (*Sander vitreus*) sampled from (*a*) Lake Ontario (●, 2002; ◇, 2003; ▲, 2004), (*b*) Lake Nipissing (○, 2005), and (*c*) Lake Winnipeg (▲, 2004). Symbols represent individual females.

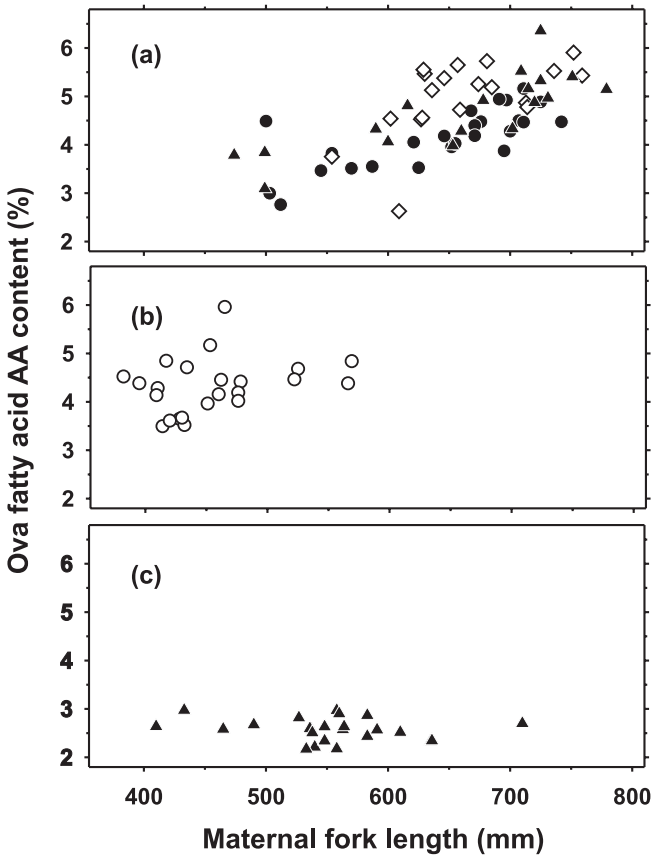
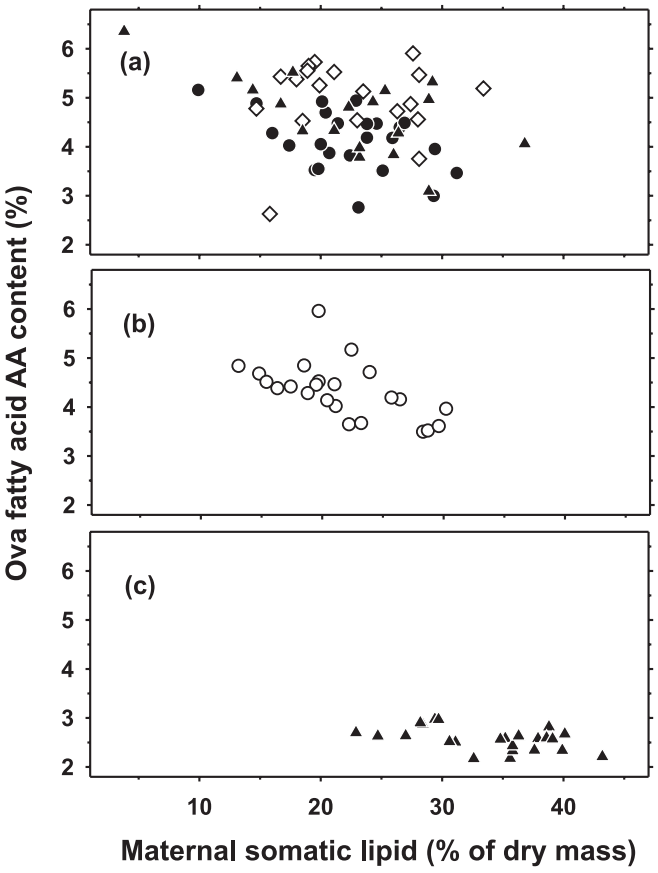


Fig. 4. Percentages of arachidonic acid (AA, 20:4(*n*-6)) in ova total lipids versus lipid content of somatic tissue (percentage of dry mass) for mature female walleye (*Sander vitreus*) sampled from (*a*) Lake Ontario (●, 2002; ◇, 2003; ▲, 2004), (*b*) Lake Nipissing (○, 2005), and (*c*) Lake Winnipeg (▲, 2004). Symbols represent individual females.



to maternal maintenance than younger, smaller females. By contrast, the commitment of Lake Winnipeg fish to reproduction at the expense of somatic reserves was comparatively low.

Ova size and lipid content

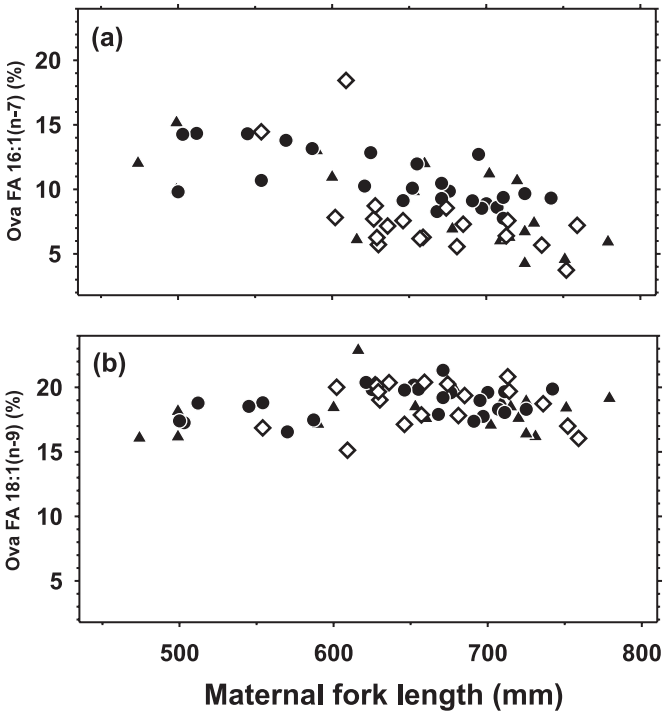
Ova size was not related to indices of maternal reserves such as body condition or somatic lipid content, although it was related to liver lipid content in one of our study populations; maternal size appeared to exert the strongest influence on ova size. Ova size is positively related to maternal size and (or) age in a wide variety of fish species (Heath and Blouw 1998; Trippel and Neil 2004; Lauer et al. 2005), as well as many other taxa (Roff 1992; Bernardo 1996). In walleye, the strength of the ova size versus maternal size relationship varies greatly among populations (Johnston and Leggett 2002). Results from the current study are consistent with this earlier research in that ova size was related most strongly to maternal size, but the strength and nature of the relationship was quite variable among the three study populations. This supports the hypothesis of greater maternal investment in reproduction with increasing age and size. Ova size may also vary seasonally in some fishes, particularly batch-spawning species (Miller et al. 1995; Evans et al. 1996; Trippel and Neil 2004), although this does not appear to be the case for synchronous spawners such as walleye (Czesny et al. 2005).

While ova size may be a valid indicator of survival potential of offspring, it does not provide qualitative information on ova composition. Furthermore, the degree of important trade-offs between various aspects of offspring size at the larval stage, such as larval length and yolk sac volume (Heyer et al. 2001), cannot be predicted based on ova size. Examination of other aspects of nutrient provisioning to offspring may increase the ability to predict offspring success based on maternal characteristics. Ova lipid content was not strongly or consistently influenced by maternal age or size. The best predictor of ova lipid content across our three study populations was liver size, expressed as HSI. As described previously, HSI is a crude index of metabolic activity devoted to vitellogenesis, and these correlations are thus consistent. On its own, ova lipid content will provide a measure of caloric reserve for the offspring, but will not indicate anything about the quality of that reserve.

Polyunsaturated fatty acids

One important aspect of the ova nutrient reserve is the FA composition of the lipids. Although we did not observe sig-

Fig. 5. Percentage of ova lipid fatty acids (FAs) as (*a*) palmitoleic acid (16:1(*n*-7)) and (*b*) oleic acid (18:1(*n*-9)) versus maternal fork length for mature female walleye (*Sander vitreus*) sampled from Lake Ontario. Symbols represent individual females (●, 2002; ◇, 2003; ▲, 2004).



nificant interannual variation in ova total lipid content, the FA composition of the ova lipid varied substantially among sample years. PCA revealed that variance in ova lipid FA profiles among the three sample years for Lake Ontario was similar in magnitude to that observed among the three populations. Overall, FA profiles varied with maternal size, but the nature of this relationship was not consistent among the populations. With a significant overall effect of maternal size on FA composition, we examined the influence of multiple maternal somatic characteristics on percentages of individual FAs within each of these populations.

Relations between DHA proportions in the ova and maternal characteristics differed among the three lakes. In Lake Ontario, DHA increased with maternal size. In Lake Nipissing, ova DHA levels increased with somatic lipid reserves, which were the lowest among the three populations. This suggests similar selection pressures in each population, with Lake Nipissing fish being limited by resources. Lake Winnipeg fish were not consistent with those in the other two lakes, as ova DHA levels declined with maternal age. In Lake Ontario walleye, the percentages of AA and EPA, as well as the EPA/AA ratio, of ova lipid FAs were all related to maternal size. A similar trend with maternal age was observed in the Lake Nipissing population. In each case, AA increased while EPA and the EPA/AA ratio declined with maternal size and (or) age. Lake Winnipeg walleye again did not conform to the other populations. They also produced ova lipids with much lower levels of AA. In that population, EPA did decline with somatic mass, but the EPA/AA ratio increased with condition. Taken together, these results suggest that there is a selective advantage in the Lake Ontario and Lake Nipissing walleye to produce ova with high DHA percentages and low EPA/AA ratios. This does not appear to be the case in Lake Winnipeg.

Benefits that may accrue as a result of more desirable PUFA percentages are most likely to be apparent during latter stages of development. Percentages of DHA did not influence survival of walleye embryos to the pigmented eye stage (Czesny et al. 2005) or to hatching (Johnston et al. 2005, 2007), but were associated with walleye survival at later stages of development (Moodie et al. 1989). Lower ratios of EPA/AA in whole-body phospholipids were associated with higher survival in juvenile walleye (Czesny et al. 1999). The Lake Ontario and Lake Nipissing walleye thus conformed to expectations formed as a result of previous studies, but the Lake Winnipeg walleye did not. Ideally, in future research, the success of offspring from individual females whose ova FA profiles are known should be followed throughout development to juvenile stages.

Monounsaturated fatty acids

We also observed strong maternal influences on MUFA percentages in Lake Ontario walleye ova. Specifically, while percentages of OA in ova lipids did not change with female size, percentages of PLA declined. Lake Nipissing fish showed somewhat different trends with MUFA. OA content of ova lipids increased with maternal age, and there was no effect of maternal characteristics on PLA. Compared with Lake Ontario, Lake Nipissing ova were poorer in OA, and the females had lower lipid reserves at the end of recrudescence.

During fish development, all FAs are catabolized to some extent, and some are preferentially conserved for incorporation into structural lipids (Rønnestad et al. 1995; Wiegand 1996*a*; Tocher 2003). OA has important structural roles in membranes, and its insertion into the *sn*-1 position of membrane phospholipids makes an important contribution to homeoviscous adaptations (Dey et al. 1993; Buda et al. 1994; Farkas et al. 2001). On the other hand, PLA is used primarily for catabolism and does not have a prominent role in structural lipids (Bell and Tocher 1989; Bell and Dick 1991; Kikuchi et al. 1999). This indicates that there is an advantage to deposition of sufficient OA in ova reserves to provide for incorporation into membranes, as well as oxidation, and that no such advantage exists for PLA. Lake Ontario walleye deposit a consistent amount of OA in their ova regardless of size, while the Lake Nipissing walleye increase ova OA reserves as they get older. A reserve of OA in the oil globule could be of value in the subsequent assembly of tissue membranes after the commencement of feeding. Similar temporary storage of FAs destined for subsequent structural purposes in neutral lipids has been proposed for developing Atlantic herring (*Clupea harengus*) (Tocher et al. 1985), cod (*Gadus morhua*) (Fraser et al. 1988), and goldfish (*Carassius auratus*) (Wiegand 1996*b*). As was the case with PUFA, the Lake Winnipeg fish did not show a similarity in maternal influences on ova MUFA deposition.

The difference between patterns of accumulation of OA and PLA in the walleye ova lipid reserve suggests that dis-

Table 4. Results of stepwise multiple regression analysis of percentages of ova lipid fatty acids or fatty acid ratios (dependent variable) against various maternal characteristics (independent variables) for each of three walleye (*Sander vitreus*) populations.

	Maternal characteristic													
	AGE		FL		SOMA		COND		SOMA-L		HSI		LIVER-L	
Fatty acid	+/–	R^2	+/–	R^2	+/–	R^2	+/–	R^2	+/–	R^2	+/–	R^2	+/–	R^2
Lake Ontario, 2002–2004 (*n* = 62)														
22:6(*n*-3)			+	0.24							+	0.05		
20:4(*n*-6)			+	0.49									+	0.05
20:5(*n*-3)			–	0.10										
EPA/AA			–	0.37										
16:1(*n*-7)			–	0.40							–	0.04	–	0.05
16:0					–	0.07								
18:1(*n*-9)											+	0.12		
18:3(*n*-3)														
Lake Nipissing, 2005 (*n* = 23)														
22:6(*n*-3)									+	0.19				
20:4(*n*-6)	+	0.36												
20:5(*n*-3)	–	0.39												
EPA/AA	–	0.45							+	0.12				
16:1(*n*-7)														
16:0														
18:1(*n*-9)	+	0.51												
18:3(*n*-3)	–	0.30												
Lake Winnipeg, 2004 (*n* = 24)														
22:6(*n*-3)	–	0.23												
20:4(*n*-6)									–	0.32				
20:5(*n*-3)					–	0.34								
EPA/AA							+	0.24						
16:1(*n*-7)														
16:0					+	0.17								
18:1(*n*-9)														
18:3(*n*-3)	+	0.09			–	0.60								

Note: For maternal characteristics retained in the final model (partial F test, $P < 0.05$), the nature of their effect (+ or –) and the proportion of variance explained (partial R^2) are indicated. Female characteristics are as follows: AGE, age (years); FL, fork length (mm); SOMA, somatic mass (g wet); COND, somatic condition (residual $\log_e$ wet mass); SOMA-L, somatic lipid concentration (percentage of dry mass); HSI, hepatosomatic index (g liver·g soma^{-1}); LIVER-L, liver lipid concentration (percentage of dry mass). EPA, eicosapentaenoic acid; AA, arachidonic acid. For the Lake Ontario population, multiple years' data were used, and the effect of interannual variation was removed prior to analyses.

crimination between the two occurs during assembly of the reserve. Discrimination among MUFA in assembling the ova nutrient reserve has previously been observed in capelin (*Mallotus villosus*) and Atlantic herring from North Atlantic waters in which discrimination against 22:1(*n*-11) was observed (Henderson et al. 1984; Henderson and Almatar 1989). The selective advantage in that case is the incompatibility of that FA with inclusion in a phospholipid bilayer for steric reasons.

General discussion

The causes of the observed, maternally related variability in FA profiles in Lake Ontario walleye ova are unknown. This variability may simply reflect differences in diet among females of different size. Since walleye continue to feed during vitellogenesis (Henderson et al. 1996), they are not entirely reliant on stored nutrients for assembly of the lipoprotein yolk and oil globule. The most variable portion of the ova reserve is the neutral lipid (Wiegand et al. 2004). The principal source of ova neutral lipid is believed to be very low density lipoprotein, the FA composition of which is strongly influenced by dietary input (Babin and Vernier 1989; Tocher 2003). However, a mechanism based on size-specific prey would not explain similar trends in Lake Nipissing, where age, not size, was the stronger predictive maternal characteristic.

Alternatively, maternally related variability in FA profiles could be an adaptive result of preferential incorporation of specific FAs and discrimination against others in the assembly of ova reserves. Such selectivity in vitellogenesis has been observed in other species (Wiegand 1996*a*; Tocher 2003). Older or larger females may commence ovarian recrudescence with greater resources of the essential FAs required for an optimal ova FA profile. Also, as the female gets older or larger, it may be advantageous, in the evolutionary sense, to put superior reserves into ova, since the number of future reproductive opportunities is declining. This enrichment of ova may occur at the expense of mater-

nal use of those resources. More detailed analysis of somatic reserves at the end of recrudescence would be necessary to substantiate this hypothesis.

Like other iteroparous fish in temperate waters, walleye must balance the direction of essential nutrients to gamete production with retention in female somatic tissue to enhance survival over winter. As well as being used in vitellogenesis, PUFA will be required by the female for maintenance of membrane viscosity and as a substrate for metabolism during colder winter conditions. A strategy of retention of essential FAs in maternal tissues, if necessary at the expense of the allocation to ova, has previously been proposed for northern pike, *Esox lucius* (Schwalme 1994). The extent to which walleye deplete and maintain somatic reserves of HUFA during recrudescence remains to be investigated.

In contrast with the trends seen with Lake Ontario and Lake Nipissing walleye, the Lake Winnipeg fish were quite different. They had low preovulatory GSI and produced small ova, but had large somatic and hepatic lipid reserves remaining after recrudescence. Furthermore, the FA profiles of these ova did not show similar relationships with maternal somatic characteristics, and the ova AA contents were quite low. Our observations of the Lake Winnipeg walleye were thus in direct contrast with our initial hypothesis.

If the strategy employed by the Lake Ontario and Lake Nipissing walleye populations is adaptive, the failure of Lake Winnipeg walleye to conform may suggest that a different set of selection pressures are operating on that population. A high availability of AA to larvae through their food supply could possibly reduce the level of AA required for inclusion in the ova reserve. Alternatively, FA resources available to adult Lake Winnipeg walleye in their food sources may be inferior to those in Lakes Ontario and Nipissing, and Lake Winnipeg females may be less able to accommodate reproductive demands for AA. Resolution of these questions will require investigation of the movement of PUFA through the food webs in the individual lakes.

The causes of variance in ova FA profiles in wild fish populations have not been investigated. The relatively consistent relationships with maternal size or age but not with indices of maternal nutrient reserve suggest that these patterns are developmental and population-specific for walleye, similar to patterns observed for egg size (Johnston and Leggett 2002). The question remains whether these relationships represent an adaptive reproductive strategy or merely differences in maternal diets among size classes, spawning years, or populations.

A growing body of research indicates that recruitment in iteroparous fish stocks is not only related to the total biomass of reproductive females but also to qualitative aspects of the spawning stock (Marshall et al. 1999; Scott et al. 1999; Murawski et al. 2001). Our results suggest that larger and (or) older females produce higher quality ova in some walleye populations, and thus, their contribution to year-class formation may be greater than their ova production alone would indicate. The implications for fisheries management are clear; if recruitment is dependent not only on the biomass of females but also the size–age composition of those females, then management plans need to consider the size-selectivity of harvest. Further refinements to stock–recruitment models will be required to quantify the sensitivity of recruitment to changes in spawning stock demographics for walleye and other species.

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