Elsevier Editorial System(tm) for Aquaculture
Manuscript Draft

Manuscript Number: AQUA-D-14-00123R1

Title: Biochemical egg quality in a captive walleye (Sander vitreus) broodstock population relative to ovulation timing following hormonal treatment

Article Type: SI : AQUA 2013 (invitation only)

Keywords: walleye; spawning period; eggs; embryogenesis; hatching success; ontogeny; total lipids; fatty acids; total amino acids; free amino acids

Corresponding Author: Ms. Sahar Mejri, MSc

Corresponding Author's Institution: ISMER (UQAR)

First Author: Sahar Mejri, MSc

Order of Authors: Sahar Mejri, MSc; Réjean Tremblay, PhD; Grant W Vandenberg, PhD; Christopher C Parrish, PhD; Céline Audet, PhD

Biochemical egg quality in a captive walleye (*Sander vitreus*) broodstock population relative to ovulation timing following hormonal treatment

Sahar Mejri[1*], Céline Audet[1], Grant W. Vandenberg[2], Christopher C. Parrish[3] and Réjean Tremblay[1]

[1] *Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski (ISMER, UQAR), 310 allée des Ursulines, Rimouski, QC, Canada, G5L 3A1*

[2] *Département des sciences animales, Pavillon Paul-Comtois, Université Laval, 2425 rue de l'Agriculture, Québec, QC, Canada, G1V 0A6*

[3] *Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1C 5S7*

***Corresponding author,** Sahar.Mejri@uqar.ca/saharmejri@gmail.com, Phone: 418-723-1986 ext 1392, Fax: 418-724-1842

Abstract

The aim of this study was to evaluate how variations in total lipids, fatty acids, and total and free amino acids in eggs affect embryonic development throughout the spawning season in cultured walleye (*Sander vitreus*). Eggs were obtained from 4-year-old females and pooled based on spawning time: they were assigned to four consecutive periods during a one-month spawning season according to the first spawning occurrence in the female broodstock. Hatching success was significantly higher at the intermediate spawning period (87.3 ± 2.4%), and no eggs hatched in the late spawning group ($p < 0.05$). Egg diameter was significantly larger for the two intermediate spawning periods, which is related to the greater larval length at hatch during these two periods. Successful development was associated with the quality of lipid reserves throughout ontogeny. For polar fatty acids, there was a specific retention of essential fatty acids (EFA), particularly of the most abundant, i.e., docosahexaenoic acid (DHA), which made up more than 40% of the polar fatty acid fraction. For total amino acids, lysine (LYS) and serine (SER) levels were significantly higher in eggs from the intermediate spawning periods and were preferentially depleted during embryogenesis. During embryogenesis, energy was derived primarily from triacylglycerols (TAG), proteins, and non-essential free amino acids. Our results suggest that the content of EFA and amino acids in eggs may explain differences in egg quality and success of larval development within a broodstock population. Our results clearly show that the timing of ovulation during the spawning period affects the success of walleye aquaculture production.

Keywords: walleye, spawning period, eggs, embryogenesis, hatching success, ontogeny, total lipids, fatty acids, total amino acids, free amino acids

1. Introduction

Walleye (*Sander vitreus*) is a valuable sport and commercial fish species in the northern United States and Canada. In the US, over one billion walleye fry and fingerlings are produced annually by public hatcheries for stocking enhancement programs; the broodstock is largely captured wild fish (Fenton et al., 1996; Malison et al., 1998; Rinchard et al., 2005). Nevertheless, efforts to raise walleye fry to marketable size remain in the early stages: more research and development is needed to ensure profitable production, including a better understanding of biochemical requirements during early life stages. The few previous studies that were done on biochemical composition during ontogeny were almost all performed on wild fish (Czesny et al., 2005; Johnston et al., 2007), but constraints are different when dealing with captive broodstock and egg rearing. For one thing, hormone treatment is generally used to induce spawning in captive female walleye (Malison et al., 1998). Such hormonal manipulations might result in different spawning periods and variable biochemical composition of eggs within the same broodstock population.

Egg size as well as egg composition (especially fatty acids and amino acids) can have a significant impact on the early life history of fish (Czesny et al., 2005). The influence of egg biochemical composition on offspring quality has been demonstrated in several teleost species (Bruce et al., 1993; Navas et al., 1997). Lipids allocated to egg production in walleye are divided between a lipoprotein yolk (LPY), which contains polar lipids and some neutral lipids, and an oil globule entirely filled with neutral lipids, principally triacylglycerols (Moodie et al., 1989). The LPY is used to satisfy the structural as well as caloric and micronutrient requirements of embryos and young larvae, and it is largely exhausted before exogenous feeding begins (McElman and Balon, 1979).

Under culture conditions, hatching success and embryonic survival have been related to

essential polyunsaturated fatty acid content (Fernández-Palacios et al., 2011; Keckeis et al., 2000; Mazorra et al., 2003; Moodie et al., 1989; Pickova et al., 1997). Amino acids (free amino acids [FAA] and protein constituents) are vital for all living organisms. During early fish ontogeny, they are used as fuel molecules, signaling factors, and substrates for the synthesis of a wide range of bioactive molecules (Finn and Fyhn, 2010). It has been suggested that amino acids are the main substrate for energy metabolism and protein synthesis in the embryos of some marine fish species, such as Atlantic cod (*Gadus morhua*) (Clarke et al., 2010; Finn et al., 1995a) and Atlantic halibut (*Hippoglossus hippoglossus*) (Finn et al., 1995b). Moreover, FAA were associated with egg viability in these species (Zhu et al., 2003). Little is currently known of the variability in the quantity and quality of egg fatty acid and amino acid profiles during the walleye's reproductive season.

This study explores intraspecific variations in total lipids, lipid class composition, and fatty acid and amino acid profiles in eggs from captive walleye broodstock. The objective was to assess variations occurring through the spawning season and to determine how they may affect walleye ontogeny from fertilization to 200 degree-days (DD) post fertilization.

2. Materials and methods

2.1. Spawning and egg production

Eggs were collected from broodstock maintained at the Station Piscicole Trois-Lacs fish farm (Wotton, Quebec, Canada). Fish were kept in a 5 m^3 circular indoor tank with a flow-through system (1 L h^{-1}) and natural photoperiod. The stimulation of sexual maturation began in April 2012 by an increase of temperature from 4.8 ± 0.4°C to 9.6 ± 1.9°C over one month. The broodstock group comprised 98 first-spawning females and 54 males (4 years old; mean weight 433 ± 78 g; mean length 36 ± 3 cm). Fish were fed with a mix of dry pellets (commercial trout

food, 45% protein, 17% lipid; 44/16 from Martin Mills Inc., Ontario, Canada) and frozen pieces of trout (*Salvelinus fontinalis*) and mackerel (*Scomber scombrus*) five times a week from June 2011 to mid-November 2011.

Since there are no external indications of ovulation, oocyte maturation was monitored weekly by sampling ovaries from 15 to 25 females. Gametes were stripped when the first signs of ovulation occurred. Eggs were classified into four groups according to the timing of ovulation (number of days after the first occurrence): early spawning period (P_1, 3 d after first occurrence), intermediate spawning periods (P_2: 5 d; P_3: 8 d), and late spawning period (P_4: 11 d). Each period included several fish, and eggs were pooled (Table 1). All females were injected once with 150 IU of human chorionic gonadotropin (hCG) on 10 May. Females that spawned on P_2, P_3, or P_4 were injected with a second dose of 500 IU of hCG on 13 May.

At each spawning period, fish were anaesthetized with MS_{222} (5 mg L^{-1}) in well-oxygenated fresh water and gametes were collected by hand stripping. Eggs were fertilized using the standard dry fertilization method (Malison and Held, 1996): eggs were collected from each female in a dry 500 mL plastic bowl and immediately fertilized with the milt of two to three males; the mixture was left undisturbed for 1–2 min. A mixture of Fuller's earth and water (3 cups Fuller's earth per 4 L of water) was added to the eggs and stirred to remove the sticky matrix and avoid egg clumping during incubation. Fertilized eggs from a single spawning period were pooled, left for 2–3 h during hardening, and then subdivided into two equal volumes and incubated in 6 L jars (15.8 cm diameter and 45.7 cm high) with a flow rate of 20 L min^{-1}. Incubators were supplied with pumped water from an external pond that had been previously drum filtered (90 µm), sand filtered (20 µm), and vacuum degassed. Temperature was monitored daily. The upwelling water flow in each incubator jar was regulated to ensure continuous gentle movement of the eggs. From two days after fertilization until just prior to hatching, formaldehyde

treatments were applied daily at a concentration of 50–100 mg of formaldehyde L^{-1} of water for 15 min to prevent fungal development.

2.2. Sample collection

For each batch produced, about 150 eggs were sampled after fertilization to determine fertilization and survival success: 30 to 40 embryos were sampled (five replicates per incubator) at 30, 60, 155, and 200 degree-days (DD) post fertilization. Three replicates were frozen in liquid nitrogen and stored at -80°C for biochemical analysis, and the two others were preserved in 1% glutaraldehyde for biometric analysis. The same sampling procedure was used at hatch and before mouth opening. Hatching success (%) was estimated using triplicate subsample counts of larvae from a well-mixed incubator, taking into account the initial number of fertilized eggs and the number of dead and viable eggs removed during incubation.

2.3. Biometric analysis

Egg diameter, oil droplet diameter, and larval length at hatch were measured with a high resolutionVHX-2000 digital microscope (Keyence, Osaka, Japan) adjusted to magnifications of 30–200x and set in high dynamic range mode with light shift.

2.4. Biochemical analysis

Lipids were extracted according to the Folch et al. (1957) procedure modified by Parrish (1999). The relative proportions of the different lipid classes (ketones [KET], triacylglycerols [TAG], free fatty acids [FFA], sterols [ST], acetone-mobile polar lipids [AMPL], and phospholipids [PL]) were determined using an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan) and were developed in a four-solvent system (Parrish, 1987; 1999). In addition, lipid extracts were separated into neutral and polar fractions by silica gel (30 × 5 mm i.d., packed with Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) hydrated with 6%

water and eluted with 10 mL of chloroform:methanol (98:2 v/v) for neutral lipids followed by 20 mL of methanol for polar lipids (Marty et al., 1992). The neutral fraction was further eluted on an activated silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid methyl esters (FAME) were prepared as described by Lepage and Roy (1984) and analyzed in MSMS scan mode (ionic range: 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan, Mississauga, ON, CA) equipped with a Valcobond VB-5 capillary column (VICI Valco Instruments Co. Inc., Broakville, ON, CA). FAME were identified by comparison of retention times with known standards (37 component FAME Mix, PUFA-3, BAME, and menhaden oil; Supelco Bellefonte, PA, USA) and quantified with tricosanoic acid (23:0) as an internal standard. Chromatograms were analyzed using integration Xcalibur 1.3 software (Thermo Scientific, Mississauga, ON, CA).

For total amino acid (TAA) analysis, samples were diluted with 2 mL distilled water and hydrolyzed with equal parts of 12 N HCl containing 10% phenol at 110 °C for 24 h. Free amino acids (FAA) and TAA were extracted and derivatized using EZ:faast^{TM} GC-FID FAA and TAA analysis kits (Clarke et al., 2010). A volume of 100 µL from each sample was mixed with 100 µL of an internal standard, norvaline (0.2 mM), and n-propanol, and passed through a sorbent tip. It was then washed with 200 µL of n-propanol for FAA analysis and 200 µL Milli-Q water for the TAA analysis. The sorbent material was ejected in an eluting medium consisting of 3:2 sodium hydroxide/n-propanol. Next, 50 µL chloroform and 100 µL iso-octane were added to the solution to form an organic layer containing the amino acids, and derivatization was completed with 1 N HCl before being run on a Varian 3800 GC-FID (Agilent Technologies, Palo Alto, CA, USA) to obtain amino acid composition with the exception of taurine and arginine. Each amino acid was quantified with a known quantity of internal standard.

2.5. Statistical analysis

Reproductive characteristics of females, egg and larva measurements, fertilization, survival, intact oil droplet and hatching successes were analyzed with one-way analysis of variance (ANOVA) followed by a posteriori Tukey multiple comparison tests when assumptions of homoscedasticity and normality were verified with Levene and Shapiro-Wilk tests, respectively. Data were transformed (log or arcsine square root) when necessary. One-way ANOVAs were used to estimate variations of total lipid classes, total fatty acids from neutral and polar fractions, total proteins, and total free amino acids according either to spawning periods (P_1, P_2, P_3, and P_4) or to DD post fertilization (30, 60, 155, and 200). Multiple linear regression analyses were used to test whether egg and oil droplet diameters could predict larval length at hatch. These analyses were performed with the SPSS 16.0 package. Permutational multivariate analysis of variance (PERMANOVA with 9999 permutations), including posteriori pair-wise comparisons, were performed on profiles of lipid classes, fatty acids, and amino acids. Assumptions of homoscedasticity were verified with a PERMDISP test, and data were transformed (arcsine square root) when necessary (Sokal and Rohlf, 1995). To analyze the similarity between spawning periods or DD post fertilization, non-metric multi-dimensional scaling (n-MDS) and SIMPER analysis were run using a Bray-Curtis similarity matrix with PRIMER 6 (v. 6.1.12) and PERMANOVA+ (v. 1.0.2). We compared variabilities between the neutral and polar lipid fractions among the different spawning periods using coefficients of variation (CV). Standard errors of the CVs across populations were estimated with a jackknife method (Efron and Gong, 1983).

3. Results

3.1. Reproductive characteristics

Total length and weight of females were similar among spawning periods ($p = 0.8$ and $p = 0.4$ for length and weight, respectively) (Table 1). However, egg diameter was greater at the intermediate (P_2, P_3) spawning periods (Table 1, $F_{\text{egg diameter (3, 120)}} = 42.2$, $p < 0.01$) while the oil droplet diameter was greater in P_1 and P_3 eggs ($F_{\text{oil droplet diameter (3, 120)}} = 4.8$, $p = 0.03$). Fertilization and survival success at 4 h post fertilization were both significantly higher in P_1 and P_2 eggs (Table 1; $F_{\text{Fertilization (3, 6)}} = 6.9$, $p = 0.02$; $F_{\text{survival (3, 6)}} = 11.6$, $p < 0.01$). Egg batches from the late spawning period (P_4) had the lowest fertilization and survival successes (49.9 ± 5.5 and 56.8 ± 6.7%, respectively). The hatching success was significantly different among spawning periods ($F_{(3, 4)} = 129$, $p < 0.01$), with the highest observed in P_2 eggs (87.3 ± 2.4%) and no hatching in P_4 eggs. Larval length at hatch was significantly higher at P_3 than at P_2 and P_1 (Table 1; $F_{\text{larval length (2, 84)}} = 10.9$, $p < 0.01$), and larval length at hatch was positively correlated with egg and oil droplet diameters ($F_{(2, 5)} = 24.5$, $p = 0.01$, $r^2 = 0.94$).

3.2. Egg biochemical composition in relation to walleye ontogeny

3.2.1. Lipids

Total lipid concentration of eggs at 30 DD post fertilization differed significantly according to the spawning period ($F_{(3, 8)} = 34.6$, $p < 0.001$; Fig. 1). Total lipids accounted for 22 ± 7% of the egg dry mass (DM), with the highest (30 ± 3% of DM) and lowest (12 ± 3% of DM) levels in eggs from the P_1 and P_4 groups, respectively (Fig. 1). Similar trends were observed for total fatty acids in both neutral and polar fractions ($F_{\text{neutral fatty acid (3, 4)}} = 642.6$; $F_{\text{polar fatty acid (3, 4)}} = 11.9$, $p < 0.001$). The major lipid classes were KET, TAG, and PL, accounting for 33, 30, and 28% of total lipids, respectively (Fig. 1). The lipid composition did not differ among eggs obtained from different spawning periods ($p = 0.22$).

The polar fraction fatty acid composition of 30 DD post-fertilization eggs did not vary with spawning period ($p = 0.17$) (Table 2). However, the neutral lipid fatty acid composition was

significantly different in P_4 eggs compared to the other three spawning periods (*Pseudo* - $F_{(3, 4)}$ = 15.2, p = 0.01). SIMPER analysis showed that the 18:1 n-9 and 16:1 n-7 contents explained most of this difference. In addition, proportions of MUFA and PUFA were significantly lower in P_4 eggs (one-way ANOVA; $F_{MUFA\ (3,\ 4)}$ = 12.2; $F_{PUFA\ (3,\ 4)}$ = 12.0, p = 0.01). We predicted that variations in the relative abundance of fatty acids among the reproductive periods would be higher in the neutral than in the polar lipid fraction, and we tested this for fatty acids of particular interest. Our comparison of CVs among the four spawning periods at 30 DD post fertilization indicates that the variability was consistently higher in the neutral than in the polar fraction as predicted, except for arachidonic acid (20:4 n-6), 18:2 n-6, 18:3 n-3, MUFA, and PUFA (Fig. 2).

3.2.2. Amino acids

Aspartic acid (ASP), cystathionine (CTH), and glutamic acid (GLU) quantitatively dominated the FAA pool in *Sander vitreus* eggs at 30 DD post fertilization, accounting for 32 ± 7, 17 ± 3, and 9 ± 2% of total FAA, respectively (Table 3). Essential amino acids (EAA) (valine [VAL], leucine [LEU], isoleucine [ILE], threonine [THR], histidine [HIS], methionine [MET], phenylalanine [PHE], lysine [LYS], and tryptophan [TRP]) accounted for 21 ± 5% of the FAA. Concerning TAA, alanine (ALA), GLU, and ASP were the dominant non-essential amino acids (NEAA), contributing an average of 37% of the TAA at 30 DD post fertilization for the four spawning periods (Table 3). VAL, LEU, and ILE were the most abundant EAA. Total FAA concentration in eggs at 30 DD post fertilization averaged 0.7 ± 0.2 mg g^{-1}, with no change among spawning periods (p = 0.07). Total protein concentration averaged 6.3 ± 2.6 mg g^{-1}, and TAA differed according to the spawning period (*Pseudo* - $F_{TAA\ (3,\ 3)}$ = 7.8, p = 0.04). SIMPER analysis showed that LYS (EAA fraction) and SER (NEAA fraction) explained more than 20 and 12%, respectively, of the differences among the four spawning periods. LYS and SER were three times higher in P_2 eggs.

3.3. Use of biochemical reserves during embryonic development

Because P_2 eggs had the highest hatching and survival successes, we only considered this group when investigating the use of biochemical reserves during embryonic development.

Total lipids decreased by almost half during embryonic development (ED) ($F_{(3, 6)} = 6.5$, $p = 0.02$), i.e., from 173.3 ± 21.6 mg g^{-1} at 30 DD to 99.7 ± 11.0 mg g^{-1} at 200 DD. Lipid class composition varied significantly during embryogenesis (*Pseudo* – $F_{(3, 4)} = 19.3$, $p = 0.03$): TAG decreased from 30.7 ± 2.4% at 30 DD to 4.7 ± 1.7% at 200 DD while PL increased from 31.8 ± 3.2% to 83.0 ± 5.7% for the same period (Fig. 3). Fatty acid proportions in the polar fraction changed during ED (*Pseudo* – $F_{(3, 4)} = 28.1$, $p = 0.03$). SIMPER analysis showed that DHA contributed the most to this difference, decreasing significantly during development. In contrast, no changes were observed in the neutral fraction ($p = 0.06$). FAA and TAA profiles varied during ED (*Pseudo* – $F_{\text{FAA } (3, 4)} = 7.0$, $p = 0.02$; *Pseudo* – $F_{\text{TAA } (3, 4)} = 8.4$, $p = 0.01$), with significant decreases in ASP and CTH from 30 to 200 DD post fertilization in the FAA fraction and significant decreases in LYS and SER from 30, 60, 155, and 200 DD post fertilization in the TAA fraction.

4. Discussion

Walleye (*Sander vitreus*) hatcheries still rely largely using broodstock composed of captured wild fish. To improve offspring growth and survival, a better understanding of the biochemical events occurring in early life stages and the impact of egg biochemical composition on subsequent ontogeny are needed. To the best of our knowledge, our study is one of a few that reports 1) evidence of an effect of egg biochemical composition on survival and 2) changes in biochemical composition during embryogenesis. Our results highlight the importance of DHA

(EFA), LYS (EAA), and SER, ASP, and CTH (NEAA) for egg viability and during larval development as well as reveal how late spawning may dramatically affect egg quality.

Variability in offspring survival within one broodstock may be related to many factors, such as spawning timing and hormonal induction (Malison et al., 1998; Migaud et al., 2013). Our data demonstrate a trend in decreasing mean egg size towards the end of the reproductive season, with the lowest egg survival and hatching success at the latest spawning period. This is in agreement with previous results obtained for walleye from Lake Ontario (Johnston et al., 2005; 2007). However, no effect of spawning timing was found on embryonic survival to the eyed stage in a walleye population from Ohio (Czesny et al., 2005). The decrease in egg size in the late-spawning batch could be due to the depletion of female energy reserves, as has been shown in Atlantic cod (Chambers and Waiwood, 1996; Kjesbu, 1989) and Atlantic halibut (Evans et al., 1996). Within a given species, it is commonly accepted that larger eggs have better survival and produce larger offspring (Bromage et al., 1994; Heath et al., 2003). However, some studies on trout and sea bass showed that eggs of varying size may exhibit similar developmental competence (Bromage et al., 1992; Cerdá et al., 1994). In our study, no relationship was found between egg size and survival or hatching success, but we found a positive correlation between egg size and larval size at hatch. Our results suggest that egg size may exert a stronger influence over post-hatch survival than embryonic survival, at least under culture conditions. Czesny et al. (2005) showed that even though egg size varied among females from an inland reservoir, it was unrelated to the egg lipid content.

Relatively little is known about the role of egg biochemical composition in early survival of walleye. Based on the reproductive results, we hypothesized that eggs with the best hatching success would have the highest amounts of total lipids, with higher proportions of essential fatty acids and amino acids. Our results clearly showed that eggs from the latest spawning period (0%

hatching success) had the lowest total lipid content, while eggs from the intermediate spawning periods (highest hatching success) had intermediate levels of total lipids. A positive effect of egg lipid content on embryonic survival and hatching was not expected because much of the lipid reserves in walleye eggs is contained in the large neutral oil droplet, which is not consumed before hatching (Johnston et al., 2007; Moodie et al., 1989).

The advantage of greater total lipid stores to hatching success is not clear. A relationship between egg total lipid content and egg viability has been observed in freshwater fishes, although contradictory reports exist concerning this relationship. High egg lipid content increased viability in roach and bream (Zhukinskiy et al., 1981) while no definite or negative effects were observed in walleye (Czesny and Dabrowski, 1998; Czesny et al., 2005), sole, sea bass, turbot (Devauchelle et al., 1982), Macquarie perch (Sheikh-Eldin et al., 1996) and common dentex (Samaee et al., 2009).

The proportions of lipid classes identified in walleye eggs are typical of fish eggs with a lipid globule (Kaitaranta and Ackman, 1981; Wiegand, 1996). Our results indicate that hatching success was probably related to the relative proportions of some fatty acids and/or amino acids. Hatching success has been associated with egg fatty acid composition in wild fish populations such as cod and walleye (Czesny and Dabrowski, 1998; Moodie et al., 1989; Salze et al., 2005), although such a relationship is not always present.

Polar fatty acid profiles did not vary with the spawning period and did not appear to influence hatching success. Fatty acid profiles at 30 DD post fertilization revealed very high levels of DHA in the polar fraction. Similar high DHA levels in the polar fraction of walleye eggs were also found by Czesny and Dabrowski (1998) and Moodie et al. (1989), suggesting its selective retention during embryogenesis, as well as by Abi-Ayad et al. (2000) and Henrotte et al. (2010) in Eurasian perch eggs. A high proportion of DHA in the polar fraction demonstrates the

importance of this fatty acid. It is likely related to special function since this compound is relatively rare at lower trophic levels in freshwater environments (Henderson and Tocher, 1987; Wiegand, 1996). Czesny and Dabrowski (1998) showed that the polar fraction of walleye egg lipids—in particular the essential fatty acids DHA, EPA, and AA—is noticeably less affected by the broodstock's nutritional status. We found stable proportions of AA + EPA compared with proportions of either fatty acid individually, which is of interest because AA and EPA are biochemical precursors in the eicosanoid synthesis pathways (Fernández-Palacios et al., 2011) and both compete for enzymes in the cyclo-oxygenase and lipoxygenase pathways, with AA being the preferred substrate (Fernández-Palacios et al., 2011). This could explain the high variability of the AA concentration in the lipid polar fraction.

We suggest that the higher levels of MUFA and PUFA in the neutral fraction for the first three spawning periods could be good indicators of offspring quality. Johnston et al. (2007) showed that the PUFA composition of neutral lipids in walleye eggs had only a minor influence on hatching success, suggesting that the relative abundance of PUFA in this fraction could be more important to offspring viability in the post-hatch period. In a marine species, the common dentex, Samaee et al. (2009) showed that high quality egg batches also had higher concentrations of total PUFA and some MUFA. Other studies showed that MUFA in the neutral fraction are preferentially utilized during embryonic development in various fish species (Fraser et al., 1988; Mourente and Vázquez, 1996; Rønnestad et al., 1994; Tocher et al., 1985; Wiegand, 1996). Indeed, in starved Eurasian perch larvae, MUFA contributed 37% of the energy from total fatty acid catabolism (Abi-Ayad et al., 2000).

Total lipids, especially TAG, decreased from 30 to 200 DD post fertilization. Such an observation suggests that TAG were used as a primary endogenous energy reserve prior to exogenous larval feeding (Falk-Petersen et al., 1989; Mejri et al., 2012; Samaee et al., 2009;

Sewall and Rodgveller, 2008). Variations in the polar fraction during embryogenesis—more precisely, the decrease of DHA—suggest that polar lipids have both structural and energetic roles. DHA assures membrane fluidity, which is required for rapid cell division and growth during embryogenesis (Wiegand et al., 2004).

Environmental factors such as temperature affect the lipid composition of fish tissues (Olsen et al., 1999). Indeed, a decrease in water temperature has been associated with an increase in PUFA content in carp tissues (Kayama et al., 1986) or with an increase in DHA content in Atlantic salmon (Olsen and Skjervold, 1995); these effects are likely related to the positive correlation between the degree of unsaturation of fatty acids and membrane fluidity. In our study, the decrease in DHA could be an adaptive mechanism to reduce membrane fluidity with the increase in temperature occurring during ED. In contrast, Abi-Ayad et al. (2004) working on pikeperch larvae, in a stable temperature environment, did not notice specific retention of DHA. In contrast to lipids, relatively little research has been conducted on the role of egg protein composition during ontogeny on subsequent offspring performance. Amino acids are important constituents of fish eggs since they are required by the embryo for protein synthesis and are a major energy source prior to hatching (Rønnestad et al., 2003). Moreover, amino acids are required to synthesize the apolipoproteins required for absorption of the oil droplet (Mani-Ponset et al., 1996; Poupard et al., 2000).

Free amino acids are more important in pelagic marine eggs than in freshwater and benthic marine eggs, where they may represent less than 5% of egg constituents. For example, in common dentex, a marine pelagophil teleost, FAA account for more than 20% of DM in eggs and play an important role during embryogenesis (Samaee et al., 2010). In freshwater eggs, an organic osmolyte pool would be disadvantageous for embryonic osmoregulation in a hypoosmotic environment (Finn and Fyhn, 2010). The significant decrease of FAA (ASP and

CTH) during ED may suggest that these components could be used as energy sources. While EAA are preferentially used for growth in fish larvae, NEAA are used as energy substrates (Abboudi et al., 2006).

Concerning total amino acids, two interesting features were noticed: 1) lysine and serine were three times higher in P_2 eggs (intermediate spawning period) than in those from the other spawning periods; 2) these two amino acids explained most of the variations occurring during ED, and they decreased significantly from 30 to 200 DD post fertilization. There is little information about the exact roles of these amino acids at this life stage, but it is known that lysine, an EAA in fish, plays an important role in the formation of collagen, which is important in early life stages for development of the skeletal system and skin (Finn and Fyhn, 2010; Ohkubo et al., 2008). Moreover, L-carnitine, which is synthesized from LYS and MET, is required for the transport of fatty acids from the cytosol into mitochondria for ß-oxidation (Brown et al., 2005; Harpaz, 2005). In their review, Rønnestad et al. (1999) noted that in fish eggs characterized by oil globules (e.g., *Sander vitreus*), 50% of the energy is derived from amino acids (predominately FAA, but with some contribution from proteins) and 50% from neutral lipids such as TAG and wax and/or steryl esters. Furthermore, there may be an interrelationship between these potential energy sources (Rønnestad et al., 1999; Rosa et al., 2003). Our findings suggest that there may be a concomitant use of free NEAA, proteins, and lipids as energy sources during walleye embryogenesis. Other limiting constituents may include the relative or absolute amounts of vitamins, macrominerals, and maternally transferred hormones, such as thyroid hormones (Brooks et al., 1997), all of which have been linked to both embryonic and post-hatch survival in fish (Hey et al., 1996).

5. Conclusion

This study shows that the timing of ovulation during the spawning period could be a strong determinant in walleye hatching success and early survival. During embryogenesis, energy is derived primarily from TAG, proteins, and non-essential free amino acids, with a possible concomitant use of DHA to reduce membrane fluidity. Even though proteins represent less than 1% of the dry mass, the depletion of LYS and SER in TAA during embryogenesis in the intermediate spawning groups suggests a critical role during walleye ontogeny. Since walleye culture is still not well developed, the data presented in this study bring useful information concerning larval protein and lipid requirements that could be used to formulate well-balanced broodstock diets.

Acknowledgements

This study was financially supported by the Société de Recherche et de Développement en Aquaculture Continentale Inc. (SORDAC), the Station Piscicole Trois-Lacs industry partner (Wotton, Quebec, Canada), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Fonds de recherche du Québec-Nature et Technologies (FRQNT). We appreciate the support of Dr. I. Ben Khemis of the National Institute of Marine Sciences and Technologies (Tunisia). We are grateful to M. Blanchet and K. Grenier for their extensive help during fieldwork and thank J. Wells for her assistance in the amino acid analysis. Finally, we would like to thank the two anonymous reviewers for their constructive comments on the manuscript.

References

Abboudi, T., Mambrini, M., Ooghe, W., Larondelle, Y., Rollin, X., 2006. Protein and lysine requirements for maintenance and for tissue accretion in Atlantic salmon (*Salmo salar*) fry. Aquaculture. 261, 369-383.

Abi-Ayad, S.M.E.A., Kestemont, P., Mélard, C., 2000. Dynamics of total lipids and fatty acids during embryogenesis and larval development of Eurasian perch (*Perca fluviatilis*). Fish Physiol. Biochem. 23, 233-243.

Abi-Ayad, S.M.E.A., Boutiba, Z., Mélard, C., Kestemont, P., 2004. Dynamics of total body fatty acids during early ontogeny of pikeperch (*Sander lucioperca*) larvae. Fish Physiol. Biochem. 30, 129-136.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J., Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 100, 141-166.

Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P., Gillespie, M., Gamble, J., 1994. Egg quality determinants in finfish: the role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. J. World Aquacult. Soc. 25, 13-21.

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Rev. Fish. Biol. Fisher. 7, 387-416.

Brown, M.R., Battaglene, S.C., Morehead, D.T., Brock, M., 2005. Ontogenetic changes in amino acid and vitamins during early larval stages of striped trumpeter (*Latris lineata*). Aquaculture. 248, 263-274.

Bruce, M.P., Shields, R.J., Bell, M.V., Bromage, N.R., 1993. Lipid class and fatty acid composition of eggs of Atlantic halibut, *Hippoglossus hippoglossus (L.),* in relation to egg quality in captive broodstock. Aquac. Res. 24, 417-422.

Cerdá, J., Carrillo, M., Zanuy, S., Ramos, J., de la Higuera, M., 1994. Influence of nutritional composition of diet on sea bass, Dicentrarchus labrax L., reproductive performance and egg and larval quality. Aquaculture. 128, 345-361.

Chambers, R.C., Waiwood, K.G., 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. 53, 1986-2003.

Clarke, M., Parrish, C.C., Penney, R.W., 2010. Free amino acids as an indicator of egg viability in Atlantic Cod (*Gadus morhua*). Bull. Aquacul. Assoc. Canada. 108-2, 6-9.

Czesny, S., Dabrowski, K., 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). Aquat. Living Resour. 11, 371-378.

Czesny, S., Rinchard, J., Dabrowski, K., 2005. Intrapopulation variation in egg lipid and fatty acid composition and embryo viability in a naturally spawning walleye population from an Inland reservoir. N. Am. J. Fish. Manage. 25, 122-129.

Devauchelle, N., Brichon, G., Lamour, F., Stephan, G., 1982. Biochemical composition of ovules and fecond eggs of sea bass(*Dicentrarchus Labrax*), sole (*Solea vulgaris*), and turbot (*Scophthalmus maximus*). in: Richter C.J.J., G.H.J.T.E. (Eds.), Reproduction Physiology of Fishes, Proc. Int. Symp. Reproductive physiology of fish, Center for Agricultural Publishing and Documentation, Wageningen, Netherlands, pp. 155-157.

Efron, B., Gong, G., 1983. A leisurely look at the bootstrap, the jackknife, and cross-validation. Am. Stat. 37, 36-48.

Evans, R.P., Parrish, C.C., Brown, J.A., Davis, P.J., 1996. Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture. 139, 139-149.

Falk-Petersen, S., Sargent, J.R., Fox, C., Falk-Petersen, I.B., Haug, T., Kjørsvik, E., 1989. Lipids in Atlantic halibut (*Hippoglossus hippoglossus)* eggs from planktonic samples in Northern Norway. Mar. Biol. 101, 553-556.

Fenton, R., Mathias, J.A., Moodie, G.E.E., 1996. Recent and future demand for walleye in North America. Fish. Res. 21, 6-12.

Fernández-Palacios, H., Norberg, B., Izquierdo, M., Hamre, K., 2011. Effects of Broodstock Diet on Eggs and Larvae. in: Holt, G.J. (Eds.), Larval Fish Nutrition. Wiley-Blackwell and sons, Inc., West Sussex, pp. 151-181.

Finn, R.N., Fyhn, H.J., 2010. Requirement for amino acids in ontogeny of fish. Aquac. Res. 41, 684-716.

Finn, R.N., Fyhn, H.J., Evjen, M.S., 1995a. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. Mar. Biol. 124, 355-369.

Finn, R.N., Rønnestad, I., Fyhn, H.J., 1995b. Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus L.*). Comp. Biochem. Phys A. 111, 647-671.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Biol. Chem. 226, 497-509.

Fraser, A.J., Gamble, J.C., Sargent, J.R., 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar. Biol. 99, 307-313.

Harpaz, S., 2005. l-Carnitine and its attributed functions in fish culture and nutrition—a review. Aquaculture. 249, 3-21.

Heath, D., Heath, J., Bryden, C., Johnson, R., Fox, C., 2003. Rapid Evolution of Egg Size in Captive Salmon. Science. 299, 1738-1740.

Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26, 281-347.

Henrotte, E., Mandiki, R.S.N.M., Prudencio, A.T., Vandecan, M., Mélard, C., Kestemont, P., 2010. Egg and larval quality, and egg fatty acid composition of Eurasian perch breeders (*Perca fluviatilis*) fed different dietary DHA/EPA/AA ratios. Aquac. Res. 41, 53-61.

Hey, J., Farrar, E., Bristow, B.T., Stettner, C., Summerfelt, R.C., 1996. Thyroid Hormones and Their Influences on Larval Performance and Incidence of Cannibalism in Walleye *Stizostedion vitreum*. J. World Aquacult. Soc. 27, 40-51.

Johnston, T.A., Miller, L.M., Whittle, D.M., Brown, S.B., Wiegand, M.D., Kapuscinski, A.R., Leggetta, W.C., 2005. Effects of maternally transferred organochlorine contaminants on early life survival in a freshwater fish. Environ. Toxicol. Chem. 24, 2594-2602.

Johnston, T.A., Wiegand, M.D., Leggett, W.C., Pronyk, R.J., Dyal, S.D., Watchorn, K.E., Kollar, S., Casselman, J.M., 2007. Hatching success of walleye embryos in relation to maternal and ova characteristics. Ecol. Freshw. Fish. 16, 295-306.

Kaitaranta, J.K., Ackman, R.G., 1981. Total lipids and lipid classes of fish roe. Comp. Biochem. Phys B. 69, 725-729.

Kayama, M., Hirata, M., Hisai, T., 1986. Effect of water temperature on the desaturation of fatty acids in carp. B. Japan. Soc. Sci. Fish. 52, 853-857.

Keckeis, H., Bauer-Nemeschkal, E., Menshutkin, V.V., Nemeschkal, H.L., Kamler, E., 2000. Effects of female attributes and egg properties on offspring viability in a rheophilic cyprinid, *Chondrostoma nasus*. Can. J. Fish. Aquat. Sci. 57, 789-796.

Kjesbu, O.S., 1989. The spawning activity of cod, *Gadus morhua L*. J. Fish. Biol. 34, 195-206.

Lepage, G., Roy, C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J. Lipid Res. 25, 1391-1396.

Malison, J.A., Held, J.A., 1996. Reproductive biology and spawning. in: Summerfelt, R.C. (Eds.), Walleye culture manual. NCRAC culture series101, Ames, pp. 11-18.

Malison, J.A., Procarione, L.S., Kayes, T.B., Hansen, J.F., Held, J.A., 1998. Induction of out-of-season spawning in walleye (*Stizostedion vitreum*). Aquaculture. 163, 151-161.

Mani-Ponset, L., Guyot, E., Diaz, J.P., Connes, R., 1996. Utilization of yolk reserves during post-embryonic development in three teleostean species: the sea bream *Sparus aurata*, the sea bass *Dicentrarchus labrax*, and the pike-perch *Stizostedion lucioperca*. Mar. Biol. 126, 539-547.

Marty, Y., Delaunay, F., Moal, J., Samain, J.F., 1992. Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. J. Exp. Mar. Biol. Ecol. 163, 221-234.

Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture. 227, 21-33.

McElman, J., Balon, E., 1979. Early ontogeny of walleye, *Stizostedion vitreum* with steps of saltatory development. Environ. Biol. Fish. 4, 309-348.

Mejri, S., Tremblay, R., Lambert, Y., Audet, C., 2012. Influence of different levels of dissolved oxygen on the success of Greenland halibut (*Reinhardtius hippoglossoides*) egg hatching and embryonic development. Mar. Biol. 159, 1693-1701.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herráez, M.P., Carrillo, M., 2013. Gamete quality and broodstock management in temperate fish. Rev. Aquac. 5, S194-S223.

Moodie, G.E.E., Loadman, N.L., Wiegand, M.D., Mathias, J.A., 1989. Influence of Egg Characteristics on Survival, Growth and Feeding in Larval Walleye (*Stizostedion vitreum*). Can. J. Fish. Aquat. Sci. 46, 516-521.

Mourente, G., Vázquez, R., 1996. Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole (*Solea senegalensis*). Fish Physiol. Biochem. 15, 221-235.

Navas, J.M., Bruce, M., Thrush, M., Farndale, B.M., Bromage, N., Zanuy, S., Carrillo, M., Bell, J.G., Ramos, J., 1997. The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. J. Fish. Biol. 51, 760-773.

Ohkubo, N., Sawaguchi, S., Nomura, K., Tanaka, H., Matsubara, T., 2008. Utilization of free amino acids, yolk protein and lipids in developing eggs and yolk-sac larvae of Japanese eel *Anguilla japonica*. Aquaculture. 282, 130-137.

Olsen, R.E., Løvaas, E., Lie, Ø., 1999. The influence of temperature, dietary polyunsaturated fatty acids, α-tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, *Salvelinus alpinus* (L.). Fish. Physiol. Biochem. 20, 13-29.

Olsen, Y., Skjervold, H., 1995. Variation in content of Ω3 fatty acids in farmed Atlantic salmon, with special emphasis on effects of non-dietary factors. Aquacult. Int. 3, 22-35.

Parrish, C.C., 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan Flame Ionization detection. Can. J. Fish. Aquat. Sci. 44, 722-731.

Parrish, C.C., 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. in: M.T., A., W.B.C. (Eds), Lipids in Freshwater Ecosystems. Springer Verlag, New York, pp. 4-20.

Pickova, J., Dutta, P.C., Larsson, P.O., Kiessling, A., 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: Comparison between two cod (*Gadus morhua*) stocks. Can. J. Fish. Aquat. Sci. 54, 2410-2416.

Poupard, G., André, M., Durliat, M., Ballagny, C., Boeuf, G., Babin, P.J., 2000. Apolipoprotein E gene expression correlates with endogenous lipid nutrition and yolk syncytial layer lipoprotein synthesis during fish development. Cell. Tissue. Res. 300, 251-261.

Rinchard, J., Dabrowski, K., Van Tassell, J.J., Stein, R.A., 2005. Optimization of fertilization success in *Sander vitreus* is influenced by the sperm : egg ratio and ova storage. J. Fish. Biol. 67, 1157-1161.

Rønnestad, I., Thorsen, A., Finn, R.N., 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture. 177, 201-216.

Rønnestad, I., Koven, W.M., Tandler, A., Harel, M., Fyhn, H.J., 1994. Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar. Biol. 120, 187-196.

Rønnestad, I., Tonheim, S.K., Fyhn, H.J., Rojas, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y., Conceição, L.E.C., 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. Aquaculture. 227, 147-164.

Rosa, R., Morais, S., Calado, R., Narciso, L., Nunes, M.L., 2003. Biochemical changes during the embryonic development of Norway lobster, *Nephrops norvegicus*. Aquaculture. 221, 507-522.

Salze, G., Tocher, D.R., Roy, W.J., Robertson, D.A., 2005. Egg quality determinants in cod (Gadus morhua L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquac. Res. 36, 1488-1499.

Samaee, S.M., Estévez, A., Giménez, G., Lahnsteiner, F., 2009. Evaluation of quantitative importance of egg lipids and fatty acids during embryos and larvae development in marine pelagophil teleosts: With an emphasis on Dentex dentex. J. Exp. Zool. Part A. 311, 735-751.

Samaee, S.M., Mente, E., Estévez, A., Giménez, G., Lahnsteiner, F., 2010. Embryo and larva development in common dentex (Dentex dentex), a pelagophil teleost: The quantitative composition of egg-free amino acids and their interrelations. Theriogenology. 73, 909-919.

Sewall, F.F., Rodgveller, C.J., 2008. Changes in body composition and fatty acid profile during embryogenesis of quillback rockfish (*Sebastes maliger*). Fish. B-NOAA. 107, 207-220.

Sheikh-Eldin, M., De Silva, S.S., Anderson, T.A., Gooley, G., 1996. Comparison of fatty acid composition of muscle, liver, mature oocytes, and diets of wild and captive Macquarie perch, *Macquaria australasica*, broodfish. Aquaculture. 144, 201-216.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The principles and practice of statistics in biological research, 3rd ed. Freeman, W.H., New York.

Tocher, D., Fraser, A., Sargent, J., Gamble, J., 1985. Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring (*Clupea harengus* L.). Lipids. 20, 69-74.

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

Wiegand, M.D., Johnston, T.A., Martin, J., Leggett, W.C., 2004. Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can. J. Fish. Aquat. Sci. 61, 110-121.

Zhu, P., Parrish, C.C., Brown, J.A., 2003. Lipid and amino acid metabolism during early development of Atlantic halibut (*Hippoglossus hippoglossus*). Aquacult. Int. 11, 43-52.

Zhukinskiy, V.N., Gosh, R.I., Konovalov, Y.D., Kim, Y.D., Kovtun, E.I., 1981. Overmaturation and resorption of mature oocytes and their physiological and biochemical features in roach and bream, Ontogenetic Diversity in Fish. Naukova Dumka, Kiev, pp. 85-125.

Figures Legends

Fig. 1. Changes in total lipid content and lipid class composition (KET: ketones; TAG: triacylglycerols; FFA: free fatty acids; ST: sterols; AMPL: acetone-mobile polar lipids; PL: phospholipids) in walleye (*Sander vitreus*) eggs at 30 degree-days post fertilization (mean ± SD). Different letters indicate statistically significant differences among spawning periods. Spawning periods were defined as the number of days following the first occurrence of ovulation: early (P_1, 3 d), intermediate (P_2, 5 d; P_3, 8 d), and late (P_4, 11 d).

Fig. 2. Variations in the proportions of selected fatty acid classes among eggs from different spawning periods at 30 degree-days post fertilization (shaded bars: neutral lipid fraction; solid bars polar lipid fraction). Values represent jackknifed means + one standard error. Results of two-tailed *t*-tests are indicated (ns: not significant; *: $p < 0.05$).

Fig. 3. Changes in the major lipid class composition (KET: ketones, TAG: triacylglycerols, PL: phospholipids) in walleye (*Sander vitreus*) eggs and larvae at 30, 60, 155, and 200 degree-days post fertilization (mean ± SD).

Table 1. Reproductive characteristics (mean ± SD) of female walleye and their eggs and larvae collected throughout the 2012-spawning season from a broodstock in captivity. Means in a row with different letters are significantly different (ANOVA: $p < 0.05$). Spawning periods within the

reproductive cycle were defined according to the number of days following the first occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d and P_3, 8 d; and late spawning: P_4, 11 d.

Table 2. Fatty acid composition of neutral and polar lipids of walleye eggs (% weight of total neutral and polar lipids ± SD) at 30 degree-days post fertilization at different spawning periods (P_1, P_2, P_3, and P_4). Spawning periods within the reproductive cycle were defined according to the number of days following the first natural occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d, and P_3, 8 d; and late spawning: P_4, 11 d. Different letters indicate significant differences among spawning periods for the neutral fraction.

Table 3. Free and total amino acid contents (% of total amino acids ± SD) of walleye eggs at 30 degree-days post fertilization at different spawning periods (P_1, P_2, P_3, and P_4). Spawning periods within the reproductive cycle were defined according to the number of days following the first natural occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d, and P_3, 8 d; and late spawning: P_4, 11 d.

Table 1. Reproductive characteristics (mean ± SD) of female walleye and their eggs and larvae collected throughout the 2012 spawning season from a broodstock in captivity. Means in a row with different letters are significantly different (ANOVA: $p < 0.05$). Spawning periods within the reproductive cycle were defined according to the number of days following the first occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d and P_3, 8 d; and late spawning: P_4, 11 d.

	Reproductive cycle			
	P_1	**P_2**	**P_3**	**P_4**
Females				
N	7	8	48	35
Length (mm)	360.00 ± 21.40	364.37 ± 38.02	370.62 ± 31.61	368.37 ± 25.90
Weight (g)	438.85 ± 49.04	419.75 ± 81.43	483.20 ± 111.88	458.40 ± 76.48
Eggs and Larvae				
Egg diameter (mm)	1.95 ± 0.05^{b}	2.01 ± 0.07^{a}	2.01 ± 0.08^{a}	1.83 ± 0.07^{c}
Oil droplet diameter (mm)	0.75 ± 0.07^{a}	0.70 ± 0.07^{b}	0.77 ± 0.04^{a}	0.73 ± 0.06^{ab}
Larval length on hatch (mm)	6.80 ± 0.25^{b}	6.85 ± 0.29^{b}	7.10 ± 0.37^{a}	-
Fertilization success (%)	75.92 ± 11.26^{a}	78.76 ± 0.34^{a}	68.16 ± 5.31^{ab}	49.92 ± 5.54^{b}
Intact oil droplet (%)	77.16 ± 4.27^{a}	75.29 ± 6.81^{a}	61.26 ± 5.15^{ab}	47.00 ± 4.24^{b}
Survival success (%)	85.18 ± 4.89^{a}	87.79 ± 5.80^{a}	71.64 ± 7.84^{ab}	56.76 ± 6.74^{b}
Hatch success (%)	56.90 ± 9.76^{b}	87.28 ± 2.43^{a}	41.01 ± 8.50^{b}	0.00 ± 0.00^{c}

Table 2. Fatty acid composition of neutral and polar lipids of walleye eggs (% weight of total neutral and polar lipids ± SD) at 30 degree-days post fertilization at different spawning periods (P_1, P_2, P_3, and P_4). Spawning periods within the reproductive cycle were defined according to the number of days following the first natural occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d, and P_3, 8 d; and late spawning: P_4, 11 d. Different letters indicate significant differences among spawning periods for the neutral fraction

Fatty acids	**Neutral fraction**				**Polar fraction**			
	Spawning periods							
	P_1	**P_2**	**P_3**	**P_4**	**P_1**	**P_2**	**P_3**	**P_4**
C14:0	1.9 ± 0.0	2.2 ± 0.3	2.1 ± 0.0	2.5 ± 0.0	1.9 ± 0.1	0.9 ± 0.3	1.1 ± 0.0	0.9 ± 0.4
C16:0	8.6 ± 0.1	9.3 ± 0.2	8.0 ± 0.1	9.5 ± 0.0	18.8 ± 0.1	18.9 ± 0.2	17.6 ± 0.5	19.4 ± 1.7
C18:0	0.8 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	1.1 ± 0.0	4.9 ± 0.1	5.2 ± 0.9	5.1 ± 0.1	5.6 ± 0.6
C19:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.0
∑SFA†	12.0 ± 3.1	13.2 ± 3.3	11.4 ± 2.9	13.9 ± 3.4	26.6 ± 6.8	26.8 ± 6.8	25.6 ± 6.3	28.0 ± 7.0
C16:1 n-7	15.1 ± 0.1	11.3 ± 3.1	14.6 ± 0.5	17.4 ± 0.2	2.4 ± 0.1	2.5 ± 0.3	2.5 ± 0.0	3.4 ± 0.7
C18:1 n-9	31.5 ± 1.0	32.5 ± 0.5	30.5 ± 0.8	18.8 ± 1.7	2.7 ± 0.1	3.3 ± 0.5	3.0 ± 0.0	8.9 ± 7.6
C20:1 n-9	1.3 ± 0.0	1.8 ± 0.0	1.5 ± 0.0	1.6 ± 0.1	1.9 ± 0.0	2.7 ± 0.5	2.6 ± 0.1	2.7 ± 0.0
∑MUFA‡	**49.5 ± 12.1^{a}**	**47.0 ± 12.0^{ab}**	**48.0 ±11.7^{a}**	**39.4 ± 8.5^{b}**	8.1 ± 1.1	9.6 ± 1.4	9.0 ± 1.3	16.0 ± 3.2
C18:2 n-6	15.9 ± 0.8	17.2 ± 0.6	16.6 ± 0.0	10.6 ± 0.2	4.8 ± 0.1	5.6 ± 0.2	5.4 ± 0.2	5.4 ± 0.4
C18:3 n-6	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
C20:3 n-6	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.9 ± 0.0	3.7 ± 0.0	3.0 ± 0.1	3.4 ± 0.2	2.9 ± 0.4
C20:4 n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:3 n-3	2.8 ± 0.0	2.6 ± 0.6	2.7 ± 0.0	3.2 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.00
C20:3 n-3	1.1 ± 0.0	0.9 ± 0.3	0.9 ± 0.0	1.4 ± 0.0	5.5 ± 0.0	4.5 ± 0.1	5.1 ± 0.4	2.6 ± 1.5
C20:5 n-3	3.2 ± 0.1	3.4 ± 0.3	3.8 ± 0.0	4.3 ± 0.1	6.6 ± 0.2	6.7 ± 0.5	7.5 ± 0.2	6.3 ± 1.0
C22:6 n-3	11.4 ± 0.0	11.6 ± 1.0	11.9 ± 0.0	10.7 ± 0.5	45.3 ± 0.9	44.6 ± 0.8	44.8 ± 0.1	40.0 ± 6.9
∑PUFAЂ	**37.1 ± 5.4^{a}**	**38.7 ± 5.8^{a}**	**38.7 ± 5.7^{a}**	**32.5 ± 6.4^{b}**	67.8 ±13.7	66.5 ± 13.5	68.2 ± 13.6	58.8 ± 12.2
∑n-3	19.2 ± 4.6	19.5 ± 4.7	20.3 ± 4.8	23.7 ± 5.4	58.3 ± 1.6	56.7 ± 1.4	58.2 ± 1.5	49.3 ± 1.3
∑n-6	17.8 ± 6.9	19.1 ± 7.5	18.4 ± 7.2	21.0 ± 8.1	9.5 ± 2.5	9.79 ± 1.9	9.9 ± 2.4	9.5 ± 3.0
Total lipids (mg g^{-1})	**94.0 ± 1.0**	**53.0 ±19.7**	**61.2 ± 0.3**	**48.1 ± 1.3**	**22.2 ± 2.9**	**14.8 ± 4.6**	**15.0 ± 1.6**	**6.9 ± 3.8**

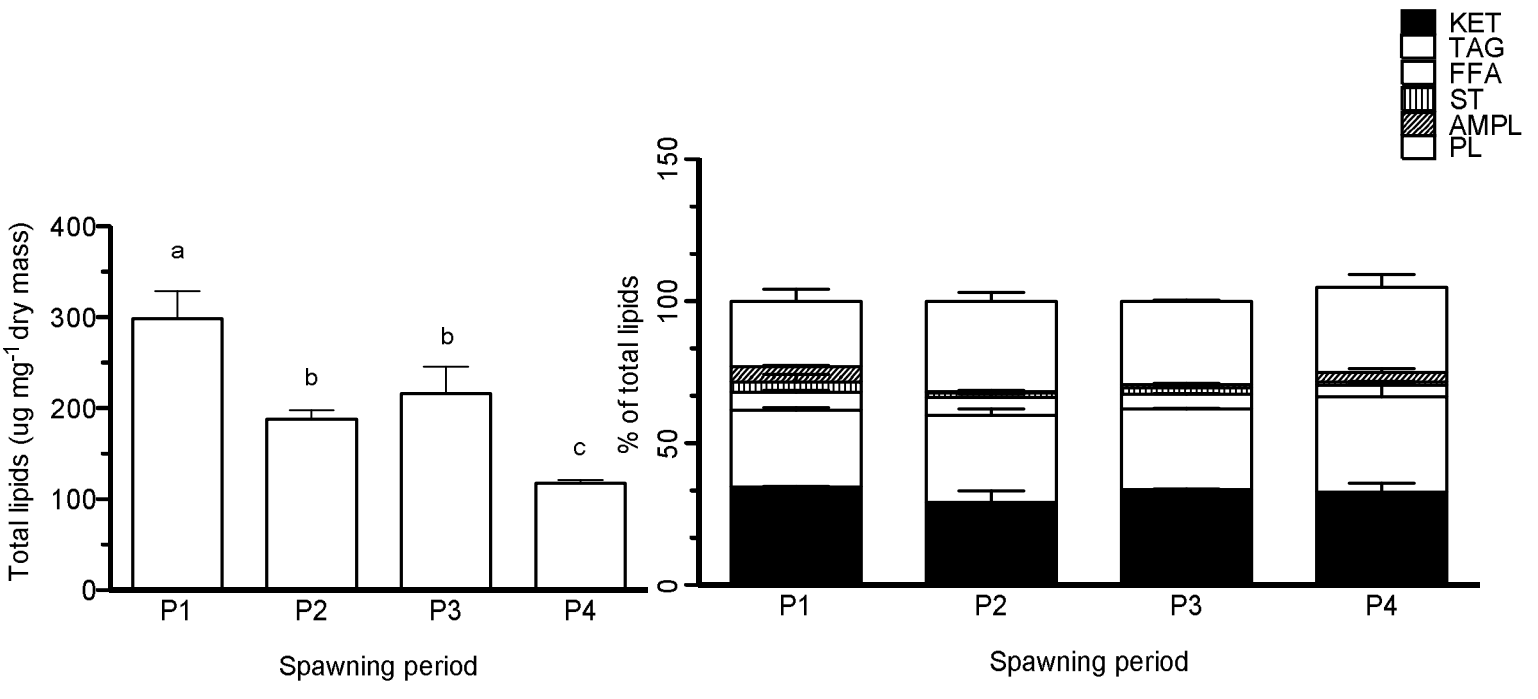
† Includes 15:0, 17:0, and 20:0; ‡ includes 15:1, 17:1, 14:1 n-5, 22:1 n-9, and 24:1; Ђ includes 18:4 n-3

Table 3. Free and total amino acid contents (% of total amino acids ± SD) of walleye eggs at 30 degree-days post fertilization at different spawning periods (P_1, P_2, P_3, and P_4). Spawning periods within the reproductive cycle were defined according to the number of days following the first natural occurrence of ovulation: early spawning: P_1, 3 d; intermediate spawning: P_2, 5 d, and P_3, 8 d; and late spawning: P_4, 11 d.

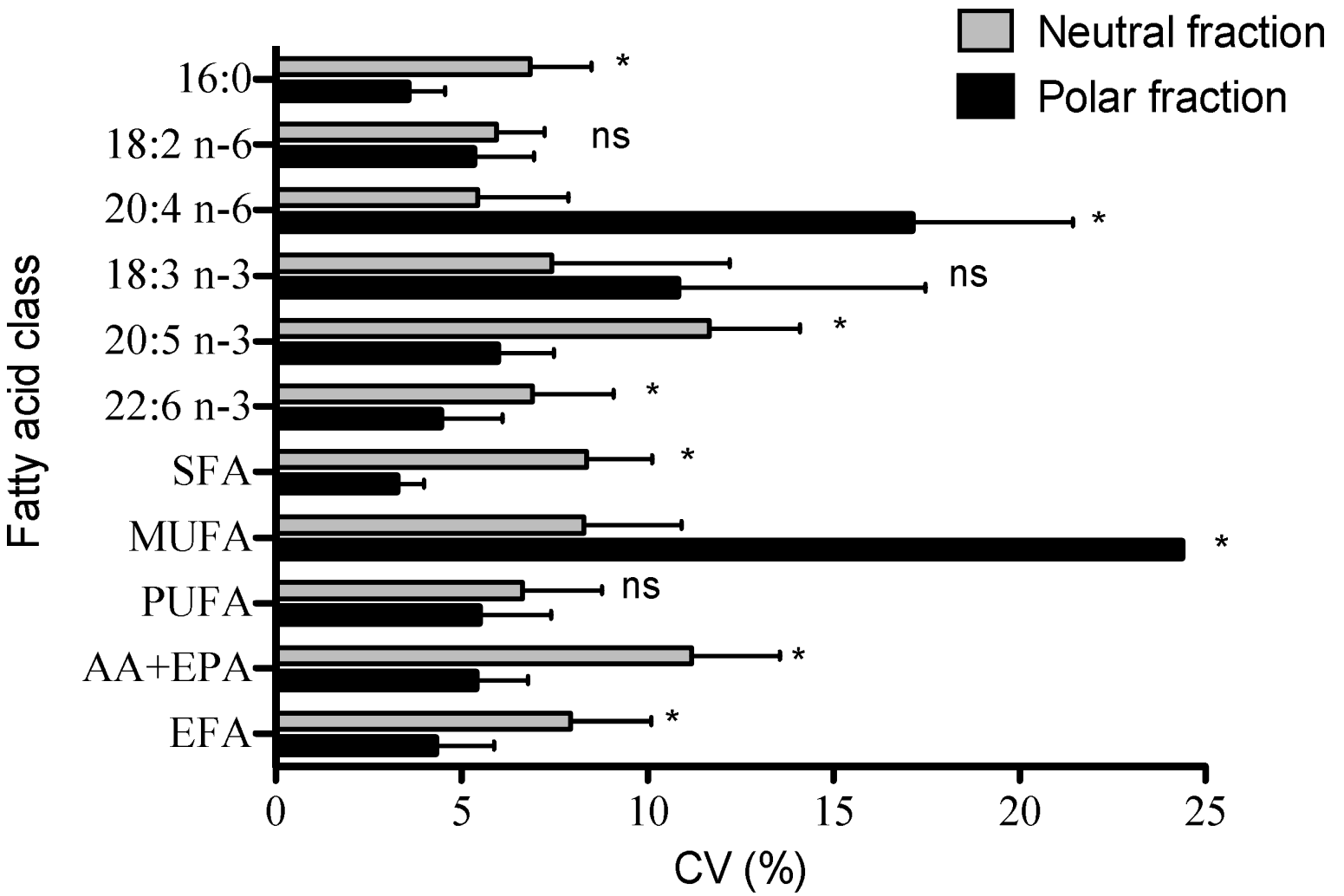
Amino acids	**Free amino acids (FAA)**				**Total amino acids (TAA)**			
	Spawning periods							
	P_1	**P_2**	**P_3**	**P_4**	**P_1**	**P_2**	**P_3**	**P_4**
Essential amino acids								
Valine. VAL	3.5	3.9 ± 0.2	3.7 ± 0.2	3.6 ± 0.4	9.3	10.8 ± 1.3	8.6 ± 0.5	8.0 ± 0.7
Leucine. LEU	3.6	4.6 ± 0.7	4.5 ± 0.3	2.6 ± 0.9	11.1	10.6 ± 0.8	11.0 ± 0.4	11.5 ± 0.0
Isoleucine. ILE	2.4	2.7 ± 0.5	2.1 ± 0.2	1.2 ± 0.7	6.8	7.5 ± 0.9	6.8 ± 0.4	6.6 ± 0.4
Threonine. THR	0.0	0.0	0.4 ± 0.5	0.6 ± 0.8	3.6	5.9 ± 0.6	3.4 ± 0.3	4.1 ± 0.1
Histidine. HIS	2.1	2.6 ± 0.4	2.0 ± 0.1	1.4 ± 0.2	0.7	0.0	1.7 ± 0.2	2.5 ± 0.2
Methionine. MET	2.2	3.0 ± 0.3	2.1 ± 0.3	0.9 ± 0.5	2.6	2.0 ± 0.1	2.8 ± 0.2	2.4 ± 0.5
Phenylalanine. PHE	1.9	2.2 ± 0.3	1.2 ± 0.2	1.7 ± 0.7	5.9	4.2 ± 0.2	4.8 ± 0.3	5.7 ± 0.4
Lysine. LYS	3.9	5.9 ± 0.5	4.5 ± 0.5	2.6 ± 2.1	5.0	15.6 ± 0.2	7.3 ± 0.2	5.3 ± 0.8
Tryptophan. TRP (TRY)	0.5	1.1 ± 0.5	0.6 ± 0.0	0.4 ± 0.6	ND	ND	ND	ND
Non- essential amino acids								
Alanine. ALA	6.4	6.8 ± 1.0	4.7 ± 0.4	2.8 ± 1.5	15.6	17.6 ± 1.5	15.0 ± 0.2	13.5 ± 0.2
Sarcosine. SAR	1.6	1.0 ± 0.7	1.2 ± 0.2	0.0	0.1	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
Glycine. GLY	2.0	2.2 ± 0.2	3.2 ± 0.2	2.1 ± 0.4	6.8	6.8 ± 1.0	6.2 ± 0.2	6.1 ± 0.2
Serine. SER	0.0	0.0	0.0	0.4 ± 0.6	0.0	6.2 ± 1.5	1.3 ± 0.6	3.4 ± 0.6
Proline. PRO	1.4	1.4 ± 0.2	1.9 ± 0.1	1.3 ± 0.9	5.3	5.9 ± 0.6	4.7 ± 0.0	5.3 ± 0.3
Thioproline. TPR	0.9	1.5 ± 1.3	1.3 ± 0.3	0.6 ± 0.2	ND	ND	ND	ND
Aspartic acid. ASP	33.6	24.2 ± 7.1	30.9 ± 0.3	39.3 ± 4.8	8.1	10.4 ± 0.4	7.9 ± 0.5	7.5 ± 0.1
Hydroxyproline. HYP	0.1	0.1 ± 0.0	0.7 ± 0.6	0.2 ± 0.2	0.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
Glutamic acid. GLU	7.8	11.4 ± 1.6	8.6 ±2.7	8.1 ± 1.1	13.8	10.2 ± 3.2	13.1 ± 0.6	11.8 ± 0.6
Glutamine. GLN	4.4	1.8 ± 2.3	3.2 ± 0.0	4.9 ± 0.2	ND	ND	ND	ND
Tyrosine. TYR	2.6	2.5 ± 0.4	2.1 ± 0.5	2.8 ± 0.9	2.75	0.8 ± 0.2	3.7 ± 0.1	3.9 ± 0.0
Cystathionine. CTH	15.3	16.6 ± 0.8	13.9 ± 0.2	19.6 ± 6.0	ND	ND	ND	ND
Cystine. C-C	0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	ND	ND	ND	ND
Σ Essential	20.5	26.3 ± 3.2	21.4 ± 0.3	15.3 ± 7.3	45.3	45.9 ± 4.4	46.8 ± 1.4	46.4 ± 0.8
Σ Non-essential	79.4	73.6 ± 5.3	76.5 ± 1.7	84.6 ± 3.2	54.6	54.0 ± 1.1	53.1 ± 1.1	52.8 ± 0.8
Σ Acidic	49.7	42.2 ± 1.0	48.5 ± 0.3	53.5 ± 2.3	23.7	18.9 ± 0.2	21.4 ± 0.0	19.7 ± 0.6
Σ Basic	6.1	8.6 ± 3.0	6.6 ± 0.7	4.0 ± 0.8	5.8	15.6 ± 0.3	9.0 ± 0.2	7.8 ± 0.8
Σ Aromatic	8.2	10.2 ± 3.2	7.3 ± 0.0	7.1 ± 7.3	9.4	4.3 ± 4.4	10.3 ± 1.4	12.1 ± 0.8
Essential/Non-essential	0.2	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.8	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0
Total ($mg.g^{-1}$)	0.7	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	8.0	3.4 ± 0.2	4.8 ± 0.2	8.9 ± 1.3

ND: not detected amino acids

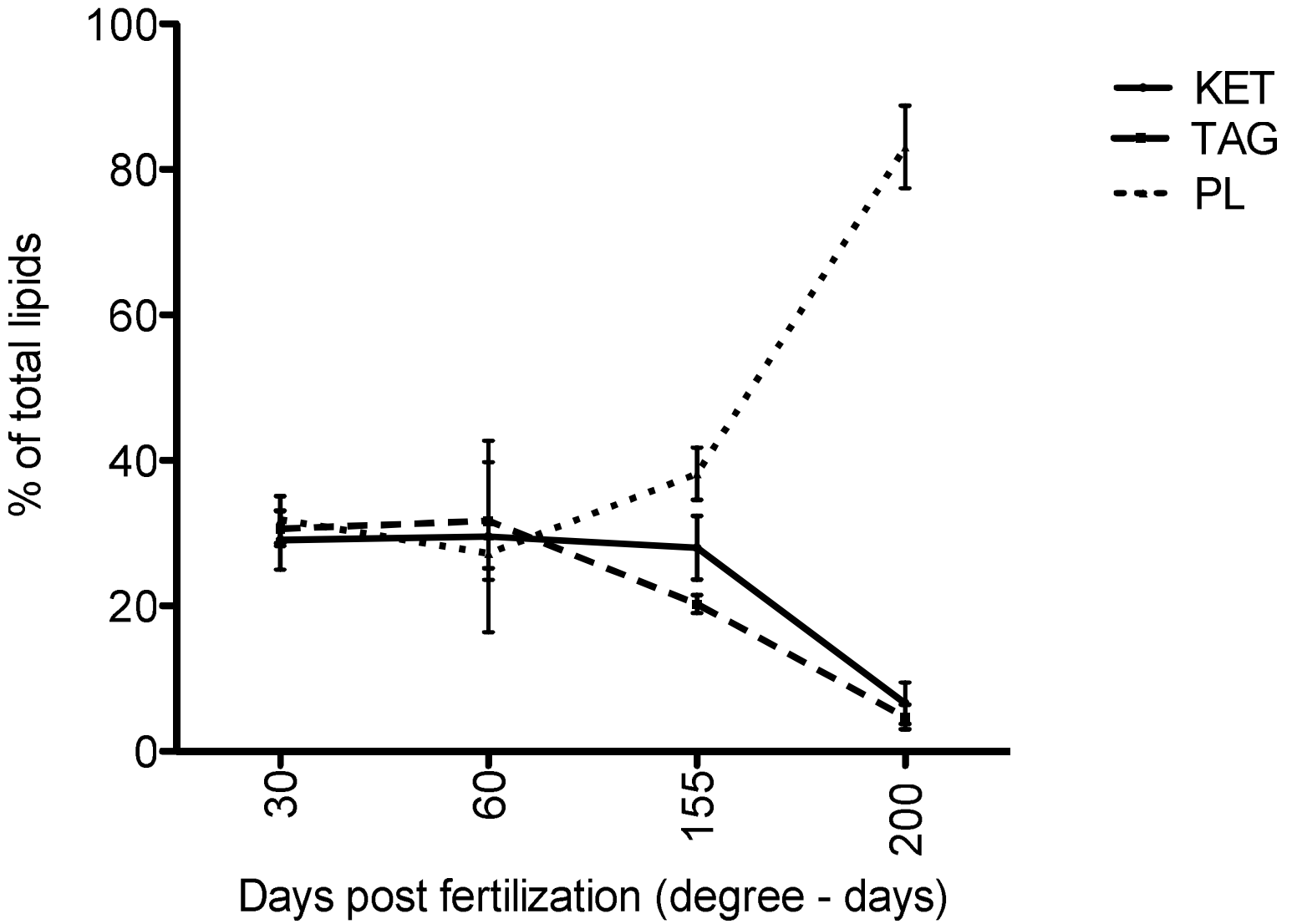
Figure(s)


Total lipids (ug mg-1 dry mass)
400
300
200
100
0
a
b
b
c
P1
P2
P3
P4
Spawning period
% of total lipids
150
100
50
0
KET
TAG
FFA
ST
AMPL
PL
P1
P2
P3
P4
Spawning period

Figure(s)


Neutral fraction
Polar fraction
16:0
18:2 n-6
20:4 n-6
18:3 n-3
20:5 n-3
22:6 n-3
SFA
MUFA
PUFA
AA+EPA
EFA
*
ns
*
ns
*
*
*
*
ns
*
*
Fatty acid class
0
5
10
15
20
25
CV (%)

Figure(s)


100
80
60
40
20
0
% of total lipids
30
60
155
200
Days post fertilization (degree - days)
KET
TAG
PL