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Fatty acids in an iteroparous fish: variable complexity, identity, and phenotypic correlates

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Running head: Fatty acid complexity and identity in yellow perch

Abstract: Ecologists often focus on summarized composition when assessing complex, multivariate phenotypes like fatty acids. Increasing complexity in fatty acid composition may offer benefits to individuals that may not be recognized by assessing mean fatty acid identity. We quantified fatty acid identity and complexity in the egg and muscle of spawning female yellow perch (*Perca flavescens* Mitchill, 1814) exposed to three overwinter thermal regimes (4, 8, and 13 °C), to evaluate i) thermal regulation of fatty acid complexity, ii) fatty acid complexity and identity differences between female muscle and eggs, and iii) relationships between fatty acid complexity and egg traits. Temperature did not alter fatty acid complexity in either tissue. Muscle contained an even distribution of 12 abundant polyunsaturated fatty acids (PUFA), while eggs exhibited a simpler phenotype of eight compounds. Fatty acid complexity in female muscle was positively correlated with potential indicators of egg quality, including size and lipid content. Preferential allocation of fatty acids to eggs by females likely contributed to a simple phenotype dominated by PUFA precursors and energy sources, while muscle represented a more complex fatty acid phenotype. Recognizing complexity could elucidate variation in reproductive condition among individuals, while identifying patterns in fatty acid allocation among populations.

Key words: fish; eggs & eggshells; muscle, temperature; reproduction; quality; aquaculture; diversity, yellow perch, *Perca flavescens*, fatty acids

35 Introduction

36 Individual organisms are composed of large numbers of tissue types, biochemical
37 compounds, behaviors, morphological traits, and other characteristics that together comprise a
38 phenome, or highly multivariate suite of phenotypic traits that determine individual fitness
39 (Houle et al. 2010). Biologists commonly focus on mechanisms controlling the function or
40 abundance of specific traits and their resulting influence on organismal performance while
41 ignoring the highly multivariate nature of most phenotypes (Laughlin and Messier 2015). Such
42 methodology may thereby overlook the simultaneous importance of phenotypic identity (e.g.,
43 mean abundances of specific compounds) and complexity (e.g., diversity or number of different
44 compounds, irrespective of identity) to individual performance (Symstad et al. 1998; Straub and
45 Snyder 2006). However, there is evidence that variation in the complexity of multivariate
46 phenotypes can influence individual performance measures, , such as predator defense and
47 resilience to stress or exertion (Price and Guglielmo 2009; Marion et al. 2015b). This suggests
48 that taking a “complexity-as-diversity” approach to quantifying multivariate traits in terms of
49 their functional number and diversity of characters could offer new insights into the role of
50 phenotypic complexity in individual performance (Marion et al. 2015b).

51 Fatty acids include a large number of molecules that serve important structural,
52 metabolic, and physiological functions in many organisms (Bell et al. 1986), thus representing a
53 complex phenotype that can have strong influences on individual fitness. In animals, total fatty
54 acid composition partially reflects molecules consumed and assimilated through the diet in
55 addition to those synthesized and metabolized *in vivo*. The fatty acid phenotype of individuals
56 can therefore be considered in terms of both fatty acid identity, which we define as the
57 multivariate fatty acid composition of an individual in terms of the identity and abundance of

fatty acids observed in their tissue (assessed using multivariate ordination techniques, see Methods), and fatty acid complexity, which we define as the diversity of different fatty acids observed in an organism's tissue regardless of identity (similar to Shannon's effective diversity in community studies, see Methods). Importantly, fatty acid allocation and use may often be preferential, which may alter the resulting complexity and identity of fatty acid composition (Kiessling and Kiessling 1993; Jeong et al. 2002; Price and Guglielmo 2009). However, fatty acid complexity, including its role in organism performance, has rarely been examined when compared to studies on fatty acid identity, even though some evidence suggests that reduced fatty acid complexity may negatively affect individual performance during stress or physical exertion (Price et al. 2008; Price and Guglielmo 2009). Therefore, examining variation in fatty acid complexity within and among individuals could clarify potential performance benefits derived from increased fatty acid complexity and assist in identifying patterns in the allocation and mobilization of both the complexity and identity of these important molecules among tissues (e.g., somatic or gonadal).

Reproductive success of fishes has been linked to the abundance of specific fatty acids for numerous species (Bell et al. 1986; Abi-Ayad et al. 2000; Tocher 2003). For example, docosahexaenoic acid (22:6n-3; DHA) concentrations in eggs have been positively linked to egg survival, hatching success, and larval mobility (Czesny and Dabrowski 1998; Tocher 2003; Fuiman and Ojanguren 2011; Fuiman and Perez 2015) and are generally important to neurological development in fishes (Tocher 2010). High levels of eicosapentaenoic acid (20:5n-3; EPA) or increased ratios of EPA to DHA or arachidonic acid (20:4n-6; AA) are related to regulation of inflammatory responses in animals (Calder 2001) and may depress larval function in fish (Henrotte et al. 2010). Few studies, however, have progressed beyond examining the role

of one or a few specific fatty acids to assess how fatty acid complexity may correspond to individual performance. Most iteroparous species allocate fatty acids to reproduction by mobilizing compounds from somatic stores well before spawning occurs (Henderson 2000), and this allocation may be preferential for specific fatty acids (Jeong et al. 2002) with implications for larval performance long after hatching (Fuiman and Perez 2015). Environmental effects, including temperature, may alter this allocation process in both somatic and gonadal tissues (Knipprath and Mead 1968; Alhazzaa et al. 2013; Feiner et al. 2016a), thereby influencing the composition and complexity of eggs and potentially their quality. Examining differences in the identity and complexity of egg and maternal somatic fatty acids in tandem could elucidate patterns in the differential allocation of fatty acids to reproduction and somatic maintenance, in addition to revealing whether differences in the allocation of a number of fatty acids (e.g., complexity) or identity of fatty acids may potentially influence egg or female quality.

We sought to develop an initial test for the concept of addressing fatty acid complexity as a potential quality indicator in fish and other taxa, while also elucidating patterns in fatty acid allocation in an important commercially and recreationally exploited freshwater fish species. Specifically, we build on a previous experiment that focused on thermal effects on fatty acid identity differences in the eggs and muscle of female yellow perch (*Perca flavescens* Mitchell, 1814), but ignored possible thermal effects on fatty acid complexity and did not seek to quantify patterns in allocation of number or identity of fatty acids between tissue types within individuals (Feiner et al. 2016a). Yellow perch are a highly important fish species in both wild fisheries and aquaculture, with well-studied reproduction cycles influenced by temperature for reproductive development and production of the ribbon-like skein matrix unique to the *Perca* genus, which binds and protects the egg mass (Newsome and Tompkins 1985; Reyes et al. 1992; Almeida et

al. 2017). Beyond skein production, yellow perch are similar to many other important freshwater fish species (e.g., walleye *Sander vitreus* [Mitchill, 1818], lake whitefish *Coregonus clupeformis* Mitchill, 1818) in several life history traits, including iteroparous spawning, allocation of energy to eggs from somatic stores of lipids, and a protracted capital spawning strategy in which lipids are allocated to gonads for several months before spawning (Henderson 2000; Kestemont et al. 2015). Using an experimental manipulation of mature female yellow perch (Feiner et al. 2016a), we sought to 1) examine the influence of temperature on patterns in fatty acid complexity in somatic (muscle) tissue and eggs in mature yellow perch, 2) understand differential allocation of both number and identity of fatty acids to maternal somatic tissue and eggs, and 3) evaluate how maternal complexity influences traits related to egg quality.

Materials and methods

Experimental design

Mature female yellow perch were exposed to three overwinter temperature treatments (4 °C, 8 °C, and 13 °C) for 150 days to evaluate the influence of overwinter thermal conditions on yellow perch reproduction. Four adult fish were randomly assigned to each of ten tanks per temperature treatment, tagged with a passive integrated transponder (PIT) tag (Oregon RFID) for individual identification, and held over winter (October to March) at their respective winter treatment temperatures. All fish were fed once per day *ad libitum* with Zeigler 5 mm floating pellets (40% protein, 10% lipid; Zeigler Bros., Inc.; see fatty acid profiles of food in Table S1 and Table S2) throughout the experiment. During spring (March and April), water temperatures were gradually warmed to mimic a natural spring warming regime, and eggs were manually

stripped from ripe, spawning-ready females (Feiner et al. 2016a). All fish were the same age and exhibited no differences in mass among treatments. The yellow perch used in the experiment were obtained from the same resident stock maintained at the Purdue University Aquaculture Research Laboratory (Feiner et al. 2016a). These fish originated from broodstock captured in the Perquimans River, NC, USA, in 1992. This stock is held and allowed to spawn naturally in outdoor ponds each spring, with occasional supplementation via indoor spawning and hatching of larvae (Andree et al. 2015; Feiner et al. 2016a). Whole skeins were stripped from females as they became ready to spawn in the spring. A subsample of eggs stripped from each female was flash frozen in liquid nitrogen for lipid and fatty acid analysis, and an additional sample was stored in 10% formalin solution for analysis of egg size.

A number of egg traits were quantified from these egg samples. Ten eggs per female were photographed under a dissecting microscope, and their diameters measured (mm) along two perpendicular axes (as yellow perch eggs are not perfectly spherical) using the program ImageJ (Rasband 1997). The mean of the two measurements was taken as the mean egg diameter for each egg, and these ten diameters were averaged to determine the mean egg diameter for each female. Egg diameter variation within each female was further determined as the coefficient of variation (CV; standard deviation / mean) of these 10 diameters. To determine mean egg dry mass, two samples of 20 to 30 eggs were placed into pre-weighed aluminum tins and dried at 70 °C for 48 hours, after which the dry mass of the sample was divided by the number of eggs in the subsample to determine mean dry mass (mg), and the mean of the two samples was determined as the mean egg dry mass for each female. Lipids were extracted from flash frozen eggs using a modified Folch method and expressed as a proportion of wet mass (Folch et al. 1957). Fatty acid composition of flash frozen egg samples was determined following the methods of Budge et al.

(2006). Briefly, lipids were extracted using the Folch method and fatty acid methyl esters prepared using transesterification reagent composed of 170 ml methanol and 2.55 ml concentrated sulfuric acid and extracted via addition of hexane (Budge et al. 2006). Fatty acid composition of each sample was then determined using gas chromatography with a Shimadzu gas chromatograph, flame ionization detector, autoinjector, and EZStart software version 7.4 (Shimadzu Corp.; for GC protocol and fatty acid standards used, please see Appendix 1 in Feiner et al. 2016a). The relative abundance (percent by mass of all fatty acids detected) of 41 fatty acids (Table 1; Table S1, S2) was quantified in each sample (see Feiner et al. 2016a for full analytical details).

In addition to egg traits, a subset of females (one randomly selected fish per tank) was euthanized via overdose of MS-222 following spawning. Each female was measured, weighed, and a dorsal muscle sample removed and flash frozen in liquid nitrogen. The muscle fatty acid composition of each female was then assessed following the same protocol as egg samples.

Data analysis

The complexity of fatty acid composition of eggs and maternal muscle among temperature treatments and tissue types was evaluated using the “complexity-as-diversity” approach based on Marion et al. (2015b). In brief, the complexity of the fatty acid signature of each individual was described using Shannon’s effective diversity qD :

$${}^qD = \left(\sum_{i=1}^K p_i^q \right)^{1/(1-q)}$$

where p_i is the proportional abundance of fatty acid i across all K fatty acids examined, and this index is weighted by diversity order q , where increasingly large q increases the influence of abundant compounds on diversity. Therefore, 0D represents fatty acid richness, whereas higher levels of q weight abundant compounds more heavily toward the calculation of qD . Although a solution does not exist for $q = 1$, the limit is exponentiated Shannon's diversity (H) (Marion et al. 2015b). Mean population diversity was estimated using the population mean abundances of each fatty acid (i.e., $\overline{p_{ij}}$, the mean of compound j in population i). Similar to community ecology, the average fatty acid diversity of an individual from a particular group is assessed as α diversity (i.e., in this experiment, α represents the average diversity of an individual from a treatment or tissue type), whereas total observed diversity across all samples within the group is referred to as γ diversity (Marion et al. 2015b). The excess diversity among individuals (i.e., the number of components, in this case fatty acids, not found in an average individual but found across individuals in the group) can then be estimated as β diversity (i.e., $\beta = \gamma - \alpha$).

We used R package *hierDiversity* (Marion et al. 2015a) to compare fatty acid complexity of female muscle and eggs among temperature treatments across levels of q from zero to six, thereby including a sufficient range so that complexity changed relatively little with additional levels of q . Because there were no significant differences in fatty acid complexity among temperature treatments in either female muscle or eggs (see Results), we pooled individuals from all three temperature treatments together for further analysis of patterns between sample types (egg and muscle) to increase sample size.

We quantified fatty acid complexity in maternal muscle and egg samples for levels of q from zero to six to evaluate patterns in the allocation of fatty acids to eggs by individual females. When significant differences in complexity were detected between sample types (i.e., differences

between eggs and muscle, as identified by non-overlapping 95% confidence intervals), we used analysis of similarity (ANOSIM) with Euclidean distances and similarity percentages (SIMPER) tests to identify fatty acids that contributed to these differences (R package *vegan*; Oskansen et al. 2013). Differences in overall fatty acid identity between tissue types were further visualized using nonmetric multidimensional scaling with Euclidean distances (NMDS; R package *vegan*), where NMDS axes were retained only if they reduced stress (a measure of agreement between observed distances among data points and their distances in the reduced dimensions of the NMDS) by at least 0.05.

Egg size, and potentially egg size variation, have been shown to be an important predictor of larval size, growth, and survival (Bagenal 1969; Marsh 1986; Andree et al. 2015). Therefore, the egg traits we measured (egg diameter, dry mass, lipid content, and diameter CV) likely provide some predictive power for the potential quality of offspring produced by each female. Several of these traits were highly correlated (e.g., egg lipid and egg dry mass: $r = 0.57$, $P = 0.06$; egg lipid and egg CV: $r = 0.67$, $P = 0.02$; and egg dry mass and egg CV: $r = 0.51$, $P = 0.07$). Therefore, we used principal components analysis on the correlation matrix of these variables (PCA; R package *vegan*) to generate orthogonal axes of egg traits, and rotated these axes so that positive values could be interpreted as improved egg quality. To examine the potential effect of female fatty acid complexity on egg quality, we then correlated each of the first two principal components (which explained 58% and 26% of the variation in egg traits, respectively; see Results) to individual female fatty acid complexity at each level of q using Pearson's correlations. Because our expectation was that egg quality should increase with increasing maternal fatty acid complexity, significance of these correlations was determined by computing one-tailed P -values with the expectation that correlations would be greater than zero.

Results

Temperature effects on fatty acid complexity

There were no differences in fatty acid complexity among temperature treatments in either egg or maternal muscle. Both egg and muscle samples were highly consistent within tissues from individual to individual across values of q ; complexity at the individual level accounted for more than 90% of the total variation in fatty acid complexity observed (i.e., a randomly selected individual would on average represent most of the fatty acid observed in the study), whereas differences among treatments generally accounted for less than 5% (and in some cases, <1%) of total fatty acid complexity (Fig. 1).

Fatty acid differences between egg and muscle

Eggs were significantly more complex than muscle tissue at low values of q , indicating a greater total fatty acid diversity in eggs based on simple presence and absence of fatty acids. However, maternal muscle consistently exhibited greater diversity of fatty acids than eggs at higher levels of q (i.e., a larger number of abundant fatty acids; Fig. 2). Specifically, four fatty acids were detected only in eggs, (15:1, 24:0, 13:0, and 15:0 *anti*) at very low abundance (<0.03% of total fatty acids), whereas muscle had no unique detections of such low-abundance compounds. However, these fatty acids occurred near the lower limit of detection in most egg samples, and, being in such low abundances, these differences are not clearly meaningful. In contrast, female muscle exhibited a more even distribution of abundant fatty acids than eggs – 11 fatty acids comprised at least 2% of the total fatty acids measured in muscle, but only eight fatty acids reached this threshold in eggs (Table 1). Of these, only 17:1 and 18:3n-3 comprised more than 2% of fatty acids in eggs, but not muscle. In contrast, 18:0, 22:5n-6, 20:4n-6, 22:5n-3, and

EPA comprised more than 2% of muscle fatty acids, but not eggs. The overall fatty acid compositions of these tissues exhibited no overlap (ANOSIM: $P < 0.001$, $R = 1.0$). Non-metric multidimensional scaling using two axes was chosen as it resulted in low stress (stress = 0.05) and revealed distinct groupings of tissue types along NMDS axis 1 while variation within tissue types was distributed along NMDS axis 2 (Fig. 3). SIMPER analyses indicated that about 75% of the differences between egg and muscle fatty acids were explained by higher levels of 18:1n-9, 18:2 *cis*, 18:3n-3, 16:1 in eggs and more 16:0, DHA, and 18:0 in muscle (Table 1, Fig. 3). Complexity of fatty acid in egg and maternal muscle at the individual level was not correlated at any level of q (range Pearson's r : -0.38 – 0.13, $P > 0.19$).

Correlates with egg phenotype

The first two principal components of a PCA on egg phenotypic traits (egg diameter, dry mass, lipid content, and diameter CV) explained 56.6% and 27.6% of the variation in egg traits, respectively (Table 2; Fig. 4). All four traits loaded positively on the first component (PC 1; component eigenvalue: 2.26); thus, we interpreted more positive PC 1 loadings to indicate higher egg quality. On the second principal component (PC 2; component eigenvalue: 1.10), egg diameter and egg mass exhibited strong and weak negative loadings, respectively whereas egg lipid and CV loaded moderately positively. Thus, PC 2 appeared to represent a tradeoff between egg size and egg size variation and lipid content. Because increased lipid stores are a strong predictor of larval survival and starvation resistance (Brooks et al. 1997, Andree et al. 2015, Kestemont et al. 2015), and PC 2 represented a tradeoff between small, lipid-rich eggs and large eggs containing less lipid, we interpreted positive PC 2 loadings to indicate increased maternal lipid allocation and potentially increased quality. Additional PCs were relatively uninformative

(explaining < 10% of the variation, eigenvalues < 0.35; Table 2), therefore, we focused further analyses on PC 1 and PC 2.

We detected positive correlations between female fatty acid complexity and egg quality on PC 1. These correlations were generally weak at low q , but strengthened as fatty acid relative abundances were weighted more heavily (Fig. 5), indicating variance in the complexity of abundant fatty acids in maternal tissues exhibited the strongest correlations with egg quality. In contrast, we found no significant correlations between maternal complexity and egg PC 2, although correlations were again generally positive. The consistency of these trends, strong correlation strengths ($r > 0.50$ in some cases), and relatively low statistical power, suggest that females exhibiting increased diversity of abundant fatty acids in their somatic tissues may have produced higher-quality eggs.

Discussion

The importance of egg and maternal fatty acids to reproductive success and larval survival has been an area of intense interest in both aquaculture and wild fish populations (Tocher 2003). However, the vast majority of studies have focused on the influence of diet on fatty acid identity and have not considered the importance of fatty acid complexity (Czesny and Dabrowski 1998; Moles et al. 2008; Fuiman and Perez 2015). This study is one of the first to move beyond the identity of fatty acids to consider the importance of fatty acid complexity to indicators of egg quality in a freshwater fish. We revealed variation in the complexity and identity of fatty acids allocated to eggs when compared to somatic fatty acid identity in spawning

females, thereby identifying potentially important avenues for future examination of these dynamics in aquatic organisms.

Neither egg nor maternal fatty acid complexity varied among temperature treatments. This result reinforces previous findings that many phenotypic traits in eggs (egg diameter, egg weight) are relatively unresponsive to overwinter temperature (Feiner et al. 2016a). Due to their importance to offspring quality, the fatty acid composition of eggs may be highly conserved through natural selection. Similar conservation of mean fatty acid concentrations and other macroscopic traits (e.g., egg size) have been reported across populations in other fish species, including percids (Wiegand et al. 2004; Feiner et al. 2016a, 2016b) and salmonids (Quinn et al. 1995; Einum and Fleming 2000). More generally, differences in fatty acid signatures in the eggs of marine and freshwater species are thought to be a product of selection due to different levels of essential fatty acids in each environment (Tocher 2010). In addition, there is the possibility that female muscle and egg fatty acid composition shifted in response to the simulated spring warming period, as fatty acid composition in fish can adapt relatively rapidly (in days or weeks) to changing temperatures (Hazel 1984; Hagar and Hazel 1985). We suggest that such rapid adaptation was unlikely to alter our results, however, for several reasons. Yellow perch allocate lipids to their eggs for months before spawning (Henderson 2000), and other studies have shown differences in the composition and performance of eggs produced by female yellow perch experiencing altered winter regimes (Farmer et al. 2015), including the observation of significantly different egg fatty acid composition among temperature treatments in an earlier analysis of these data (Feiner et al. 2016a). Therefore, we suggest our results are more likely the result of conservation of fatty acid complexity in the face of different overwinter thermal regimes, particularly in eggs, rather than rapid acclimation to spring warming.

Organisms exposed to thermal, resource, or physical stress have exhibited preferential depletion of certain fatty acids and altered fatty acid composition, including increased mobilization of long chain polyunsaturated fatty acids (PUFAs) (Knipprath and Mead 1968; Raclot and Groscolas 1995; Price and Guglielmo 2009). Yellow perch require cold winter temperatures to spawn successfully (Hokanson 1977; Farmer et al. 2015) – thus, it was expected that female yellow perch held at warmer temperatures would exhibit decreased fatty acid complexity as a sign of thermal stress. However, we saw no change in complexity across temperature treatments. Fatty acid content of diets greatly influences somatic fatty acid composition in fish (Budge et al. 2006) – as all fish in this study were fed a controlled diet, this may have limited detectable variability in fatty acid composition among temperature treatments. In addition, yellow perch are thermal generalists (Kestemont et al. 2015). Potentially, our temperature treatments were not sufficiently extreme to provoke a thermal stress response in this species. Further experimentation altering temperature to a sufficient extent to modify fatty acid content in tissues of the target species likely is needed to understand how fatty acid mobilization changes with thermal stress.

Fatty acid complexity and identity differed between eggs and maternal muscle, clearly suggesting preferential allocation of fatty acids during oogenesis and vitellogenesis in yellow perch. Eggs were primarily dominated by relatively fewer main fatty acids, including 18:1n-9, 18:2n-6, and 18:3n-3, DHA, as well as shorter chain monounsaturated (16:1, 17:1) and saturated (16:0) fatty acids. These C₁₈ molecules are essential fatty acids for freshwater fish, serving as precursors for the synthesis of other long chain PUFAs, including DHA, EPA, and AA (Tocher and Glencross 2015) and have been observed to serve these roles during larval development in percids (Czesny and Dabrowski 1998; Abi-Ayad et al. 2000; Cejas et al. 2004). Other abundant

egg fatty acids, including 18:1n-9, 16:0, 17:1, and 16:1, comprise up to 40% of the fatty acids catabolized for energy use during embryogenesis and early development in larval fish (Abi-Ayad et al. 2000; Tocher 2003; Cejas et al. 2004; Czesny et al. 2009). Use of these PUFA appears to be most intense during the switch to exogenous feeding, highlighting the importance of these fatty acids to larval survival through what is usually considered a period of high mortality risk (Abi-Ayad et al. 2000).

Preferential mobilization of fatty acids is thought to be related to their chemical structure, with more unsaturated or shorter chain acids being more easily and efficiently mobilized than longer chain or more saturated molecules (Raclot and Groscolas 1993). The patterns of allocation observed here are consistent with this paradigm – relatively few long chain fatty acids were abundant in eggs, while several C₂₀ and C₂₂ fatty acids were abundant in muscle tissue. Thus, while female muscle was a complex mixture of structural and energetic C₁₈, C₂₀, and C₂₂ PUFA (Bell et al. 1986; Tocher 2003), eggs primarily contained energy sources and the essential precursors for long chain fatty acids that serve important roles in larval development and survival (Henderson 1996; Tocher 2010), potentially mediated through the relative efficiency of fatty acid mobilization. As fatty acids allocated to offspring may influence larval performance for days or weeks after hatching, these patterns of allocation may have significant influences on larval survival (Beckmann et al. 2014). By characterizing patterns of complexity between tissue types, we were able to identify and highlight these differences in fatty acid allocation, and suggest that examinations of freshwater fish should also consider the abundances of essential C₁₈ fatty acids and important energy sources when determining the influence of fatty acid composition on offspring success.

In most fish species, larger eggs and larvae with increased lipid reserves are considered higher quality (Brooks et al. 1997; Venturelli et al. 2009; Fuiman and Ojanguren 2011; Olin et al. 2012; Andree et al. 2015). We observed positive correlations between fatty acid complexity of individual females and the quality eggs they produced, as determined by egg size, lipid content, and egg size variability, suggesting that females with more complex fatty acid profiles produced higher-quality eggs. Higher numbers or evenness of fatty acids may signify a diverse reserve of lipids to use during the energetically expensive production of eggs, allowing females to preferentially mobilize long chain PUFA for somatic use while allocating C₁₈ and short chain monounsaturated and saturated acids to eggs (Tocher 2003; Hauville et al. 2015). Due to limited sample size, and because we did not examine the viability or survival of eggs or larvae, we are unable to infer the eventual reproductive success of these females. Further experiments examining egg survival, larval growth, and other measures of reproductive success in relation to fatty acid composition and complexity are needed to clarify the importance of fatty acid complexity to individual success, thereby providing biologists with a crucial tool to predict and detect individual performance, environmental quality, and recruitment success in fishes.

Many other studies have noted differences in fatty acid identity among tissue types in fish. Eggs are often enriched in 18:3n-3 and 18:1n-9 compared to muscle tissue, and the composition of visceral fat and liver fatty acids may vary dramatically (Beckmann et al. 2014; Hauville et al. 2015). Percids, like many determinate-spawning iteroparous species, draw on both visceral fat and muscle somatic lipid stores to allocate energy toward reproduction over a period of several months, and the relative contributions of these somatic lipids could influence egg composition (Henderson et al. 2000). As we did not measure fatty acids in visceral fat, we may be overlooking an important component of fatty acid allocation to eggs. However, muscle

and fat fatty acid identity were qualitatively similar in the closely related Eurasian perch (*P. fluviatilis* L., 1758) (Jankowska et al. 2010). Therefore, our general conclusions may be unlikely to change – preferential allocation of fatty acids to eggs appears to result in a relatively simple egg phenotype dominated by PUFA precursors and energy sources, while the abundant fatty acids in somatic stores are likely more complex due to fatty acid synthesis and allocation of lipids from the diet (Tocher 2003).

This study represents an initial attempt to understand the role fatty acid complexity, in addition to identity, may play in determining the reproductive performance of fish. By identifying variation in fatty acid complexity among tissue types, we identified important influences of preferential allocation of fatty acids to reproduction in this species. However, we acknowledge that our ability to make general inferences are limited, as our study used a domesticated strain of yellow perch in laboratory conditions, likely reducing potential variation in fatty acid complexity that might be observed in wild populations with access to a broader range of habitats and resources. Fatty acid identity also significantly varies among species (e.g., Alfaro et al. 2006; Czesny et al. 2011), suggesting the potential for taxonomic variability in fatty acid diversity as well. Thus, this study offers a conceptual model of how fatty acid complexity might be considered in future ecological studies. For example, additional investigations of fatty acid dynamics during early life in wild individuals or other species could elucidate allocation patterns of fatty acids to reproduction and their importance to larval growth and development. Moreover, developing relationships between somatic complexity and indicators of egg quality could potentially provide an avenue to assess spawning stock quality and potential recruitment success, as well as investigate the impact of stressors (e.g., changes in resource availability; Schaeffer et al. 2000) on wild populations.

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Draft

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Tables

Table 1. Mean relative abundance (percent by mass of total fatty acids measured $\pm$ SD) of fatty acids in egg and muscle tissues in adult female yellow perch *Perca flavescens* Mitchill, 1814 ranked by fractional contribution (Prop. Cont.) to difference in total fatty acid composition between tissues. *P*-value for significance of contribution (SIMPER analysis), and ranks on both nonmetric multidimensional scaling axes (MDS1 and MDS2) are also shown.

Fatty acid	Egg	Muscle	Prop. Cont.	Cumulative Cont.	<i>P</i>	MDS1	MDS2
18:1n-9	25.600 $\pm$ 1.546	9.297 $\pm$ 1.337	0.1925	0.1925	<0.001	-0.080	-0.004
16:0	4.809 $\pm$ 0.786	17.344 $\pm$ 1.141	0.148	0.3405	<0.001	0.095	-0.002
22:6n-3	13.917 $\pm$ 2.111	26.163 $\pm$ 1.675	0.1446	0.4851	<0.001	0.051	0.000
18:2 <i>cis</i>	20.901 $\pm$ 2.255	12.308 $\pm$ 0.975	0.1014	0.5865	<0.001	-0.044	0.007
18:3n-3	7.461 $\pm$ 3.364	0.986 $\pm$ 0.143	0.0765	0.663	<0.001	-0.134	-0.032
16:1	9.762 $\pm$ 1.790	3.737 $\pm$ 0.544	0.0712	0.7342	<0.001	-0.077	0.009
18:0	1.038 $\pm$ 0.422	4.499 $\pm$ 0.723	0.0408	0.775	<0.001	0.107	-0.009
17:1	2.697 $\pm$ 0.391	0.212 $\pm$ 0.099	0.0294	0.8044	<0.001	-0.144	0.009
20:5n-3	1.810 $\pm$ 0.257	4.239 $\pm$ 0.653	0.0286	0.833	<0.001	0.069	0.003
22:5n-3	1.143 $\pm$ 0.519	3.455 $\pm$ 0.513	0.0273	0.8603	<0.001	0.086	-0.009
20:4n-6	0.941 $\pm$ 0.298	3.152 $\pm$ 0.500	0.0261	0.8864	<0.001	0.089	-0.009
22:5n-6	0.900 $\pm$ 0.218	3.038 $\pm$ 0.279	0.0253	0.9117	<0.001	0.092	0.000
16:0 <i>iso</i>	0.055 $\pm$ 0.010	1.406 $\pm$ 0.305	0.0159	0.9276	<0.001	0.156	0.011
18:1n-7	2.601 $\pm$ 0.404	3.526 $\pm$ 0.405	0.0112	0.9388	<0.001	0.026	-0.001
15:0	1.118 $\pm$ 0.113	0.212 $\pm$ 0.031	0.0107	0.9495	<0.001	-0.115	-0.006
21:0	0.678 $\pm$ 0.079	1.555 $\pm$ 0.214	0.0103	0.9598	<0.001	0.066	-0.001
17:0 <i>iso</i>	0.651 $\pm$ 0.443	0.422 $\pm$ 0.425	0.0061	0.9659	0.082	-0.028	0.007
20:3n-6	0.189 $\pm$ 0.362	0.423 $\pm$ 0.618	0.0055	0.9714	0.096	0.086	0.096
18:3n-6	1.006 $\pm$ 0.124	0.650 $\pm$ 0.112	0.0042	0.9756	<0.001	-0.036	0.007
14:0	0.685 $\pm$ 0.118	1.014 $\pm$ 0.178	0.004	0.9796	<0.001	0.032	0.000
20:3n-3	0.340 $\pm$ 0.278	0.089 $\pm$ 0.057	0.0034	0.983	0.006	-0.097	-0.075
22:4n-6	0.260 $\pm$ 0.078	0.517 $\pm$ 0.078	0.0031	0.9861	<0.001	0.056	-0.006
20:1n-9	0.315 $\pm$ 0.110	0.565 $\pm$ 0.120	0.003	0.9891	<0.001	0.050	-0.001
23:0	0.087 $\pm$ 0.080	0.204 $\pm$ 0.117	0.0018	0.9909	0.007	0.071	-0.017
18:2 <i>trans</i>	0.170 $\pm$ 0.141	0.123 $\pm$ 0.047	0.0015	0.9924	0.016	-0.029	0.016
24:1	0.074 $\pm$ 0.027	0.175 $\pm$ 0.087	0.0015	0.9939	<0.001	0.061	-0.012
20:2	0.245 $\pm$ 0.115	0.254 $\pm$ 0.036	0.0011	0.995	0.033	0.007	-0.017
20:0	0.110 $\pm$ 0.091	0.137 $\pm$ 0.021	0.0009	0.9959	<0.001	0.015	0.013

22:2	0.101 ± 0.064	0.113 ± 0.018	0.0007	0.9966	0.023	0.010	0.023
22:0	0.044 ± 0.035	0.018 ± 0.043	0.0005	0.9971	0.016	-0.074	-0.085
<i>hydroxy</i> 14:0	0.053 ± 0.029	0.013 ± 0.018	0.0006	0.9977	0.002	-0.102	0.032
22:1	0.033 ± 0.017	0.020 ± 0.039	0.0004	0.9981	0.003	-0.035	0.016
15:0 <i>iso</i>	0.048 ± 0.007	0.012 ± 0.013	0.0004	0.9985	<0.001	-0.104	0.006
14:1	0.089 ± 0.031	0.099 ± 0.027	0.0004	0.9989	0.37	0.006	0.009
15:1	0.029 ± 0.021	0.000 ± 0.000	0.0003	0.9992	<0.001	-0.182	0.056
12:0 (OH)	0.002 ± 0.005	0.025 ± 0.074	0.0004	0.9996	0.362	0.167	0.170
24:0	0.015 ± 0.010	0.000 ± 0.000	0.0001	0.9997	<0.001	-0.169	-0.013
13:0	0.009 ± 0.016	0.000 ± 0.000	0.0001	0.9998	0.028	-0.156	-0.017
15:0 <i>anti</i>	0.009 ± 0.008	0.000 ± 0.000	0.0001	0.9999	0.002	-0.183	0.054
12:0	0.005 ± 0.004	0.001 ± 0.002	0.0001	1	<0.001	-0.140	-0.029

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Table 2. Standardized eigenvectors for each variable on principal components analysis for egg quality in yellow perch *Perca flavescens* Mitchill, 1814 including four egg quality variables (egg lipid content as proportion wet mass, diameter [mm], dry mass [mg], and diameter coefficient of variation [CV]). The proportion of variance explained by each principal component (PC) and each PC's eigenvalue are also shown.

Variable	PC1	PC2	PC3	PC4
Egg lipid	0.567	0.250	0.699	-0.357
Egg diameter	0.274	-0.827	0.279	0.403
Egg dry mass	0.568	-0.254	-0.616	-0.482
Egg CV	0.529	0.434	-0.232	0.691
Proportion variance	0.566	0.276	0.085	0.073
Eigenvalue	2.263	1.104	0.340	0.292

Figure Captions

Fig. 1. Differences in mean individual fatty acid complexity among overwinter temperature treatments in yellow perch *Perca flavescens* Mitchill, 1814 eggs (top panels) and maternal muscle (bottom panels) at different weights q . Thick lines in left hand panels denote mean fatty acid complexity for each temperature treatment (solid black, 13 °C; dashed black, 8 °C, solid gray, 4 °C) and outer thin lines of same type represent 95% confidence intervals. Bars in left-hand panels display total proportion of effective fatty acids found within an average individual (light gray), within temperature treatments (gray), and among all individuals (dark gray). There were no differences in complexity among temperature treatments, and within-individual variation accounted for nearly all observed variation in fatty acid complexity.

Fig. 2. Differences in mean individual fatty acid complexity between yellow perch *Perca flavescens* Mitchill, 1814 egg and maternal muscle samples at different weights q . Thick solid lines in top panel denote mean fatty acid complexity in egg (black) and female muscle (gray) samples, and outer thin dashed lines of same color represent 95% confidence intervals. Bars in bottom panel display total proportion of effective fatty acids found within an average individual and sample type (light gray), within sample types (gray), and between tissues (dark gray). Up to 20% of variation in complexity was observed between treatments.

Fig. 3. Differentiation of fatty acid composition between egg (open symbols) and muscle (closed symbols) of spawning female yellow perch *Perca flavescens* Mitchill, 1814 using nonmetric multidimensional scaling. Vectors display loadings of seven fatty acids most influential to

610 between-tissue differences as identified by SIMPER analysis (refer to Table 1 for loadings of all
611 fatty acids).

612 **Fig. 4.** Principal components analysis on metrics of yellow perch *Perca flavescens* Mitchill,
613 1814 egg quality, where vectors for phenotypic egg traits (egg dry mass, egg diameter, egg lipid
614 content, and egg diameter coefficient of variation [CV]) are multiplied by two for clarity and
615 positive loadings along PC 1 and PC 2 are interpreted to signify higher quality eggs (e.g.,
616 increased size and/or lipid content). The percent of total variance that each PC explained is
617 denoted in parentheses on each axis.

618

619 **Fig. 5.** Correlations between maternal yellow perch *Perca flavescens* Mitchill, 1814 muscle fatty
620 acid complexity and egg phenotypic principal components, where egg principal component 1
621 (Egg PC 1) is positively related to egg lipid, mass, diameter, and CV (i.e., more positive PCs
622 indicate higher quality) and egg PC 2 is positively related to lipid content and CV and negatively
623 related to egg diameter and mass. Left panels represent example relationship between maternal
624 muscle fatty acid complexity ($q = 3$) and egg PCs. Right hand panels show Pearson's
625 correlations (r) between egg PCs and female muscle complexity at different weights q , where the
626 solid line denotes the mean correlation estimate, the shaded area denotes the 95% confidence
627 interval, and the dashed line denotes zero (no correlation). One-tailed P -values (expecting
628 positive correlations) for each correlation coefficient are provided in each plot.

Fig. 1. Differences in mean individual fatty acid complexity among overwinter temperature treatments in yellow perch *Perca flavescens* Mitchell, 1814 eggs (top panels) and maternal muscle (bottom panels) at different weights q . Thick lines in left hand panels denote mean fatty acid complexity for each temperature treatment (solid black, 13 °C; dashed black, 8 °C, solid gray, 4 °C) and outer thin lines of same type represent 95% confidence intervals. Bars in left-hand panels display total proportion of effective fatty acids found within an average individual (light gray), within temperature treatments (gray), and among all individuals (dark gray). There were no differences in complexity among temperature treatments, and within-individual variation accounted for nearly all observed variation in fatty acid complexity.

184x152mm (300 x 300 DPI)

Fig. 2. Differences in mean individual fatty acid complexity between yellow perch *Perca flavescens* Mitchill, 1814 egg and maternal muscle samples at different weights q . Thick solid lines in top panel denote mean fatty acid complexity in egg (black) and female muscle (gray) samples, and outer thin dashed lines of same color represent 95% confidence intervals. Bars in bottom panel display total proportion of effective fatty acids found within an average individual and sample type (light gray), within sample types (gray), and between tissues (dark gray). Up to 20% of variation in complexity was observed between treatments.

88x165mm (300 x 300 DPI)

Figure 3. Differentiation of fatty acid composition between egg (open symbols) and muscle (closed symbols) of spawning female yellow perch *Perca flavescens* (Mitchill 1814) using nonmetric multidimensional scaling. Vectors display loadings of seven fatty acids most influential to between-tissue differences as identified by SIMPER analysis (refer to Table 1 for loadings of all fatty acids).

79x57mm (300 x 300 DPI)

Figure 4. Principal components analysis on metrics of yellow perch *Perca flavescens* (Mitchill 1814) egg quality, where vectors for phenotypic egg traits (egg dry mass, egg diameter, egg lipid content, and egg diameter coefficient of variation [CV]) are multiplied by two for clarity and positive loadings along PC 1 and PC 2 are interpreted to signify higher quality eggs (e.g., increased size and/or lipid content). The percent of total variance that each PC explained is denoted in parentheses on each axis.

79x59mm (300 x 300 DPI)

Figure 5.

184x177mm (300 x 300 DPI)