Finishing diet, genetics, and other culture conditions affect ovarian adiposity and caviar yield in cultured white sturgeon (*Acipenser transmontanus*)

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Abstract

The on-going and precipitous decline in sturgeon abundance and increased demand for caviar have promoted the development of sustainable sturgeon aquaculture. One common problem that confounds caviar farming efficiency is highly variable roe yield associated with the proliferation of ovarian fat: sturgeon females with fatty ovaries tend to produce a smaller yield of inferior quality roe compared to those with lean ovaries. Here, we evaluated the effects of finishing diet, farm origin, age at maturity, tank, and genotype on ovarian adiposity and caviar yield in cultured white sturgeon (*Acipenser transmontanus*). We conducted a feeding trial in which fish aged 5+ years at two different commercial farms were either maintained on a high fat production diet or received a low fat finishing diet for 19 and 33 months before caviar harvest; at this time fish were aged 7 or 8 years, respectively. Measurements of ovarian fattiness, caviar yield, and morphology were collected at the time of harvest. Microsatellite genotyping was performed to estimate relatedness among experimental fish and subsequent heritability of these traits. Mean caviar yield normalized by body weight increased from 7.6-8.6% to 8.0-9.2% and mean caviar yield scaled by ovary weight increased from 55.5-59.8% to 61.3-64.6% in white sturgeon fed the high and low fat diets, respectively. All females were classified post hoc according to degree of ovarian fattiness: low, medium, or high. Regardless of diet, farm, or age, females in the high ovarian adiposity group had larger body size, higher condition factor, but lower caviar yields than those in the low fattiness group because of the presence of large ovarian fat lobes and adipocytes surrounding individual oocytes. Furthermore, only 11.8-17.2% of female white sturgeon given the low fat finishing diet displayed a high degree of ovarian fattiness compared to 18.0-33.2% of those on the high fat diet. Robust negative correlations were found between measurements of ovarian adiposity and caviar yield further illustrating this inverse relationship observed by caviar farmers. The fixed effects of diet, farm, age, and tank all significantly influenced ovarian adiposity and caviar yield except farm origin did not significantly impact ovarian fat lobe weight. Heritability values for measurements of ovarian adiposity and caviar yield were moderate and ranged from 0.20-0.39, however heritability of ovarian tissue weight was slightly lower ($h^2=0.11$). Our study demonstrates that finishing diet, farm origin, age at maturity, tank and genotype significantly affect and may be manipulated to increase caviar yield in cultured white sturgeon.

Key words: ovarian adiposity, caviar yield, white sturgeon, finishing diet, heritability

1. Introduction

Commercial sturgeon farming is a burgeoning industry that developed to meet international demand for caviar and to reduce fishing pressure on dwindling wild sturgeon stocks. Historical over-exploitation and subsequent decline of natural sturgeon populations prompted the 1997 listing of all species by the International Union for Conservation of Nature (IUCN) under the Convention on International Trade in Endangered Species (CITES). Such a designation protects imperiled sturgeon species by limiting and regulating international trade. Recently, the contribution of sturgeon aquaculture to the global caviar market has been rising while caviar produced by capture fisheries is comparatively close to zero; approximately 260 tons of caviar was harvested from farmed sturgeon worldwide in 2012 and it is hypothesized that total output will double or triple in 10 years (Bronzi and Rosenthal 2014). In western North America, the primary production fish is white sturgeon (*Acipenser transmontanus*). White sturgeon are well suited for caviar farming as a large sturgeon species with superior caviar and meat quality, fast growth at 18-21°C, and tolerance of high culture density (Van Eenennaam et al., 2004). However, a substantial investment is required to rear white sturgeon for caviar as females raised in an aquaculture setting do not reach sexual maturity and are therefore not appropriate for caviar harvest until a minimum of 7 to 8 years of age (Doroshov et al., 1997). The optimization of culture conditions to maximize caviar yield while minimizing producer-incurred cost is therefore critical for successful sturgeon farming.

One aspect of caviar production that undermines efficiency is highly variable roe yield (Figure 1) associated with the proliferation of fat in the ovaries of mature female sturgeon. Adipose tissue is present both in variable sized lobes attached to the lateral portion of each ovary and in the interstices between individual eggs (Scarnecchia et al., 2007). Some caviar processors in North America observe that sturgeon with lean ovaries tend to produce a greater yield of superior quality caviar than those with fatty ovaries (S. Bishop, unpublished data; Figure 2). Additionally, more labor and a longer processing time are required to clean and separate eggs from fatty ovarian tissue (Scarnecchia et al., 2007; Ovissipour et al., 2015). Until recently,

published accounts of low caviar yield due to high ovarian adiposity were not available. In a 2008 study that examined variation in caviar yield in relation to oocyte polarization index (PI; the final stage of maturation; Chapman and Van Eenennaam, 2007) or egg diameter in white sturgeon ($n=97$), the authors reported that roe yield correlated with neither of the expected two variables (Van Eenennaam et al., 2010). Instead, they noted that the largest females had the greatest amounts of ovarian fat and the lowest caviar yields (Van Eenennaam et al., 2010). Ovissipour et al. (2015) also found that caviar yield, as a percentage of ovary weight, ranged widely from 39-75% in white sturgeon due to the amount of adipose tissue incorporated into the ovaries. The etiology of ovarian adiposity is unclear, however the amount of adipose tissue in the ovaries has been known to decrease as the gonads approach first maturity and during subsequent reproductive cycles in wild and cultured Acipenseriform fishes: Chinese paddlefish (*Psephurus gladius*; Liu et al., 1995), Chinese sturgeon (*A. sinensis*; Wei et al., 1997), shovelnose sturgeon (*Scaphirhynchus platyrhynchus*; Colombo et al., 2007; Wildhaber et al., 2007), Russian sturgeon (*A. gueldenstaedtii*; Hurvitz et al., 2007), and paddlefish (*Polyodon spathula*; Scarnecchia et al., 2007). These observations suggest that ovarian fat may serve as an energy source for gonadal development or for overwintering and spawning migrations.

While no studies have been published, environmental, physiological, and genetic factors are likely to influence the accumulation of adipose tissue in the ovaries of cultured white sturgeon. Diet fat content is frequently manipulated in fish aquaculture to optimize body fat concentration (e.g., *Oncorhynchus mykiss*; Jobling et al., 1998; Lee and Putnam 1973); a low fat diet may reduce sturgeon ovarian fattiness and thus improve caviar yield. The fat content of some cultured salmonids increases with age (*O. mykiss*; Kiessling et al., 1991). Farmed white sturgeon females typically reach full sexual maturity at 7 and 8 years of age (Doroshov et al., 1997). It may therefore be prudent to test whether adipose tissue and roe production differ between cultured age 7 and age 8 white sturgeon females. It is unknown to what extent genetics impacts ovarian adiposity and caviar yield in white sturgeon, however genotype does have an effect on bodily fat composition in other fish species (Garcia-Celdran et al., 2015a; Garcia-Celdran et al., 2015b; Gjerde and Schaeffer 1989; Kocour et al., 2007; Quinton et al., 2005; Rye and Gjerde 1996). Heritability of bodily fat composition is generally intermediate to high, ranging from 0.19 (fat in *Salmo salar*; Quinton et al., 2005) to 0.58 (mean percentage of fat in *Cyprinus carpio*; Kocour et al., 2007), indicating that genetics play a significant role and the fat

content of farmed fish may be manipulated via selective breeding. Furthermore, because there is considerable variance in both ovarian adiposity and roe yield (Ovissipour et al., 2015; Van Eenennaam et al., 2010; Figure 1) in white sturgeon, these traits should be amenable to heritability analyses.

The purpose of the present study was two-fold. We first evaluated the effect of reducing dietary fat on ovarian adiposity and roe yield in a feeding trial. We reared a cohort of white sturgeon females aged 5+ years from multiple full- and half-sibling families for 19-33 months prior to caviar harvest that were either maintained on a high fat production diet or fed a low fat finishing diet at two farms in California. The amount of adipose tissue and caviar yield were characterized at the time of harvest. The morphological and caviar yield characteristics between groups of females with different degrees of ovarian fattiness was also assessed. Secondly, we more broadly investigated factors that may influence ovarian adiposity and caviar yield. On a sub-group of the same females, we also genotyped individuals to quantify their relatedness. This use of microsatellite analysis permits the construction of a genomic relationship matrix (VanRaden 2008). In this linear mixed model, the fixed effects were farm, diet, tank, and age at maturity and the random effect was genotype. The sub-objectives were to: 1) examine the correlation between high ovarian adiposity and low caviar output, 2) test the significance of fixed effects that contribute to, and 3) estimate the narrow sense heritability of ovarian adiposity and caviar yield in farmed white sturgeon. The results of this study will guide aquaculturalists in refining rearing practices to minimize the proliferation of ovarian fat and thereby improve roe yield and quality.

2. Methods

2.1. Fish husbandry and feeding trials

On April 20, 2006, 6 sires and 4 dams were spawned to create 21 full- and half-sibling white sturgeon families that were pooled together during hatch into a single communal tank. Fin clips from sires and dams were not collected at the time of spawn. Progeny were randomly distributed between two farms in California: Farm A (Sterling Caviar LLC; Wilton, CA) and Farm B (The Fishery; Galt, CA). Each farm started with approximately 200,000 of these larvae

that were initially fed a semi-moist commercial diet (52/16, as percent protein/fat content). At 2 months of age, fry were weaned onto a dry diet (42-44/14-18) from different feed manufacturers. Fish were maintained on the dry diet and reared in larger and more numerous tanks as growth progressed. At 3-4 years of age with a body weight of 7-10 kg, fish were sexed by direct examination of the gonads through a small abdominal incision (Chapman and Van Eenennaam, 2012); identified females were segregated into separate tanks.

Feeding trials began when fish were 5+ years of age in 2011. Six tanks at each farm were devoted to this study. Three tanks per farm were randomly assigned and maintained on the high fat (HF) production diet; the remaining three tanks at each farm were fed a low fat (LF) finishing diet. While the primary ingredients and nutritive content of the HF and LF diets were very similar in both farms, respectively, Farm A and Farm B purchased feeds from two different manufacturers; limited tank and fish availability precluded the optimal design of providing diets from both manufacturers at both farms. Therefore, any variance in ovarian adiposity and caviar yield originating from differences in feed manufacturers was included in farm-associated variance.

Farm A: Prior to this study, all tanks were maintained on the HF Skretting USA diet (42/18). In preparation for feeding trials, all tanks were fed a 50/50 mix of HF Skretting USA and either HF or LF EWOS Canada, Ltd. for a two week weaning period. On September 14, 2011, 30 fish from each of the six tanks were randomly selected for weighing to determine the average body weight (± 0.1 kg) at the initiation of the diet shift. Feed for the feeding trial was purchased from EWOS Canada, Ltd and the digestible energy was HF: 17.3 MJ kg⁻¹, 44% protein, and 17% fat; LF: 15.5 MJ kg⁻¹, 47% protein, and 9% fat. Fish were held in 9.2 m diameter circular fiberglass tanks that were 1.3 m deep and capable of holding a total volume of 86.7 m³ of water. Stripped well water from a head pond flowed into tanks at 350 L/min/tank and dissolved oxygen levels were maintained at >6.5 ppm with an automated oxygen injection system. Annual tank water temperatures ranged from 17-23 °C and individual tanks were flushed 3-4 times per week to remove accumulated feces and uneaten food. There was one demand feeder per tank and all feeders were filled with 40% of the daily ration in the morning and 60% in the evening. Feeding rates varied from 0.20-0.26% tank biomass/day. The number of fish stocked and density for the six tanks ranged from 231-259 fish and 63-72 kg/m³, respectively.

Farm B: Prior to this study, all tanks were maintained on the HF Skretting USA diet (42/18). In preparation for feeding trials, the three tanks that would eventually receive the LF Skretting USA diet were fed a 50/50 mix of the HF/LF Skretting USA diet for a two week weaning period. On September 12, 2011, 30 fish from each of the six tanks were randomly selected for weighing to determine the average body weight (± 0.1 kg) at the initiation of the diet shift. Feed for the feeding trial was purchased from Skretting USA and the digestible energy was HF: 17.2 MJ kg⁻¹, 42% protein, and 18% fat; LF: 14.4 MJ kg⁻¹, 47% protein, and 8% fat. Fish were held in 14.6 m diameter circular concrete tanks that were 1.8 m deep and capable of holding a total volume of 290 m³ of water. Pond water flowed into tanks at a rate of 1,136 L/min and dissolved oxygen levels were maintained at >6 ppm with diffused pure oxygen; dissolved oxygen was recorded at least weekly. Annual tank water temperatures ranged from 10-28 °C and individual tanks were flushed daily to remove accumulated feces and uneaten food. There were three demand feeders per tank and the daily feed ration was offered once per day in the afternoon. Feeding rates varied from 0.20-0.40% of the tank biomass based upon the growth charts developed by the farm. The feed rate was adjusted daily, depending on water temperature, feeding behavior, and water quality. The number of fish stocked and density for the six tanks ranged from 628-659 fish and 30-43 kg/m³, respectively.

2.2. Sample collection

Experimental fish were reared at Farm A and B until the fall of 2012 when all 6.5-year-old females were inspected for the presence of darkly pigmented oocytes indicative of sexual maturity and potential candidates for caviar harvest in the spring. Farm A females were examined in December of 2012 and Farm B females were examined in January of 2013. An oocyte sample was obtained via a small abdominal incision and insertion of a catheter (Van Eenennaam et al., 2004). Fish with black eggs were marked with color-coded cable ties placed below the dorsal fin corresponding to the farm, diet, and replicate tank from which they originated. These gravid females were transferred to a communal coldwater facility with water temperatures of 8-10°C to prevent ovarian atresia (Doroshov et al., 1997; Webb et al., 2001); replicate tanks from each farm were combined but diet treatments were maintained in four separate tanks throughout the winter and spring holding period. Remaining immature females

were left to develop at Farm A and Farm B under the same conditions for another year. Mature females were kept at the coldwater facility until caviar harvest in April of 2013 when fish were approximately 7 years of age ($n=799$ total); this group is henceforth known as the age 7 class. The following measurements were collected from all fish at the time of harvest: fork length (FL; ± 0.5 cm), body weight (BW; ± 0.05 kg), total ovary weight (OW; ± 0.05 kg), and caviar weight “in-the-tin” (± 1.0 g). Condition factor (K) was computed as:

$$K = (BW/FL^3) \times 10^5 \quad (1)$$

Gonadosomatic index (GSI) was calculated as:

$$GSI = (OW/BW) \times 100 \quad (2)$$

Caviar weight in-the-tin normalized by both body weight and ovary weight was also determined. An ovarian adiposity score was assigned to every female: high, medium, or low fattiness. A high fattiness score was recorded when the caviar weight in-the-tin was less than 50% of the ovary weight, between 50-65% of the ovary weight for a medium fattiness score, and more than 65% of the ovary weight for a low fattiness score (Ovissipour et al., 2015). For a sub-sample of 30 fish per tank per farm ($n=360$ total), after roe had been removed, fat lobes were dissected from the ovaries and both were measured individually: fat lobe weight and ovarian tissue weight. Fin clip tissue samples were also excised from the sub-sampled fish for microsatellite genotyping and heritability analyses. Mature females at Farm A and Farm B were similarly identified again in October 2013 and February 2014, respectively, cultured at the coldwater facility, and harvested in the spring of 2014 when fish were approximately 8 years of age ($n=1,307$ total); this group is henceforth known as the age 8 class. However, to maintain culture densities at Farm A, fish in one HF diet tank and one LF diet tank were split between the remaining two HF and LF tanks, respectively, so two fewer tanks were sub-sampled this year ($n=300$ total). Drought conditions in California and the large number of 8-year-old females to process limited the number of fish that could be held at the coldwater facility at a given time and so these females were processed over an extended period: February to June of 2014.

2.3. *Microsatellite genotyping*

DNA was extracted from fin clips of sub-sampled fish and genotyped at 11 polymorphic sturgeon-specific microsatellite loci: AciG 2, AciG 35, AciG 53, AciG 110, AciG 140, As015, Atr 107, Atr 109, Atr 117, and Atr1173 (Börk et al., 2008; Rodzen and May 2002; Zhu et al., 2005). DNA extraction, microsatellite genotyping, and analysis methods are described in Gille et al. (2015). Due to the polyploid nature of the white sturgeon genome, microsatellites were scored as dominant data: either present (score of 1) or absent (score of 0).

2.4. *Statistical methods*

For all experimental fish in the feeding trials, a Student's *t*-test was used to compare percentage of mature females as well as measurements of body weight, ovary weight, and caviar yield between the HF and LF diets and within the age 7 and age 8 classes for each farm; replicate tanks were pooled. The null hypothesis was that treatment means were equal. A 5% level of probability ($P < 0.05$) was considered significant. The same methods were used to compare fat lobe weight between the two diets in the sub-sampled age 7 and age 8 fish. The least squares mean and standard error for body weight, condition factor, caviar yield and caviar yield normalized by body weight by farm, age, and diet and adjusted for tank was also predicted for each ovarian adiposity fattiness category (groups with low, medium, and high adiposity scores) using R (Endelman 2011; Hadfield 2010; R Core Team 2016).

Correlations between ovarian adiposity and caviar yield were estimated for sub-sampled fish after correcting for the effects farm, diet and tank (for model see Equation 3 below). The methodology is a straightforward extension of univariate linear models, a model that provides for a covariance between the residuals of observations made for each trait on the same animal (Brown and Prescott 2015). The software used to fit this model is one of the public-domain packages available in R, the package MCMCglmm (Hadfield 2010).

It was not possible to do traditional log likelihood parentage assignment and pedigree reconstruction for the purpose of heritability analysis because fin clips were not collected from the sires and dams used for spawning in 2006. Instead, we constructed a realized relationship matrix among all sub-sampled progeny using dominant microsatellite data with the A.mat

function in the R package rrBLUP (ver. 4.3; Endelman 2011). We then tested the hypothesis that age at maturity, farm, diet, tank, and genotype affected ovarian adiposity and caviar yield in subsampled experimental fish. In this linear mixed model, the fixed effects were age, farm, diet, and tank (nested within farm and diet) and the random effect was molecular relatedness:

$$Y_{ijklm} = \mu + \text{farm}_i + \text{diet}_j + \text{tank}_{ijk} + \text{age}_l + a_{ijklm} + e_{ijklm} \quad (3)$$

where Y_{ijklm} is the individual phenotypic observation of caviar yield or ovarian adiposity, μ is the general mean, farm_i is the effect of the farm ($i = 2$, Farm A or Farm B), diet_j is the effect of diet ($j = 2$, HF or LF), tank_{ijk} is the effect of tank ($k = 1, 2, 3$) nested within farm_i and diet_j , age_l is the effect of age ($l = 2$, aged 7 or 8 years), a_{ijklm} is the additive genetic effect, and e_{ijklm} is the vector of residual effects. Feed manufacturer is included in the effect of farm while the effect of diet refers to fat content. The significance of each fixed effect was tested by constructing models with and without each fixed effect. The mixed.solve function in rrBLUP (ver. 4.3; Endelman 2011) was used to calculate the restricted maximum likelihood (REML) solutions to the mixed models for all phenotypic measurements of caviar yield and ovarian adiposity. The difference between the REML of the models with and without each fixed effect was compared by a chi-square test.

2.5. Heritability

The mixed.solve function in rrBLUP (ver. 4.3; Endelman 2011) was also used to return BLUP solutions of the additive genetic variance and residual variance of each phenotype for subsampled fish. Narrow-sense heritabilities of phenotypic traits were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \quad (4)$$

where h^2 is narrow-sense heritability, σ_a^2 is the additive genetic variance, and σ_e^2 is the residual variance.

3. Results

3.1. Feeding trials

Feeding trial results are presented in Table 1a and Table 1b. Upon initiation of the feeding trial, Farm A females weighed (mean $\pm$ standard error) 24.0 ± 0.4 kg and Farm B females weighed 16.3 ± 0.3 kg. The percentage of mature females harvested for caviar at 7 or 8 years of age was not significantly different between the HF and LF diets at both farms, indicating that diet fat was not a contributing factor to age at maturity. At age 7, Farm A had (mean $\pm$ standard error) $31.0 \pm 3.1\%$ and $28.5 \pm 1.6\%$ mature females in the LF and HF treatments, respectively, while Farm B had $14.4 \pm 4.3\%$ and $18.2 \pm 3.1\%$. At age 8, Farm A had $56.6 \pm 1.3\%$ and $53.8 \pm 5.1\%$ mature females in the LF and HF treatments, respectively, while Farm B had $43.3 \pm 2.8\%$ and $42.5 \pm 1.6\%$.

Age 7 females that received the LF diet for 19 months were, on average, 2 kg smaller than their cohort that was maintained on the HF diet (Table 1a). A similar trend was observed in age 8 females that were continued on the LF diet for an additional year at Farm A, however age 8 females at Farm B fed the LF diet weighed approximately 5 kg less than fish in the HF diet group (Table 1a). Females given the LF diet were generally leaner and had a lower condition factor compared to HF diet fish although this trend was only significant by t-test at Farm B (Table 1a). The percentage of fish with high fat ovaries was elevated in the HF diet tanks and ranged from 18.0-33.2% but was only 11.8-17.2% in the LF diet tanks (Table 1a). Conversely, the percentage of fish with desirable low fat ovaries was 41.2-55.3% in the LF diet tanks compared to 23.5-33.7% in the HF diet tanks (Table 1a). Differences in percentages of fish with high or low fat ovaries between diets were only significant by t-test at Farm B (Table 1a). Females on the LF diet had smaller ovaries than those that received the HF diet, even when ovary weight was normalized by body weight (i.e., GSI); GSI differences among diet groups were statistically significant for age 7 females at Farm A and for age 8 females at Farm B (Table 1b). Caviar yield did not appear to be influenced by the diet treatment except in age 8 females at Farm B where fish given the LF diet produced 0.2 kg less caviar than HF diet fish (Table 1b). However, caviar yield normalized by body weight was greater for females fed LF diets at both

ages and was significant by t-test at Farm B (Table 1b). Caviar yield normalized by ovary weight was significantly higher for the groups of fish shifted to the LF diet except for age 8 females at Farm A ($P=0.06$; Table 1b). Caviar yield normalized by ovary weight at Farm A was 63-64% and 58-60% for the LF and HF groups, respectively, and at Farm B were 61-65% and 56-58% for the LF and HF groups, respectively (Table 1b).

Of sub-sampled fish, females reared on the HF diet had more prominent ovarian fat lobes than those supplied with the LF diet, however the difference between diet groups was not significant by t-test in age 8 females at Farm A (Table 2). The most notable disparity between diets was in age 8 fish at Farm B: the mean fat lobe weight of females in HF diet tanks was nearly twice that of females in the LF diet tanks (Table 2).

When fish were separated by degree of ovarian fattiness (i.e., low, medium, and high adiposity scores) post hoc, females with high fat ovaries were significantly larger and had the highest condition factor compared to those with low fat ovaries regardless of farm, age, and diet; data for females with medium fat ovaries fell in between (Figures 3a and 3b). Although larger in body weight, females with high fat ovaries yielded significantly less caviar than smaller females with lean ovaries (Figure 4a). Females with lean ovaries demonstrated a 3% increase in caviar yield normalized by body weight over the high fat ovary group (Figure 4b).

3.2 Ovarian adiposity and caviar yield correlations

Correlations among measures of ovarian adiposity (total ovary weight, fat lobe weight, ovarian tissue weight, ovary weight scaled by body weight, and fat lobe weight scaled by total ovary weight) and caviar yield (caviar weight in-the-tin, caviar yield scaled by body weight, caviar yield scaled by total ovary weight) are presented in Table 3; 95% confidence limits of the correlation values are presented in Table 4. Positive correlations were observed among all ovary weight measurements and all caviar yield measurements and ranged from 0.35-0.87 and 0.28-0.72, respectively. The most robust correlation that confirms the inverse relationship between ovarian adiposity and caviar yield witnessed by caviar farmers was between ovarian fat lobe weight and caviar yield when both were normalized by total ovary weight: -0.77. There was also a strong negative correlation between unscaled ovarian fat lobe weight and caviar yield normalized by total ovary weight: -0.64. Other negative correlations ranging from -0.35-(-0.04)

were observed between ovarian adiposity and caviar yield when one or the other was normalized by total ovary weight.

3.3 Treatment effects and heritability

Farm, diet, tank, and age had large effects on ovarian adiposity and caviar yield in subsampled fish ($P<0.05$; Table 5). The one exception was that the effect of farm was not significant on ovary fat lobe weight ($P=0.309$; Table 5). Ovary weight, ovary fat lobe weight, ovary weight/body weight, fat lobe weight/ovary weight, caviar weight in-the-tin, caviar yield/body weight, and caviar yield/ovary weight were moderately heritable (h^2 ranged from 0.20 to 0.39; Table 6) while the heritability of ovarian tissue weight was lower ($h^2=0.11$; Table 6).

4. Discussion

The association between highly variable roe yield and the proliferation of adipose tissue in the ovaries of sturgeon has frequently been observed by caviar processors but never formally studied. Here, we performed a feeding trial to examine the effects of a low fat finishing diet compared to a high fat production diet on body size, ovarian adiposity, and caviar yield in more than 2,000 mature female white sturgeon. On a subset of these same fish, we evaluated the significance of farm, diet, age, tank, and genotype on the same phenotypes. The sustained production of high quality caviar from sturgeon aquaculture is dependent upon understanding and optimizing the key factors that influence ovarian adiposity and roe yield. Additionally, there is very little published data on caviar yield in white sturgeon. A commonly cited value for caviar yield for all sturgeon has been 10% of total body weight (Billard 2009). Williot et al. (2001) reported caviar yields in cultured Western European species (including white sturgeon) as 10-15% of total body weight. Ours is the first comprehensive study to reveal the range of caviar yield in white sturgeon to be less than 1% and as high as 22% of total body weight with an average yield of 7.6-9.2% of total body weight.

The effect of diet on white sturgeon caviar production has been seldom studied likely due to the large body size and late age of maturity inherent to the species. Zhang et al. (2011) found that diet had no significant effect on egg yield in white sturgeon, however the feeding trial was

performed in non-replicated tanks that contained a small sample size of 15-21 fish and lasted for only 11 months. The feeding trial presented here incorporated hundreds of fish in three tank replicates and ran for 19-33 months. With increased power, statistical analyses revealed that a low fat finishing diet substantially decreased the amount of adipose tissue in the ovaries of white sturgeon and simultaneously increased caviar yield. Although fish also grew to be larger when maintained on the high fat diet, caviar yield, when normalized for body weight, was higher in females that received the low fat diet. These two pieces of experimental evidence suggest that caviar farmers should initially culture fish on a higher fat production diet to allow for faster body growth but then shift to a lower fat finishing diet at least 19-33 months prior to caviar harvest to reduce the proliferation of ovarian fat and thereby improve egg yield. However, it is important that conversion to a low fat diet not be made too early as slowed body growth may delay sexual maturation and therefore age at harvest. More importantly, the slower growth of fish fed the lower fat diet would also allow caviar farmers to stock a greater number of smaller, higher yielding females per tank and thus increase the amount of caviar produced and profit per tank. The low fat finishing diet treatment also resulted in an increase in the percentage of females with lean ovaries that not only produced more superior quality roe but were much faster to process (S. Bishop and B. Renschler, unpublished data). Fedorovkykh et al. (2015) also reported difficulties in processing roe from fatty ovaries of Russian sturgeon when fish were fed diets with over 18% fat content. Post hoc analyses revealed that fish in the low ovarian fattiness group were consistently smaller and leaner regardless of farm, age, or diet. Efforts should be undertaken to determine whether leaner individuals merely consumed less, were outcompeted by larger, more aggressive females with fatty ovaries, or whether particular sires or dams generated female offspring with lean or fatty ovaries. It is also conceivable that a physiological issue prevented females with high levels of ovarian fat from utilizing the fat deposited in the ovaries for energy.

This study corroborates observations made by sturgeon farmers as there were strong negative correlations, as great as -0.77, between measurements of ovarian fat and roe yield. Correlation data underscore the importance of preventing high ovarian fattiness in sturgeon aquaculture. Adjusting rearing protocols to limit the proliferation of adipose tissue in the ovaries should have a direct and substantial impact on the amount of caviar that can be harvested from white sturgeon.

Investigation of farm, diet, tank, and age fixed effects revealed that all treatments significantly contribute to ovarian adiposity and caviar yield. It is not surprising that phenotypic measurements were affected by farm origin as culture conditions at Farm A and Farm B were dissimilar, such as temperature. Temperature is a controlling factor in sturgeon metabolism that promotes maximal growth when optimized (Cech et al., 1984; Hung et al., 1993). While specific water temperatures and feeding rates were not described, Steffens and Jahnichen (1995) observed lipid deposition in the gonads of mature bester (*Huso huso* x *A. ruthenus*) kept in warm water tanks on an intensive feeding treatment. To counteract ovarian fattiness, bester females intended for breeding were subsequently removed to ponds with lower temperatures and fed an adjusted diet. Here, water temperatures at Farm A were consistently higher and more often in the optimum range for white sturgeon which likely contributed to the larger size of these females in the feeding trial. In addition to culture conditions, diet manufacturers differed between Farm A and B (EWOS Canada, Ltd and Skretting USA, respectively) and while nutritive content was virtually identical, proportions of ingredients and exact recipes were proprietary. Farm origin significantly affected measurements of ovarian fat and egg yield because of variation associated with specific farm culture conditions; further examination of these farm features is therefore warranted.

Results of the feeding trial and linear mixed model analyses were congruent and showed that diet is a key factor that can influence both ovarian adiposity and roe yield in cultured white sturgeon. Specifically, a production diet with high fat content will increase the amount of ovarian adipose tissue that displaces the amount of potentially harvestable roe. Caprino et al. (2008) found that female white sturgeon fed a diet coated with either squid oil or a blend of soybean and fish oils had very high caviar yields. While no history or details of culture conditions, fish ages, or pre-study diets were given, this study is worthy of note as caviar yield from 20 females (33-34 kg) was 14-15% of body weight and 86-89% of ovary weight. These consistent high yields are very curious as they have only been observed in the very best female white sturgeon processed in California during past studies (the top 1% of many thousands of individuals; Figure 1) with absolutely no adipose lobes or interstitial adipose tissue between individual eggs. Within this study, only 17 of 2,106 fish processed (top 0.8%) produced such yields. If the data presented by Caprino et al. (2008) is accurate, then the white sturgeon industry should further investigate the Italian culture protocols, specifically those related to diets fed.

Despite randomization of individuals among tanks and other precautions, tank effects are commonly encountered in aquaculture experiments (e.g., Bagley et al., 1994; Herlinger et al., 1999; Hastings et al., 2013). A significant tank effect was not unexpected here as sturgeon females were kept in the twelve total experimental tanks for only 19-33 months and were transferred to communal tanks at a coldwater facility months before caviar harvest. It is also nearly impossible to maintain all of the large experimental tanks (Farm A used 9.2 m diameter tanks and Farm B used 14.6 m diameter tanks) at the exact same hydrology and density conditions. Furthermore in 2013, fish from one HF tank and one LF tank at Farm A were divided between the remaining two diet-specific tanks, respectively, to maintain adequate culture densities. These changes would have attenuated experimental tank associated variation. However, there are other environmental factors that could explain ovarian adiposity and caviar divergence among tank groups. For example, it is common practice in finfish aquaculture to adjust stocking densities and to grade according to size to limit aggression and the formation of dominance hierarchies, reduce stress, and prevent disease (Ashley 2007). Mitigation of aggression and unequal access to food maximizes growth rates among aquaculture species and thereby improves culture efficiency (Knights 1987; Holm et al., 1990; Wipf and Barnes 2011). Stocking densities and fish sizes in this study were maintained within a fairly narrow margin but were not identical among tanks. It is possible that differences in competition, stress, and subsequent growth rates among tanks significantly influenced the proliferation of adipose tissue and caviar yield in white sturgeon cultured here. While often unknown and unpredictable, every effort should be made to minimize tank variation in aquaculture studies so that meaningful treatment effects are not obscured.

Age had a significant impact on all measures of both ovarian adiposity and caviar yield and results of the feeding trials indicated that age 8 females produced slightly more caviar and had slightly larger fat lobes than age 7 females. These findings are consistent with what is known about Acipenseriform fish biology. Under optimal culture conditions, white sturgeon grow more rapidly compared to their wild counterparts and thus mature at a younger age (Van Eenennaam et al., 2004) and so it follows that females that mature at age 7 would be smaller and therefore yield fewer eggs and have less ovarian adipose tissue than those that are mature a year later at age 8. It is also important to note that the accumulation of ovarian fat in Acipenseriform fishes is not linear with age. Scarnecchia et al. (2007) discovered that gonadal fat bodies in female paddlefish

proliferate and are most abundant during the first few spawning events but are largely depleted by middle-age (approximately 25 years of age). As ovarian fat wanes, body size and caviar yield increase and in this way reproductive effort is maximized with age. However, achieving the age of optimum caviar output would be cost prohibitive for sturgeon farmers as it would involve multiple reproductive cycles and rearing fish for many years longer than culture practices currently dictate (Scarnecchia et al., 2007). While age at harvest can be somewhat manipulated, it seems a much more effective strategy to focus on adjusting those aquaculture factors that have a more immediate and direct effect on ovarian adipose tissue and caviar yield in young sturgeon such as a low fat finishing diet.

The moderate heritability values reported here are favorable for sturgeon aquaculture because they indicate that selection for lean ovaries and high caviar yield will respond to breeding and improve offspring. However, implementation of selective breeding programs will be challenging given the slow growth and late age of maturation of most sturgeon species. Many common aquaculture practices will also need to be amended in order to identify the most desirable sires and dams. For example, multiple full- and half-sibling families and unrelated progeny are often combined in a single tank during the hatching process making pedigree tracking impossible. To perform individual parent selection, caviar farmers would need to rear full-sibling families in separate tanks or use physical or genetic tagging methods to discern full-sibling families and evaluate ovarian adiposity and caviar yield in offspring across multiple years. Furthermore, not all sturgeon are capable of spawning annually so controlled crosses of select broodstock may not be feasible. While logistics would be difficult, heritability data and the successes of selection programs in other aquaculture species (Gjedrem and Baranski 2009; Ponzoni et al., 2007; Ponzoni et al., 2008) suggest that selective breeding would be a worthwhile future endeavor for caviar farmers to improve production efficiency and economic profitability.

5. Conclusions

The proliferation of ovarian adipose tissue profoundly and negatively impacts roe yield and therefore caviar farming efficiency and profitability in cultured white sturgeon. The study presented here demonstrates how the culture conditions of feed fat content, farm origin, age at maturity, tank, and genotype are all of vital importance and can be optimized and manipulated to

minimize ovarian adiposity while simultaneously maximizing caviar yield. We discovered that white sturgeon females fed a low fat finishing diet 19-33 months before caviar harvest had less ovarian fat, were smaller in size, and produced a greater yield of caviar compared to those maintained on a high fat production diet. Furthermore, there was a greater percentage of females with a high degree of ovarian fattiness in the high fat production diet group than in the low fat finishing diet group. Ovarian adiposity and caviar yield were strongly and inversely correlated which corroborates observations made by caviar farmers. We found that diet, farm origin, age at maturity, and tank all significantly affected ovarian adiposity and caviar yield. Estimated heritability values for measurements of ovarian adiposity and caviar yield were moderate indicating that selective breeding could be an effective means to improve these traits.

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Table Captions

Table 1a. Mean ($\pm$ standard error) fork length, body weight, condition factor, and percentage of fish with high and low fat ovaries of dietary treatments compared by Student's t-test for 7- and 8-year-old fish in feeding trials at Farms A and B. Replicate tanks were combined. * $P < 0.05$.

Table 1b. Mean ($\pm$ standard error) ovary weight, gonadosomatic index (GSI), caviar yield, caviar yield normalized by body weight (BW), and caviar yield normalized by ovary weight (OW) compared by Student's t-test for 7- and 8-year-old fish in feeding trials at Farms A and B. Replicate tanks were combined. * $P < 0.05$.

Table 2. Mean ($\pm$ standard error) ovarian fat lobe weight of dietary treatments compared by Student's t-test for 7- and 8-year-old sub-sampled fish at Farms A and B. Replicate tanks were combined. * $P < 0.05$.

Table 3. Correlations among measurements of ovarian adiposity (OW = total ovary weight; OFL = ovary fat lobe weight; OT = ovary tissue weight; GSI = gonadosomatic index or percentage of ovary weight scaled by body weight; OFL/OW = percentage of ovary fat lobe weight scaled by total ovary weight) and caviar yield (CY = caviar weight in-the-tin; CY/BW = percentage of caviar weight in-the-tin scaled by body weight; CY/OW = percentage of caviar weight in-the-tin scaled by total ovary weight).

Table 4. Confidence levels of ovarian adiposity and caviar yield correlations. The upper 95% limit is shown above and the lower 95% limit is listed below the diagonal.

Table 5. Significance (P values) of fixed effects on ovarian adiposity and caviar yield measurements.

Table 6. Narrow-sense heritability (h^2) of ovarian adiposity and caviar yield measurements.

Figure Captions

743

744 Figure 1. Percent caviar yield by total body weight of female white sturgeon ($n=9,708$) harvested
745 at Farm A from 2012-2014 (S. Bishop and B. Renschler, unpublished data).

746

747 Figure 2. White sturgeon ovaries classified as (top) high fattiness with prominent lobes and
748 interstitial adipose tissue and a caviar yield of less than 50% of the total ovary weight; and
749 (bottom) low fattiness with minimal lobes and interstitial adipose tissue and a caviar yield of
750 greater than 65% of the total ovary weight (photo credit: F. Conte). *****Please print this figure in***
751 ***color.***

752

753 Figure 3. Caterpillar plots of (a) mean body weight (kg) and (b) condition factor (K) of each
754 fattiness category (adiposity score of low, medium or high) by farm (A or B), age (7 or 8), and
755 diet (H or L). *****Please print this figure in color.***

756

757 Figure 4. Caterpillar plots of (a) mean caviar yield (kg) and (b) mean caviar yield scaled by body
758 weight (shown as a percentage) of each fattiness category (adiposity score of low, medium or
759 high) by farm (A or B), age (7 or 8), and diet (H or L). *****Please print this figure in color.***

760

Figure 1

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765

Figure 2

Figure 3

Figure 4

770 **Table 1a**

	Age	Diet	<i>n</i>	Fork length (cm)	Body weight (kg)	Condition factor (K)	High fat ovaries (%)	Low fat ovaries (%)
Farm A	7	HF	112	152.15 ± 0.91	32.22 ± 0.65	0.91 ± 0.01	25.44 ± 5.62	32.48 ± 7.22
		LF	126	150.36 ± 0.72	30.84 ± 0.50	0.90 ± 0.01	11.81 ± 2.47	51.04 ± 5.99
	8	HF	79	160.97 ± 1.10	38.33* ± 0.69	0.92 ± 0.01	17.95 ± 5.45	33.71 ± 2.46
		LF	95	159.43 ± 0.85	36.48* ± 0.61	0.90 ± 0.01	12.51 ± 3.82	48.54 ± 3.64
Farm B	7	HF	315	140.76 ± 0.49	26.02* ± 0.33	0.92* ± 0.00	33.18* ± 3.06	23.52* ± 2.47
		LF	246	139.34 ± 0.58	24.31* ± 0.36	0.89* ± 0.00	17.21* ± 2.24	41.15* ± 2.97
	8	HF	564	152.04* ± 0.44	33.21* ± 0.35	0.93* ± 0.00	24.70* ± 3.88	30.77* ± 4.00
		LF	569	147.76* ± 0.41	28.60* ± 0.29	0.87* ± 0.00	12.05* ± 1.25	55.25* ± 4.05

771
772

773 **Table 1b**

	Age	Diet	<i>n</i>	Ovary weight (kg)	GSI	Caviar yield (kg)	Caviar yield (%BW)	Caviar yield (%OW)
Farm A	7	HF	112	4.25* ± 0.12	13.13* ± 0.21	2.43 ± 0.07	7.58 ± 0.17	58.03* ± 1.07
		LF	126	3.87* ± 0.09	12.51* ± 0.17	2.46 ± 0.06	8.00 ± 0.16	64.05* ± 0.92
	8	HF	79	5.01 ± 0.13	13.08 ± 0.22	2.95 ± 0.08	7.80 ± 0.20	59.83 ± 1.23
		LF	95	4.70 ± 0.12	12.85 ± 0.21	2.96 ± 0.09	8.08 ± 0.19	63.02 ± 1.14
Farm B	7	HF	315	3.76* ± 0.07	14.29 ± 0.15	2.04 ± 0.04	7.87* ± 0.11	55.53* ± 0.72
		LF	246	3.47* ± 0.07	14.15 ± 0.16	2.08 ± 0.04	8.60* ± 0.12	61.28* ± 0.70
	8	HF	564	5.06* ± 0.07	15.10* ± 0.13	2.81* ± 0.04	8.58* ± 0.09	57.73* ± 0.54
		LF	569	4.13* ± 0.05	14.39* ± 0.11	2.61* ± 0.03	9.23* ± 0.09	64.59* ± 0.50

774

775

776 **Table 2**

	Age	Diet	<i>n</i>	Fat lobes (kg)
Farm A	7	HF	90	0.86* ± 0.06
		LF	90	0.57* ± 0.05
	8	HF	60	0.87 ± 0.07
		LF	58	0.70 ± 0.08
Farm B	7	HF	90	0.95* ± 0.07
		LF	90	0.63* ± 0.05
	8	HF	90	1.02* ± 0.08
		LF	90	0.64* ± 0.05

777
778

779 **Table 3**

	OW	OFL	OT	GSI	OFL/OW	CY	CY/BW	CY/OW
OW		0.75	0.69	0.67	0.43	0.53	0.20	-0.29
OFL			0.55	0.55	0.87	0.19	-0.17	-0.64
OT				0.43	0.35	0.26	0.01	-0.35
GSI					0.37	0.39	0.54	-0.18
OFL/OW						-0.04	-0.40	-0.77
CY							0.49	0.28
CY/BW								0.72
CY/OW								

780

781

782 **Table 4**

	OW	OFL	OT	GSI	OFL/OW	CY	CY/BW	CY/OW
OW		0.79	0.74	0.70	0.50	0.57	0.27	-0.23
OFL	0.72		0.62	0.61	0.89	0.26	-0.12	-0.60
OT	0.66	0.52		0.50	0.42	0.33	0.07	-0.29
GSI	0.61	0.51	0.38		0.44	0.45	0.59	-0.11
OFL/OW	0.38	0.86	0.29	0.31		0.03	-0.33	-0.74
CY	0.46	0.12	0.19	0.33	-0.12		0.56	0.35
CY/BW	0.13	-0.26	-0.08	0.48	-0.46	0.44		0.75
CY/OW	-0.37	-0.69	-0.42	-0.25	-0.80	0.21	0.68	

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785 **Table 5**

Phenotype	Fixed effects (<i>P</i> values)			
	Age	Farm	Diet	Tank
OW	0	0.011	<0.001	<0.001
OFL	0	0.309	<0.001	<0.001
OT	0	<0.001	<0.001	<0.001
GSI	0	<0.001	<0.001	0
OFL/OW	0	0.004	<0.001	0
CY	0	0.006	<0.001	<0.001
CY/BW	0	<0.001	<0.001	<0.001
CY/OW	0	0.008	<0.001	0

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788 **Table 6**

Phenotype	h^2
OW	0.33
OFL	0.26
OT	0.11
GSI	0.34
OFL/OW	0.27
CY	0.39
CY/BW	0.20
CY/OW	0.22

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791 Statement of relevance impact - Gille et al.
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793 Study findings will increase sturgeon aquaculture profits.
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