

PROJECT TERMINATION REPORT

Part I: Summary

PROJECT TITLE: Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar

REPORT GIVEN IN YEAR: 2011

PROJECT WORK PERIOD: September 1, 2007 – August 31, 2011

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Technical Advisor:	Fred Conte	University of California, Davis
Industry Advisor:	Peter Struffenegger	Sterling Caviar, LLC

REASON for TERMINATION: Objectives completed and funding ended.

PROJECT OBJECTIVES:

The long-term goal of this study is to optimize the yield and quality of sturgeon caviar by developing less invasive, faster, and better predictors of maturity than currently used oocyte polarization index (PI). The main objective is to correlate predictors of maturity with instrumental and biochemical assays conducted at different stages of ovarian maturity in white sturgeon, including resorption of ovarian follicles (ovarian follicular atresia). The specific objectives of this study are the following:

- 1) Determine how currently utilized morphological characteristics (oocyte PI, ovarian follicle size, gonadosomatic index, age and live weight) correlate with caviar quality and yield (years 1-2);
- 2) Determine how plasma sex steroid, total calcium, and plasma protein levels and crude chemical composition of eggs change with maturity (years 1-2);
- 3) Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish (years 1-2);
- 4) Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe (years 1-2);
- 5) Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian follicular atresia to avoid sacrificing fish with inferior quality roe and use them during the next production cycle (year 1);
- 6) Conduct training and outreach programs at field sites in Idaho and California (years 3-4).

PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Determine how currently utilized morphological characteristics (oocyte PI, ovarian follicle size, gonadosomatic index, age and live weight) correlate with caviar quality and yield. The analysis of morphological characteristics and caviar harvested during 2008, 2009, 2010, and 2011 revealed relationships of caviar yield with body weight (BW), stage of maturity (oocyte PI), and egg diameter (ED) for age 7 fish in California ($P < 0.05$), however the coefficients of determination (R^2) were less than 0.10. There was a significant ($P < 0.0001$) relationship between gonadosomatic index (GSI) and caviar yield for both age 7 and 8 cohorts in California, with R^2 values of 0.46 and 0.52, respectively. Unexpectedly, the stage of maturity (oocyte PI) was not a main factor affecting roe yield due to apparent confounding and interactive effects of other variables, particularly the highly variable caviar yield caused by extensive ovarian adiposity which was pronounced in larger fish within age cohort. Three categories of ovarian adiposity (high fat (HF), medium fat (MF) and low fat (LF)) corresponded well to the caviar yield per body weight (CY%BW) and caviar production per fish (CW). Generally, processed caviar yield was less than 50% of the ovarian weight for the HF category, within 50-65% for the MF category, and more than 65% for the LF category.

We examined the relationship between oocyte PI and caviar yield (% BW) within each category of ovarian adiposity and found there was a relationship in the medium fat group ($P = 0.01$); however the R^2 was only 0.0387. In California females, the three fattiness groups did not differ by GSI ($P > 0.05$, one-way ANOVA and Tukey test), but the means for caviar weight and caviar yield per both BW and ovary weight (OW) were significantly different. The HF group had the highest mean BW, smallest eggs (ED), and the lowest caviar yields per fish BW and OW (CY-BW and CY-OW). The best producers of roe were the females in the LF group; while they had significantly lower mean BW and condition factor (K), they produced the highest yield of caviar (CY% BW and CY% OW).

The caviar harvest data from Idaho included fewer fish, but the comparisons with California revealed differences between the stocks of the two states. The Idaho fish were similar in BW but much older, had a lower K (0.73 versus 1.03), smaller ovaries, and a lower GSI compared to California fish. Despite the lower GSI in the Idaho stock, the mean caviar weight and the caviar yield per body weight were similar in both states because the Idaho fish ovaries were much leaner. The mean yield of caviar as a percent of ovarian weight was 71% in Idaho compared to 58% in California stock. The Idaho fish were much older (15-21 years) and produced larger eggs (3.4 mm diameter compared to 3.1 mm in California fish). Other factors to consider are generally cooler water in Idaho and the fact that fish were kept in the dirt canals for several years prior to this study having access to a single demand feeder and various species of fish living in the canal. This restricted feeding and more naturalistic environment could also be a factor in the leaner ovaries and lower condition factor. It should be noted that the Idaho producers have observed more fatty ovaries in the younger year-class fish reared in the concrete raceways and fed prepared diets more regularly.

Though the main effect of the oocyte PI was not obvious in this study, this analysis does not invalidate the importance of detecting maturational stage by non-invasive methods. Using the oocyte PI even though weakly correlated to caviar yield to determine the optimal time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain. As well, the quality of caviar was a critical component of this project. In the past, caviar processors have noted that using the same percent of salt throughout the processing season

resulted in egg batches becoming too salty or not salty enough. It has long been assumed that the variation in saltiness following a standardized salt treatment could be associated to variation in egg maturity (oocyte PI) between females. If females could be harvested at more homogenous oocyte PI's then the standardized percent of salt used would result in less variation in quality of the caviar, in terms of saltiness.

Objective 2. Determine how plasma sex steroid, total calcium, and plasma protein levels and crude chemical composition of eggs change with maturity. Blood plasma calcium and total protein was measured repeatedly in fish in 2007-2008. Plasma calcium and total protein did not differ among stages of maturity in these fish. These parameters were not measured in the following years of the study. Blood plasma T and E2 and crude chemical composition of eggs did not change with increasing maturity (measured by decreasing oocyte PI) at the time of caviar harvest. Blood plasma T and E2 were significantly lower in fish with atretic ovaries compared to fish with normal ovaries.

Objective 3. Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish. SW-NIR spectra were collected in California in 2008 and 2009 and Idaho in 2009 to assess the ability of SW-NIR to predict stage of maturity (oocyte PI). For California, a model was developed using the intensity spectra collected on the abdomen resulting in 84% of the fish with high PI values and 62% of the fish with low PI values correctly classified. For a similar analysis done using the intensity spectra for Idaho collected on the abdomen, 66% of the fish with high PI values and 82% of the fish with low PI values were correctly classified. SIMCA models were also built from one year (2008 or 2009) in an attempt to predict the PI value of the other year. However results from these models are poor and lack the ability to predict from one year to the next. It is unclear at this time whether the transferability of the model is precluded by differences in the eggs from one year to the next or by instrumental irreproducibility. Other factors such as limited year classes (individual fish variation) and limited sample size when compared to typical SW-NIR models may also contribute to the lower predictability of the models developed in this study. Though sturgeon gonadal development is synchronous, we tested whether there were differences in the stage of maturity as seen by oocyte PI and SW-NIR spectra across and between gonadal lobes. There is no difference between anterior, middle, or posterior scans on a given fish, and the differences in oocyte PI were not biologically significant.

Objective 4. Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe. We applied a rapid and less invasive vibrational spectroscopy method to collect spectral features of sturgeon female plasma and quantify and predict the actual PI values with the aid reference values and partial least squares regression chemometric models. Two types of partial least squares regression (PLSR) models were established and the advantages/disadvantages of both models were compared. The Plan A PLSR model was constructed from spectra from fish plasma from the years of 2007, 2008 and 2009 randomly selected with a leave-one-out cross validation to challenge the rigorousness of the calibrated model. The Plan B PLSR model was constructed similarly to the Plan A model except that 5 fish samples from the 2010 and 2011 data sets were randomly selected and used for model establishment and leave-one-out cross validation. The PI values of all fish in 2010 and 2011 were predicted based upon the Plan A model, while the remaining fish not used in model development from 2010 and 2011 were predicted based upon the Plan B model. Strictly speaking, only the Plan A PLSR model is "truly" predicting the PI values of the samples in the year of 2010 and 2011, and the Plan B PLSR model is only cross

validating additional samples from 2010 and 2011. This is because Plan B incorporates spectral features of the fish sampled in 2010 and 2011 (n=5 each) into the calibration model, whereas Plan (A) is based upon fish from prior years only. FT-IR can successfully predict oocyte PI with approximately 90% accuracy. Model maintenance is necessary and a total of 10 fish per year.

Objective 5. Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian atresia. Plasma sex steroid analysis, SW-NIR of abdomen and ovarian follicles, and FT-IR of plasma appear to be reliable predictors of atresia with variable success rates depending on the tool chosen. The use of plasma sex steroids analyzed by radioimmunoassay (RIA) resulted in 95% of the females with normal ovarian follicles (non-atretic) and 93% of the atretic females correctly classified (Talbot et al. 2010). Logistic regression equations were developed to predict the probability that a female white sturgeon has normal ovaries given a specific concentration of T or E2. In California, females with a circulating concentration of T less than 35.1 ng/ml should not be harvested as they have a 95% probability of having initiated ovarian atresia. The SW-NIR spectroscopy resulted in a model that correctly predicted 115 body scans out of 120 body scans from atretic fish for a correct classification of 96%. The validation of this model using a leave-one-out technique showed that 55% of the body scans were correctly classified as atretic (Servid et al. 2010). Similarly, the classification rates of atretic scans on ovarian follicles were high for the model development (96%), and the validation of the model showed that 58% of the atretic scans were correctly classified. The FT-IR was capable of identifying atretic females with 70% accuracy (Lu et al. 2010).

Objective 6. Conduct training and outreach programs at field sites in Idaho and California. The studies were conducted at aquaculture facilities in both Idaho and California with extensive support and participation of our industry partners. Industry partners were involved in field collection of all biological samples for laboratory analysis and on-site SW-NIR spectroscopy. The results of the project revealed that FT-IR can be used to successfully predict oocyte PI and analysis of plasma sex steroids was the most reliable predictor of follicular atresia. These laboratory techniques are available for the aquaculture industry; however, given that oocyte PI did not correlate well with caviar yield, none of the current techniques were pursued for instrument-to-fish analysis. An outreach publication to describe the different methods to determine follicular atresia in female sturgeon is currently being written. To improve quality and yield of caviar in farmed white sturgeon, it is essential to correctly assess the stage of ovarian maturity and avoid harvesting females with atretic ovarian follicles.

IMPACTS:

Relevance: Currently, the only means to assess ripeness of white sturgeon females and properly time caviar harvest is measurement of oocyte PI that requires surgical biopsy. This technique is accurate but invasive and stressful to fish, time consuming, and not an effective tool for handling a large number of female fish. The large caviar farms that harvest thousands of females cannot determine oocyte PI for every fish resulting in decreased caviar yield and quality due to follicular atresia (phagocytosis of ovarian eggs). Even the early stage of atresia causes a reduction in the firmness, flavor, and shelf life of caviar, and sometimes the complete loss of the product. Specifically, the sturgeon industry was interested in new methods to predict sturgeon maturity (oocyte PI) as an alternative to the surgical biopsy and identify females in the early stages of atresia.

Response: To achieve our goal, we needed a better understanding of the biochemical and physiological changes that occur during gonadal maturation and early atresia, and how these changes correlate with roe quality. The methods should be non-invasive, minimally stressful to fish, and quick. Ideally, they would allow female fish to be sorted in the fall during late vitellogenesis into groups based upon the degree of ripeness, followed by a quick examination of groups for early atresia just prior to selection for caviar harvest. In this project, we examined the ability of non-invasive SW-NIR, FT-IR, and immunochemical assays to identify early atresia in females and replace the surgical biopsy procedure currently used to determine ripeness in sturgeon.

Results: The radioimmunoassay of plasma steroids (Talbot et al. 2011), spectral analysis of plasma by FT-IR (Lu et al. 2011), and abdominal and follicular scans by SW-NIR (Servid et al. 2011) were suitable for detecting early atresia with 55-93% accuracy depending on the tool, and the FT-IR was determined to be the most accurate in determining maturational stage (oocyte PI) and may be used in lieu of the surgical biopsy technique (90% accuracy). The analysis of caviar harvest found that the stage of maturity manifested by the oocyte PI does not necessarily correlate with higher caviar yields. However, using oocyte PI even though weakly correlated to caviar yield to determine the time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain. Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster growing fish. It should be noted that this analysis does not invalidate the importance of detecting maturity stage by non-invasive methods. Using the same percent of salt throughout the processing season has resulted in some egg batches becoming too salty or not salty enough. The variable saltiness is hypothesized to be associated with variation in egg maturity (PI) between females. If females could be harvested at more homogenous PI's then the standardized percent of salt used would result in less variation in quality of the caviar in terms of saltiness.

Collaborators: This collaborative project included researchers from Eastern Oregon University (EOU), Washington State University (WSU), Montana State University (MSU), University of California, Davis (UCD), Bozeman Fish Technology Center (BFTC), University of Idaho Extension (UIE), and industry collaboration in California (Sterling Caviar, LLC) and Idaho (Fish Breeders of Idaho and Blind Canyon Aqua Ranch).

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RECOMMENDED FOLLOW-UP ACTIVITIES:

Sturgeon farmers in California and Idaho observe highly variable roe yield in mature sturgeon, associated with accumulation of fat in the ovaries. Environmental, genetic and developmental factors can all affect caviar yield and quality, but as of yet the role of these factors is not well understood. Understanding these effects is essential for sustained production of high quality sturgeon caviar in the Western Region. We proposed a collaborative study, with participation of four states of the Region and four sturgeon farms, aimed to investigate these effects on ovarian adiposity, roe yield and caviar quality in farmed sturgeon. This recent WRAC proposal was funded for the first year. An outreach publication to describe the different methods to determine follicular atresia in female sturgeon is currently in preparation. To improve quality

and yield of caviar in farmed white sturgeon, it is essential to correctly assess the stage of ovarian maturity and avoid harvesting females with atretic ovarian follicles.

More robust PLS-DA analyses compared to SIMCA of the eggs and spectra collected on the abdomen is underway and will be completed this fall. A blind study was conducted to test whether a model built on spectra collected during the atresia study in California could be used to predict atresia in Idaho fish. Analyses are currently underway.

SUPPORT:

YEAR	WRAC-USDA Funding	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Federal	Other	Total	
2008	100,001	0	18,500	79,986	0	98,486	198,487
2009	100,001	0	18,500	79,226	0	102,726	202,727
2010	100,001	0	19,000	71,023	0	90,023	190,024
2011	100,000	0	19,000	70,156	0	89,156	189,156
TOTAL	400,003	0	75,000	300,391	0	380,391	780,394

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Publications:

Lu, X., Webb, M., Talbott, M., Van Eenennaam, J., Doroshov, S., and B. Rasco. 2011. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) utilizing fourier transform infrared spectroscopy (FT-IR). *Aquaculture* 315: 298-305.

Lu, X., Webb, M., Talbott, M., Van Eenennaam, J., Palumbo, A., Linares-Casenave, J., Doroshov, S., Struffenegger, P., and B. Rasco. 2010. Distinguishing ovarian maturity of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: a potential tool for caviar production management. *Journal of Agricultural and Food Chemistry* 58: 4056-4064.

Lu, X. MS. 2009. Determining Sexual Maturity in White Sturgeon (*Acipenser transmontanus*) to Maximize Yield and Quality of Caviar. Masters Thesis, Washington State University.

Servid, S.A., and Cavinato, A.G. Non invasive characterization of maturity status to optimize caviar yield and quality in white sturgeon. 2010-2011. *Eastern Oregon Science Journal*, Vol. XXI, in print.

Servid, S.A., Talbott, M.J., Van Eenennaam, J.P., Doroshov, S.I., Struffenegger, P., Webb, M.A.H., and A.G. Cavinato. 2011. Rapid noninvasive characterization of ovarian follicular atresia in cultured white sturgeon (*Acipenser transmontanus*) by near infrared spectroscopy. *Aquaculture* 315:290-297.

Talbott, M., Van Eenennaam, J.P., Linares-Casenave, J., Doroshov, S.I., Guy, C.S., Struffenegger, P., and M.A.H. Webb. 2011. Plasma sex steroid concentrations during onset of ovarian atresia in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture* 315: 283-289.

Talbott, M.J. 2010. Determining Morphological and Biochemical Parameters Associated with Ovarian Follicular Atresia and Caviar Quality and Yield in Cultured White Sturgeon (*Acipenser transmontanus*). Masters Thesis, Montana State University.

Vixie, B. MS. 2011. Stress Response and Implications for White Sturgeon (*Acipenser transmontanus*) Cultivation: Qualitative Detection of Cortisol in Caviar and Roe by FT-IR and Differentiating Between Soft and Hard Roe by SEM. Masters Thesis, Washington State University.

Papers Presented:

Cavinato, A.G., Servid, S., Van Eenennaam, J., Doroshov, S., Lu, X., Rasco, B., Talbott, M., Sealy, W. and M. Webb. Non-invasive means to determine maturity in white sturgeon females. 2011. 242nd American Chemical Society National Meeting, Denver, CO, August 2011. (Invited presentation).

Cavinato, A.G. Adapting research to meet regional needs. 2009. 237th American Chemical Society National Meeting, Salt Lake City, UT, March 2009. (Invited presentation).

Lu, X., Webb, M., Doroshov, S., and Rasco, B. Distinguishing vitellogenin, sex steroids and lipids in blood plasma and atresia of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: a potential tool for caviar production. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, Illinois. July 17-21, 2010.

Lu, X., Callahan, G., and Rasco, B. Infrared spectroscopy, a novel and less invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females by predicting polarization index and follicle diameter. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, Illinois. July 17-21, 2010.

Lu, X., Wang, J., and Rasco, B. Rapid determination of maturity in cultured white sturgeon (*Acipenser transmontanus*) females using plasma steroid concentrations by Fourier transform infrared spectroscopy and partial least squares model. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, Illinois. July 17-21, 2010.

Lu, X., Webb, M., Doroshov, S., and Rasco, B. Distinguishing vitellogenin, sex steroids and lipids in blood plasma and atresia of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: a potential tool for caviar production. Pacific Fisheries Technologists 61th Annual Meeting, poster presentation. Seattle, Washington. Feb 21-24, 2010.

Lu X., Callahan G., and Rasco B. Infrared spectroscopy, a novel and less invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females by predicting polarization index and follicle diameter. Pacific Fisheries Technologists 61th Annual Meeting, poster presentation. Seattle, Washington. Feb 21-24, 2010.

Lu, X. and Rasco, B. The use of Fourier transform infrared spectroscopy to predict maturity stages and caviar quality in white sturgeon female. Institute of Food Technologists 2009 Annual Meeting, oral presentation. Anaheim, California. Jun 6-9, 2009.

Lu, X. and Rasco, B. The use of Fourier transform infrared spectroscopy to predict ovarian atresia and caviar quality in white sturgeon (*Acipenser transmontanus*). Aquaculture America 2009 Meeting, oral presentation. Seattle, Washington. Feb 15-18, 2009.

Lu, X. and Rasco, B. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier transform infrared spectroscopy and multivariate analysis. Pacific Fisheries Technologists 60th Annual Meeting, oral presentation. Portland, Oregon. Feb 22-25, 2009.

Sealey, W., Webb, M.A.H., Doroshov, S.I., Cavinato, A., Rasco, B. Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar. Idaho Aquaculture Association. Twin Falls, Idaho, 12 June 2010.

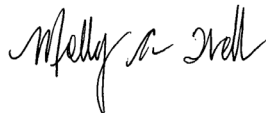
Servid, S.A., Cavinato, A.G. "Novel non-invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females" 241th American Chemical Society National Meeting, Anaheim, CA, March 2011.

Servid, S.A., Cavinato, A.G. "Rapid and noninvasive characterization of ovarian follicular atresia in cultured white sturgeon by near infrared spectroscopy", 239th American Chemical Society National Meeting, San Francisco, CA, March 2010.

Talbott, M.J., Van Eenennaam, J.P., Linares-Casenave, J., Doroshov, S.I., Guy, C.S., and Webb, M.A.H.. 2009. Investigating non-invasive methods to predict follicular atresia and spawning readiness in white sturgeon. Oral presentation at the AFS 139th Annual Meeting, August 30-September 3, 2009, Nashville, Tennessee.

Van Eenennaam, J., Linares-Casenave, J., Talbot, M., Webb, M., Struffenegger, Conte, F., and Doroshov, S. 2010. The relationship between white sturgeon caviar yield and stage of maturity. Oral presentation at Aquaculture 2010, March 1-5, 2010, San Diego, California.

SUBMITTED BY:



PI Name

Date

APPROVED:

Project Monitor (Technical Advisor)

Date

PROJECT TERMINATION REPORT

Part II: Detail

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PROJECT OBJECTIVES:

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- 1) Determine how currently utilized morphological characteristics (oocyte PI, ovarian follicle size, gonadosomatic index, age and live weight) correlate with caviar quality and yield (years 1-2);
- 2) Determine how plasma sex steroid, total calcium, and plasma protein levels and crude chemical composition of eggs change with maturity (years 1-2);
- 3) Evaluate short wavelength near infrared spectroscopy (SWNIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish (years 1-2);
- 4) Evaluate Fourier transform infrared spectroscopy (FTIR) as a method to predict fish maturity from spectral measurements of blood and roe (years 1-2);
- 5) Using SWNIR and plasma steroids, determine whether it is possible to detect the early signs of follicular atresia to avoid sacrificing fish with inferior quality roe and use them during the next production cycle (year 1);
- 6) Conduct training and outreach programs at field sites in Idaho and California (years 3-4).

TECHNICAL SUMMARY AND ANALYSIS:

During the first year of the project, biological samples from two groups of sturgeon held at Sterling Caviar, LLC were collected to address Objectives 1 through 5. The first group of sturgeon was sampled to specifically address whether it is possible to detect early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe (Objective 5; referred to as the *Atresia Study*). The second group of females were harvested for caviar to address whether stage of maturity can be assessed less-invasively using the proposed tools (SW-NIR, FT-IR, plasma steroids measured by radioimmunoassay (RIA)) and to determine if there is a correlation between oocyte PI and caviar quality and yield (Objectives 1-4; herein referred to as the *2008 Caviar Study*). In 2009, females in both California and Idaho were segregated by stage of maturity in the fall by calculating oocyte PI, and these segregated groups were harvested at different times in the spring when each group would have fully grown eggs to determine whether our alternative sampling techniques (FTIR, SWNIR, and plasma steroids) could accurately determine oocyte PI. This study is herein referred to as the *2009 Caviar Study*. In 2010, females in both California and Idaho were segregated into two groups (low oocyte PI and high oocyte PI) using FT-IR in the fall. The FT-IR currently appears to have the highest predictive power for determining stage of maturity. Fish were harvested in the winter and spring accordingly and comparisons were made between the actual oocyte PI and the calculated PI predicted from FT-IR. This study is herein referred to as the *2010 Caviar Study*. Also, in 2010 we sampled females at caviar harvest and at spawning for an additional SWNIR study to determine the homogeneity of the gonad (referred to as the *Homogeneity Study*). In 2011, we repeated the 2010 study design except that the FT-IR analysis was not completed at the time of harvest and was compared post-facto. An additional objective was to evaluate the effect of fish pre-selection by the oocyte PI in December on the caviar yield in February and May. This report summarizes the data collected by objective.

Objective 1. Determine how currently utilized morphological characteristics (oocyte PI, ovarian follicle size, gonadosomatic index, age and live weight) correlate with caviar quality and yield. The caviar yield of the low PI fish harvested in February 2011 in California was not different from the control group ($P > 0.05$, Student test), and all other parameters did not differ between the two groups (Table 1). The high PI fish harvested in May were significantly heavier (body weight; BW) and had significantly heavier ovaries (ovary weight; OW), compared to the control group ($P < 0.05$, Student test). However, there was no difference in gonadosomatic index (GSI), caviar yield, and all other parameters (Table 1). This is similar to the previous observations during the harvest in California in 2010 (Table 2). For example, in both 2010 and 2011, the low PI fish (i.e. more mature in December) harvested in February appeared to have higher PI and smaller eggs at harvest, compared with the high PI fish harvested in May (Tables 1 and 2).

All harvest data from 2008 through 2011 in California, separated by age (7 and 8 year old), were compared (Table 3). The 8 year old females were almost 5 kg heavier but had similar condition factor and PI compared to the 7 year old fish. The two age groups did differ by the egg size, GSI, ovary and caviar weight, and caviar yield, as a percent of fish BW and OW (Table 3). Thus 8 year old females were more productive compared to 7 year old fish, and one factor that could attribute to this increase in productivity is whether or not some of the fish were on their 2nd ovarian cycle (were mature or atretic at age 6 or 7 and were mature again at age 8).

Regression analyses of each age class did reveal some significant relationships of the caviar yield with BW, egg PI and egg diameter for age 7 fish (Figures 1-3), but the R^2 values were less than 0.01. There was a significant ($P < 0.0001$) relationship between GSI and caviar yield for both age groups, but even this regression had an R^2 value of only 0.46 for 7 year old and 0.52 for 8 year old fish (Figure 4).

Unexpectedly, this analysis revealed that fish maturity (stage of GV migration) was not a main factor affecting roe yield in the Sterling Caviar, LLC population. The lack of a strong relationship between the oocyte PI and caviar yield was due to the highly variable caviar yield caused by extensive ovarian adiposity, particularly in larger fish. Adipose tissue develops before sex differentiation in the medial region of the sturgeon ovary and proliferates into the ovarian stroma towards the ovigerous lamellae. We observed two regions of fatty tissue in the ovaries of sampled fish: clusters of fat surrounding eggs in the germinal part of the ovary and the more significant and discrete portions of fat along the sides of ovaries. The subjective method for evaluation of ovarian adiposity does not allow direct measurement of the effect of fat content on caviar yield, but we can discern its effect by examining the percent of the ovaries weight that ends up as tinned caviar. The remainder of the ovary after caviar screening contains the fatty adipose tissue and the germinal tissue with several generations of younger follicles. Three categories of ovarian adiposity, high fat (HF), medium fat (MF) and low fat (LF), corresponded well to the caviar yield per body weight (CY%BW) and caviar production per fish (CW).

Data for the three categories are shown in Table 4. Generally, processed caviar yield was less than 50% of the ovarian weight for the HF category, within 50-65% for the MF category, and more than 65% for the LF category. Whether there was a relationship between PI and caviar yield (% BW) within each category of ovarian adiposity was also examined, and there was a relationship in the medium fat group ($P = 0.01$) but the R^2 was only 0.0387 (Figure 5).

Although the three fattiness groups did not differ by GSI ($P > 0.05$, one-way ANOVA and Tukey test), the means for caviar weight and caviar yield, per both BW and OW, were significantly different (Table 4). The HF group had the highest mean body weight, smallest eggs, and the lowest caviar yields per fish (CW), body weight and ovarian weight (CY-BW, and CY-OW). The best producers of roe were the females in the LF group: while they had significantly lower mean body weight and condition factor, they produced the highest yield of caviar CY%BW and CY% OW (Table 4).

The caviar harvest data from Idaho included fewer fish ($n = 81$), but the comparisons with California revealed differences between the stocks of two states. The Idaho fish were similar in BW but much older, had a lower condition factor (0.73 versus 1.03), smaller ovaries and a lower GSI compared to California fish (Table 5). Despite the lower GSI in Idaho stock, the mean caviar weight and the caviar yield per body weight were similar in both states because the Idaho fish ovaries were much leaner. The mean yield of caviar as a percent of ovarian weight was 71% in Idaho stock compared to 58% in California stock (Table 5). The Idaho fish were much older (15-21 years) and produced larger eggs (3.4 mm diameter compared to 3.1 mm in California fish; (Table 5). Other factors to consider are generally cooler water in Idaho and the fact that fish were kept in dirt canals for several years prior to this study having access to a single demand feeder and various species of fish living in the canal. This restricted feeding and more natural environment could also be a factor in the leaner ovaries and lower condition factor. It should be noted that the Idaho producers have observed more fatty ovaries in the younger year-class fish reared in the concrete raceways and fed prepared diets more regularly.

Objective 2. Determine how plasma sex steroid, total calcium, and plasma protein levels and crude chemical composition of eggs change with maturity. Blood plasma calcium and total protein was measured repeatedly in fish in 2007-2008. Plasma calcium and total protein did not differ among stages of maturity in these fish. These parameters were not measured in the following years of the study. Blood plasma T and E2 and crude chemical composition of eggs did not change with increasing maturity (measured by decreasing oocyte PI) at the time of caviar harvest. Blood plasma T and E2 were significantly lower in fish with atretic ovaries compared to fish with normal ovaries.

Objective 3. Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish. Spectra acquired during the California (CA) and Idaho (ID) samplings conducted in 2008 (CA 08) and 2009 (CA 09 and ID 09) were independently analyzed as well as merged to generate a larger dataset that comprises fish from both facilities. Spectra were acquired using a ProSpectra spectrophotometer equipped with a fiber optic probe (D-Squared Development Inc., La Grande, OR). The probe contains four illuminating tungsten lamps and one central collection fiber-bundle. Spectra were collected in diffuse reflectance mode over a wavelength range from 600 to 1100 nm. Before spectra were acquired on the sample, dark and reference spectra were obtained. For studies on live anesthetized fish, the probe was placed on the abdominal area and moved around in correspondence to the placement of the ovary. Five independent spectra were collected on each fish during each sampling. For studies on eggs, during each sampling approximately 30 cm³ of eggs were surgically removed from each female and transferred in a teflon holder for spectral acquisition. Five spectra were collected on each egg batch. To ensure that different eggs would be sampled after each spectrum, eggs were stirred. All spectra were the average of 50 scans with 200 ms exposure time for each sample. A total of 1725 spectra were collected on eggs and 1879 spectra were collected on the abdominal area of anesthetized fish.

Data analysis for SW-NIR was performed using Delight Version 3.2.1 software (D-Squared Development Inc., La Grande, OR) and PLS Toolbox (Eigenvector Research, Wenatchee, WA). The datasets from CA 08 and 09 as well as ID 09 were analyzed independently and as a combined file using Partial Least Squares Discriminant Analysis (PLS-DA) and Soft Independent Modeling of Class Analogy (SIMCA). In PLS-DA, a partial least square regression calculates a response variable (either 0 or 1) which expresses membership to a given class. In the SIMCA approach, multiple PCA models are generated for each class in the calibration set. Unknown samples are then compared to each class model and assigned to classes based on their analogy to the calibration samples. This report contains only SIMCA results while the PLS-DA analysis is ongoing and will be reported in the future. When merging spectra collected on California sturgeon with spectra collected on Idaho sturgeon, differences originally attributed to physiological or chemical variations in the eggs were actually an artifact due to the side of the reference block on which the reference spectrum was acquired. When all absorbance spectra were calculated based on the same reference, the large spectral differences were no longer present. This led us to reanalyze all the data using the intensity spectra (not transformed based on the reference) and absorbance spectra (transformed on one reference) in order to determine whether instrumental artifacts would affect the results of the analysis, and the best classification (i.e. using either intensity or absorbance spectra) is reported.

SIMCA Analysis of Egg Scans (CA 2008 Egg Scans). SIMCA analyses were conducted on intensity and absorbance spectra collected on eggs. Table 6 shows SIMCA results from analyses done on the intensity values from CA 08 spectra. Spectra were classified as either high

(>0.13) or low (<0.13) PI. The SIMCA model was built from PCA models based on high and low PI values. Using this method several different SIMCA models with different numbers of principal components and different thresholds were constructed to optimize prediction. Cross validation was performed for each analysis by removing all spectra collected on a given fish, building the model without these spectra, and then using the model to predict the class of the removed spectra. Based on input from the industry, when over 60% of spectra collected on a given fish were predicted as having high or low PI, the fish was classified in that category. These fish were then tallied to give the results seen in the following tables.

Results from analysis on the CA 08 intensity spectra collected directly on the eggs revealed 64% of the fish with high PI values were predicted in the high category, and 68% of the fish with low PI values were predicted in the low PI category. Conversely, 32% of the fish with low PI values were predicted in the high category, and 36% of the fish with high PI values were predicted in the low category.

SIMCA Analysis of Egg Scans (CA 09 Egg Scans). Results for a similar analysis of the CA 09 intensity spectra collected directly on the eggs can be seen in Table 7. Sixty-four percent of the fish with high PI values were predicted in the high category, and 83% of the fish with low PI values were predicted in the low PI category. Conversely, 17% of the fish with low PI values were predicted in the high category and 36% of the fish with high PI values were predicted in the low category.

SIMCA Analysis of Egg Scans (Combined CA 08 and 09 Egg Scans). Results for a similar analysis of the combined CA 08 and 09 intensity spectra collected directly on the eggs can be seen in Table 8. Sixty-seven percent of the fish with high PI values were predicted in the high category, and 71% of the fish with low PI values were predicted in the low PI category. Conversely, 29% of the fish with low PI values were predicted in the high category and 33% of the fish with high PI values were predicted in the low category.

Next, a SIMCA model was built from one year in an attempt to predict the PI values of fish from another year. Best results were achieved when predicting the CA 08 fish based on the CA 09 model with 86% of all fish with high PI, and 54% of all fish with low PI correctly classified (Table 9). It is unclear at this time whether the transferability of the model is precluded by differences in the eggs from one year to the next or by instrumental irreproducibility.

SIMCA Analysis of Egg Scans (ID 09 Egg Scans). Results for a similar analysis of the ID 09 intensity spectra collected directly on the eggs can be seen in Table 10. Sixty percent of the fish with high PI values were predicted in the high category, and 75% of the fish with low PI values were predicted in the low PI category. Conversely, 25% of the fish with low PI values were predicted in the high category, and 40% of the fish with high PI values were predicted in the low category.

SIMCA Analysis of Abdominal Scans (CA 08 Abdominal Scans). Spectra (intensity and absorbance) collected on the abdominal area of the fish were analyzed by SIMCA. Table 11 summarizes SIMCA results for CA 08 dataset. Sixty four percent of the fish with high PI values were predicted in the high category, and 64% of the fish with low PI values were predicted in the low PI category. Conversely, 36% of the fish with low PI values were predicted in the high category and 36% of the fish with high PI values were predicted in the low category.

SIMCA Analysis of Abdominal Scans (CA 09 Abdominal Scans). Results for the analysis of the CA 09 intensity spectra collected on the abdominal area can be seen in Table 12. A total of 62.5% of the fish with high PI values were predicted in the high category, and 77% of the fish

with low PI values were predicted in the low PI category. Conversely, 23% of the fish with low PI values were predicted in the high category and 37.5% of the fish with high PI values were predicted in the low category.

SIMCA Analysis of Abdominal Scans (Combined CA 2008 and 2009 Abdominal Scans).

Results for the analysis of the CA 08 and 09 intensity spectra collected on the abdominal area can be seen in Table 13. Eighty four percent of the fish with high PI values were predicted in the high category, and 62% of the fish with low PI values were predicted in the low PI category. Conversely, 38% of the fish with low PI values were predicted in the high category, and 16% of the fish with high PI values were predicted in the low category.

SIMCA models were also built from one year (2008 or 2009) in an attempt to predict the PI value of the other year. However results from these models are poor and lack the ability to predict from one year to the next.

SIMCA Analysis of Abdominal Scans (ID 2009 Abdominal Scans). Results from analysis on the ID 09 intensity spectra (Table 14) show that 66% of the fish with high PI values were predicted in the high category, and 82% of the fish with low PI values were predicted in the low PI category. Conversely, 18% of the fish with low PI values were predicted in the high category, and 34% of the fish with high PI values were predicted in the low category.

Comparison of Results Obtained on Eggs vs Abdominal Scans. Results for CA 08 indicate that the ability to predict the PI value is comparable whether spectra are collected on eggs or through the abdomen. For the CA 09 dataset, results for spectra collected on eggs are slightly better than abdominal scans. However, for the combined CA 08 and 09 data and the ID 09 data, analyses of spectra collected on the abdomen showed better results than similar analyses conducted on egg spectra.

Under the current experimental setup, intensity spectra provided better results than absorbance spectra. This is most likely due to irreproducibility in the acquisition of the reference spectrum. Improved results were achieved when analyzing the combined CA 08 and 09 files both for egg and abdomen spectra compared to each individual year. For spectra collected on eggs, PI predictions of CA 08 using the CA 09 model was feasible, while predictions of CA 09 from the CA 08 model were poor.

Homogeneity Study. For the homogeneity study, five fish were scanned at three different locations on each ovarian lobe. These scans were then analyzed to determine if there were differences in the spectra depending on the location of the scan. When viewing all five fish in a score plot, absorbance spectra cluster by fish. These score plots show that there is no difference between anterior, middle, or posterior scans on a given fish. A similar analysis was conducted on intensity spectra with the same results. A SIMCA analysis with cross validation of the absorbance and intensity spectra was also done. These SIMCA models were built from PCA models based on spectra collected on the anterior, middle and posterior ovary locations. Models built on either absorbance or intensity lack the ability to differentiate between the three positions on the ovary.

Objective 4. Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe. We applied a rapid and less invasive vibrational spectroscopy method to collect spectral features of sturgeon female plasma and quantify and predict the actual PI values with the aid reference values and partial least squares regression chemometric models. Two types of partial least squares regression (PLSR) models were established and the advantages/disadvantages of both models were

compared. The Plan A PLSR model was constructed from spectra from fish plasma from the years of 2007, 2008 and 2009 randomly selected with a leave-one-out cross validation to challenge the rigorousness of the calibrated model. The Plan B PLSR model was constructed similarly to the Plan A model except that 5 fish from the 2010 and 2011 data sets were randomly selected and used for model establishment and leave-one-out cross validation. The PI values of all fish in 2010 and 2011 were predicted based upon the Plan A model, while the remaining fish not used in model development from 2010 and 2011 were predicted based upon the Plan B model. Strictly speaking, only the Plan A PLSR model is “truly” predicting the PI values of the samples in the year of 2010 and 2011, and the Plan B PLSR model is only cross validating additional samples from 2010 and 2011. This is because Plan B incorporates spectral features of the fish sampled in 2010 and 2011 (n=5 each) into the calibration model (referred to as model maintenance), whereas Plan (A) is based upon fish from prior years only. FT-IR can successfully predict oocyte PI with approximately 90% accuracy. The prediction of oocyte PI in the fall using FT-IR can be seen in Tables 15 and 16. Segregating fish into groups of oocyte PI in the fall for caviar harvest in the spring did in general standardize the stage of maturity at harvest. Model maintenance will require a total of 10 fish added the model per year.

Objective 5. Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian atresia. Plasma sex steroid analysis, SW-NIR of abdomen and ovarian follicles, and FT-IR of plasma appear to be reliable predictors of atresia with variable success rates depending on the tool chosen. The use of plasma sex steroids analyzed by radioimmunoassay (RIA) resulted in 95% of the females with normal ovarian follicles (non-atretic) and 93% of the atretic females correctly classified (Talbot et al. 2010). Logistic regression equations were developed to predict the probability that a female white sturgeon has normal ovaries given a specific concentration of T or E2. In California, females with a circulating concentration of T less than 35.1 ng/ml should not be harvested as they have a 95% probability of having initiated ovarian atresia. The SW-NIR spectroscopy resulted in a model that correctly predicted 115 body scans out of 120 body scans from atretic fish for a correct classification of 96%. The validation of this model using a leave-one-out technique showed that 55% of the body scans were correctly classified as atretic (Servid et al. 2010). Similarly, the classification rates of atretic scans on ovarian follicles were high for the model development (96%), and the validation of the model showed that 58% of the atretic scans were correctly classified. The FT-IR was capable of identifying atretic females with 70% accuracy (Lu et al. 2010).

Objective 6. Conduct training and outreach programs at field sites in Idaho and California. The studies were conducted at aquaculture facilities in both Idaho and California with extensive support and participation of our industry partners. Industry partners were involved in field collection of all biological samples for laboratory analysis and on-site SW-NIR spectroscopy. The results of the project revealed that FT-IR can be used to successfully predict oocyte PI, and analysis of plasma sex steroids was the most reliable predictor of follicular atresia. These laboratory techniques are available for the aquaculture industry; however, given that oocyte PI did not correlate well with caviar yield, none of the current techniques were pursued for instrument-to-fish analysis. An outreach publication to describe the different methods to determine follicular atresia in female sturgeon is currently being written. To improve quality and yield of caviar in farmed white sturgeon, it is essential to correctly assess the stage of

ovarian maturity and avoid harvesting females with atretic ovarian follicles.

IMPACTS:

Relevance: Currently, the only means to assess ripeness of white sturgeon females and properly time caviar harvest is measurement of oocyte polarization index (PI) that requires surgical biopsy. This technique is accurate but invasive and stressful to fish, time consuming, and not an effective tool for handling a large number of female fish. Because the large caviar farms that harvest thousands of females cannot determine PI in every fish, this can result in decreasing caviar yield and quality due to follicular atresia (phagocytosis of ovarian eggs). Even the early stage of atresia causes a reduction in the firmness, flavor, and shelf life of caviar, and sometimes the complete loss of the product. Specifically, the sturgeon industry was interested in new methods to predict sturgeon maturity (PI) as an alternative to the surgical biopsy, and be able to identify females in the early stages of atresia.

Response: To achieve our goal, we needed a better understanding of the biochemical and physiological changes that occur during gonadal maturation and early atresia, and how these changes correlate with roe quality. The methods should be non-invasive, minimally stressful to fish, and quick. Ideally, they would allow female fish to be sorted in the fall during late vitellogenesis into groups based upon the degree of ripeness, followed by a quick examination of groups for early atresia, just prior to selection for caviar harvest. In this project, we examined the ability of non-invasive SW-NIR, FT-IR, and immunochemical assays to identify early atresia in females and replace the surgical biopsy procedure currently used to determine ripeness in sturgeon.

Results: The radioimmunoassay of plasma steroids (Talbot et al. 2011), spectral analysis of plasma by FT-IR (Lu et al. 2011), and abdominal and follicular scans by SW-NIR (Servid et al. 2011) were suitable for detecting early atresia with 55-93% accuracy depending on the tool, and the FT-IR was determined to be the most accurate in determining maturational stage (oocyte PI) and may be used in lieu of the surgical biopsy technique (90% accuracy). The analysis of caviar harvest found that the stage of maturity manifested by the oocyte PI does not necessarily correlate with higher caviar yields. However, using oocyte PI even though weakly correlated to caviar yield to determine the time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain. Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster growing fish. It should be noted that this analysis does not invalidate the importance of detecting maturity stage by non-invasive methods. Using the same percent of salt throughout the processing season has resulted in some egg batches becoming too salty or not salty enough. The variable saltiness is hypothesized to be associated with variation in egg maturity (PI) between females. If females could be harvested at more homogenous PI's then the standardized percent of salt used would result in less variation in quality of the caviar in terms of saltiness.

Collaborators: This collaborative project included researchers from Eastern Oregon University (EOU), Washington State University (WSU), Montana State University (MSU), University of California, Davis (UCD), Bozeman Fish Technology Center (BFTC), University of Idaho Extension (UIE), and Hagerman Fish Culture Experiment Station (HFCES), and industry collaboration in California (Sterling Caviar, LLC) and Idaho (Fish Breeders of Idaho and Blind Canyon Aqua Ranch).

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

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SUBMITTED BY:



PI Name

Date

APPROVED:

Project Monitor (Technical Advisor)

Date

APPENDIX I: Tables and Figures:

Abbreviations in Tables and Figures

BW - body weight;
 K- Fulton condition factor;
 PI- polarization index of oocyte;
 ED - egg diameter;
 OW - ovarian weight (both ovaries);
 GSI - gonadosomatic index;
 CW- caviar weight (eggs separated from stroma and packed in tins);
 CY % BW – caviar yield normalized by body weight;
 CY % OW – caviar yield normalized by ovarian weight

Table 1. Caviar production metrics (means ± SD) of California sturgeon stocks (Sterling Caviar LLC) in spring 2011. Mixed age (7 & 8 yr) stocks were preselected as Low and High PI Groups in December 2010 and processed during February and May 2011, respectively. Controls were randomly sampled fish with unknown PI from cohorts in the same tank. Asterisks indicate significant difference between means in preselected groups and controls (P<0.05, Student t-test).

¹ One fish not included in the analyses, because of a data input error.

Processed 2/9&10/11	Low PI (n=29¹)	Control (n=30)
BW, kg	34.5 ± 8.5	31.9 ± 5.1
K	0.94 ± 0.10	0.96 ± 0.11
PI	0.143 ± 0.04	0.160 ± 0.04
ED, mm	3.08 ± 0.14	3.02 ± 0.12
OW, kg	4.0 ± 1.4	3.6 ± 0.7
GSI, %	11.3 ± 2.0	11.4 ± 1.6
CW, kg	2.4 ± 0.9	2.1 ± 0.5
CY % of BW	6.8 ± 1.8	6.7 ± 1.5
CY % of OW	59.7 ± 11.4	58.3 ± 12.2
Processed 5/11&13/11	High PI (n=30)	Control (n=30)
BW, kg	39.5 ± 8.7	31.8 ± 6.9 *
K	0.99 ± 0.11	0.98 ± 0.12
PI	0.116 ± 0.03	0.107 ± 0.04
ED, mm	3.16 ± 0.08	3.16 ± 0.10
OW, kg	5.1 ± 1.3	4.0 ± 1.2 *
GSI, %	12.9 ± 2.1	12.3 ± 2.0
CW, kg	2.8 ± 0.7	2.5 ± 0.8
CY % BW	7.3 ± 1.4	7.7 ± 1.7
CY % OW	57.1 ± 11.6	62.3 ± 10.0

Table 2. Caviar production metrics (means \pm SD) of California sturgeon stocks (Sterling Caviar LLC) in spring 2010. Seven year old stocks were preselected as Low and High PI Groups in December 2009 and processed during February and May 2010, respectively. Controls were randomly sampled fish with unknown PI from cohorts in the same tank. Asterisks indicate significant difference between means of low and high PI groups and their controls ($P < 0.05$, Student t-test).

Processed 2/3&10/10	Low PI (n=30)	Control (n=20)
BW, kg	36.3 \pm 7.7	37.8 \pm 5.1
K	1.02 \pm 0.11	0.99 \pm 0.07
PI	0.204 \pm 0.05	0.188 \pm 0.04
ED, mm	3.03 \pm 0.12	3.01 \pm 0.07
OW, kg	4.2 \pm 1.4	4.0 \pm 1.3
GSI, %	11.4 \pm 2.0	10.5 \pm 2.7
CW, kg	2.5 \pm 0.7	2.3 \pm 0.8
CY % of BW	6.8 \pm 1.8	5.9 \pm 1.9
CY % of OW	59.7 \pm 13.0	57.7 \pm 13.9
Processed 5/11&13/10	High PI (n=30)	Control (n=20)
BW, kg	41.8 \pm 6.9	43.1 \pm 6.5
K	1.07 \pm 0.10	1.12 \pm 0.08
PI	0.122 \pm 0.04	0.126 \pm 0.04
ED, mm	3.07 \pm 0.12	3.15 \pm 0.09 *
OW, kg	4.8 \pm 1.0	5.1 \pm 1.5
GSI, %	11.6 \pm 1.9	11.8 \pm 2.3
CW, kg	2.4 \pm 0.8	3.0 \pm 1.0 *
CY % of BW	6.0 \pm 2.2	6.9 \pm 2.4
CY % of OW	51.0 \pm 15.2	57.7 \pm 14.0

Table 3. Caviar production metrics (means \pm SD) of 7 and 8 year old sturgeon females (Sterling Caviar LLC) for 2008 through 2011 caviar processing seasons (pooled samples). Asterisks indicate significant difference between means of two age groups ($P < 0.05$, Student t-test).

Age of stocks	Seven year (n=286)	Eight year (n=113)
BW, kg	35.4 \pm 7.4	40.3 \pm 7.5 *
K	1.03 \pm 0.11	1.02 \pm 0.12
PI	0.131 \pm 0.06	0.133 \pm 0.05
ED, mm	3.08 \pm 0.11	3.17 \pm 0.14 *
OW, kg	4.4 \pm 1.3	5.6 \pm 1.7 *
GSI, %	12.4 \pm 2.5	13.7 \pm 2.9 *
CW, kg	2.5 \pm 0.8	3.4 \pm 1.1 *
CY, % of BW	7.1 \pm 2.1	8.4 \pm 2.2 *
CY, % of OW	57.1 \pm 12.0	61.1 \pm 10.9 *

Table 4. Caviar production metrics (means \pm SD) for the fish (Sterling Caviar, LLC) with high, medium and low fat ovaries. Records were collected during four years (2008-2011) of caviar processing. Different letters indicate significant differences ($P < 0.05$) between the means of three groups (one-way ANOVA, Tukey test)

	Females with high fat ovaries (n=100)	Females with medium fat ovaries (n=167)	Females with low fat ovaries (n=132)
% of total #	25.1	41.8	33.1
BW, kg	40.0 \pm 7.8 <i>a</i>	36.1 \pm 7.7 <i>b</i>	35.2 \pm 6.9 <i>b</i>
K	1.05 \pm 0.10 <i>a</i>	1.04 \pm 0.12 <i>a</i>	0.99 \pm 0.11 <i>b</i>
PI	0.142 \pm 0.06 <i>a</i>	0.127 \pm 0.05 <i>b</i>	0.130 \pm 0.05 <i>ab</i>
ED, mm	3.06 \pm 0.14 <i>a</i>	3.12 \pm 0.11 <i>b</i>	3.13 \pm 0.13 <i>b</i>
OW, kg	5.1 \pm 1.7 <i>a</i>	4.7 \pm 1.5 <i>ab</i>	4.4 \pm 1.4 <i>b</i>
GSI, %	12.7 \pm 2.9 <i>a</i>	13.0 \pm 2.6 <i>a</i>	12.5 \pm 2.7 <i>a</i>
CW, kg	2.1 \pm 0.8 <i>a</i>	2.7 \pm 0.9 <i>b</i>	3.1 \pm 1.0 <i>c</i>
CY, % of BW	5.4 \pm 1.5 <i>a</i>	7.5 \pm 1.6 <i>b</i>	8.9 \pm 2.0 <i>c</i>
CY, % of OW	42.2 \pm 6.6 <i>a</i>	58.0 \pm 4.2 <i>b</i>	70.7 \pm 4.1 <i>c</i>

Table 5. Comparison of caviar production metrics means \pm SD in California and Idaho during a period of project (2008-2011, pooled data). Asterisks indicate significantly different means (Student t-test, $P < 0.05$).

State stocks	California Age = 7-8 year (n=399)	Idaho Age = 15-21 year (n=81)
BW, kg	36.8 \pm 7.7	37.7 \pm 9.3
K	1.03 \pm 0.11	0.73 \pm 0.08 *
PI	0.132 \pm 0.05	0.162 \pm 0.04 *
ED, mm	3.11 \pm 0.13	3.40 \pm 0.15 *
OW, kg	4.7 \pm 1.5	3.7 \pm 1.5 *
GSI, %	12.8 \pm 2.7	9.7 \pm 2.1 *
CW, kg	2.7 \pm 0.9	2.6 \pm 1.1
CY, % of BW	7.4 \pm 2.2	6.9 \pm 2.0
CY, % of OW	58.2 \pm 11.9	71.0 \pm 12.3 *

Table 6. California 2008 intensity spectra collected on eggs based on high PI (> 0.13), 4 principal components and thresholds of 1.5. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	64 (14)	32 (16)
	Low	36 (8)	68 (34)

Table 7. California 2009 intensity spectra collected on eggs based on low PI (<0.13), 6 principal components and thresholds of 3. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	64 (64)	17 (7)
	Low	36 (44)	83 (34)

Table 8. California 2008 and 2009 intensity spectra collected on eggs based on low PI (<0.13), 1-4th and 7th principal components and thresholds of 2.5. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	67 (94)	29 (29)
	Low	33 (47)	71 (71)

Table 9. SIMCA prediction results for California 2008 intensity spectra collected on eggs. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses. This model was built using California 2009 spectra with PCA models based on high and low PI values. The high model contained 5PCs and the low model had 2PCs.

CA 08 egg intensity spectra		Actual	
		High	Low
Predicted as	High	86 (19)	46 (22)
	Low	14 (3)	54 (26)

Table 10. Idaho 2009 intensity spectra collected on eggs based on high PI (>0.13), 7 principal components and thresholds of 2.5. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	60 (26)	25 (3)
	Low	40 (17)	75 (9)

Table 11. California 2008 intensity spectra collected on the abdominal area based on high PI (>0.13), 4 principal components and thresholds of 1.5. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	64 (14)	36 (25)
	Low	36 (8)	64 (44)

Table 12. California 2009 intensity spectra collected on the abdominal area based on high PI (>0.13), 5 principal components and thresholds of 2.25. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	62.5 (70)	23 (10)
	Low	37.5 (42)	77 (34)

Table 13. California 08 and 09 intensity spectra collected on the abdominal area based on low PI (<0.13), 4 principal components and thresholds of 2. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	84 (114)	38 (45)
	Low	16 (22)	62 (74)

Table 14. Idaho 2009 intensity spectra collected on the abdominal area based on high PI (>0.13), 5 principal components and thresholds of 3. The table shows percentages of fish that were classified in each category. The actual number of fish in each category is shown in parentheses.

		Actual	
		High	Low
Predicted as	High	66 (33)	18 (2)
	Low	34 (17)	82 (9)

Table 15. Idaho oocyte polarization index (PI) prediction results for FT-IR of blood plasma using both Model A and Model B. Red and blue values indicate predicted oocyte PI values that are 2 standard deviations from the mean.

Fish ID (Idaho 2011)	Reference PI	Model A Prediction	Model B Prediction
94A2B	0.1417	0.1928±0.0158	0.1320±0.0163
95635	0.2386	0.2109±0.0234	0.2292±0.0241
44A6F	0.1840	0.1208±0.0329	0.1719±0.0113
1515F	0.1691	0.1836±0.0215	0.1732±0.0132
30F1C	0.1526	0.1939±0.0352	0.1684±0.2071
86476	0.1677	0.1593±0.0323	0.1493±0.0236
C793E	0.1333	0.1975±0.0332	0.1493±0.0182
60C34	0.1281	0.1648±0.0128	0.1397±0.0184
C2568	0.1294	0.1192±0.0112	0.1215±0.0145
03514	0.1169	0.1738±0.0276	0.1438±0.0164
3331D	0.2102	0.1759±0.0255	0.2041±0.0248
44B57	0.2259	0.2539±0.0156	0.2431±0.0262
56818	0.1622	0.2013±0.0268	0.1532±0.0198
07C7B	0.1860	0.1242±0.0142	0.1638±0.0215
D1F04	0.2574	0.2031±0.0193	0.2626±0.0216
52879	0.0922	0.1423±0.0192	0.0897±0.0131
86C2E	0.2358	0.1968±0.0185	0.2232±0.0158
30E28	0.2130	0.1767±0.0136	0.2023±0.0109
54C11	0.1485	0.1982±0.0198	0.1632±0.0155
D6D03	0.1870	0.1793±0.0319	0.1786±0.0227

Table 16. California oocyte polarization index (PI) prediction results for FT-IR of blood plasma using both Model A and Model B. Red and blue values indicate predicted oocyte PI values that are 2 standard deviations from the mean.

Fish ID (California 2011)	Reference PI	Plan A Prediction	Plan B Prediction
1	0.1436	0.1976±0.0183	0.1369±0.0133
2	0.1178	0.1395±0.0291	0.1269±0.0146
3	0.1302	0.1629±0.0135	0.1428±0.0172
4	0.1478	0.1932±0.0241	0.1834±0.0145
5	0.1151	0.1465±0.0234	0.1098±0.0130
6	0.1666	0.1538±0.0262	0.1449±0.0175
7	0.2213	0.2031±0.0248	0.2197±0.0169
8	0.1330	0.1525±0.0118	0.1292±0.0120
9	0.1800	0.2034±0.0314	0.1809±0.0301
10	0.2074	0.2415±0.0247	0.1809±0.0258
11	0.1227	0.1391±0.0106	0.1159±0.0194
12	0.2095	0.2209±0.0128	0.1987±0.0183
32	0.1026	0.1326±0.0192	0.0995±0.0117
33	0.0778	0.1031±0.0144	0.0801±0.0104
34	0.1872	0.2016±0.0295	0.1691±0.0210
44	0.1629	0.1703±0.0210	0.1752±0.0178
45	0.1014	0.1589±0.0311	0.1069±0.0156
61	0.0725	0.1031±0.0129	0.0693±0.0125
62	0.1568	0.1747±0.0216	0.1352±0.0173
63	0.0985	0.1425±0.0193	0.0921±0.0128
64	0.1244	0.1538±0.0149	0.1313±0.0151
65	0.1138	0.0913±0.0253	0.1229±0.0176
66	0.1231	0.1019±0.0138	0.1335±0.0187
67	0.0774	0.1231±0.0204	0.0798±0.0174
68	0.0708	0.0703±0.0111	0.0821±0.0157
69	0.1406	0.1576±0.0301	0.1629±0.0218
70	0.1406	0.1838±0.0253	0.1493±0.0215
71	0.1151	0.0813±0.0129	0.1093±0.0103
72	0.1047	0.1562±0.0136	0.1021±0.0192
73	0.1896	0.2130±0.0251	0.1769±0.0204
74	0.1374	0.1823±0.0164	0.1425±0.0143
75	0.1409	0.1908±0.0215	0.1467±0.0139

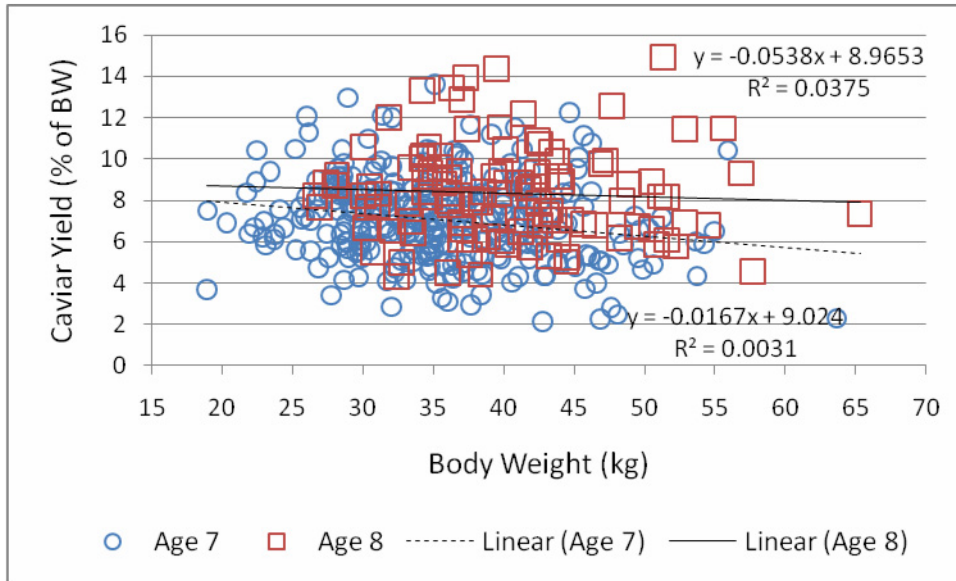


Figure 1. The relationship of body weight to caviar yield for age 7 (n=286) and age 8 (n=113) white sturgeon processed for caviar during 2008 through 2011. Slope for age 7 fish was significant (P = 0.001).

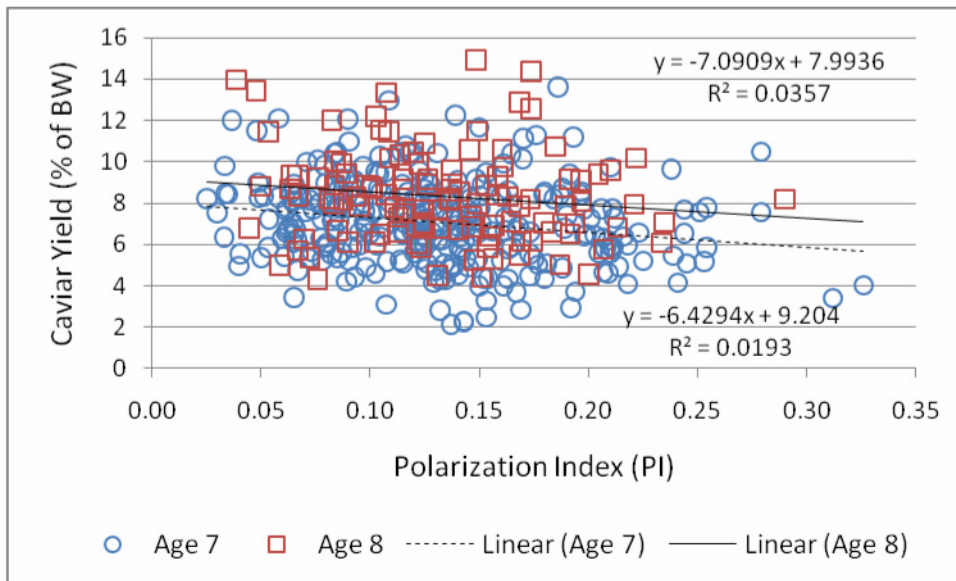


Figure 2. The relationship of polarization index (PI) to caviar yield for age 7 (n=286) and age 8 (n=113) white sturgeon processed for caviar during 2008 through 2011. Slope for age 7 fish was significant (P = 0.0008).

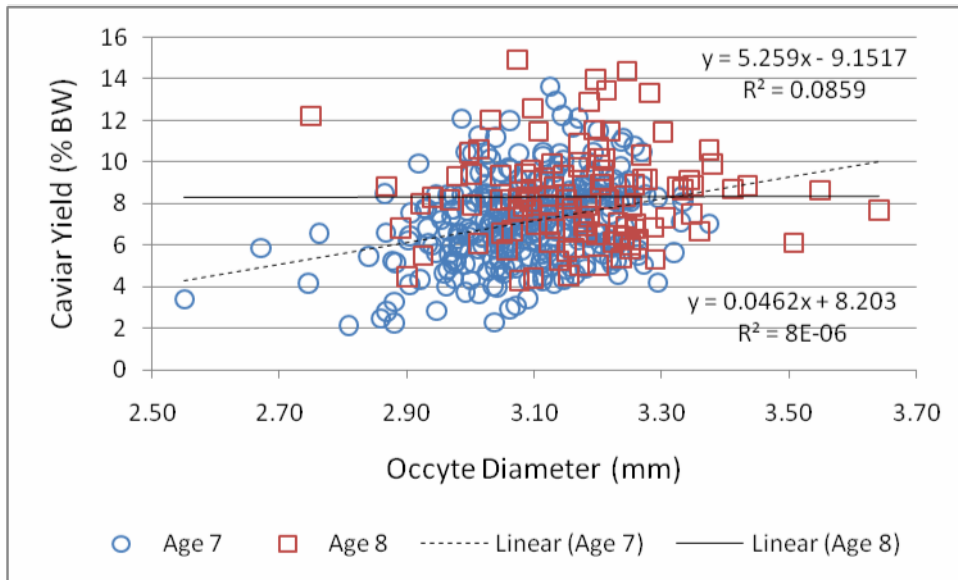


Figure 3. The relationship of oocyte diameter to caviar yield for age 7 (n=286) and age 8 (n=113) white sturgeon processed for caviar during 2008 through 2011. Slope for age 7 fish was significant ($P < 0.0001$).

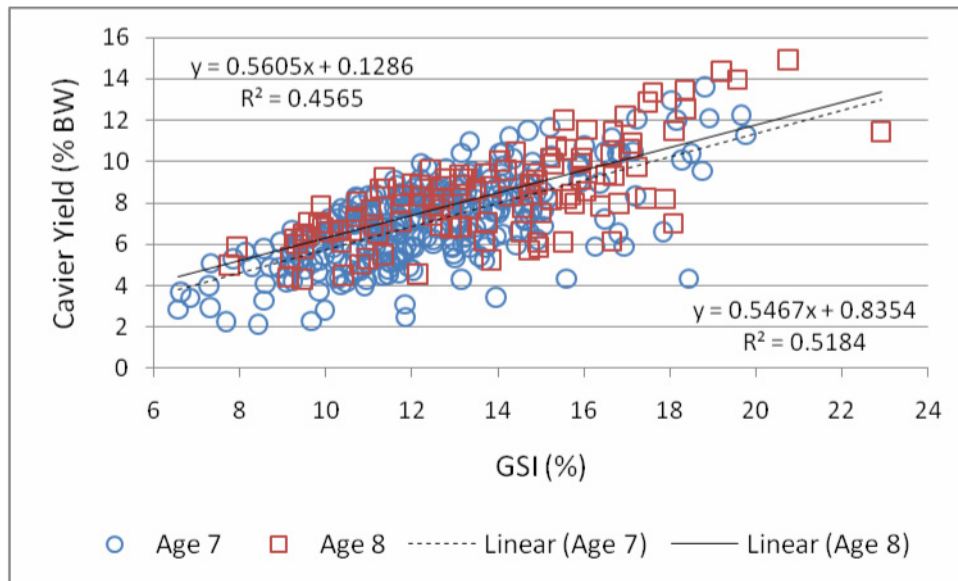


Figure 4. The relationship of gonadosomatic index to caviar yield for age 7 (n=286) and age 8 (n=113) white sturgeon processed for caviar during 2008 through 2011. Slopes for both age groups were significant ($P < 0.0001$).

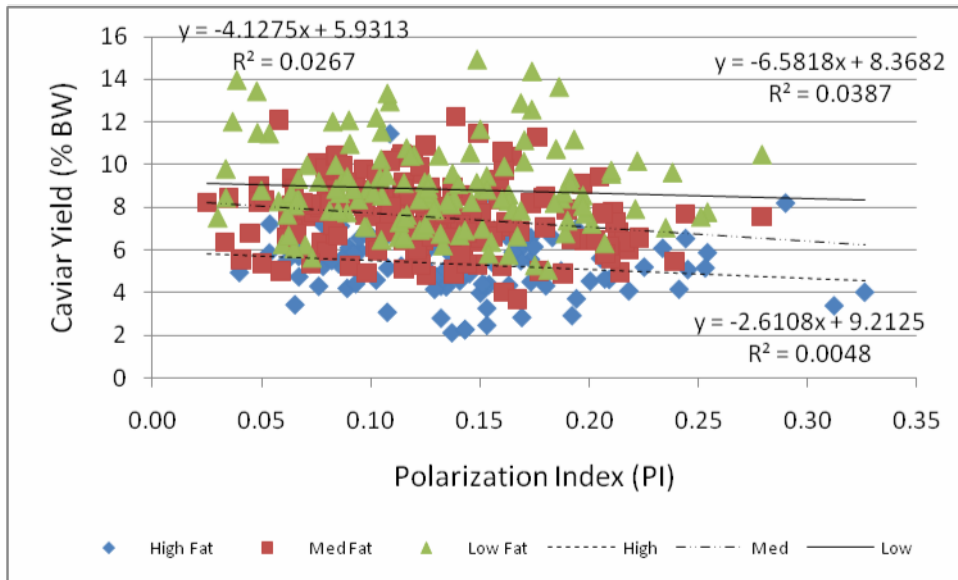


Figure 5. The relationship of polarization index (PI) to caviar yield for all California white sturgeon processed for caviar during 2008 through 2011, and assigned the category of low, medium or high ovarian fat. Slope for medium fat fish was significant ($P = 0.01$, $R^2 = 0.0387$).