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ARTICLE

Distinguishing Ovarian Maturity of Farmed White Sturgeon (*Acipenser transmontanus*) by Fourier Transform Infrared Spectroscopy: A Potential Tool for Caviar Production Management

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Fourier transform infrared spectroscopy (FT-IR, 4000−400 cm^{-1}) was applied to blood plasma of farmed white sturgeon (N = 40) to differentiate and predict the stages of ovarian maturity. Spectral features of sex steroids (~3000 cm^{-1}) and vitellogenin (~1080 cm^{-1}) were identified. Clear segregation of maturity stages (previtellogenesis, vitellogenesis, postvitellogenesis, and follicular atresia) was achieved using principal component analysis (PCA). Progression of oocyte development in the late phase of vitellogenesis was also monitored using PCA based on changes in plasma concentrations of sex steroid and lipid content. The observed oocyte polarization index (PI, a measure of nuclear migration) was correlated with changes in plasma sex steroid levels revealed by FT-IR PCA results. A partial least squares (PLS) model predicted PI values within the range 0.12−0.40 (R = 0.95, SEP = 2.18%) from differences in spectral features. These results suggest that FT-IR may be a good tool for assessing ovarian maturity in farmed sturgeon and will reduce the need for the invasive ovarian biopsy required for PI determination.

KEYWORDS: FT-IR; white sturgeon; plasma; sex steroids; vitellogenin; oocyte polarization index

INTRODUCTION

Global demand for caviar exceeds the supply from capture fisheries, and aquaculture is the primary source for meeting this demand (*1*). Wild sturgeons are overexploited and suffer declines in reproduction caused by anthropogenic changes to reproductive habitat (*2*). Many species or populations of chondrosteans worldwide are listed as threatened or endangered, and some may be close to extinction (*3*). In contrast to capture fisheries, sturgeon aquaculture offers a sustainable production of traditional food products, including caviar. Improving methods for cultivating sturgeon will hopefully take some pressure off of the wild fishery and improve the overall health of the wild stocks. Caviar production system is very complex, because of late sexual maturity and long ovarian cycles in sturgeon. It is based on management of multiage cohorts, with asynchronous maturation of individual fish within each age group (*4*, *5*). An important technical consideration in this production system is how to determine when to harvest the fish to obtain the maximum yield of fully grown oocytes. Currently the only way to accurately predict harvest time is an invasive abdominal biopsy for sampling oocytes and determining oocyte size and polarization index (PI) (*5*).

During the late phase of vitellogenesis, the nucleus (germinal vesicle, GV) of the fully grown oocyte migrates to the animal pole, and its position in the cytoplasm indicates spawning readiness (*6*). Oocyte PI is a ratio of the distance of the germinal vesicle from the animal pole to the oocyte animal−vegetal axis diameter, with a low PI value indicating maturational competence (*4*). Optimally, female fish are harvested for caviar when the oocyte PI is less than 0.10, a value which has been correlated with spawning success (*7*). In some aquaculture operations, farmers take PI determinations only on a relatively small number of female brood fish that are most likely to be sexually mature and ready for spawning the next spring. Utilization of this time-consuming method on large numbers of females for caviar production is impractical, and lack of alternative methods of determining stage of maturity could result in lower caviar yield and poor caviar quality.

Other assessments of fish maturity involve plasma sex steroids which can be good biomarkers for predicting female maturity (*8*). Several sex steroids are present in the sturgeon plasma matrix, including 17β-estradiol, testosterone, 11-ketotestosterone, progesterone, and 17α, 20β-dihydroxy-4-pregnen-3-one (*9*−*11*). The secretion of sex steroids is regulated in sturgeon by two pituitary

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gonadotropins, GTH I and GTH II (*12*), which are, respectively, the orthologues of FSH and LH of higher vertebrates (*13*). Plasma 17β-estradiol rises at the onset of vitellogenesis, reaches peak level in midvitellogenesis and decreases in late vitellogenesis. However, both 17β-estradiol and its precursor testosterone remain elevated until spawning at normal development (*12*, *14*−*16*). In contrast, plasma concentration of these steroids rapidly decreases in fish undergoing ovarian follicular atresia. The follicular atresia during late phase of vitellogenesis is common in farmed sturgeon, if fish are maintained under grow-out water temperatures (> 18 °C) during the winter and spring (*14*, *16*, *17*). Webb et al. (*8*) used plasma concentrations of 17β-estradiol, 11-ketotestosterone and testosterone, to classify the stage of gonadal maturity in wild white sturgeon. Currently, the most common methods for measuring sex steroid concentrations in fish plasma are radioimmunoassay (RIA) (*8*, *16*, *18*) and enzyme linked immunosorbent assay (ELISA) (*19*).

Plasma proteins may also serve as biomarkers for ovarian maturation in white sturgeon. For example, plasma concentrations of the egg yolk precursor protein vitellogenin, a high molecular weight glycolipophosphoprotein synthesized in the liver, may be used to identify vitellogenic females and monitor ovarian maturation (*20*). The expression of vitellogenin is dependent on the synthesis of sex steroids and is directly stimulated by 17β-estradiol. Vitellogenin concentrations rise steadily in plasma following the onset of estrogen synthesis in sturgeon (*15*, *20*). Plasma vitellogenin concentrations can increase over one million fold, to mg mL^{-1}, in mature female fish compared with immature females (*21*). However, vitellogenin concentrations decrease when visible signs of follicular atresia (marbled follicles) are present (*15*, *17*). The decrease in plasma vitellogenin is also observed in the late phase of ovarian development in white sturgeon (*20*). Radioimmunoassay (*21*), Western blotting (*22*), ELISA (*23*), alkali-labile phosphorus (ALP) (*24*), and total plasma calcium (*20*) are used for vitellogenin quantitation in blood plasma.

This study was conducted to determine whether Fourier transform infrared spectroscopy (FT-IR) could be used to monitor changes in one or more of these components and then to correlate these changes with biological predictors of ovarian maturity in sturgeon. FT-IR detects biochemical components of complex matrixes for qualitative identification and quantitative estimation based on deformation, bending and ring-vibrations of covalent bonds within the spectral range of 4000−400 cm^{-1}. Utilization of an attenuated total reflectance (ATR) cell with FT-IR spectroscopy allows detection of a wide variety of constituents, with little or no sample preparation. FT-IR spectroscopy combined with multivariate analysis has been widely utilized for identification of proteins, lipids and carbohydrate moieties (*25*−*29*). Recently, FT-IR was used in fish ecotoxicology to detect nonylphenol (*30*) and 17β-estradiol (*31*) in rainbow trout liver. To our knowledge, little research to date has been conducted using FT-IR to detect sex steroids or vitellogenin in blood plasma, for prediction of physiological or reproductive status. The objectives of this study were to utilize FT-IR spectroscopy combined with multivariate analysis to detect changes in the concentrations of sex steroids, vitellogenin and nonpolar lipids in white sturgeon plasma collected at different stages of sexual maturity. It is also necessary to determine whether changes in these spectral features could be correlated with widely used biological parameters such as the oocyte PI (*5*, *6*).

MATERIALS AND METHODS

Study Site and Sampling Description. The white sturgeon (*Acipenser transmontanus*) females used in this study were reared at Sterling Caviar (Wilton, CA) in the flow-through outdoor tanks (9.1 m diameter, 1.5 m depth) under constant temperature (ca. 20 °C) and natural photoperiod. Tanks were oxygenated to maintain dissolved oxygen at or slightly above air saturation. In the fall, gravid females were transferred to a cold-water site (3.7 m diameter by 0.9 m deep tanks) to prevent ovarian atresia at this temperature-sensitive developmental stage of sturgeon (*14*, *17*). Fish were held until caviar harvest in outdoor, flow-through tanks supplied with 10−13 °C water from Lake Amador. In both warm-water and cold-water facilities fish were fed pelleted sturgeon diet manufactured by EWOS at 0.03−0.09% body weight per day.

Twenty sturgeons with late vitellogenic or atretic ovaries were surgically biopsied in April 2007 for a preliminary study (each PI value was only measured once). Another 20 randomly chosen females (fork length 132−156 cm, weight 25−40 kg) were individually tagged with passive integrated transponder (PIT) tags in September 2007 and biopsied in September 2007. Fifteen of these fish had late vitellogenic ovaries, and five females had ovaries in either previtellogenic or postvitellogenic (approaching maturation) stage of development. Eleven females in the late vitellogenic group were repeatedly sampled in November 2007. Ovarian biopsies in late vitellogenic fish were performed to measure the oocyte PI and assess the stage of oocyte development. At each biopsy, fish were anesthetized using a 100 ppm tricaine methanesulfonate (MS-222) bath and bled and the ovary was biopsied. In addition, fork length, weight, and PIT tag number were recorded.

Sample Preparation. During biopsy, blood was collected from the caudal vasculature and centrifuged. Separated plasma (3.5 mL from each fish) was shipped frozen by overnight courier to Washington State University (Pullman, WA) for spectral analysis. Oocyte PI values were measured at Bozeman Fish Technology Center, US Fish and Wildlife Services (Bozeman, MT) (*7*, *8*).

FT-IR Spectroscopy Samples. Ten microliters of plasma was applied to a glass slide and air-dried under laminar flow at room temperature (ca. 20 °C) for 1 h to obtain a uniform dry film. A second 10 μL of plasma was applied on top of the dried film and air-dried under a fume hood for 24 h until the film was visibly dry and homogeneous. Triplicate slides were prepared for each plasma sample. Sex steroid standards (17β-estradiol, 17α,20α-dihydroxy-4-pregnen-3-one, testosterone, progesterone, and 11-ketotestosterone) were purchased from Sigma-Aldrich Co. (St. Louis, MO) or were provided by Bozeman Fish Technology Center, US Fish and Wildlife Services (Bozeman, MT). Purified white sturgeon vitellogenin (concentration 26 μg/mL in PBS) was provided by the University of California, Davis (Davis, CA) (*32*). The samples of plasma from a previtellogenic sturgeon before and after estrogen treatment were collected at the University of California, Davis (Davis, CA), stored at −80 °C and sent to Washington State University (Pullman, WA) for FT-IR spectra analysis to detect sex steroids and vitellogenin.

FT-IR Spectroscopy. FT-IR spectra were collected using a Thermo Nicolet Avatar 360 FT-IR spectrometer (Thermo Electron Inc., San Jose, CA). The glass slide with dried plasma spot was placed in direct contact with an attenuated total reflection (ATR) zinc selenide (ZnSe) crystal and spectra taken (4000 to 400 cm^{-1}). Thirty spectra were collected at room temperature for each plasma sample, ten for each slide (N = 3). FT-IR spectra were also obtained for each sex steroid standard (in powder form, 5 mg powder per analysis) and for white sturgeon vitellogenin (in PBS). The resolution of FT-IR instrument was set at 4 cm^{-1} with each spectrum composed of a mean of 36 separate scans.

Data Analysis. FT-IR data analysis was conducted using OMNIC (Thermo Electron Inc.) and Delight version 3.2.1 (Textron Systems, Wilmington, MA) software. The data preprocessing, such as binning and smoothing followed by second derivative transformation (*33*), magnifies and aids in the visualization of small differences among spectra. Binning reduces the number of data points in a spectrum by a few points into one and often eliminates the imbalance problem associated with many array-based spectrophotometers (*34*). Smoothing eliminates high frequency instrumental noise by adjacent data points (*35*). Second derivative transformation separates overlapping absorption bands, eliminates baseline offsets, increases the apparent spectral resolution and provides an estimate of the number of overlapping bands within a region (*25*). Data preprocessing algorithms, such as binning (2 cm^{-1}) and smoothing (Gaussian function over 10 cm^{-1}), were employed, followed by a second derivative transformation with a gap value of 12 cm^{-1}. After data preprocessing, principal component analysis (PCA) and partial least-squares (PLS) models were

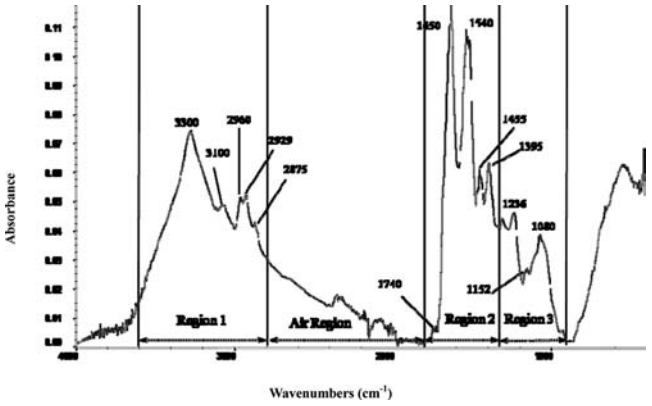

Figure 1. Typical FT-IR absorbance spectrum (before data preprocessing) for white sturgeon (*Acipenser transmontanus*) plasma.

developed. Due to the noise resulting from air, PCA and PLS analysis was based on the combination of the wavenumbers of 3600 to 2700 cm^{-1} and 1800 to 900 cm^{-1}.

Principal component analysis (PCA) is one of the most valuable interpretations derived from applied linear algebra and is a common multivariate statistical method for interpreting spectral data variance (*36*). The principle of the PCA method determines which major factors affect the differences in the observed spectral features among samples and constructs a model to segregate plasmas based upon selected sample variances (*37*). The first principal component (PC1) contributes more than the second principal component (PC2) to the variation observed between spectra, etc. PCA models can be used to sort plasmas by treatment differences and to confirm which wavenumbers provide the greatest contribution to the plasma variance. PCA is based upon a second derivative analysis.

The partial least squares (PLS) model is a multivariate regression method to establish a relationship between reference value data and predicted value data; the larger the *R* value, the better and more rigorous the model (*38*). To establish a PLS model, the first step is to choose the optimal number of latent variables (*35*). Too many latent variables decrease the precision of the model due to data overfitting. Too low a number of latent variables will reduce the utility of the model since not all of the relevant data is used for its construction (*33*, *38*). Being able to establish a robust model and spectral library for predicting a physiological parameter such as sturgeon PI requires sufficient reference data and valid correlations between the reference value (PI) and the predicted value based upon spectral features determined.

RESULTS AND DISCUSSION

Spectral Features of Sturgeon Plasma, Steroid Hormones and Vitellogenin. A typical FT-IR spectrum of white sturgeon plasma is presented in **Figure 1**. This spectrum can be separated into three basic regions: from 3600 cm^{-1} to 2700 cm^{-1}, 1800 cm^{-1} to 1300 cm^{-1} and 1300 cm^{-1} to 900 cm^{-1}. The first two regions are mainly protein and lipid regions and the third region contains spectral features associated with nucleic acid, polysaccharides and phospholipids. The region between 1800 cm^{-1} and 900 cm^{-1} (sometimes referred to as the "fingerprint" region) often contains features for phospholipids, proteins and nucleic acids in complex biological matrixes (*39*).

A summary of FT-IR absorption band assignments (between 4000 cm^{-1} and 400 cm^{-1}) is presented in **Table 1**. These band assignments are important for determining how the relative amounts of various biochemical components in fish plasma are changing as the fish progresses through vitellogenesis.

Figure 2 provides spectra of various steroids that are critical biological indicators of fish maturity. Steroid hormones are lipids with unique spectral features (see) at wavenumbers from 3150 cm^{-1} to 2850 cm^{-1} (**Table 1**, **Figures 1** and **2**) (*29*). There are

Table 1. Absorption Band Assignments in the FT-IR Spectral Region[a]

wavenumber (cm^{-1})	spectral assignment
~3300	N−H stretching of proteins and O−H stretching of polysaccharides
~3100	olefinic =CH stretching vibration: lipids, cholesterol esters
~2960	CH_3 asymmetric stretch of methyl groups: mainly lipids
~2929	CH_2 asymmetric stretch of methylene groups: mainly lipids
~2875	CH_3 symmetric stretch of methyl groups: mainly lipids
~1740	C=O of ester functional groups: triglycerides, cholesterol esters
~1650	C=O stretch of amides of proteins: amide I
~1540	N−H bend and C=O stretch of amides of proteins: amide II
~1455	CH_2 bending: mainly lipids, with little contribution from proteins
~1395	COO^- symmetric stretch: fatty acids and amino acids
~1236	P=O asymmetric stretch: mainly nucleic acids and phospholipids
~1152	CO−O−C asymmetric stretch: glycogen and nucleic acids
~1080	P=O symmetric stretch: mainly nucleic acids and phospholipids

[a] See refs *27*−*31*, *37*, *44*, *45*, *54*−*60*.

distinct spectral features around 3000 cm^{-1} for all of the steroid hormones examined in this study (**Figure 2**). Three peaks (~2960 cm^{-1}, 2929 cm^{-1} and 2850 cm^{-1}) are associated with steroid hormones in plasma.

The pure sturgeon vitellogenin (with PBS) spectrum is shown in **Figure 3**. A clear peak around 1080 cm^{-1} represents vitellogenin (**Figure 3**).

Figure 4 shows a comparison of the FT-IR spectra of plasma from the same sturgeon before and after estrogen treatment. Changes in the sex steroid region (~3000 cm^{-1}), lipid region (~1700 cm^{-1}) and vitellogenin region (~1080 cm^{-1}) were clearly manifested. This result combined with spectral analysis of standards (**Figure 2** and **Figure 3**) strongly supports detection of sex steroids and vitellogenin regions on the FT-IR spectra.

Sturgeon plasma is a very complex biochemical matrix containing sex steroids, polar and nonpolar lipids (*4*) and proteins including lipophosphoglycoproteins such as vitellogenin (*40*) immunoglobulins and gonadotropins. In the plasma of female white sturgeon, the concentrations of 17β-estradiol, testosterone, and 11-ketotestosterone changed as the fish became sexually mature similar to what has been described in previous studies (*15*, *18*, *41*−*43*). The levels of these have been found to differ significantly in wild and farmed sturgeon at various stages of sexual maturity (previtellogenic, vitellogenic, postvitellogenic and atretic periods). Amiri et al. (*15*) demonstrated that plasma concentrations of 17β-estradiol from previtellogenesis through atresia were less than 0.6 ng mL^{-1} in immature female cultured sturgeon hybrid, the bester (*Huso huso* × *Acipenser ruthenus*), while in the vitellogenic individuals, the level of 17β-estradiol increased to 2−4 ng mL^{-1} and then decreased rapidly to 1−2 ng mL^{-1} when the fish entered postvitellogenesis. Amiri et al. (*15*) also reported that serum concentrations of testosterone were 5−15 ng mL^{-1} in immature fish, and then increased to 20−80 ng mL^{-1} when fish went into vitellogenesis. Linares-Casenave et al. (*17*) reported that plasma concentration of testosterone in white sturgeon (*Acipenser transmontanus*) remained elevated (100−150 ng mL^{-1}) whereas 17β-estradiol steadily declined to 0.5 ng mL^{-1} from late vitellogenesis to spawning.

We identified the vibrational spectral features of sex steroid hormones in fish blood plasma (**Figures 1** and **2**). There are three distinctive major peaks for sex steroids (11-ketotestosterone, 17α,20α-dihydroxy-4-pregnen-3-one, 17β-estradiol, testosterone and progesterone) around 3000 cm^{-1} which were also present in sturgeon blood plasma (**Figure 1**). These steroid features are distinct from those of nonpolar lipid (~1700 cm^{-1}) and proteins (~1650 cm^{-1} (amide I) and 1540 cm^{-1} (amide II)), both of which

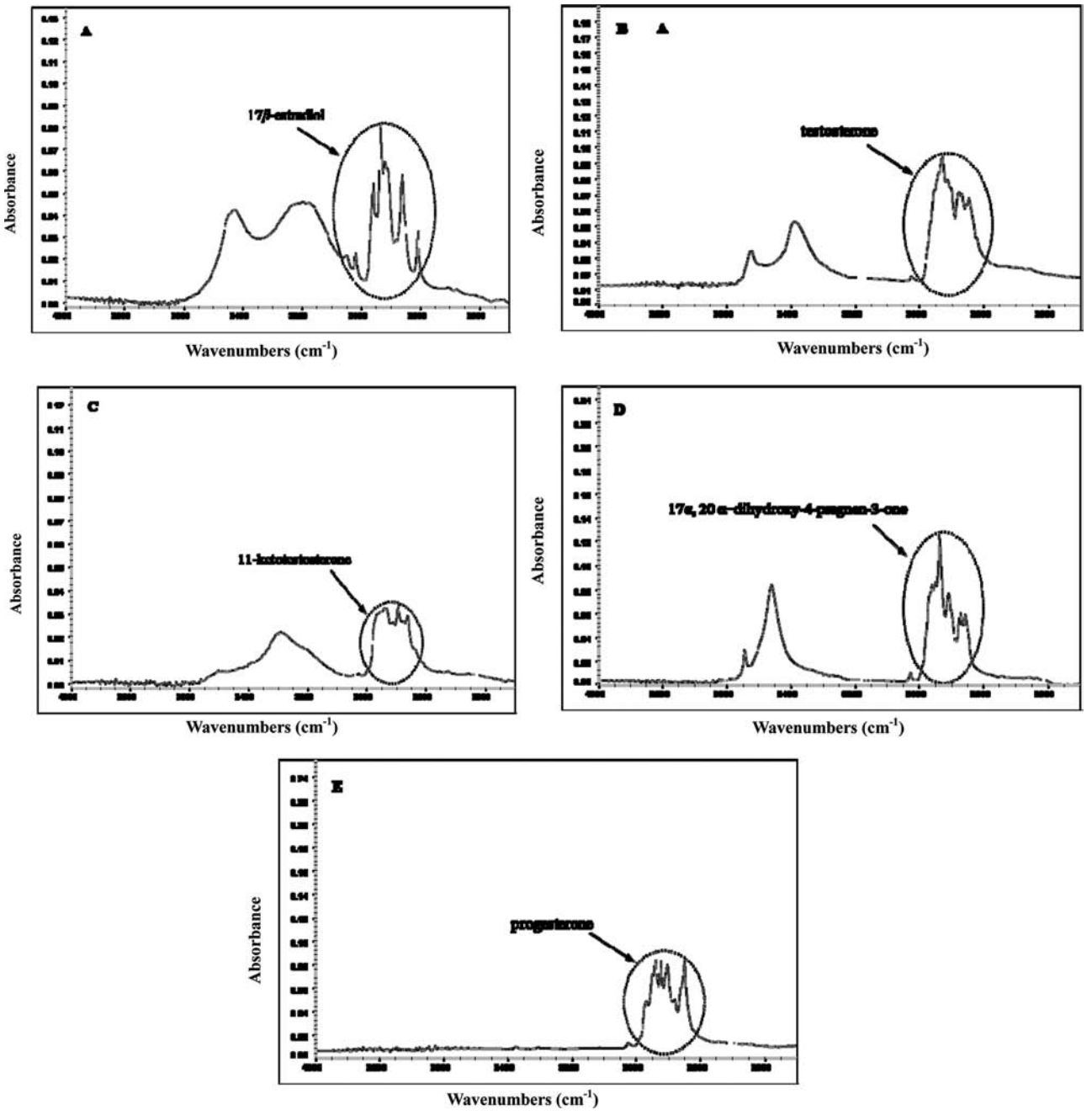

Figure 2. Typical FT-IR spectra for sex steroids: (**A**) 17β-estradiol, (**B**) testosterone, (**C**) 11-ketotestosterone, (**D**) 17α,20α-dihydroxy-4-pregnen-3-one, and (**E**) progesterone.

were found to be important for establishing either PCA or PLS models (**Figures 5**, **7**, and **8**) in this study.

Change in vitellogenin content is also apparent in FT-IR spectra as the sturgeon become sexually mature. The spectral features of sturgeon vitellogenin appear around 1080 cm^{-1} and are indicative of phospholipids and glycosides (*44*, *45*) (**Figure 3**). Fish vitellogenin is a large molecule (native mw ~400 kDa in sturgeon (*46*)) composed of protein backbone containing phosphorylated serine moieties, a high lipid content (~20%), and a high level of carbohydrates (*4*, *47*, *48*). Hiramatsu et al. (2002) isolated and confirmed that lipovitellin, β'-component and phosvitin are the corresponding yolk proteins to sturgeon vitellogenin (*47*).

Vitellogenin plays an important role in the development and growth of the sturgeon ovarian follicles. The transcription and translation of vitellogenin is initiated by 17β-estradiol binding to the estrogen receptors on the membrane of the liver cells (*20*). Vitellogenin binds calcium and other cations during its transport from liver to the ovary (*48*). Vitellogenin is internalized by the oocyte via receptor-mediated endocytosis and degrades into two yolk proteins, lipovitellin and phosvitin, which then crystallize to form yolk platelets in the egg cytoplasm (*4*, *47*). Teleost fish have several different forms of vitellogenin and yolk proteins. The vitellogenin induction is shown in **Figure 4** showing results for the same fish before and after estrogen treatment. In this study, a sturgeon in a previtellogenic period one year before breeding had low levels of both estrogen and vitellogenin. After estrogen treatment, the concentration of vitellogenin in the plasma increased (**Figure 4**). Moberg et al. (*41*) induced (with estrogen implants) hepatic synthesis of vitellogenin in immature white sturgeon, but there was no vitellogenin uptake by the oocyte. Fujii et al. (*49*) indicated that the vitellogenic period corresponded

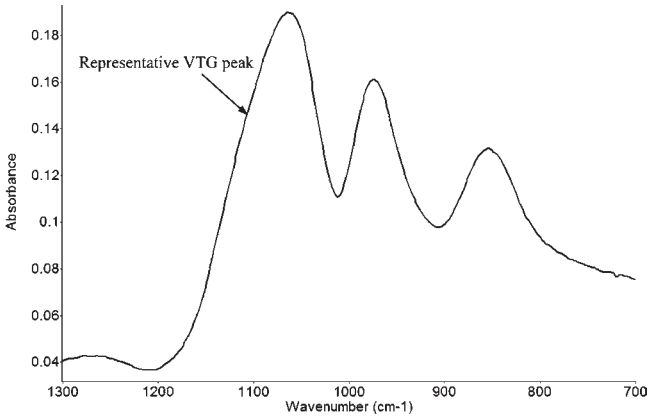

Figure 3. Typical FT-IR spectra for pure white sturgeon female vitellogenin (with phosphate buffered saline).

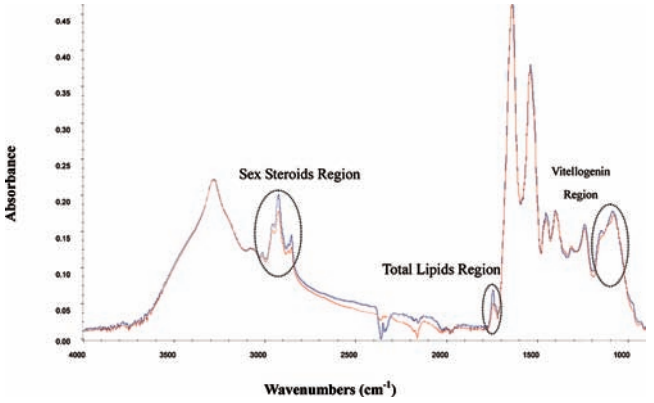

Figure 4. Comparison of FT-IR spectra for plasma from the same white sturgeon female before (red color) and after (blue color) estrogen treatment.

to high 17β-estradiol levels in maturing bester (*Huso huso* × *Acipenser ruthenus*) sturgeon and the same situation was found in Russian sturgeon *Acipenser gueldenstaedtii* and stellate sturgeon *Acipenser stellatus* by Barannikova et al. (*42*).

PCA of Sturgeon Maturity Stages. Figure 5a shows a representative example of separation, using 2-D PCA, between the spectral features of plasma sampled from the same fish in September and then again in November 2007. Loading plots were used to examine which spectral region contributed most to this segregation. From **Figures 5b** and **5c**, PC1 was around 3000 cm^{-1}, and PC2, around 1700 cm^{-1}, both of which are the steroid hormones and lipid regions. PC1 contributed about 61% to the variation between samples, and PC2, about 13%.

During different maturity phases, the chemical components in white sturgeon plasma will change, to some extent, especially steroid hormone level, total lipid level and the level of specific proteins, such as vitellogenin. Principal component analysis based model was also used to sort plasma samples from different fish at different stages of sexual maturity. A clear separation of fish sampled in April is shown in **Figure 6**. The spectra of plasma from individuals with atretic ovaries were clearly separated from those with vitellogenic, postvitellogenic and previtellogenic ovaries, indicating that each ovarian stage had distinct chemical signature based on blood metabolites.

Our study indicated that it is possible to use Fourier transform infrared spectroscopy (FT-IR) coupled with multivariate analysis to distinguish and segregate population by maturity stages (**Figures 5** and **6**). First, several groups of fish at different maturity periods (previtellogenesis, vitellogenesis, and late vitellogenesis)

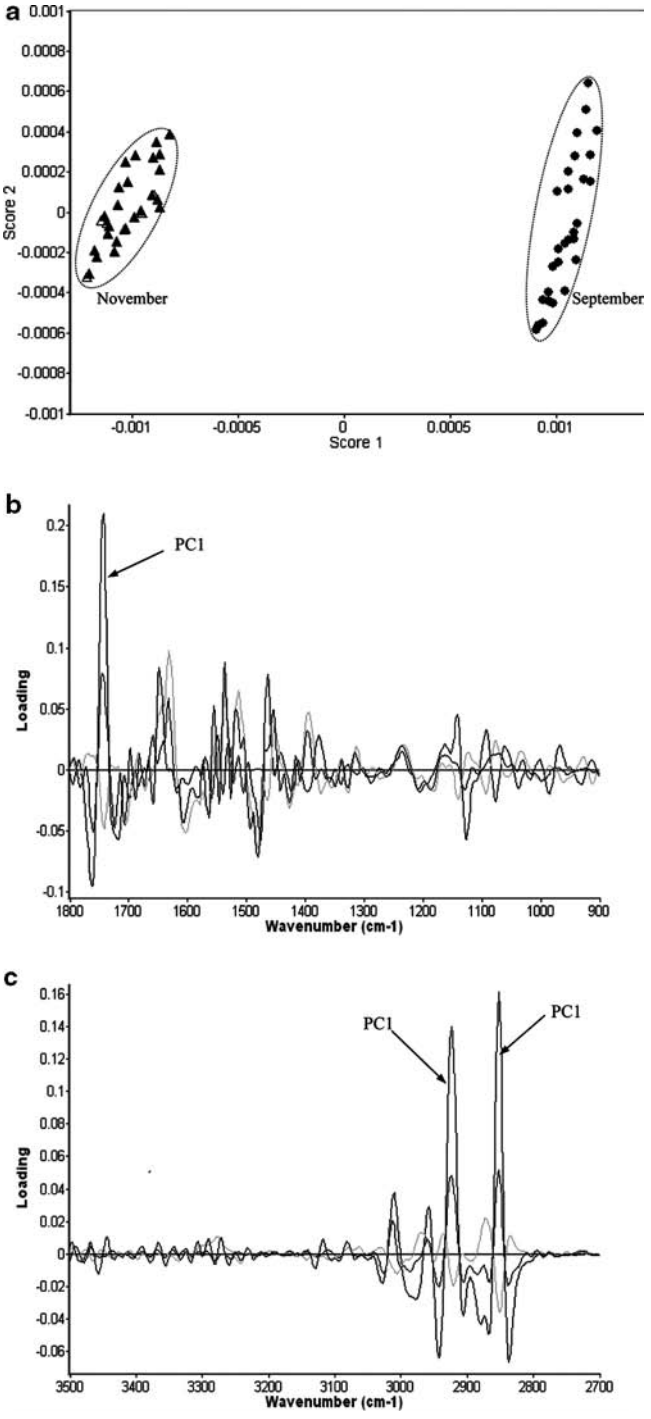

Figure 5. (**a**) Representative two-dimensional PCA clustering results for plasma from white sturgeon at different months during sexual maturation ($N = 11$). (**b**) Representative loading plot of first principal component (PC1) obtained from PCA results of FT-IR spectra of plasma from the same white sturgeon female from 1800 cm^{-1} to 900 cm^{-1}. (**c**) Representative loading plot of first principal component (PC1) obtained from PCA results of FT-IR spectra of plasma from the same white sturgeon female from 3500 cm^{-1} to 2700 cm^{-1}.

can be distinguished, as well as fish undergoing follicular atresia. Furthermore, FT-IR can be used to monitor the progression of a single fish through late vitellogenesis. The plasma concentrations of sex steroids and lipids appear to be the primary factors, as indicated by high values of loading plots, which contributed to principal component analysis (PCA) cluster segregation. These findings support earlier research that plasma

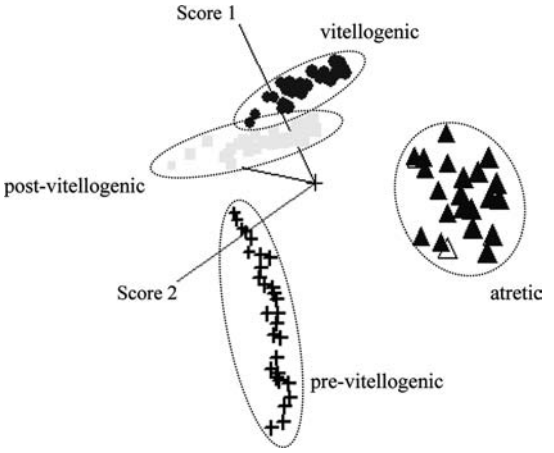

Figure 6. Representative three-dimensional PCA clustering results for stages of maturity of white sturgeon using plasma samples from different fish ($N = 5$).

steroid hormones are important biomarkers for predicting sturgeon maturity stages as determined by steroid measurement by radioimmunoassay. Doroshov et al. (*50*) and Moberg et al. (*12*) indicated that plasma concentrations of 17β-estradiol can be used to discriminate vitellogenic stages in sturgeon. Webb et al. (*8*) reported that plasma testosterone and 17β-estradiol were the best predictors of white sturgeon sex and stage of maturity. Webb et al. (*43*) pointed out that, by using plasma concentrations of testosterone, 11-ketotestosterone and 17β-estradiol, it was possible to correctly classify 61% previtellogenic females, 86% vitellogenic females, 92% postvitellogenic females, 55% postovulatory females and 29% atretic females. Malekzadeh Viayeh et al. (*51*) demonstrated that plasma testosterone and 17β-estradiol plus either age, total length, fork length or weight were good predictors for determining sturgeon maturity stage.

Polarization Index and the PCA/PLS Model. Table 2a shows the oocyte PI values for 20 sturgeons at one time period, and the values were within the range of 0.084 and 0.332. Eleven fish were tracked from September to November to monitor changes in the PI values along with fish maturity stages. As sturgeon become more mature, PI values decrease steadily (**Table 2b**).

Figure 7a shows discrete PCA clusters for different PI ranges reflecting fish maturity levels. The loading plots were used to check which region accounts for PC1. From **Figures 7b** and **7c**, the regions around 3000 cm^{-1} and around 1700 cm^{-1} contributed the most to the PCA separation and correspond to the steroid hormone indicating that there is a relationship between steroid hormone level and PI value.

Partial least squares (PLS) models were developed to predict the actual PI value of white sturgeon females (**Figure 8**). Validation results for prediction of PI values from spectral features (6 latent variables) between 0.12 and 0.40 from white sturgeon females resulted in a high correlation coefficient of determination ($R = 0.95$) and a relatively low standard error of prediction (SEP = 2.18%) based on the regions of 3600 cm^{-1} to 2700 cm^{-1} and 1800 cm^{-1} to 900 cm^{-1} of the FT-IR spectra.

Aquaculturists have traditionally used surgical biopsy to measure the oocyte PI value to determine whether the fish are ready for spawning induction (*6*,*52*,*53*). As the sturgeon progress through vitellogenesis, the PI value decreases steadily from about 0.4 to less than 0.1 (**Table 2**), and the decrease of PI value is accompanied by an increase in size of follicle and changes of oocyte pigmentation (*5*−*7*) reflecting the morphological changes occurring before the egg maturation.

Table 2. Oocyte Polarization Index (PI) Values of Sturgeons in 2007[a]

(**a**) April

sample ID	PI (2007−04−09)	sample ID	PI (2007−04−09)
1	0.1067	11	0.1181
2	0.1736	12	0.1311
3	0.0857	13	0.1277
4	0.1513	14	0.1102
5	0.3109	15	0.1129
6	0.3317	16	0.1768
7	na[b]	17	0.0962
8	na[b]	18	0.0657
9	0.1185	19	0.0840
10	0.1439	20	0.2083

(**b**) September and November

sample ID[c]	PI (2007−09−10)	PI (2007−11−16)	PI difference
182F	0.2310 (0.053)	0.1736 (0.048)	0.0574
205E	0.2294 (0.027)	0.1360 (0.020)	0.0934
2E7C	0.2749 (0.081)	0.2411 (0.059)	0.0338
3D08	0.2814 (0.065)	0.2137 (0.034)	0.0677
4458	0.3884 (0.054)	0.1919 (0.014)	0.1965
401A	0.1866 (0.075)	0.2236 (0.050)	−0.0370
483D	0.2892 (0.069)	0.2289 (0.041)	0.0603
4B34	0.3000 (0.055)	0.1955 (0.020)	0.1045
6D7E	0.2307 (0.042)	0.1291 (0.027)	0.1016
795C	0.2371 (0.036)	0.1618 (0.050)	0.0753
7E07	0.3161 (0.068)	0.1862 (0.030)	0.1299

[a] Each PI value was only measured once. [b] When sturgeons enter the atretic period, the PI value measurement is not available because follicles are too soft. [c] These eleven fish are tracked to monitor the PI value changes from September to November.

Based on the results in **Figure 7**, the interrelatedness of PI values and plasma concentration of sex steroids and lipids was shown as the fish eggs progress in the GV migration during late vitellogenesis. Here, the plasma concentration of steroids and lipids comprises the first principal component being the biochemical feature most responsive to maturity changes and thereby useful for segregating the fish by different oocyte PI regions (**Figure 7a**). This figure also indicates changes in plasma sex steroid hormones at different maturity stages through the vitellogenesis (*5*,*6*,*43*). The PI decreases in normally developing egg by the displacement of GV to the animal pole. The follicular atresia is irreversible degeneration of follicular wall and the oocyte (*17*). When atresia starts, GV migration is arrested and PI often increases since GV is no longer supported by the cell skeleton. The atresia may happen at any stage of GV migration. The relationship between PI value and steroids in plasma could be manifested in **Figures 7b** and **7c** by the loading plot analysis in which sex steroid and total lipid are the most important factors in PCA.

The PLS model using FT-IR measurement of blood plasma was established as an alternative to the traditional method for determining PI values (**Figure 8**). When we randomly selected samples (making an even interval on the reference data), a robust model using 6 latent variables could be developed ($R = 0.95$). The latent variables are the factors responsible for the model establishment and involved a combination of the steroid region (around 3000 cm^{-1}), lipid region (around 1700 cm^{-1}) and vitellogenin region (around 1080 cm^{-1}).

Fourier transform infrared spectroscopy could be a potential tool for sturgeon farms according to our current research results. Compared with the traditional biopsy for sex maturity determination, FT-IR has some unique advantages. Although FT-IR requires

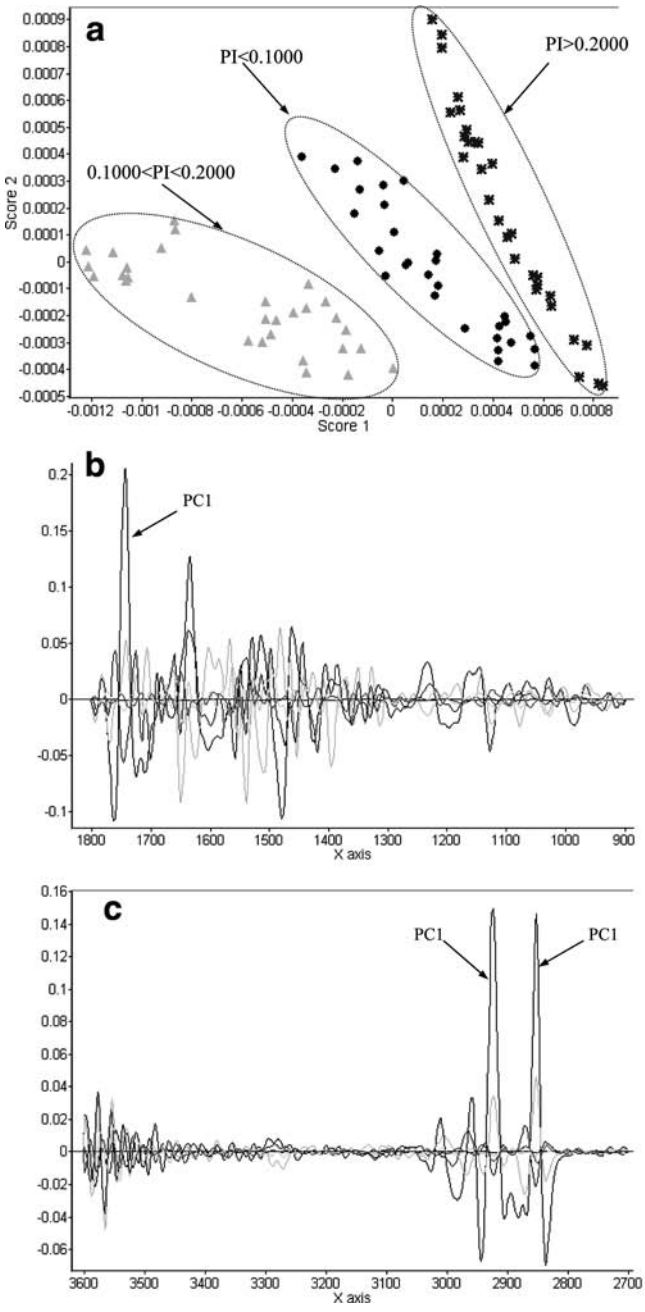

Figure 7. (**a**) Representative two-dimensional PCA clustering results for fish plasma based on PI values: PI value < 0.1000 (spawning in 2 weeks), 0.1000 < PI value < 0.2000 (spawning in 1 month) and PI value > 0.2000 (spawning in 2 months) (N = 15). (**b**) Representative loading plot of first principal component (PC1) obtained from PCA results of FT-IR spectra of plasma based on different PI values from 1800 cm^{-1} to 900 cm^{-1}. (**c**) Representative loading plot of first principal component (PC1) obtained from PCA results of FT-IR spectra of plasma based on different PI values from 3600 cm^{-1} to 2700 cm^{-1}.

a small sample of blood, it is a minimally invasive method, with little risk of infection since a sterile needle is used for blood collection. Furthermore, FT-IR coupled with multivariate analysis can accurately assess maturity by using a composite picture of chemical "fingerprints" of the reproductive state. However, this method requires the use of a laboratory; therefore each sampled fish has to be tagged. Tagging is not a problem for a small population, such as brood fish for hatchery breeding, but significantly increases the cost of labor in the case of a large population, such as selection of fish for caviar in a commercial aquaculture facility.

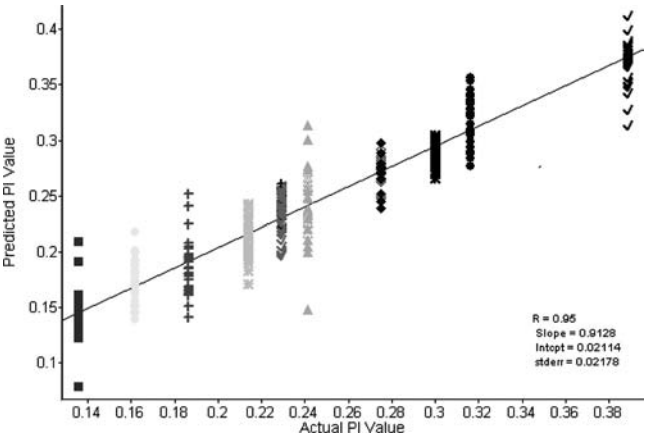

Figure 8. Comparison between the actual and predicted PI values (0.12 < PI < 0.40) of white sturgeon females over the course of a harvest season. Each cluster of data along the X axis represents one sturgeon plasma sample with corresponding PI values selected from **Table 2a**,**b** (N = 11 fish, each sampled twice). This figure represents one of the eight PLS models constructed for these data. Key: R, the correlation coefficient; Intept, intercept; stderr, the standard deviation of the errors of prediction.

In conclusion, biochemical components in white sturgeon plasma, including sex steroids and vitellogenin, can be detected by FT-IR spectroscopy and can predict ovarian maturation stage in female sturgeon. Combined with multivariate analysis (PCA, PLS), FT-IR provides a new assessment method for segregating fish by maturity stages (previtellogenesis, vitellogenesis, post-vitellogenesis), identifying follicular atresia, and predicting the oocyte PI values which is currently only possible by traditional surgical biopsy. Further work needs to be conducted to improve the accuracy of the predictive models to ensure that they accurately reflect the biochemical changes that occur over the entire ovarian cycle of farmed sturgeon.

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