



Rapid noninvasive characterization of ovarian follicular atresia in cultured white sturgeon (*Acipenser transmontanus*) by near infrared spectroscopy

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ABSTRACT

We report a rapid and noninvasive method based on short wavelength near infrared (SW-NIR) spectroscopy to detect onset of atresia in farmed white sturgeon (*Acipenser transmontanus*) (N=10). The only current means to assess follicular atresia is by direct oocyte examination which requires a surgical biopsy of the ovary. In this study, abdominal scans were collected noninvasively by SW-NIR on anesthetized females using a diffuse reflectance fiber optic probe. In addition, to further verify the ability to detect spectroscopical changes related to onset of atresia, during each sampling roughly 30 cm³ of ovarian follicles was surgically removed from each female and transferred to a Teflon holder for spectral acquisition. Comparison of spectra collected on normal or atretic fish or ovarian follicles was conducted using Principal Component Analysis (PCA). Principal components suggest that the best indicator of atresia onset is a decrease in the intensity of the lipid bands at 930 nm. Prediction models for atresia were constructed using Soft Independent Modeling of Class Analogy (SIMCA) with leave-one-out cross validation. Seventy one percent of all atretic spectra and 65% of normal spectra collected on ovarian follicles were correctly classified. Exclusion of spectra from two potential outlier fish improved the predictability of normal ovarian follicles to 76%. Similarly, 72% of all atretic spectra and 59% of all normal spectra collected noninvasively in whole fish were correctly classified. Exclusion from the model of spectra from the same two outlier fish improved prediction of atresia from 72% to 75% as well as improving the prediction of normal spectra from 59% to 62%. This study represents the first example of using a noninvasive approach based on SW-NIR to detect onset of atresia in female sturgeon. Upon further development, this approach may potentially replace the need for surgical biopsy to detect ovarian regression. The availability of the proposed spectroscopic approach would grant sturgeon growers a powerful tool to follow more closely the maturation cycle with the goal of producing a consistently uniform product, standardize processing conditions, and maximize caviar yield by harvesting fish when the ovarian follicles have the appropriate firmness and a larger size.

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1. Introduction

Efforts to protect wild sturgeon from aggressive harvest for caviar production have recently resulted in domestically farmed caviar. However, producers lack simple and reliable methods to produce a consistently uniform product and maximize caviar yield by harvesting fish when the ovarian follicles have the appropriate firmness and a larger size. Currently, the only means to assess ripeness and follicular atresia (reabsorption of ovarian follicles, hence a metric of egg quality) in white sturgeon (*Acipenser transmontanus*) females is by

direct oocyte examination which requires a surgical biopsy of the ovary. This technique is stressful, time consuming, and not an effective tool for handling a large number of female fish. Hence, ripeness is assessed once in the fall to identify maturing females later in the winter and spring, and follicular atresia is assessed macroscopically just prior to harvest. Phagocytic changes may occur within the oocyte during the early onset of follicular atresia that cannot be detected visually. This approach results in an improper timing of harvest, decreasing caviar yield and quality, and increasing the rate of follicular atresia. Atresia causes a reduction in the firmness, flavor, and shelf life of caviar after processing, and sometimes the complete loss of the product. New methods to predict whether a female has initiated follicular atresia are needed. Ideally, these methods should be noninvasive, if possible, and quick. Noninvasive or minimally invasive

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methods such as ultrasound imaging and endoscopy have been reported to identify sex of sturgeon as well as determine gonadal development (Damon-Randall et al., 2010). Successful application of ultrasound is limited by the presence of adipose tissue which can prevent a clear image from being obtained. Species specific interpretation of ultrasound images by trained personnel may be required because of variations in abdominal wall thickness and bony plates. Endoscopy has also been used to identify fish sex and measure gonad volume, fecundity, and reproductive stage (Bryan et al., 2007). The authors applied endoscopy with logistic regression to predict reproductive stages in shovelnose sturgeon and were able to correctly predict all five stages in 58% of the fish when viewing the gonadal structure through the urogenital duct wall and 49% of the fish when inserting the endoscope through a small ventral incision. However, these techniques have not been used to detect onset of atresia.

Short wavelength near infrared (SW-NIR: 700–1100 nm) spectroscopy has been increasingly applied in studies involving the optical properties of biological tissues (Munck, 2007) and the noninvasive diagnosis of tumors (Xu and Povoski, 2007). For instance, oxygen saturation can be measured by correlating the absorption ratio of oxy- to deoxyhemoglobin (Dullenkopf et al., 2004), and glucose concentration in blood can be determined by measuring photon absorption at glucose-specific wavelengths (Li et al., 2009; Sieg et al., 2005; Pickup et al., 2005). Hematomas and deep vein thromboses can be detected based upon the condition of higher hemoglobin concentration in the affected tissues. Similarly, tumors can be detected because the higher vascular development around them results in higher hemoglobin concentrations (Cheng et al., 2003; Heffer et al., 2004). Because NIR light can penetrate several centimeters of tissues, it has been recently used for optical imaging for diagnosis of human breast cancer (Demos et al., 2004; Chen et al., 2005) and other tumor tissues (Gurfinkel et al., 2000).

Many SW-NIR applications involve diffuse reflectance measurements in which near infrared light is launched into the tissue by several illuminating fiber optics located at the end of a flexible probe. The light is diffusely reflected through the different tissue layers and is collected by an optical fiber located in the center of the probe. The light penetration depth in a specific sample is a function of the geometry of the optical probe and the scattering and absorption characteristics of the sample. Typical penetration depth within biological tissues is at least 10 mm or longer depending upon the tissue studied. When light enters the tissues, it is either absorbed or scattered, based on wavelength and differences in chemical composition of the tissue. Since different types of molecules vibrate at different frequencies, different compounds in the tissue can be identified based on the frequencies of light that are detected by the instrument. For example, in the 700–1100 nm wavelength range, second and third overtones of the OH, CH and NH vibrational transitions are detected in addition to the combination bands from other types of vibration (Weyer, 1985). Band position varies with the degree of uniqueness of the spectra for different organic molecules (Cavinato et al., 1992).

Because near infrared light can travel over long path lengths and be implemented in a non-destructive and noninvasive approach by using fiber optics, it is possible to illuminate a fish through the skin and scales and obtain chemical information on tissue composition that can be correlated to fish development and health status. In fish, NIR bands are related to three major components: the methylene CH stretch of fat, the OH stretch of water, and the NH stretch of protein. In addition to specific spectral features, scattering is also observed. Scattering varies with wavelength as λ^{-4} and, therefore, decreases as wavelength increases. Based on these spectral characteristics, noninvasive methods based on SW-NIR have been developed for measuring fat content in salmon (Solberg et al., 2003; Folkestad et al., 2008), detecting quality defects in foods including pathogens and spoilage (Al-Qadiri et al., 2008; Al-Holy et al., 2005; Lin et al., 2004) and for

determining fish health status (Boethin et al., 2007). Visible and SW-NIR spectroscopies were also applied for detecting bruises and internal defects in salmon based on the differences in absorbance between oxy- and deoxyhemoglobin (Lin et al., 2003), and for determining gonadal maturation status of Chinook salmon (Davis et al., 2006). SW-NIR can detect chemical differences in the composition of testes and ovaries, e.g., the greater lipid content of ovarian follicles versus sperm. These differences affect scattering and absorbance of SW-NIR light that can be detected and used to successfully segregate fish based on sex and maturity before external secondary sexual characteristics are evident. The authors determined that SW-NIR light has a penetration depth through the abdominal wall of sturgeon of approximately 20 mm (data not published), thus guaranteeing that the underlying gonadal tissues can be sampled noninvasively through abdominal areas with thicknesses of 20 mm or less. In addition, rapid acquisition of spectra (in the order of milliseconds) means a reduced period of time in which the fish is held under anesthesia and/or out of the water.

In this study, SW-NIR spectroscopy was used for rapid and noninvasive detection of ovarian follicular atresia in farmed white sturgeon. Using Principal Component Analysis (PCA) and Soft Independent Modeling of Class Analogy (SIMCA), we demonstrate the potential of this method to detect onset of follicular atresia both in live fish and extracted ovarian follicles. Using this noninvasive technique, sturgeon farmers will be able to improve caviar yield by harvesting fish that have not initiated follicular atresia and saving atretic fish for the caviar harvest in the next ovarian cycle.

2. Materials and methods

2.1. Study sites and experimental design

All samples were collected from adult white sturgeon reared at Sterling Caviar LLC, California. For a description of study sites and overall experimental design see Talbott et al. (this volume).

2.2. Spectra collection

A schematic of the experimental setup used for spectral acquisition is shown in Fig. 1. Briefly, the abdominal area is scanned using a custom probe placed in direct contact with the fish skin. The probe contains four 5 W illuminating halogen lamps and one central

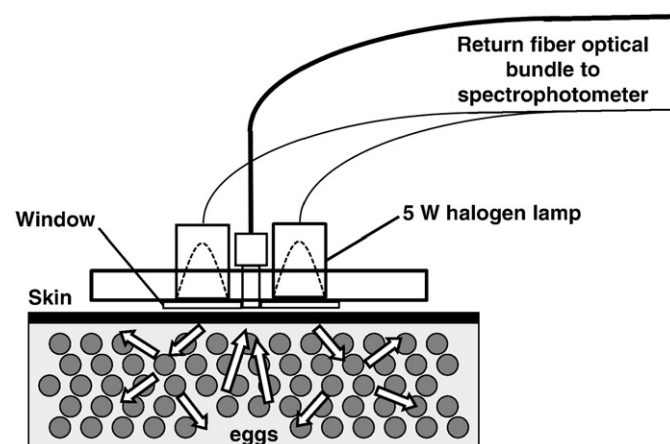


Fig. 1. Schematic of optical arrangement for spectral acquisition. An optical probe containing four 5 W halogen lamps illuminates the abdominal area. As light enters the ovarian sac it is in part absorbed and in part reflected by the ovarian follicles. Back-scattered light is collected by a central pick up fiber bundle and detected by the spectrophotometer.

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