Forensic Science International 229 (2013) 128–132

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Can fatty acid and mineral compositions of sturgeon eggs distinguish between farm-raised versus wild white (*Acipenser transmontanus*) sturgeon origins in California? Preliminary report

Edward J. DePeters [a,*], Birgit Puschner [b], Scott J. Taylor [a], Jeff A. Rodzen [c]

[a] Department of Animal Science, University of California, Davis, CA 95616, United States
[b] Department of Molecular Biosciences and the California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, CA 95616, United States
[c] California Department of Fish and Wildlife, Wildlife Forensics Laboratory, Sacramento, CA 95814, United States

ARTICLE INFO

Article history:
Received 23 July 2012
Received in revised form 30 March 2013
Accepted 7 April 2013
Available online 4 May 2013

Keywords:
Sturgeon
Wild
Farmed
Fatty acid
Roe

ABSTRACT

The objective was to investigate the potential of using fatty acid and mineral compositions of sturgeon eggs to distinguish their source, either farm-raised or wild fish. Trafficking of illegally obtained wild white sturgeon eggs is a major concern to the California Department of Fish and Game, but there is no forensic method to separate wild and farm-raised white sturgeon eggs. The extension of these findings in future work will be to use the fatty acid and mineral compositions as forensic indicators of caviar produced legally from farm raised sturgeon compared with illegal caviar produced from sturgeon poached from the wild. Samples (10) of sturgeon eggs were collected from a commercial aquaculture facility in the Sacramento Valley. Eggs from wild sturgeon (9) were obtained primarily from confiscations of illegally caught sturgeon by fish and game law enforcement personnel. The total lipid content of sturgeon eggs was analyzed for fatty acid composition. The most notable difference was the higher concentration ($P < 0.001$) of C18:2n6 in farm raised eggs (6.5 mg/100 g total lipid) than wild eggs (0.6 mg/100 g total lipid) while other differences between fatty acids were smaller. Eicosapentaenoic acid (C20:5n3) was higher ($P < 0.02$) in farm-raised (5.56 mg/100 g) than wild (4.49 mg/100 g). Docosahexaenoic acid (C22:6n3), C18:1 cis 9&10, and C20:4n6 were not different for origin of the eggs. Concentration of selenium was markedly higher ($P < 0.001$) in eggs from wild sturgeon (10.0 mg/kg dry weight) than farm-raised sturgeon (2.7 mg/kg dry weight). Concentrations of iron, zinc, copper, phosphorus, sulfur, calcium, and potassium did not differ between farm-raised and wild eggs. Arsenic concentration in wild eggs was 3.3 mg/kg dry weight whereas arsenic was not detected in the farm-raised eggs. Fatty acid and mineral compositions of eggs differed significantly between farm-raised and wild sturgeon and these should be investigated further as biological markers for forensic identification of caviar origin.

1. Introduction

The objective was to initiate preliminary research to determine if the fatty acid and mineral compositions of sturgeon eggs from commercially, farm-raised sturgeon differed from eggs obtained from sturgeon poached from the wild. If fatty acid and mineral compositions are uniquely different, a future goal will be to use fatty acid and mineral compositions to distinguish caviar produced from farm-raised sturgeon compared with caviar produced from wild sturgeon illegally captured. Ludwig [1] reviewed the issues related to identification of acipenseriformes species as it relates to the trade of food products. Fatty acid composition of sturgeons and paddlefishes was a viable option to identify wild versus farm raised fish. Thus, this study was initiated to explore the possibilities of using fatty acid and mineral compositions of sturgeon eggs as biological markers for farm-raised and wild sturgeon eggs.

The white sturgeon population in California has a large temporal size variation due to the long generation time of the species and a correlation with high and low rainfall years, but it is generally believed that the population is in a slow, long term decline [2]. Sturgeon populations both in California and abroad face a wide variety of challenges, many of which are still poorly understood. While comprehensive review of these factors is outside the scope this letter, they are reviewed in detail [3–5].

A major threat to California's white sturgeon population is the illegal harvest and commercialization of this resource. Caviar is

* Corresponding author. Tel.: +1 49 530 752 1263; fax: +1 49 530 752 0175.
E-mail address: ejdepeters@ucdavis.edu (E.J. DePeters).

0379-0738/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.forsciint.2013.04.003

sold on the "black market," and illegal fishing of white sturgeon is attractive to poachers. Officials in the Law Enforcement Division of the California Department of Fish and Game (CDFG) identified the illegal fishery of white sturgeon as a high priority for enforcement.

Unfortunately, there was no forensic technique available to discriminate wild and farm-raised caviar in California. The captive white sturgeon broodstock used in California aquaculture is only two or three generations removed from the wild source population. The frequency distribution of STR DNA (Short Tandem Repeats of DNA) profiles of the captive stocks has not diverged significantly from the wild population (J. Rodzen unpublished data; CDFG), thus DNA techniques are not useful for forensic identification of wild and farm raised caviars in California. Thus, an alternative approach for identifying the source of eggs must be developed.

Both the fatty acid and mineral compositions of sturgeon eggs reflect the environment of the fish. The interspecies differences in fatty acid composition of sturgeon lipids were attributed to differences in feeding strategies [6], which basically recognizes that the lipid composition of the prey species influences the fatty acid composition of lipids in sturgeon. The fatty acid composition of adipose tissue [7,8] and milk [9] lipids in non-ruminants (e.g. pig, domestic cat, and human) reflected the diet fed, and the same should be true for lipids in fish such as sturgeon. In contrast, the fatty acid composition of ruminant (e.g. cattle) adipose [7] and milk [10,11] did not reflect the fatty acid composition of the diet. The main reason for the differences in fatty acid composition of milk and adipose lipids between non-ruminants and ruminants is biohydrogenation of unsaturated fatty acids in the reticulo-rumen of ruminants by the anaerobic bacteria [12]. Biohydrogenation of unsaturated fatty acids prior to the small intestine does not occur in most non-ruminants, which suggests that the fatty acid composition of body lipids, in particular the neutral lipids, reflects the fatty acid composition of the diet. Thus, we hypothesize that the fatty acid composition of sturgeon eggs will reflect feeding habits in the wild or the fatty acid composition of diets fed in captivity.

Previously the nutrient composition of sturgeon caviar was investigated to provide a farm-raised product that was equivalent to caviar produced in the wild [6]. Production of caviar based on wild populations threatens survival of wild populations [13]. Production of farm-raised caviar with a composition and organoleptic properties similar to wild caviar is important for consumers, for the success of farm production systems, and for survival of wild populations. Czesny et al. [14] used fatty acid analyses to distinguish the origin of sturgeon eggs from wild and farmed animals. The linoleic acid (C18:2n6) content of farm- raised white sturgeon eggs was fourfold higher than eggs from wild animals [14]. However, a closer look at the data reveals that for one set of farm raised eggs the C18:2n6 content (2.5 wt%) was not different from one group of wild eggs (3.1 wt%) for Lake sturgeon but was different from Sacramento River sturgeon eggs (0.3 wt%). They reported a higher C18:1 cis 9 content in wild than farmed eggs although this was not significant in all cases. Interestingly the composition of long chain n3 fatty acids (eicosapentaenoic acid, C20:5n3 and docosahexaenoic acid, C22:6n3) was lower in eggs from wild than farmed-raised sturgeon [14]. Gessner et al. [15] found difference in n3 and n6 fatty acids for caviar produced by aquaculture versus wild origin.

Mineral content, specifically cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), of sturgeon caviar did not differ between farm and wild origins [6,16]. Likewise, for other minerals, including phosphorus (P), sulfur (S), and magnesium (Mg), no differences were observed in content of fresh caviar based on species and origin [17]. However, arsenic (As), bromine (Br), and strontium (Sr) concentrations varied with respect to what the authors described as either "brackish" or "fresh" water caviar origins [17]. Selenium (Se) was not measured as its concentration could be related to pollution or diet [6]. However, a previous study reported that Se accumulated in gonad tissue of sturgeon [18]. Interestingly, juvenile sturgeon was reported to accumulate potentially toxic minerals during their time in the Suwanne River prior to migration to the Gulf of Mexico. Tissue accumulations of Cd, chromium (Cr), Cu, mercury (Hg), nickel (Ni), Pb, and Zn were observed in one to three year old sturgeon [19]. Thus, we proposed that the concentration of selected minerals associated with environment, for example either water or diet, would be reflected in sturgeon eggs.

The objective was to determine if the fatty acid and mineral compositions of sturgeon eggs could be used to distinguish between eggs from either farm-raised or wild sturgeon, sturgeon eggs were obtained from a commercial farm in the San Joaquin Valley and from sturgeon caught in the wild.

2. Materials and methods

2.1. Sturgeon egg collection and analytical procedures

Farm-raised sturgeon eggs (roe) were collected from the one commercial aquaculture facility in the Sacramento Valley that harvested eggs from white sturgeon. Farm-raised caviar samples were collected over a period of twelve months at three different times depending on the harvest of roe. These samples originated from different individual fish. Eggs were stored frozen (−20 °C) until delivered to the laboratory where the eggs were freeze-dried prior to analysis. The wild sturgeon eggs were obtained from two sources. Nine samples were provided by CAFG from samples confiscated from poachers by Fish and Game wardens. Because of the difficulty of catching poachers by wardens one sample was obtained from a local fisherman from a legal catch to provide a total of 10 wild samples of roe. These wild egg samples were stored and processed similar to the farm-raised eggs. The objective of this initial study was to compare 10 wild samples and 10 farm-raised samples of sturgeon eggs.

For the farm-raised sturgeon, a purchased, commercial diet was fed. One sample of diet was analyzed at Cumberland Valley Analytical laboratories and found to contain (dry matter basis) 48% crude protein, 8.4% neutral detergent fiber, 8.7% ash, 1.92% Ca, 1.28% P, 0.22% Mg, 0.92% K, 0.59% Na, 325 ppm Fe, 74 ppm Mn, 196 ppm Zn, and 16 ppm Cu. The crude fat content (dry matter basis) was 15.7% [6]. The ingredients in the diet from highest to lowest proportion were: fishmeal, corn gluten meal, poultry meal, wheat, fish oil, canola meal, feather meal, canola oil, poultry oil, linseed meal, dried beans, shrimp meal, peas, a vitamin premix, and a mineral premix. Fatty acid composition [22] of the total lipid (g/100 g dry matter) for the major fatty acids was 2.64 C16:0, 4.77 C18:1 cis 9&10, 2.25 C18:2n6, 10.5 C20:5n3, and 0.83 C22:6n3. Total fatty acid content of the diet was 17 g/100 g dry matter [22].

Sturgeon egg fatty acid composition was determined by gas chromatography of methyl esters. Duplicate 100 mg aliquots of frozen eggs were weighed into 125 × 18 mm glass tubes and lyophilized at −40 to −50 °C condensor temperature and 28 °C shelf temperature for 15 h. Fatty acid methyl esters were produced using an adaptation of the one step methylation procedure [20].

Two ml of 0.5 mg/ml of nonadecanoic acid (C19, NuChek Prep, Elysian, MN) in heptane were added as an internal standard along with three ml of 10% methanolic HCl. The tubes were tightly sealed with screw cap Teflon lined tops and heated at 90 °C for 2 h. After cooling in an ice bath, 10 ml of 6% potassium carbonate were added followed by vortexing for 10 min. The tubes were centrifuged at 500 × *g* for 5 min to separate the solvent layers and the upper heptane layer was transferred into a 13 mm × 100 mm glass culture tube containing one gm of sodium sulfate. The samples were vortexed briefly and allowed to sit for 15 min before centrifuging again at 500 × *g* for 5 min and finally transferred into two ml auto sampler vials for the gas chromatograph.

The methyl esters were separated and quantified using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector and a Supelco 2560, 100 m capillary column containing a 0.25 mm inside diameter and a 0.20 μm film thickness. Hydrogen was used as the carrier gas with a linear flow rate of 27 cm/s and a column head pressure of 33 psi. One microliter of the ester mixture was injected using a Hewlett Packard 7673 auto injector and subjected to a split vent flow rate of 100 ml/min. The injector temperature was set at 210 °C and the detector temperature set at 220 °C with the column temperature initially set at 70° C for 10 min followed by a programmed increase to 175 °C at 20 °C per minute for 29 min and finally to 225 °C at 5 °C/min for 12 min.

One gram of egg sample was weighed out, 3 ml of concentrated trace metal grade nitric acid added for wet decomposition of organic matter, diluted to 10 ml, and analyzed for arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), zinc (Zn), nickel (Ni), thallium (Tl), and vanadium (V) by