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Discrimination of wild and domestic origin of sturgeon ova based on lipids and fatty acid analysis

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Abstract

This study was designed to discriminate different origins of sturgeon eggs (wild or domestic) based on their biochemical composition. Fatty acid profiles of neutral and phospholipid fractions of three populations of white sturgeon, *Acipenser transmontanus* (two domestic and one wild) and one population of wild lake sturgeon *Acipenser fulvescens* ova lipids were analyzed. Palmitic acid (16:0) was the dominant saturated fatty acid in both neutral and phospholipid fractions of egg lipids regardless of species or population origin. Levels of palmitoleic ($16{:}1n-7$) and docosahexaenoic ($22{:}6n-3$) acids were species specific irrespective of fish origin. Palmitoleic acid was found at a significantly ($P < 0.05$) higher level in lake sturgeon egg neutral lipids than in white sturgeon. The opposite was the case for $22{:}6n-3$. Other fatty acids, such as stearic (18:0) and oleic ($18{:}1n-9$) acids, were origin specific rather than species specific. Stearic acid was found at significantly lower levels in wild fish egg neutral lipids than in domesticated fish ova, whereas $18{:}1n-9$ showed the opposite trend. Phospholipid fatty acids were much less variable between species and among populations. We demonstrated that sturgeons' environment, thus their diet along with species specific characteristic life history (i.e., freshwater or marine origin) play an important role and markedly influence fatty acid composition of their eggs. Thus, egg fatty acid profile can be a viable tool in discrimination of different sturgeon populations with respect to caviar source and can ultimately be used to protect endangered wild populations of sturgeon.

Keywords: Sturgeon; Eggs; Fatty acids; Caviar; Conservation

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1. Introduction

In many parts of the world, conservationists look upon captive breeding and stock enhancement as ''the sturgeon's only chance of survival'' (Dumont, 1995). In recent years technology of controlled reproduction and rearing in sturgeons have made enormous progress (Doroshov et al., 1997). Captive propagation of sturgeon brings not only a game-ranching industry (Wells, 1997), but more importantly, caviar from fish farms undercut incentives for poachers of sturgeon. Remaining wild stocks of sturgeon can then be preserved.

In their timely communication, DeSalle and Birstein (1996) described accurate methods for identification of sturgeon and paddlefish caviar using the polymerase chain reaction (PCR). They concluded that some of the products on the US food markets are misrepresentations of the species listed on labels. The caviar may originate from illegal catches or represent species, which already may be extinct or threatened. In North America, several species and/or populations never recovered from over-fishing and river damming at the end of the last century (Bemis and Findeis, 1994).

In the context of both wild and cultured sturgeon caviar on the markets, a method is required to distinguish the origin. Siberian sturgeon, for example, is intensively cultured throughout Europe. Sturgeon farms in California culture white sturgeon and already supply ''farmed'' caviar to food markets. A method able to differentiate caviar from cultured and wild fish can complement the PCR method of species identification and help in the protection of endangered species or populations. Chen et al. (1995) successfully used the stepwise discriminant analysis to compare fatty acid profiles of muscle lipids to distinguish wild and domesticated Gulf sturgeon. In this study, we attempted to use egg lipids and their fatty acid profiles to identify sturgeon species and origin.

2. Materials and methods

2.1. Egg collection

Wild lake sturgeon (*Acipenser fulvescens*) were captured during their spawning run in the Wolf River (Wisconsin) near Shawano Dam. Location and method of capture were previously described by Folz et al. (1983). Eggs from eight ovulating females ($n = 8$, 162 ± 12.6 cm TL) were stripped, placed in separate plastic vials (7-ml volume) and immediately frozen in liquid nitrogen. Ovulated eggs from cultured white sturgeon (*Acipenser transmontanus*) were collected at the University of California, Davis ($n = 2$, 30 ± 1.42 kg), and Sea Farm, CA ($n = 3$, 34.3 ± 8.6 kg). Wild white sturgeon were captured in the Sacramento River, CA ($n = 3$, 36.7 ± 3.8 kg). All samples of eggs were immediately frozen and kept at -83°C until analysis. Domesticated broodstock white sturgeon from UC, Davis were fed commercial diets designated for salmonid fish, which contained 40–42% protein, 10–12% fat, 1–3% fiber, and 9–12% ash (Nelson's Sterling Silver Cup Trout Production, Nelson and Sons, Murray, UT) whereas white sturgeon from Sea Farm, CA received ''sturgeon diet'' containing 36–38% protein, 6–8% fat,

1–3% fiber, 9–12% ash. This diet was manufactured by the same company. Both of these feeds contained plant oils in addition to fish meal and fish oils.

2.2. Lipids and fatty acid analysis

Total lipids were extracted from sturgeon eggs according to the procedure of Folch et al. (1957). Crude lipid extracts were separated into polar (phospholipids) and neutral (mostly triglycerides) lipids and the amounts of these two fractions were determined as previously described by Czesny and Dabrowski (1998). Fatty acid methyl esters were prepared from both fractions of lipids according to the method described by Metcalfe and Schmitz (1961). The gas chromatogaph analyses of the fatty acids methyl esters were performed on a Varian 3300 Gas Chromatograph equipped with glass column (custom made 304.8 mm × 6.35 mm OD × 2 mm ID), packed with 10% SP 2330 on 100/120 Supelcoport (Varian Chromatography Systems, Walnut Creek, CA). The carrier gas was helium at a pressure of 80 psi. The thermal gradient was 175°C for 26 min, then increased by 2°C/min to 205°C and held at 205°C for 30 min. The individual fatty acids were identified by comparing their retention times with the retention times of known fatty acids, available in mixtures of external standards (Nu-Chek-Prep, Elysian, MN). The fatty acids were quantified (% by weight) by comparing areas of their peaks with that of the peak of a known amount of an added internal standard, C 19:0 (nonadecanoate).

2.3. Statistical analysis

Data were analyzed by one-way ANOVA, and significant differences between groups were identified by Scheffe's *F*-test (SPSS 7.5 for Windows, SPSS, Chicago, IL). The percentage data were subjected to arcsin transformation. Normality and homogeneity of variance were confirmed for all data prior to statistical analysis. Rejection level for all statistical analysis was set at $\alpha = 0.05$.

3. Results

Ova of two sturgeon species had significantly ($P < 0.05$) different lipid levels ($10.8 \pm 0.9\%$ of wet weight, $n = 8$ and $13.5 \pm 0.8\%$, $n = 8$, for lake sturgeon and white sturgeon, respectively). These differences were attributed only to the neutral lipid concentration, since phospholipids were found at similar levels of approximately 2.5% in all sampled populations. As such, white sturgeon eggs were significantly richer in neutral lipids than lake sturgeon eggs regardless of the population's origin. At the same time there were no differences among white sturgeon populations, even though two were domesticated and one wild.

Fatty acid profiles of both neutral and phospholipids are presented in Tables 1 and 2. The 16:0 was the most abundant saturated fatty acid (SAFA) in both fractions of egg lipids, regardless of species or population origin. The analysis of fatty acids in neutral lipids revealed that $16{:}1n-7$ and $22{:}6n-3$ were species specific despite the popula-

Table 1
Fatty acid composition of sturgeon roe neutral lipids (weight %). Means within a row sharing a letter in common did not differ ($P < 0.05$)
ND — not detected.

Fatty acid	White sturgeon Sea Farm in California ($n = 3$)	White sturgeon domesticated Univ. California ($n = 2$)	White sturgeon wild Sacramento River ($n = 3$)	Lake sturgeon wild Wolf River ($n = 8$)
Saturated				
14:0	3.5 ± 0.3^{a}	3.1 ± 0.0^{ab}	1.9 ± 0.3^{b}	3.2 ± 0.6^{a}
16:0	13.6 ± 0.5	13.8 ± 0.2	14.9 ± 1.5	14.2 ± 1.3
18:0	1.9 ± 0.1^{a}	1.8 ± 0.2^{ab}	1.4 ± 0.1^{bc}	1.3 ± 0.2^{c}
Monoenoic				
16:1$n-7$	5.6 ± 0.2^{a}	4.9 ± 0.2^{a}	6.4 ± 0.9^{a}	9.0 ± 1.1^{b}
18:1$n-9$	20.1 ± 1.0^{a}	21.5 ± 0.7^{ab}	27.1 ± 1.7^{b}	26.4 ± 2.8^{b}
20:1$n-9$	0.9 ± 0.1^{a}	0.8 ± 0.0^{a}	0.3 ± 0.0^{b}	0.5 ± 0.1^{c}
n − 6				
18:2$n-6$	2.5 ± 0.2^{a}	11.9 ± 1.9^{b}	0.3 ± 0.0^{c}	3.1 ± 0.4^{a}
20:4$n-6$	1.8 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.3 ± 0.5
22:4$n-6$	0.5 ± 0.0^{a}	0.4 ± 0.0^{ab}	0.6 ± 0.1^{a}	0.2 ± 0.1^{b}
22:5$n-6$	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.6 ± 0.1^{b}	0.2 ± 0.1^{a}
n − 3				
16:3$n-3$	0.1 ± 0.1	0.1 ± 0.1	ND	0.1 ± 0.1
16:4$n-3$	0.8 ± 0.0^{a}	0.6 ± 0.1^{a}	1.1 ± 0.1^{ab}	1.4 ± 0.2^{b}
18:3$n-3$	0.4 ± 0.0^{a}	1.1 ± 0.5^{ab}	0.2 ± 0.1^{a}	1.5 ± 0.4^{b}
18:4$n-3$	1.3 ± 0.3^{a}	1.0 ± 0.0^{a}	1.1 ± 0.2^{a}	0.5 ± 0.1^{b}
20:4$n-3$	0.6 ± 0.0^{a}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{b}	0.4 ± 0.1^{ab}
20:5$n-3$	5.5 ± 0.5^{a}	4.6 ± 0.5^{ab}	3.1 ± 0.8^{bc}	1.9 ± 0.6^{c}
22:5$n-3$	2.0 ± 0.1^{a}	1.6 ± 0.0^{ab}	1.1 ± 0.1^{bc}	0.8 ± 0.4^{c}
22:6$n-3$	10.7 ± 0.8^{a}	9.0 ± 0.3^{a}	9.2 ± 0.8^{a}	4.8 ± 1.3^{b}
SAFA	19.1 ± 0.2	18.7 ± 0.0	18.2 ± 1.6	18.6 ± 1.7
MUFA	26.6 ± 1.0^{a}	27.2 ± 0.5^{a}	33.8 ± 2.3^{ab}	35.9 ± 3.7^{b}
PUFA	27.0 ± 0.5^{a}	33.9 ± 2.2^{b}	19.6 ± 1.4^{c}	17.1 ± 2.5^{c}
Total $n-6$	5.5 ± 0.2^{a}	15.1 ± 1.3^{b}	3.4 ± 0.2^{c}	5.3 ± 0.6^{a}
Total $n-3$	21.4 ± 0.6^{a}	18.6 ± 0.9^{ab}	16.2 ± 1.6^{b}	11.5 ± 2.2^{c}
$n-3/n-6$	3.9 ± 0.2^{a}	1.2 ± 0.1^{c}	4.8 ± 0.7^{a}	2.2 ± 0.4^{b}

tion's origin. The same was true for total $n-3$ fatty acids. 16:1$n-7$ was found at significantly ($P < 0.05$) higher levels in lake sturgeon egg neutral lipids than in white sturgeon. The opposite was the case for 22:6$n-3$, which was at half the level in lake sturgeon egg neutral lipids compared to white sturgeon. Other fatty acids such as 18:0, 18:1$n-9$ or 20:1$n-9$ were origin specific rather than species specific. 18:0 and 20:1$n-9$ were found at significantly lower levels in wild fish egg neutral lipids than in domesticated or farmed, whereas 18:1$n-9$ showed the opposite trend.

Consequently, higher levels of 16:1$n-7$ and 18:1$n-9$ in wild than in domesticated or farmed fish ova neutral lipids elevated total monounsaturated fatty acids (MUFAs) proportions. On the other hand, wild fish ova neutral lipids were characterized by

Table 2
Fatty acid composition of sturgeon roe phospholipids (weight %). Means within a row sharing a letter in common did not differ ($P < 0.05$)
ND — not detected.

Fatty acid	White sturgeon sea farm in California ($n = 3$)	White sturgeon domesticated Univ. California ($n = 2$)	White sturgeon wild Sacramento River ($n = 3$)	Lake sturgeon wild Wolf River ($n = 8$)
Saturated				
16:0	13.9 ± 0.6^{a}	13.3 ± 0.0^{ab}	13.3 ± 0.9^{ab}	12.1 ± 0.6^{b}
18:0	5.3 ± 0.1^{a}	4.6 ± 0.5^{ab}	3.5 ± 0.1^{b}	4.7 ± 0.6^{a}
Monoenoic				
16:1$n-7$	0.9 ± 0.1^{a}	1.2 ± 0.0^{ab}	1.4 ± 0.5^{ab}	1.4 ± 0.2^{b}
18:1$n-9$	10.5 ± 0.5	10.3 ± 0.5	11.4 ± 0.7	11.7 ± 0.7
20:1$n-9$	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
n − 6				
18:2$n-6$	0.6 ± 0.1^{ab}	2.5 ± 0.6^{c}	ND^{b}	1.3 ± 0.5^{a}
20:4$n-6$	3.8 ± 0.2	3.9 ± 0.1	3.8 ± 0.3	4.7 ± 0.8
22:4$n-6$	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.3
22:5$n-6$	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.2	0.4 ± 0.3
n − 3				
18:3$n-3$	ND	ND	ND	ND
18:4$n-3$	0.9 ± 0.2	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.2
20:4$n-3$	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.1^{a}	0.5 ± 0.2^{b}
20:5$n-3$	6.7 ± 0.2^{a}	6.0 ± 0.7^{ab}	4.2 ± 1.1^{b}	4.2 ± 0.7^{b}
22:5$n-3$	1.6 ± 0.5	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.5
22:6$n-3$	15.1 ± 0.3	13.8 ± 0.4	15.2 ± 0.9	12.5 ± 2.3
SAFA	19.2 ± 0.5^{a}	17.9 ± 0.5^{ab}	16.8 ± 0.8^{b}	16.8 ± 0.9^{b}
MUFA	11.5 ± 0.4^{a}	11.6 ± 0.6^{a}	12.9 ± 0.3^{ab}	13.2 ± 0.7^{b}
PUFA	29.9 ± 0.3	29.5 ± 1.2	26.7 ± 1.6	27.6 ± 2.8
Total $n-6$	5.4 ± 0.2^{a}	7.6 ± 0.7^{ab}	5.3 ± 0.5^{a}	8.2 ± 1.0^{b}
Total $n-3$	24.5 ± 0.2^{a}	22.0 ± 0.6^{ab}	21.4 ± 2.1^{ab}	19.4 ± 2.6^{b}
$n-3/n-6$	4.5 ± 0.4^{a}	2.9 ± 0.2^{bc}	4.1 ± 0.8^{ac}	2.4 ± 0.4^{b}

significantly lower proportions of total polyunsatureted fatty acids (PUFAs) compared to domesticated or farmed fish. It was mainly due to the fact that neutral lipids of domesticated white sturgeon eggs from University of California contained almost 12% of linoleic acid (18:2$n-6$), which was approximately fourfold higher then in farmed white sturgeon (2.5%) or wild lake sturgeon (3.1%). Furthermore, only 0.3% of this tatty acid was found in egg neutral lipids of wild white sturgeon eggs from Sacramento River. Lower levels of eicosapentaenoic acid (20:5$n-3$, EPA) and docosahexaenoic acid (22:6$n-3$, DHA) in neutral lipids of eggs from wild fish contributed also to overall higher PUFAs proportions in domesticated and farmed fish compared to the wild ones.

Phospholipid fatty acids were much less variable between species and among populations. The most notable was the difference in linoleic acid (18:2$n-6$) levels. It was found at $2.5 \pm 0.6\%$ in phospholipids of white sturgeon eggs from the University of

California domesticated population, whereas in other analyzed groups this fatty acid was either at significantly ($P < 0.05$) lower levels or below detection level. EPA was at a significantly lower level in phospholipids of wild fish eggs than in domesticated or farmed ones, however, it was not a species specific characteristic.

In an attempt to use fatty acid profiles of sturgeon eggs as a tool to identify and distinguish the studied species and populations we performed stepwise discriminant analysis. However, no conclusive answer was found.

4. Discussion

We found sturgeon eggs to contain rather high levels of total lipids compared to teleost fish like roach (*Rutilus rutilus*) or perch (*Perca fluviatilis*), in which lipid egg content is less than 5% (Kaitaranta and Ackman, 1981). Similarly high values of egg total lipids were reported, however, for other fish species, such as whitefish (*Coregonus lavaretus*) (Kaitaranta, 1980) and walleye (*Stizostedion vitreum*) (Czesny and Dabrowski, 1998). Phospholipid content of sturgeon eggs were similar among studied populations indicating the more conservative and essential character of this fraction of lipids compared to neutral lipids. Gallagher et al. (1998) demonstrated that the eggs of wild striped bass (from Shubenacadie River) had a higher neutral lipid and significantly lower polar lipid content than did eggs from cultured population. However, this was not the case in domesticated walleye population which had lower phospholipid level in their eggs compared to the wild stocks (Czesny and Dabrowski, 1998). Notwithstanding, it appears that phospholipids, as structural units needed during embryogenesis, are accumulated and conserved during vitellogenesis to a greater extent than neutral lipids.

The most dominant SAFA in both fractions of lipids was palmitic acid, 16:0. This fatty acid is the most plentiful SAFA in the eggs and other tissues of most fish species (Ashton et al., 1993; Harrell and Woods, 1995; Silversand et al., 1996; Czesny and Dabrowski, 1998). Muscle of Gulf sturgeon (*A. oxyrinchus desotoi*) contained large quantities of palmitic acid, where it contributed over 60% of total SAFA (Chen et al., 1995). Moreover, the same authors reported that most saturated fatty acids in the muscle of Gulf sturgeon were at higher levels in cultured fish compared to wild ones. Although this trend was not apparent in neutral lipids of white sturgeon eggs analyzed in our study, SAFA were at a significantly higher level in phospholipids of white sturgeon from California, compared to wild conspecifics from Sacramento River.

We found the amounts of total monoenes in neutral and phospholipids to be higher in wild than in cultured fish egg lipids. The same tendency was also found in the total lipids of ovulated eggs of wild and cultured turbot (Silversand et al., 1996). Neutral lipids of walleye eggs contained significantly higher levels of MUFAs in a wild population (Lake Erie) compared to domesticated broodstock (London State Fish Hatchery, London, OH), whereas the opposite was true for phospholipids where MUFA were lower in wild population (Salt Fork Reservoir) than in domestic stock (Czesny and Dabrowski, 1998). However, studies on fatty acid composition of chinook salmon (*Oncorhynchus tshawytscha*) eggs shown the opposite (Ashton et al., 1993). Chinook

stocks from Big Qualicum and Robertson Creek had higher proportions of monoenes compared to cultured conspecifics.

The pattern of differences in MUFA content in the lipids appears to be species and tissue specific since contrary to eggs lipids of sturgeon (this study) or turbot, MUFA in muscle lipids of turbot were found at higher levels in farmed fish than in wild ones (Sérot et al., 1998). Noteworthy is the species specific and origin specific pattern of individual monoenes concentrations. 16:1$n-7$ was almost twice as high in lake sturgeon egg neutral lipids compared to white sturgeon, while no differences were observed in regard to the origin of the latter. This fatty acid was at similarly high concentrations in both wild and domesticated white sturgeon eggs indicating its high levels in their diets. More interestingly, 18:1$n-9$ was found at higher levels in both wild populations of white and lake sturgeon than in both domestic white sturgeon populations. It is indicative of an origin specific pattern rather then species specific, implying that 18:1$n-9$ is likely to be at higher levels in natural diets of both species of sturgeon compared to commercial diets used in culture conditions.

The amount of 18:2$n-6$ was much greater in domesticated white sturgeon egg neutral lipids from UCD than in any other population studied. This is in agreement with data reported for other fish species (Chen et al., 1995; Harrel and Woods, 1995; Silversand et al., 1996). It is a commonly observed phenomenon since most commercial salmonid diets, used to raise sturgeon in captivity, are rich in linoleic acid. In many cases this single fatty acid, at a very elevated level can be an indicator of the origin of sturgeon eggs. In some cases, however, even though fish are in commercial farms they may be fed a fish oil based diet and then the level of linoleic acid in their eggs will stay at the level characteristic to wild fish (Table 1).

The changes in relative concentrations of individual fatty acids among fish populations from different environments affect proportions of $n-3$ and $n-6$ fatty acid families. The eggs of wild chinook salmon contained significantly more of $n-3$ fatty acids in total lipids and in phospholipids than these of cultured fish. Consequently, the ratio between $n-3$ and $n-6$ fatty acids in neutral and phospholipids was higher in wild chinooks (Ashton et al. 1993). The opposite was true for macquarie perch (*Macquaria australasica*) where $n-3/n-6$ ratio was lower in the lipids of mature oocytes of wild fish compared to captive ones (SheikhEldin et al., 1996). In this case, the lower levels of $n-6$ fatty acids in oocytes from tank-reared fish contributed to this difference. The effect of maternal diet with respect to $n-3/n-6$ ratio was very profound in our study. Neutral lipids of white sturgeon from Sea Farm contained the highest level of $n-3$ FAs but very low level of $n-6$ FAs, whereas these of wild lake sturgeon exhibited the opposite trend. Consequently, the former had a $n-3/n-6$ ratio of 4.5 while the latter had only 2.4.

There are two major points that arise from this present work. One point is directly relevant to the study of ''marine and freshwater'' lipids extending our knowledge concerning the distinction in their PUFA contents (Morris, 1984). PUFAs are dominated by the $n-3$ series in tissues of marine fish, although the response to the temperature, known as ''homeoviscous adaptation'', in phospholipid fraction may not only compensate for compositional differences, but lead to the identity of thermal environments (Dey et al., 1993). Because sturgeon maturation (gametogenesis) can be completed entirely in

marine or freshwater environments, a bridge between the dietary pool and ovarian fatty acid composition must reflect either ''marine'' or ''freshwater'' characteristics. Linoleic and linolenic acids in both fractions illustrated predominance of those fatty acids in freshwater food chains (Henderson and Tocher, 1987), whereas polyunsaturate levels also reflect the metabolic requirement for long chain $n-3$ PUFA.

The second point is relevant to the distinction of the domesticated (cultured) and wild fish based on their ova fatty acids. Wild and cultured Gulf of Mexico sturgeon can be discriminated based on fatty acid profiles of their flesh (Chen et al., 1995). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) were also used to identify sturgeon caviar with respect to its origin (Chen et al., 1996). Egg lipid profiles of domesticated and wild marine fish, striped bass, were significantly different and the $n-3/n-6$ ratio (10.99 and 1.27, respectively) was sufficient to discriminate the origin of the females (Harrel and Woods, 1995). Our results complement the discriminatory power of the PCR method to identify sturgeon species ova. Our results add important characteristics of egg biochemical composition to identify sturgeon species ova. In that way, the conservation of wild stocks can be reinforced in parallel to the enhancement of stocks with fish produced in an aquaculture setting. Farmed sturgeon can substitute for wild sturgeon caviar. We realize that this approach may not satisfy opponents of the ''use it or lose it'' (Wells, 1997) philosophy, but the sturgeon survival or disappearance is now only a matter of decades.

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