Artificial reproduction of sturgeon: the correlation between the lipid composition of caviar and it fish breeding quality

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Abstract. In our research we study of the correlation between the biochemical composition of unfertilized sturgeon caviar and the results of its incubation. We studies besters (bester is a hybrid of beluga and sterlet) and stellate sturgeon caviar. We find out that for both bester and stellate sturgeon, the dependence of the fish breeding quality of eggs on the lipid composition was identical. It was shown that the lipid composition of caviar has the greatest influence on the fish breeding qualities of caviar. Also it was shown that such biochemical characteristics of the caviar as protein level does not correlate with fish breeding quality of the caviar. The recommendations to use the caviar with high level of the ratio phospholipids/total lipids and high level of phosphatidylcholine and low level of monoacylglycerols, diacylglycides or lysophosphatidylcholines for artificial reproduction were made.

1 Introduction

Sturgeon fish is one of the most valuable biological resource of Sea of Azov. The age of Azov sturgeon populations is estimated at millions of years. This indicates their protection in interspecific competition. However, the population of this rare fish decreases greatly [1-2]. There are several reasons of this fact. They are: poaching, water pollution, and so on. But according our point of view the main reason of this phenomenon is decreasing of natural reproduction. The traditional spawning grounds of beluga and stellate sturgeon were on the upper Don. The Tsimlyansk dam was built in 1952, and it was the reason of the loss of these spawning grounds. So, the catch of sturgeon fish at the Sea of Azov after 1952 decreases considerably.

For example, in 1925-1941, catches averaged about 3 thousand tons of sturgeon per year (the largest catch was in 1937 - 7270 tons). In 1944-1954 catches amounted to about 1.5-2.5 thousand tons per year.

After Tsimlyansk dam appearance there was a decrease in sturgeon catch from 1200 tons in 1956 to 500 tons in 1969. The problem became more actual in 1968, the Fedorovskaya dam was built on the Kuban River. It is necessary to add, that modern

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approaches in hydro energetics could avoid the problem of spawning grounds degradation [3-4].

So, artificial reproduction is used for save the population of sturgeon fish in the Sea of Azov. Artificial reproduction requires caviar of high fish breeding quality [5-10]. Especially it is actual for sturgeon caviar, because sturgeon caviar with bad fish breeding quality could be used for food purposes.

But in resent years the fish breeding quality decreases. This problem is actual both for wild sturgeon fish (for example for stellate sturgeon caught in natural reservoirs) and "domestic" sturgeon fish (bester from broodstock kept at fish hatcheries).

The aim of our research was to find out the way to predict fish breeding quality of the caviar.

2 Method of research

The object of research was the caviar of stellate sturgeon caught in natural reservoirs and bester from broodstock kept at fish hatcheries.

We studied eggs at the 4th stage of development (2nd division) and at the hatching of prelarvae. In bester, the results of egg development were additionally assessed at stages 17-18 and 25-26.

Fertilization of eggs was carried out using a semi-dry method. In this case, only good quality sperm was used. The eggs were incubated in apparatuses "Osetr", produced in Russia. Eggs from each female were placed in a separate box of the incubator in an amount of no more than 1.5 kg. During the incubation period, regular hydrochemical monitoring was carried out according to the following indicators: water temperature, pH, oxygen content dissolved in water and biogenic composition, permanganate and dichromate oxidizability.

General chemical analysis of the caviar was carried out using traditional methods. They included the determination of moisture - by drying at $T = 105^0C$, crude protein - by colorimetric determination of nitrogen, ash - by burning the test material in a muffle furnace at a temperature of 500^0 C, fat - by extracting lipids from biosamples using Soxhlet method.

When studying various lipid fractions, they were extracted using the Folch method.

Thin layer chromatography was used to separate lipids into classes.

In the experiments, caviar from 14 female sturgeon and 28 bester was used.

Statistical processing of the results, correlation, regression and cluster analyzes were carried out using the Statistica 5.5 and Origin 6.1 programs.

3 Results and discussion

According to correlation analysis data, the results of incubation largely depend on some indicators of the lipid composition of unfertilized eggs. Thus, there is a strong positive relationship between the fertilization of eggs and the ratio of phospholipids/total lipids, as well as a medium strength relationship with the content of phosphatidylcholines and phospholipids (positive), di- and monoacylglycerols (negative) in unfertilized eggs.

The final result of incubation (the release of prelarvae) is significantly influenced by the level of some lipids in unfertilized eggs. Strong relationships were noted with the content of phosphatidylcholines (positive), diacylglycerols and lysophosphatidylcholines (negative).

The results of the correlation analysis for stellate sturgeon caviar are presented at Table 1 and Table 2.

Table 1. The correlation between fertilization and lipid composition of unfertilized stellate sturgeon caviar.

Lipid compound	Correlation coefficient
phospholipids/total lipids	0.806
Phospholipids	0.659
Phosphatidylcholine	0.691
Lysophosphatidylcholine	—0.317
Phosphatidylethanolamine	—0.303
Sphingomyelin	—0.321
Phosphatidylserine	0.240
Neutral lipids	—0.319
Monoacylglycerols	—0.629
Diacylglycerols	—0.684
Triacylglycerols	0.386
Cholesterol	—0.211
Cholesterol esters	0.495
Free fatty acids	—0.422

Table 2. The correlation between the release of prelarvae and the lipid composition of unfertilized stellate sturgeon caviar.

Lipid compound	Correlation coefficient
phospholipids/total lipids	0.617
Phospholipids	0.442
Phosphatidylcholine	0.716
Lysophosphatidylcholine	—0.692
Phosphatidylethanolamine	—0.632
Sphingomyelin	—0.399
Phosphatidylserine	0.493
Neutral lipids	—0.135
Monoacylglycerols	—0.689
Diacylglycerols	—0.787
Triacylglycerols	0.661
Cholesterol	—0.401
Cholesterol esters	0.658
Free fatty acids	—0.532

The results of correlation analysis for bester are presented at Tables 3 and 4.

Table 3. The correlation between fertilization and lipid composition of unfertilized bester caviar

Lipid compound	Correlation coefficient
phospholipids/total lipids	0.71
Phospholipids	0.63
Phosphatidylcholine	0.69
Lysophosphatidylcholine	—0.39
Phosphatidylethanolamine	—0.13
Sphingomyelin	—0.34
Phosphatidylserine	0.23
Neutral lipids	—0.32
Monoacylglycerols	—0.63
Diacylglycerols	—0.71
Triacylglycerols	0.32
Cholesterol	—0.2
Cholesterol esters	0.51
Free fatty acids	—0.38

Table 4. The correlation between the release of prelarvae and the lipid composition of unfertilized bester caviar.

Lipid compound	Correlation coefficient
phospholipids/total lipids	0.64
Phospholipids	0.47
Phosphatidylcholine	0.76
Lysophosphatidylcholine	—0.72
Phosphatidylethanolamine	—0.43
Sphingomyelin	—0.22
Phosphatidylserine	0.42
Neutral lipids	—0.22
Monoacylglycerols	—0.71
Diacylglycerols	—0.78
Triacylglycerols	0.66
Cholesterol	—0.21
Cholesterol esters	0.73
Free fatty acids	—0.55

As for another biochemical components, we does not find considerable correlation between protein level in the caviar and it fish breeding quality.

The acquired data can be interpreted as follows:

Cholesterol esters serve as a reserve of free cholesterol, which has been shown in existing literature to possess the ability to counteract bacterial and parasitic hematoxins. An elevation in non-esterified fatty acids is a highly dynamic indicator of lipid metabolism status, signifying the presence of stressors affecting the body. Excessive free fatty acids

play a pathogenic role in metabolic disturbances as they readily combine with various proteins, including enzymes. Elevated levels of non-esterified fatty acids within cells lead to the disruption of tissue respiration processes and oxidative phosphorylation uncoupling.

Simultaneously increased concentrations of lysophosphatidylcholine and diacylglycerols create a synergistic effect. These compounds act as activators of protein kinase C, collectively prolonging the activity of this kinase [11]. Activation of protein kinase C results in reduced concentrations of certain substances, notably phosphatidylcholine. Furthermore, protein kinase C stimulates enzymes, disrupting the synthesis of basement membrane components and altering vascular wall permeability.

It should be noted that alterations in phospholipid composition, such as a decline in major phospholipid fractions, particularly phosphatidylethanolamine, along with an increase in lysophosphatidylcholine, monoacylglycerols, and diacylglycerols, are associated with radiation damage (both acute and chronic) and are indicative of oxidative stress responses according to some researchers [12]. We find out the same changes of lipid compound in the caviar with bad breeding quality.

Phosphatidylcholine serves as an activator for natural antioxidants, specifically tocopherol. A decrease in phosphatidylch concentration within the plasma membrane is likely to disrupt its interaction with antioxidants, leading to increased oxygen species and subsequent lipid peroxidation. This process results in elevated membrane viscosity caused by reduced liquid hydroph lipids in bilayer sections, the formation of intermolecular cross-links, and increase in ordered lipids with restricted mobility within the bilayer. Additionally, it leads to a rise in negative on the membrane surface due to secondary products of lipid peroxidation containing carbonyl carboxyl groups.

Biological membranes (cell membranes, mitochondria, endoplasmic reticulum, and lysosomes) demonstrate induced permeability to various ions, non-electrolytes, and macromolecules as a consequence of lipid peroxidation. Furthermore, properties of membrane proteins such as Ca^{+2}-ATPase transporters, Na^{+}/K^{+} ATPase pumps cytochromes P-460 enzymes,and phospholipases undergo alterations due to peroxidative damage.

However, lipid peroxidation is not the most dangerous consequence of the activity of antioxidants decreasing and an increase in reactive oxygen species concentration. To neutralize toxic forms of oxygen, organisms have developed various protective mechanisms, which can be divided into several types. The first type of defense systems is based on the activity of special enzymes, for which the decomposition of reactive oxygen species is the main and, in some cases, the only function. In the second type of defense systems, cellular metabolites are used to destroy toxic forms of oxygen, which, as a rule, usually perform other functions in the cell. The third defense mechanism involves the destruction of the source of toxic forms of oxygen itself, by launching a self-destruction program (apoptosis) in the cell. It is likely that evolution create this mechanism for other purposes, but it is useful for reactive oxygen species detoxification.

Considering the exceptional danger of reactive oxygen species, in the course of evolution a more radical and effective way to combat this evil has been developed. This mechanism involves destroying the source of reactive oxygen species itself.

Therefore, in cells that “felt” an increased content of reactive oxygen species in the environment, the mechanism of programmed cell death or apoptosis is triggered.

The complex mechanics of life in multicellular organisms includes not only the developmental processes of growth and reproduction, but also mechanisms that cause cells to instantly cease to exist. This phenomenon, when entire groups of cells, or even entire organs, die in response to an invisible command, is called apoptosis.

Cells that produce hydroxyl radicals not only damage themselves, but also become dangerous to surrounding tissue. Hence, the elimination of cells responsible for generating reactive oxygen species and their immediate microenvironment is imperative.

Consequently, an upsurge in hydroxyl radical formation should trigger a cascade leading to the programmed cell death (apoptosis) in hydroxyl radical-producing cells and their neighboring counterparts. Mitochondria exhibit heightened susceptibility to elevated levels of reactive oxygen species. Subcellular processes akin to apoptosis also operate at the mitochondrial level. The degeneration of mitochondria during reticulocyte maturation into erythrocytes, induced by mitochondrial lipoxygenase activation, exemplifies programmed mitochondrial demise, termed "mitoptosis." Analogous to apoptosis, this phenomenon involves the opening of pores in the inner mitochondrial membrane to purge the mitochondrial population of those generating substantial amounts of reactive oxygen species. Research has demonstrated [13] that heightened reactive oxygen species concentrations induce mitochondrial demise, irrespective of whether these species result from mitochondrial metabolic activity or other sources.

So, literature data allows as to make a hypothesis that the caviar with low breeding quality contains more reactive oxygen species than the caviar with high breeding quality. In this case it is possible to separate “breeding” caviar from “food” caviar using electronic paramagnetic resonance. If this assumption will find experimental confirmation, the time of caviar analysis will decrease greatly.

4 Conclusions

- The lipid composition of unfertilized stellate sturgeon and bester caviar determines its fish-breeding qualities.
- The percentage of fertilization of stellate sturgeon eggs is directly proportional to the ratio of phospholipids/total lipids, and the percentage of prelarvae yield is directly proportional to the level of phosphatidylcholines and inversely proportional to the level of diacylglycerols and lysophosphatidylcholines.
- The percentage of fertilization of bester eggs is directly proportional to the ratio of phospholipids/total lipids and phosphatidylcholine level, and the percentage of prelarvae yield is directly proportional to the level of phosphatidylcholines and inversely proportional to the level of diacylglycerols and lysophosphatidylcholines.

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