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Lipid composition of oocytes and tissues of sturgeons depending on conditions of detention

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Abstract. Shortage of sturgeon spawners has led to the need for reservation and long-term detention of spawners, creation of their own brood stocks. The issues of assessing the functional state of producers and the reproductive products and offspring become topical. The wintering conditions of fish in cages at the natural course of temperatures negatively affect their physiological state. During this period, the fish actively move in the cages, but do not feed. The detention of sturgeon spawners in the pools with regulating the parameters of the aquatic environment makes it possible to stabilize the cycle of maturation of the gonads and increase the number of fish-producing females. The lipid composition of caviar affects its fish-breeding quality. The main components of sturgeon caviar fats are triglycerides and lipid substances, which are represented by phospholipids that are actively involved in generative metabolism during the maturation of gonads. The main fraction of oocyte lipids is represented by triglycerides, whose level in the oocytes of fish from recirculating water installation (RWI) is higher in comparison with cage fish. It was found that sterlet oocyte lipids are mainly represented by triglycerides. Their concentration in the oocytes of fish kept in RWI is higher in comparison with fish kept in cages. Against the higher amount of triglycerides in fish roe from RWI, a decrease in the level of phospholipids and cholesterol was noted. The aging conditions of producers affect the composition of the main lipid fractions. As a result of the research, a relationship was established between the content of polyunsaturated fatty acids in the tissues of aquatic organisms and environmental conditions. The amount of saturated and monounsaturated fatty acids in the muscles of fish kept in RWI was higher than in the tissues of fish kept in cages. Increasing proportion of polyunsaturated fatty acids in the eggs of fish kept in cages indicates a fairly high utilization of monounsaturated fatty acids.

Keywords: spawners, sterlet, oocytes, reproduction, lipid status, detention, rearing

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Научная статья

Липидный состав ооцитов и тканей осетровых рыб в зависимости от условий содержания

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Аннотация. Дефицит производителей осетровых рыб привел к необходимости резервирования и длительного содержания производителей, создания собственных маточных стад. Актуальными становятся вопросы оценки функционального состояния производителей и полученных от них половых продуктов и потомства. Условия зимовки рыб в садках при естественном ходе температур негативно отражаются на физиологическом состоянии рыб. В этот период рыба активно двигается в садках, однако не питается. Содержание производителей осетровых рыб в бассейнах при регулировании параметров водной среды позволяет стабилизировать цикл созревания половых желез и увеличить количество рыбоводно-продуктивных самок. Липидный состав икры оказывает влияние на ее рыбоводные качества. Основными компонентами жиров икры осетровых рыб являются триглицериды и липоидные вещества, которые представлены фосфолипидами, принимающими активное участие в генеративном обмене при созревании гонад. Основная фракция липидов ооцитов представлена триглицеридами, уровень которых в ооцитах рыб из установки замкнутого водоснабжения (УЗВ) выше в сравнении с рыбами садкового содержания. Выявлено, что липиды ооцитов стерляди представлены в основном триглицеридами. Их концентрация в ооцитах рыб, содержащихся в УЗВ, выше в сравнении с рыбами садкового содержания. На фоне более высокого количества триглицеридов в икре рыб из УЗВ отмечено снижение уровня фосфолипидов и холестерина. Условия выдерживания производителей влияют на содержание основных фракций липидов. В результате исследований установлена связь между содержанием полиненасыщенных жирных кислот в тканях гидробионтов и условиями внешней среды. В мышцах рыб, содержащихся в УЗВ, количество насыщенных и мононенасыщенных жирных кислот было выше, чем в тканях рыб, содержащихся в садках. Увеличение доли полиненасыщенных жирных кислот в икре рыб, содержащихся в условиях садков, свидетельствует о достаточно высокой утилизации мононенасыщенных жирных кислот.

Ключевые слова: производители, стерлядь, ооциты, воспроизводство, липидный статус, содержание, выращивание

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Introduction

Currently, the sturgeon population of the Volga-Caspian Sea is in critical condition. The moratorium introduced in Russia on the commercial fishing of sturgeon in the Caspian basin is gradually undergoing the remaining part of the population, the artificial reproduction of species that allow the production of its stocks. Hatchery juveniles, released in the natural habitat, obtained from broodstocks formed at the enterprise and kept for a long time in aggressive conditions under the constant influence of stress factors.

An unstable hydrochemical regime, an unbalanced diet, and high stocking densities have a negative impact on the physiological state of breeders and, subsequently, on their offspring (Geraskin et al., 2004) [1]. The most pronounced changes are observed in biochemical parameters, including in the lipid composition of gonads and oocytes (Mizenko, 1979; Shatunovsky, 1980) [2, 3].

Oocyte lipids have a higher degree of unsaturation than fats in muscles and other fish tissues. Polyunsaturated fatty acids in the body of fish are involved in the adaptation of the body to environmental conditions, are involved in the synthesis of endohormones that regulate the processes of growth, maturation, and reproduction (Ostroumova, 2012) [4]. In this regard, the study of the lipid and fatty acid composition of sturgeon oocytes under various conditions of keeping

will allow us to assess the state of spawners, the quality of offspring, and correct the process of feeding and keeping them.

Material and methods of research

The research was carried out for a number of years at the fish-breeding enterprises of the Astrakhan region during the period of the fish-breeding campaign and in the laboratories under Astrakhan State Technical University. The objective of the research was to analyze unfertilized oocytes obtained from starlet females stimulated with gonadotropic drugs. Oocytes were obtained by cutting the oviduct (Podushko, 1986) [5]. The lipid composition of oocytes and fish tissues was studied by conventional methods (Bligh and Dyer, 1959; Rzhavskaya, 1976; Alekseev et al., 1981) [6–8]. The fatty acid composition of lipids was determined by the thin layer chromatography.

Research findings and discussions

The tissues of sturgeons kept in fish cages are characterized by a high supply of phospholipids and triglycerides. Fish kept in closed water supply at a constant water temperature and their feeding activity were quite high. This provided a high rate of metabolic processes and, accordingly, the accumulation of lipids in the muscles (Fig. 1).

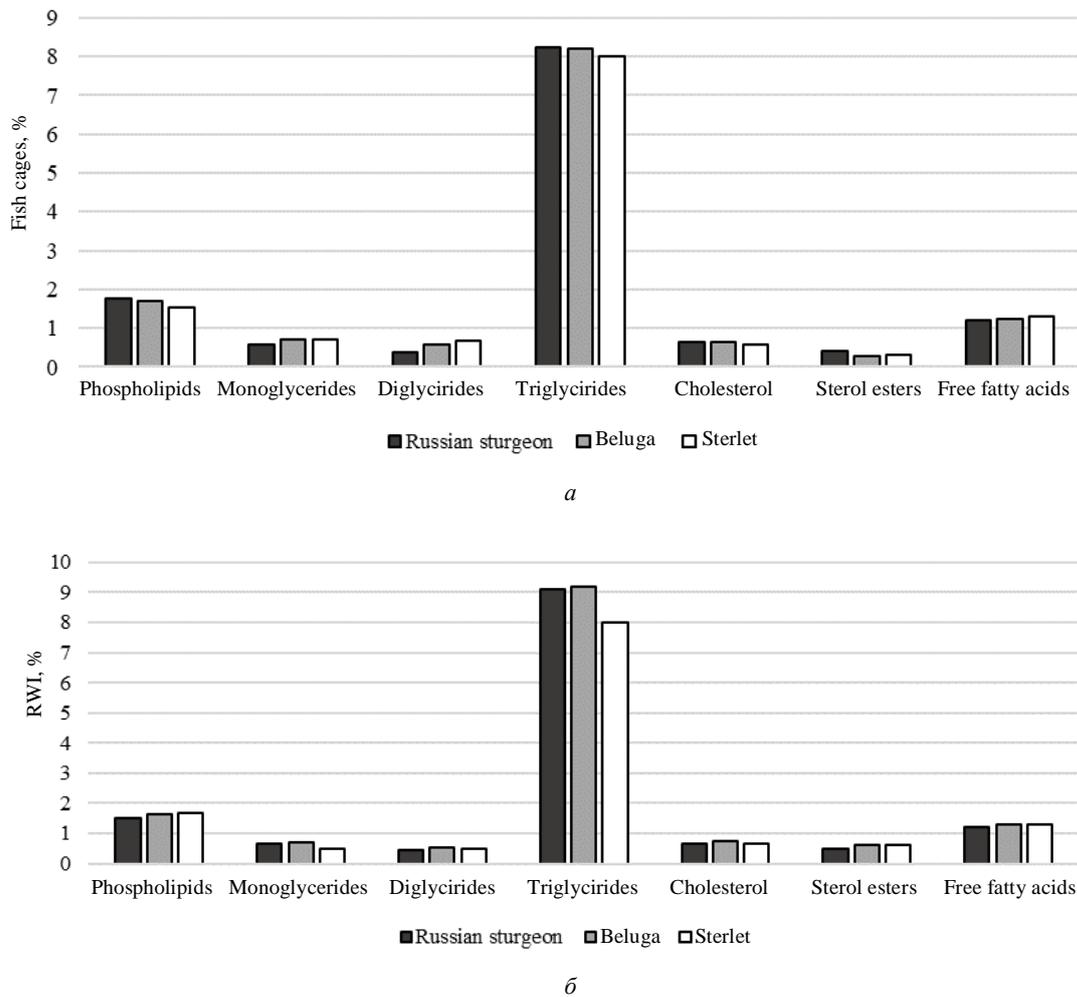


Fig. 1. The composition of lipids in the muscle tissue of sturgeon kept under various conditions, %:
 a – fish cages; b – recirculating water installation (RWI)

The research findings indicate that the lipids of sterlet's oocytes are mainly represented in the triglycerides. A similar situation is observed in other sturgeon species. The concentration of lipids in the oocytes of fish kept in a recirculating water installation (RAS) was $82.09 \pm 1.2\%$, while that of fish kept in cages was $64.57 \pm 1.13\%$ of the total lipids. On comparative basis, the concentration for fish kept in cages was slightly lower. Oocytes of Russian sturgeon females reared in RAS were characterized by the level of triglycerides $80.09 \pm 1.12\%$, beluga sturgeon 79.71 ± 1.11 . With cage keeping, a slight decrease was noted – by 2% in Russian sturgeon females and less than 1% in beluga.

Apart from higher amount of triglycerides in fish kept in RWI, a decrease in the level of phospholipids and cholesterol was observed. The opposite result was observed in the oocytes of starlet kept for a long time in unstable hydro chemical conditions - cages in a natural reservoir. The maintenance of the spawner under conditions of unstable temperature conditions led to an increase in the synthesis of phospholipids

and cholesterol in the case of a decrease in water temperature, or to digression with a short-term increase in temperature. Thus, phospholipids are used to maintain energy metabolism in fish kept in cages at different periods of the annual cycle (wintering, spawning, etc.) (Ostroumova, 2012) [4].

The muscle tissue of fish kept in recirculation plants was characterized by a large amount of saturated and monounsaturated fatty acids, in comparison with the tissues of cage reared fish. This is due to their use as an energy source. An increase in the content of polyunsaturated fatty acids in the muscles of fish from cages indicates adaptive expenditure and an increase in lipid fluidity due to an increase in double bonds and a decrease in fat saturation. During the maturation of reproductive products from muscle tissue, lipid transport occurs and some fatty acids do not accumulate in oocytes. The analysis of monounsaturated fatty acids showed their smaller amount in the tissues of cage fish. A high level of polyunsaturated fatty acids in such fish indicates their adaptive expenditure. The

fluidity of lipids increases due to light increase in double bonds and decrease in fat saturation.

The lipid status of fish is also affected by feeding. Thus, sterlet, which consumed only natural food,

was dominated by more than 20% polyunsaturated fatty acids. About 15% are essential acids of the $\omega 3$ class (Fig. 2).

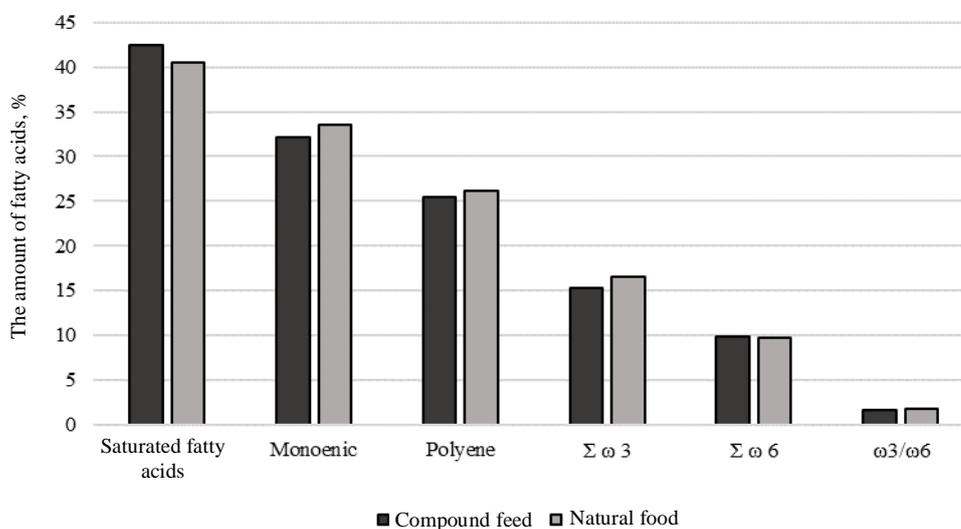


Fig. 2. The amount of fatty acids in the composition of total lipids of sterlet tissues, %

Comparing fish from the replacement group grown under industrial conditions and caught from the natural environment, it was revealed that in the total lipids of fish kept under temperature control conditions, the level of phospholipids was higher and amounted to 41.3%. In sterlet from the natural population, this value did not exceed 37.3%. The phospholipid fraction of sterlet grown from caviar was distinguished by a high content of fatty acids of the linolenic series – 17.4–18.5%.

In the RWI, starlet specimens were kept under conditions with slight changes in water temperature during winter-spring period (7–10 °C), while in the cages, water temperature dropped to 1–3 °C during the winter period. As a result of this temperature, food consumption for fish kept in RAS was quite high indicating a high rate of metabolic process, high energy required for movements and formation of reproductive systems. This intensive feeding led to the accumulation of lipid resources in the body.

On the contrary, for fish kept in cages, a decrease in water temperature led to use of more energy for metabolism process and formation of reproductive products, which, in turn, led to a decrease in the level of lipids in the body.

Thus, the earlier temperature condition subjected to fish affected the content of the main lipid fractions (phospholipids and triglycerides) which are actively involved in not only energy of metabolism process but also the reproductive systems. On the other hand, stable temperature condition for the gas regime in RAS did not affect the lipid metabolism of fish. Re-

serve fats and phospholipids were consumed in accordance with fish body requirements and for fish kept in cages, fat metabolism was more intense, which contributed to an increase in the proportion of phospholipids in the muscles.

Findings from the research indicate a close relationship between the content of polyunsaturated fatty acids in the tissues of aquatic organisms and environmental conditions. An increase in the content of polyunsaturated fatty acids in the muscles of fish from cages indicates more energy use and increase in lipid fluidity due to an increase in energy use during metabolism and reproductive processes and a decrease in saturated fats. During maturation of the gonads, lipids are transported from the muscles, while at the same time, some fatty acids are mobilized from the body, but do not accumulate in oocytes (Lovern, 1942, Malkolm Lav, 1976) and are probably consumed in the metabolic process [9, 10].

The amount of saturated and monounsaturated fatty acids in the muscles of fish kept in RWI was higher than in the tissues of fish kept in cages, which is due to their use of energy sources (Kreps, 1981; Shulman and Yuneva, 1990; Nordgarden et al., 2003; Weber et al., 2003; Murzina et al., 2012; Kalchenko et al., 2013) [11–16]. The fatty acid composition of lipids in fish oocytes kept under different conditions was somewhat different. The provision of oocytes obtained from females in RWI with monounsaturated fatty acids was higher than oocytes from fish kept in cages (Fig. 3).

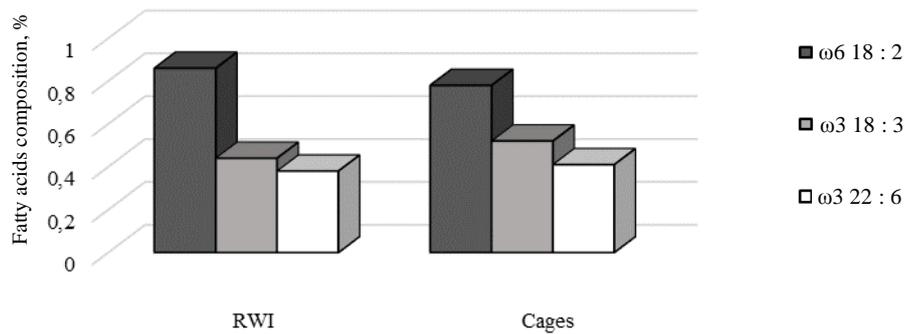


Fig. 3. Fatty acid composition of unfertilized sterlet oocytes contained in different conditions

This may be explained by the fact that monounsaturated fatty acids, having a high oxidation rate, were spent to a greater extent on maintaining energy needs and therefore they did not accumulate in the oocytes of fish from cages. In this case, the accumulation of polyene fatty acids (40.16-49.3% of the total fatty acids) was the most active.

Thus, the development of thermal adaptation in fish is largely associated with the degree of lipid unsaturation (Kreps, 1981; Sidorov, 1983) [11, 17]. An increase in the proportion of polyunsaturated fatty acids in the eggs of fish kept in cages indicates a fairly high utilization of monounsaturated fatty acids. Of the polyene

fatty acids, an important role in metabolism is played by docosahexaenoic acid (22:6ω3) which is most sensitive to changes in the physiological state. The 22:6ω3 is synthesized from 20:5 ω3 (eicosapentaenoic acid), which is involved in reproductive function.

A high level of saturated and unsaturated C₁₈ fatty acids in oocytes is noted compared to low concentration of C₁₆ fatty acids. Of the ω9 fatty acids, oleic acid (18:1ω9) predominated, providing the permeability of cell membranes and being a powerful internal antioxidant. The ratio of essential fatty acids in the oocytes of both groups (RWI and cages) was approximately the same as indicated (Table).

The level of fatty acids in the oocytes of sturgeons kept under various conditions

Fatty acid	RWI, %			Fish cages, %		
	Sterlet	Russian turgeon	Beluga	Sterlet	Russian sturgeon	Beluga
Palmitic	1.41	1.12	1.65	1.61	1.23	2.10
Stearic	1.12	1.27	1.45	1.32	1.31	1.87
Oleic	2.12	1.92	1.5	1.86	1.96	1.92
Linoleic	0.31	0.63	0.4	0.63	0.86	0.78
Linolenic	0.46	0.44	0.34	0.64	0.52	0.66
Arachidonic	0.58	0.64	0.50	0.98	0.91	0.62
Eicosapentaenoic	0.23	0.08	0.12	0.61	0.52	0.31
Docosahexaenoic	0.41	0.38	0.2	2.15	1.25	0.9

However, the influence of environmental factors in cage conditions contributed to a higher accumulation of docosahexaenoic acid in oocytes. The proportion of 22:6ω3 fatty acid in this case was 12.78% of the total fatty acids. In oocytes of fish kept in RWI, these indicators were 3-5 times lower. Research findings indicate the special role of docosahexaenoic acid in the adaptation of fish to environmental conditions. Its accumulation in tissues with a negative change in environmental factors indicates an increase in the intensity of lipid metabolism.

The precursor of prostaglandins involved in the reproductive activity of the organism is arachidonic fatty acid (20:4ω6). During maturation of the gonads, its concentration in oocytes was higher than in tissues. Unfavorable conditions during the winter, negatively affected its synthesis of arachidonic fatty acid (20:4ω6) in the body of spawners kept in cages. In the oocytes of females kept in cages, the proportion of 20:4ω6 acid was 6.54% of the total fatty acids, while it

was higher by 1.73% in the oocytes of females kept in RAS. Fish breeding and biological indicators of fish with low levels of arachidonic acid were low. The number of fish that matured in cages was 76.38%, maturation was more effective in RAS – 84.83%, with a high percentage of fertilization – 87.38%. The level of fatty acids in the oocytes of sturgeons kept under various conditions, %.

Conclusion

A decrease in water temperature when keeping sturgeon spawners in the pre-spawning period stimulates active synthesis and accumulation of docosahexaenoic (22:6ω3) fatty acid in the body. The level of arachidonic (20:4 ω6) acid in oocytes decreases by 1.5 times, which negatively affects the fish-breeding and productive state of Russian sturgeon females by 8.5%, beluga - by 12.7%, sterlet - by 7.5%. The percentage of fertilization is reduced by 4-17% in various sturgeon species.

From the analysis of the findings, it should be noted that the environment of keeping spawners affect the state of lipid metabolism. In one case, they stimulate the synthesis of polyene fatty acids, in particular, do-

cosahexaenoic ω 3 fatty acid, which is responsible for the adaptive abilities of the body, while on the other hand, they inhibit the arachidonic ω 6 fatty acid necessary for ovulation.

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