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UDC 69.25.99 **Research article**

Intoxication by nitrogen compounds in sturgeon juveniles grown in recirculation aquaculture system

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Abstract

Background and Aim. Studies conducted in recirculation aquaculture system (RAS) show that the immunity of juvenile sturgeon can vary significantly depending on several factors, including water quality, housing conditions, nutrition, and stress levels. Juvenile sturgeon are more vulnerable to disease and intoxication than adults, and their immune systems are still developing. The aim of this research was to study the intoxication of juvenile sturgeon by nitrogenous compounds, such as ammonia, nitrites and nitrates, which are formed during fish metabolism and pose a significant threat.

Materials and Methods. Materials for the study included juvenile sturgeon grown in a RAS (*Acipenser baerii* and *Acipenser Gueldenstaedtii*) and recycled water samples from ponds 3, 1 and 8 where the juveniles were reared. During the study, the following methods were used: clinical examination of individuals exhibiting physiological deviations, pathoanatomic autopsy of dead sturgeon specimens, and toxicological analysis of pathological material and recycled water using ultrasound, with results compared to nominal indicators.

Results. The hydrochemical analysis of recycled water in the RAS, revealed a slight deviation from optimal values, although levels remained within maximum permissible concentration. However, juvenile sturgeon can be poisoned even at these levels, because their bodies are very sensitive to minimal changes in the hydrochemical parameters of the water. This was confirmed by the results of clinical examinations, and pathoanatomical autopsies.

Conclusion. Sturgeon are more susceptible to poisoning early in their development because their detoxification systems are less efficient. Water control problems in RAS can be exacerbated by the accumulation of ammonia and nitrite concentrations to toxic levels. Therefore, it is important to closely monitor the recirculating water in RAS.

Keywords: sturgeon; intoxication; nitrogen compounds; RAS; nitrification.

Introduction

Recirculation aquaculture systems play a key role in fisheries by providing stable conditions for fish growth and development. However, high stocking densities and limited water resources, combined with increased pollution levels and inadequate filtration systems, can lead to the accumulation of toxic substances, particularly nitrogen compounds produced during fish metabolism.

Nitrogen compounds such as ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2) and nitrate (NO_3) can have toxic effects on fish by interfering with their physiological processes. In recirculating aquaculture system, low concentrations of these compounds are constantly present due to the two-step mechanism of action of the nitrifying microflora. Typically, during the startup phase of biofilters, a transient accumulation of nitrite may occur, because the energy yield of the chemical reaction of the oxidation of ammonium to nitrite is much higher than that of the chemical reaction of the oxidation of nitrite to nitrate. Feed, feed waste, fish excreta and decomposition of organic matter are the main sources of nitrogen compounds in aquaculture systems [1, 2].

Ammonia is one of the main types of toxic nitrogen compounds found in water, where its concentration can increase significantly due to the metabolic activity of fish and the decomposition of organic waste. Ammonia is present in water in two forms: free ammonia (NH3) and ionized ammonium ($NH4^+$). Free ammonia is highly toxic to fish as it can penetrate cell membranes and disrupt the acid-base balance, leading to damage to internal organs, respiratory failure and death by severe intoxication. Ammonium salts are less toxic to fish due to the low aggressiveness of the ammonium ions and, their effect is mainly due to the presence of free ammonia. With increasing pH and water temperature, the amount of free ammonia usually increases. Ammonia is also formed in the body of the fish as a by-product of nitrogen metabolism and is excreted through the gills. The mechanism of ammonia poisoning in fish is therefore quite complex. It depends on the concentration of exogenous and endogenous ammonia and the variability of the physico-chemical properties of the water. Ammonia has hemolytic and local effects and is a typical neurotoxic agent [3].

In juvenile sturgeon, ammonia toxicity initially manifests as neural damage, with increased activity of acetylcholinesterase and superoxide dismutase, indicating oxidative stress and neurotoxicity. This is accompanied by a decrease in appetite due to changes in the expression of genes regulating hunger and satiety: appetite stimulating factors(agrp, npy) decrease, while inhibitory factors(pomc, cart, crf) increase. At high concentrations of ammonia, there is a significant decrease in feed intake and deterioration in physiological state, as reflected in hepatosomatic and viscerosomatic parameters [4].

Nitrite is an intermediate product of ammonia oxidation during the nitrification process, while nitrate is the end product. Although nitrites are less stable in water, they are more toxic to fish. Nitrite suppresses hemoglobin levels and can cause methemoglobinemia, which reduces the blood's ability to carry oxygen, leading to oxygen deprivation. In addition, acute exposure to nitrites in sturgeons causes ion imbalance (hyperkalemia, increased chloride content, low sodium content), as well as damage to the liver and cardiovascular system. As the effects of nitrites intensify, behavioral disorders are noted, expressed as increased breathing, unnatural and sluggish swimming, and loss of balance. Despite this, sturgeon exhibit a relative tolerance to nitrites due to the ability to regulate blood plasma nitrite levels in below environmental concentrations[5].

Nitrates in moderate amounts are not particularly toxic to fish. However, high concentrations in water can negatively affect fish health and have long-term consequences, disrupting the functioning of the entire aquatic ecosystem. The toxicity of nitrates depends on the size of the fish, with the susceptibility increasing with increasing fish weight. The LC50 level for nitrates decreases in larger fish, indicating an increased risk for adult sturgeon in recirculating systems. Endocrine disorders resulting from chronic exposure to nitrates can cause an increase in the level of sex steroids (testosterone, estradiol) and disrupt endocrine function, as well as alter secondary stress responses [6]. Therefore, this study aims to identify pathological changes in juvenile sturgeon caused by nitrogen compound poisoning. In addition, the research aims to develop a set of preventive measures tailored to the progression and severity of the intoxication.

Materials and Methods

The studies were conducted between September 2024 and February 2025. The materials for the study were reared juvenile sturgeon weighing 100-150 g, grown in a RAS (*Acipenser baerii* and *Acipenser ruthenu*s) and recycled water samples from rearing basins No. 3 and 8, as well as from quarantine basin No. 1, where juveniles were kept at a stocking density of 80 kg/m^3.

The study began with a clinical inspection of hydrobionts. During the clinical examination, attention was paid to external changes and the general condition of the fish. At autopsy, the condition of the internal organs and any pathological changes were examined. Additionally, the amount of nitrogen compounds in the circulating water of the pond where the suspected fish were landed was determined and compared with nominal indicators [7].

Nessler's reagent was used to determine the amount of total ammonia in water. A positive reaction with Nessler's reagent results in a yellowish reddish-brown colour change in the solution. First, the proportion of ammonium ions relative to non-ionised ammonia was determined, followed by calculation of the total ammonia concentration (mg/L). The Griess reagent was used to determine nitrite and nitrate concentrations in the water. A pink color indicates the presence of nitrite and a yellow colour indicates the presence of nitrate [8-10].

Ammonia in pathological material was determined by a qualitative method using three indicator papers moistened with alkaline solution of lead acetic acid, alkaline solution of copper sulphate and wet litmus paper. A blue colour change in the litmus and copper paper indicates the presence of ammonia in the organ homogenate. The homogenate was placed in a corked conical flask. *Z. Svobodova's* micro diffusion method is used for the quantitative determination of ammonia in fish, and K. Schreckenbach and others describe the *Müller-Weisenhirtz* and *Keller* methods based on the use of indophenol as an efficient indicator [11, 12].

Results and Discussion

Table 1 presents the results of nitrogen compound analysis in water samples collected from rearing basins No. 3, 1 (quarantine), and 8 in the RAS.

Table 1 – Results of Analyses Nitrogen Compounds in RAS

Nitrogen compounds	Maximum Permitted Concentration	Pool No.3	Pool No.1 (quarantine)	Pool No.8
1	2	3	4	5
NO_2, mg/L	0.25-0.3	0.289±0.043	0.392±0.019	0.369
NO_3, mg/L	Not less than 40	7.50±0.21	6.80±0.06	13.50±0.14
NH_4, mg/L	0.01-0.86	0.25±0.02	0.20±0.01	0.36±0.01

The table shows that the amount of nitrogenous compounds slightly deviates from the norm, particularly No_2, which exceeds the norm by 15-24%. However, juvenile sturgeon can be affected even by such deviations because their bodies are very sensitive to even minimal changes in the hydrochemical parameters of water.

The pH and temperature of the aqueous media will affect the balance between ammonia and ammonium. Nitrite levels increase during the biofilter loading phase and during overloading. Low pH tends to increase nitrite. If there are no denitrifying plants, nitrate will build up in the water [13].

Nitrites bind to the haemoglobin in the blood to form methaemoglobin, which reduces the ability of the blood to carry oxygen. Elevated levels of methaemoglobin can be the cause of anemia and other blood disorders. Nitrates can cause kidney damage, manifesting as nephritis or other diseases. Oxygen deprivation causes fish to behave abnormally, such as struggling to swim or becoming aggressive, making them susceptible to infection [14].

Intoxication with nitrogen compounds, particularly ammonia, leads to severe neurological, metabolic and behavioral changes. The main pathologies are as follows: neurotoxicity, oxidative stress in the brain, decreased appetite, and tissue damage. Autopsies of dead sturgeon fish revealed changes indicating the effects of toxins on the fish. Pathological changes are clearly visible: body and gill surfaces covered with mucus, reddened gills, necrotic areas, muscle weakness, hepatomegaly (enlarged liver), signs of nephritis, such as reddening and inflammation of kidney tissue, focal haemorrhages (Fig. 1).

Figure 1 – Autopsies of dead juvenile sturgeon fish

At high concentrations, ammonia causes degeneration, vacuolization and necrobiosis of gill epithelium, disturbed perfusion of internal organs, and sometimes hepatic necrobiosis. Erythrocyte haemolysis, nervous system dysfunction and degenerative changes in internal organs are more pronounced at low concentrations [15].

The clinical signs of poisoning by nitrogen compounds can manifest themselves in a variety of ways and depend on the degree and duration of exposure to the toxins. In the early stages, restlessness gradually increases and the fish become more sensitive to mechanical and light stimuli. This is followed by clonicotonic convulsions characterized by violent movements and trembling of the gills. The fish lose their balance and open their mouths wide (hypoxia), spread their gills and gill covers, and touch the bottom of the water. Exposure of juvenile sturgeon to ammonia, especially at high concentrations (15 mg/l), is associated with pronounced damage to nerve fibers in the brain. This is accompanied by increased acetylcholinesterase activity, a marker of neurotoxicity. Significantly increased superoxide dismutase (SOD) activity in the fish brain indicates development of oxidative stress and cell damage [16].

Nitrogen compounds cause a significant decrease in daily and total feed intake and a decrease in the fat index, hepatic and visceral indices, indicating metabolic disorders and general fatigue of the organism. More than 1500 genes involved in appetite regulation are altered by ammonia. Appetite-stimulating factors (agrp and npy) are suppressed, while appetite-suppressing factors (pomc, cart, crf) are activated.

Even at levels close to the maximum permissible concentration, the general health of aquatic organisms deteriorates, immunity is weakened, lethargy is observed, excessive mucus is secreted from the gills, and there are problems with feed consumption and digestion. The lack of feed utilization, in turn, leads to an excessive load on the mechanical and biological filtration system, and economic losses for the company [17].

Conclusion

Juvenile sturgeon, like other fish, are very sensitive to the toxic effects of nitrogenous compounds. As our results have shown, deviations of up to 15-24% can lead to negative effects on the fish, even death. High concentrations of ammonia can cause stunted growth, decreased immunity, and increased mortality. In the early stages of development, sturgeons are more susceptible to poisoning because their detoxification system is less effective. Problems with regulating water flow in the RAS may worsen due to an increase in the concentration of ammonia and nitrites to toxic levels. It is important to control the water circulation in the RAS.

Methods for Monitoring and Controlling Nitrogen Compound Concentrations:

1. Filtration and biofiltration systems. One of the most effective methods of the control of nitrogen compounds in the recirculating water of a wastewater treatment system is the use of bio-

filtration. In biofilters, water toxicity is significantly reduced by microbiological processes of ammonia oxidation (nitrification) and the subsequent conversion of nitrites to nitrates. However, for biofilters to operate effectively, it is necessary to maintain optimal conditions for the development of beneficial microorganisms. This requires constant monitoring of water parameters.

2. Recycled water changes and aeration. Regular partial water changes and improved aeration are also important measures to control nitrogen levels. This assists in maintaining proper oxygen levels and removing excess ammonia and other toxins. However, this process must be balanced, taking into account the needs of the sturgeon, as excessive water changes can cause stress to the fish.

3. The use of chemical agents. In some cases, to rapidly reduce levels of toxic nitrogen compounds in water by binding ammonia and other compounds and preventing their accumulation in water bodies, chemicals such as zeolites or special aquaculture additives are used.

4. To increase the sturgeon’s resistance to poisoning, several measures should be taken. These include continuous monitoring of water quality, optimization of stocking density (since overcrowding accelerates the accumulation of nitrogen compounds), enrichment of the diet with balanced protein and energy levels (which helps reduce ammonia formation during metabolism), and the use of ultraviolet radiation and ozone treatment, which effectively eliminate nitrite and ammonia from the water, and reduce their toxicity to fish [18].

Controlling nitrogen compounds concentrations is essential for maintaining the health of juvenile sturgeons. An integrated approach including biofiltration, regular water replacement, and the use of specific technologies can significantly mitigate the harmful effects of ammonia, ammonium, nitrites, and nitrates, thereby contributing to the success of sturgeon aquaculture.

Authors' Contributions

BA, NA, and SB: conceptualized and framed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. GN, SB and BA: carried out the final revision and proofreading of the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Тұйық сумен қамтамасыз ету қондырғылары жүйесінде өсірілетін жас бекіре балықтарының азот қосылыстарымен улануы

Бексултан А.Е., Гинаятов Н.С., Сариев Б.Т., Бригида А.В., Ниматов А.И.

Түйін

Алғышарттар мен мақсат. Тұйық сумен қамтамасыз етілген қондырғылар жүйесінде (ТСҚеҚ) жас бекіре балықтарының иммунитетінің деңгейі бірқатар факторларға, соның ішінде судың сапасына, азықтандыру нормаларына және стресстің болуына байланысты айтарлықтай өзгеруі мүмкін. Жас бекіре тұқымдас балықтар ересек балықтарға қарағанда ауру мен интоксикацияға бейім, олардың иммундық жүйесі әлі де дамып келеді. Зерттеудің мақсаты жас бекіре тұқымдас балықтардың азотты қосылыстармен, мысалы, аммиак, нитриттер және балықтың метаболизмі кезінде пайда болатын нитраттармен улануын зерттеу болып табылды.

Материалдар мен әдістер. Зерттеу материалы ретінде ТСҚеҚ жүйесінде өсірілген жас бекіре (*Acipenser baerii* және *Acipenser gueldenstaedtii*) және бекіре балықтары өсірілген №3, 1 және 8 бассейндердегі айналмалы су үлгілері алынды. Зерттеу барысында физиологиялық ауытқуы бар дараларды клиникалық тексеру, летальді жағдайдағы бекіре тұқымдас балықтарды патанатомиялық жарып-сою, сонымен қатар патологиялық материалды токсикологиялық талдау мен айналмалы суды номиналды көрсеткіштермен салыстыру әдістері қолданылды.

Нәтижелер. Айналмалы судың гидрохимиялық талдауының нәтижелері бойынша аммоний қосылыстарының нормадан сәл ауытқуы анықталды, бірақ ол шекті рұқсат етілген концентрация деңгейінде. Дегенмен, жас бекіре балықтары үшін осы көрсеткіштер улануды туғызуы мүмкін, себебі жас бекіре балықтарының ағзасы судың гидрохимиялық көрсеткіштерінің ең аз өзгерістеріне де өте сезімтал, бұл клиникалық тексерудің нәтижелерімен, сондай-ақ өлексенің беткі қабатында токсиндердің әсерін көрсететін өзгерістерді анықтайтын патологиялық сараптама нәтижелерімен расталады.

Қорытынды. Дамудың алғашқы кезеңдерінде бекіре тұқымдас балықтар улануға бейім, өйткені олардың детоксикация жүйесі онша тиімді емес. ТСҚеҚ-дағы суды бақылау проблемалары аммиак пен нитрит концентрациясының улы деңгейге дейін жиналуымен қиындауы мүмкін. ТСҚеҚ-дағы айналымдағы суды бақылау маңызды.

Кілт сөздер: жас бекіре балықтары; интоксикация; азот қосылыстары; ТСҚеҚ; нитрификация.

Отравление азотными соединениями молоди осетровых рыб, выращиваемых в установках замкнутого водоснабжения

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Аннотация

Предпосылки и цель. Исследования, проведенные в условиях установок замкнутого водоснабжения (УЗВ), показали, что уровень иммунитета молоди осетра в УЗВ может значительно варьировать в зависимости от ряда факторов, включая качество воды, условия содержания, питание и наличие стресса. Целью исследований было изучение интоксикации молоди осетра азотистыми соединениями, такими как аммиак, нитриты и нитраты, образующихся в процессе метаболизма рыбы, представляет собой серьезную проблему.

Материалы и методы. Материалом для исследования послужили молодь осетровых, выращенная в УЗВ (*Acipenser baerii* и *Acipenser Gueldenstaedtii*), и пробы оборотной воды из бассейна № 3, 1 и 8, где выращивалась молодь. В ходе исследования использовались методы клинического обследования особей с физиологическим отклонением, патоанатомического вскрытия погибших особей осетровых, а также токсикологического анализа патологического материала и оборотной воды в УЗВ в сравнении с номинальными показателями.

Результаты. По результатам гидрохимического анализа оборотной воды было выявлено незначительное отклонение от нормы по аммонийным соединениям, но оно находится на уровне предельно допустимой концентрации. Однако молодь осетровых рыб может быть отравлена этими показателями, так как организм молоди осетровых рыб очень чувствителен даже к минимальным изменениям гидрохимических параметров воды, что подтверждается результатами клинического осмотра, а также патоанатомического вскрытия.

Заключение. На ранних стадиях развития осетровые более восприимчивы к отравлению, поскольку их система детоксикации менее эффективна. Проблемы контроля воды в УЗВ могут усугубляться накоплением концентраций аммиака и нитритов до токсичных уровней. Важно контролировать оборотную воду в системе УЗВ.

Ключевые слова: осётр; интоксикация; соединения азота; УЗВ; нитрификация.