CHAPTER «BIOLOGICAL SCIENCES»

ECOSYSTEM CONNECTIONS AND FISH HEALTH

Eduard Kompanets[1]
Viktoria Lavrynenko[2]

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Abstract. Ecosystems are subject to many human influences. The balance between species is disturbed due to interference with the aquatic environment. Due to environmental pollution, its impact on fish and other aquatic organisms changes. This affects ecosystem connections. Changes in the environment also change the adaptive capacity of fish, leading to impaired health. Also, there is a need to study the protective capabilities of fish from the naturally occurring opportunistic species Aeromonas hydrophila, which causes infections in them.

In natural hydrobiocenoses, fish, as well as pathogens of its diseases (aeromonads) are components of food chains formed by evolution. Literature sources prove that aeromonads are normally present in microbial associations of benthic microflora as a normal saprophytic component of hydroecosystems.

These bacteria feed on organic residues that are concentrated at the bottom of water bodies and perform a sanitary function, like other similar types of microorganisms. The health of fish depends on their ability to adapt to the environment. Usually in the wild, fish are rarely susceptible to disease. Local populations for a long time of coexistence have formed a certain balance with other species, including parasitic. The balance is reflected by a certain rate of abundance between species. Imbalance due to fishing from the reservoir, or, conversely, with an artificial increase in numbers, leads to changes in the aquatic environment. Changes in the habitat of fish affect themselves. Fish health is changing.

[1] Candidate of Agricultural Sciences, Associate Professor at Department of Ecology, Drahomanov National Pedagogical University, Ukraine
[2] Candidate of Agricultural Sciences, Associate Professor at Department of Ecology, Drahomanov National Pedagogical University, Ukraine

In nature, such a disease as aeromonosis is an ecological concept. Violation of the ecological conditions of the species leads to stress, and reduced immunity in fish, leads to fish disease. In aeromonad infections with weak symptoms in carp, a decrease in biological parameters was observed: growth, body weight, fatness and survival (57.1%). The number of blood cells in diseased fish decreased, especially leukocytes and lymphocytes. The percentage of T- and B-lymphocytes in the blood of carp-infected carp increased. The introduction of the bacterium stimulated the immune response – an increase in the percentage of T-lymphocytes. The percentage of B cells did not increase significantly. In diseased fish, the percentage and number of low-activity T-lymphocytes increased, which corresponded to the presence of an immune response to the bacterium. The values of antibacterial activity of blood serum (BASC) in both groups of fish did not change.

1. Introduction

The study of ecosystem relationships is important in understanding the phenomenon of parasitism. This issue is becoming relevant due to disturbances in habitats that occur under the influence of human economic activity, as well as large-scale changes caused by global warming. Diseases that have a natural focus, among which is aeromonosis, are important.

In the structure of ecosystems, potential pathogens of human and fish diseases create natural isolates with other types of microorganisms in the soils at the bottom of reservoirs. The bottom is rich in organic residues and is the beginning of food chains of detritophagous and saprophytic. Microorganisms perform the function of organic processors. Studies show that potentially pathogenic organisms in natural conditions can exist as saprophytic forms.

Krushelnytska and others (2012) studied the soils of reservoirs for the presence of five species of conditionally dangerous bacteria and found them there. Identified E. Coli (24%), St. aureus (21%), Cl. perfringens (21%), Aeromonas hydrophila (13%) and Pr. vulgaris (11%) [11]. Therefore, it proves the thesis about the natural communities of saprophytic microbes and not the absolute pathogenicity of microorganisms.

With the deterioration of living conditions, the physiological state of fish changes, the immune state of the organism changes and resistance to pathogens decreases [8].

At a disease, together with the main pathogenic organism the group of similar organisms which is layered on infectious process and aggravates it operates. A study by Krushelnytska and others (2012) of the composition of the associative microflora from fish with aeromonosis identified four species of microorganisms: Aeromonas hydrophila (31.6%), E. coli (31.6%), St. aureus (26%) and Pr. vulgaris (10,5%) [11]. The results prove the complexity of the aeromonad infection. Along with the main pathogen Aeromonas hydrophila, non-specific types of microbes take part in the infectious process.

The ecological component is manifested in the marked seasonality of fish morbidity. Aeromonoses naturally appear mainly in the spring, when water temperatures rise in reservoirs. The prevalence of the disease is associated with physiological changes in fish, in the warm seasons, when external living conditions change [15; 17; 24].

The problem of the study is to identify the ecosystem ties and causes that lead to poor health in fish, and to study some components of protection of fish from the naturally occurring opportunistic species Aeromonas hydrophila.

2. Ecosystem components and immunity

Ecosystems are structural and functional elements of the biosphere. For the hydrobiosphere, respectively, these are aquatic ecosystems [18; 19].

An ecosystem is a collection of living organisms, interconnected by biotic bonds through the flow of matter and energy, and inanimate components of their environment that are involved in metabolic processes. An ecosystem is also a combination of living organisms and the environment, which are characterized by a certain stability and have a well-functioning internal cycle of substances. The aquatic ecosystem inextricably combines the inanimate environment (abiotic components – water, sediments and physical and chemical environmental factors) and biota – a multicomponent complex of groups and populations of plants, animals and microorganisms.

Associations of living organisms in an ecosystem have their own composition, number of species and individuals that correspond to some average environmental conditions. Organisms in this environment are interdependent and are preserved due to the possibility of constant reproduction in certain places of this environment. This pays tribute to the aquatic environment as an abiotic factor that determines the existence of all aquatic organisms [18; 19].

The ecological environment consists of abiotic and biotic components that ensure the existence of life in general, and the coexistence of species directly [2; 3; 22]. In this division, the biotic component is more diverse, as it must constantly adapt to changes in the abiotic. Earth conditions are subject to daily, seasonal, annual and perennial cycles in each geographical area. This happens all the time. This has happened before, is happening now and will happen in the future. The geological history of the planet entails changes in biota and is happening constantly.

For the full existence of the biotic component evolutionarily developed rules that ensure the viability of organisms, their development, the transfer of hereditary information for the successful continuation of development over time. Food chains, chemical and energy transformation are the basis of any life. Each ecosystem has biomass accumulators, biomass consumers and recyclers. But, each such system, also evolutionarily, has fuses that control the balance between species and the internal balance of the species. For interspecific balance there are predators and various parasitic organisms, for intraspecific balance – various forms of social relations (behavior) [19]. For parasites, there is a concept of «natural hearth», which characterizes the constant presence of these species in the food chains of this or several compatible ecosystems [18]. From the point of view of «rules of creation of ecosystems» there are no «bad» species, there are not very adapted, or those which now do not have the regulator.

Each ecosystem that develops in a certain geographical area is unique not only in its species composition, included in the food chain, but also in the ratio of species. Because ecosystems are open, there is a constant flow of species on their borders, due to both «planned» migration and attempts to conquer conditionally free areas. When the external conditions (ecotope) change very slowly, a situation of «permanence» is created, when the ecosystem has more stability. The ecosystem can change its territory and area over time, gradually «moving» to a new place – a zone of greater comfort. When external conditions change «quickly», there are fluctuations in the amount of biomass accumulation, which affects its consumers throughout the food chain and introduces an imbalance. Smooth movement does not occur due to very radical changes. Some species are disappearing due to unsuitable living conditions. Others, who could live in these conditions, but their food chain has collapsed, are also disappearing due to hunger.

Stress creates an additional burden on the body and its defense system – immunity. Immunity is weakened and opens up «additional opportunities» for parasitic organisms, which are also trying to find a new refuge. In this situation, there is a possibility of «parasitic» symptoms, even in species that have not previously shown «aggression». They only need to get into the internal very nutritious environment of the macroorganism. Insufficient resistance of the immune system and lack of control can have two finals. One – the capture of the whole host, sepsis and destruction. Acute course of the disease. The second is the confrontation between the macroorganism and the potential «invader» organism. Manifestations of such confrontations can have a different course, from the manifestation of external clinical signs of the disease, to the complete absence of manifestations. Such diseases are called atypical, asymptomatic [8; 13; 16].

Thus, predators and parasitic organisms in ecosystems develop as a priority when there is an imbalance – an increase in the number and biomass of species that are the basis of the food chain. And, complement each other. In the conditions of sharp changes of environmental conditions and decrease in number and biomass of species, the stress factor and decrease in immunity has the greater advantage. Such conditions are more favorable for parasites.

Aquatic ecosystems, due to their rapid metabolism, are less stable than terrestrial-aerial. The specificity of aquatic ecosystems is related to the environment itself, which is water. Water is the basis of life and it is the habitat of aquatic organisms. Water is much thicker than air, and much more labile than solid land. It is easier to maintain «soaring» (levitation) and movement of small organisms throughout its thickness – from surface to bottom. Other aquatic organisms, including fish, can also move freely in the water column. This creates both food connections and the possibility of contact with conditionally dangerous organisms.

3. In nature disease is an ecological concept

In nature, there are many different types and forms of parasites. Consider their ecological function in ecosystems. These are adapted (adapters) to life, or nutrition (c) on other organisms, species in ecosystems. Internal parasites exist in the host ecosystem and contact through it with the external ecosystem for reproduction. This is a necessary condition for all parasitic organisms. External parasites – are temporary (in warm-blooded animals and humans – mosquitoes, mites, lice, fleas, bedbugs) and permanent (in fish – fingerprints, girodactylus and others). They use the host mainly as a means of food.

More adapted to a particular host species of parasites exist in it so invisibly that they do not cause him significant harm. They live as long as their master lives. Such a passive form of coexistence is beneficial to the parasite, but the host has no benefit from it. The parasite feeds on the food of the host, or himself. In this ecosystem, there is a balance between the host's immune system and the parasite's outer coverings and secretions.

Parasites-aggressors, on the contrary, wait for the moment for penetration into internal system of an organism for fast reproduction and the owner becomes a link of transfer of the next generations of parasites to the following owners. The more of them, the better for the parasite. The fate of the owner in this case is not important. The process stops only when the frequency of contacts with potential hosts decreases. Such parasitic organisms constitute a natural outbreak to control the number of host species. If the host species itself has not coped with its number, it is «helped» by the species controller. This position is true for both plants and animals and microorganisms. The species-controllers themselves cannot exceed their own number, as they depend on the number of the host species. More often the species-controllers are microorganisms. The balance between the host species and the parasite species is very unstable and is based on immunology. Only those who have become ill and have acquired immunity, or those who have isolated themselves from others and avoided contact, survive. Survival of the parasite between periods of infection is carried out either in the environment or at the expense of host carriers that have previously become ill.

Cities in humans, agricultural production in fields and greenhouses, livestock, fish farming violate all natural «norms» of numbers and contribute to the «correction» of such an unnatural situation with the help of numerous species-controllers. It should be understood that the violation of established laws in nature will always have appropriate consequences. Chemicals will poison water, soils, surrounding organisms and consumers. However, the effect will always weaken due to the emergence of new forms of organisms resistant to destruction. Adaptation to chemicals will occur constantly. This is a natural process. You can't win here. It is easier to create more natural forms of management in aquaculture, the agricultural sector and to abandon intensive industrial livestock and fish farming in favor of grazing.

Diseases most often occur where the principle of maximum species size for a given area or volume (population and species constancy) is violated [19]. Cities in humans, agricultural production in fields and greenhouses, livestock, fish farming violate all natural «norms» of numbers and contribute to the «correction» of such an unnatural situation with the help of numerous species-controllers. It should be understood that the violation of established laws in nature will always have appropriate consequences. Chemicals will poison water, soils, surrounding organisms and consumers. However, the effect will always weaken due to the emergence of new forms of organisms resistant to destruction. Adaptation to chemicals will occur constantly. This is a natural process. You can't win here. It is easier to create more natural forms of management in aquaculture, the agricultural sector and to abandon intensive industrial livestock and fish farming in favor of grazing.

4. Activity of immunocites in aeromonads infection

The aquatic environment is often exposed to anthropogenic impacts, resulting in deteriorating ecological status. In natural reservoirs and aquaculture facilities, along with the acute and chronic course of diseases, there are asymptomatic (atypical or latent) manifestations of diseases that adversely affect the growth, fatness of fish and increase the natural and technological rates of their death. Such diseases are very little studied [1; 4; 6; 7; 9; 14; 20; 23].

When conducting experiments to determine the virulence of different strains of aeromonads, there are situations when infected fish did not show signs of disease in the form of acute or chronic course, but its death was observed within a few weeks under all conditions equal to control specimens. Microbiological samples from the tissues of the liver and kidneys of such fish were found in a small number of strains previously introduced aeromonads, which allowed to consider their participation in this process. In addition, similar to this situation were observed both in natural conditions and in the conditions of pond fisheries [8; 10; 25]. Due to this reaction of fish in experiments and the presence of similar phenomena in reservoirs, it was decided to study it in the laboratory.

Materials and methods of research. In carp, body weight and length, Fulton's fattening rate were measured, relative gain, mean weight ratio, and fish survival at the end of the experiment were determined. Experimental two-year-old carp were infected with a culture of the bacterium Aeromonas hydrophila, isolated from fish infected with aeromonosis, at a dose of LD_{50}. Control fish were injected with sterile saline. The selection of material was performed in fish at the appearance of clinical signs of the disease in a weakly expressed form: slight redness in the thoracic and abdominal fins, weak motor activity. The fish were kept separately in the pools for 30 days. The water temperature during the experiments was in the range of 11-16°C. Experimental carp were fed compound feed ДО-111-9 3 times a day at a rate of 3% by weight.

The number and percentage of T lymphocytes in the blood of carp was determined in the reaction of direct rosette formation of cells with untreated sheep erythrocytes. The number of B-lymphocytes in the blood was determined by rosette formation using erythrocytes, which were treated with antibodies and complement. The functional activity of lymphocytes was determined by the presence of the number of receptor zones on the surface of their membranes, to which indicator erythrocytes adhered [12; 21]. Low- (3-4) and highly active (5-10) T- and B-lymphocytes of blood were determined by the number of indicator erythrocytes attached to lymphocytes.

The antimicrobial activity of blood serum (BASB) was determined by the micromethod [5].

Results and discussion. The initial mass of carp in the experimental and control groups were almost the same. After 30 days, the ratio of the average weight of bacteria infected with the bacterium decreased. In control carp observed better growth and weight gain than in individuals with vague symptoms of aeromonosis. The fattening rate in control fish was higher than in experimental carp.

In infected Aeromonas hydrophila fish observed significantly lower survival (57.1%) than in the control group of carp (96%). The death of diseased fish throughout the experiment occurred gradually, without the effect of an outbreak. In these carp from the internal organs isolated pathogen A. hydrophila.

Table 1

Indicators of mass, survival and growth in experimental and control carp at the beginning of the experiment

Groups of fish	Planted		
	fish	average weight of fish, g	ratio among. mass, %
control	175	190,8±6,2	100
with aeromonosis	182	193,1±7,9	101,20

Table 2

Indicators of mass, survival and growth in experimental and control carp at the end of the experiment

Groups of fish	Caught			The ratio of average weight, %	Relative increase, %
	fish	survival, %	average weight of fish, g		
control	168	96,0	230,5±9,8	100	120,81
with aeromonosis	104	57,1	201,7±9,1	86,46	104,45

Blood cell counts in diseased fish decreased. A particularly marked decrease was observed in leukocytes and lymphocytes. Clinically healthy fish of the control group had 10.64 and 9.81 thousand cells. mm^3, respectively. In diseased carp, they were significantly ($P<0.001$) lower, 4.89 and 4.31 thousand cells. mm^3, respectively. The number of erythrocytes in the blood of infected fish decreased slightly, 0.57 thousand cells. mm^3, compared with the control of 0.67 thousand cells. mm^3.

In immunological studies in the blood of uninfected carp, the percentage of both T- and B-lymphocytes, 26.4% and 16.7%, was less than those infected with aeromonads. The introduction of the bacterium stimulated the immune response and the percentage of T-lymphocytes increased to 43.1% ($P<0.001$). The percentage of B cells increased not so much – up to 20.0%.

But when we studied the quantitative indicators, the decrease in the total number of lymphocytes in diseased carp more than twice, led to a general decrease in T- and B-immunocytes in the blood of these fish (1.74 and 0.82 thousand mm^3 cells). In the control group of fish, they were higher, 2.62 and 1.54 thousand cells. mm^3, respectively.

Table 3

Comparative table of biological, hematological and immunological parameters in experimental and control fish (n = 30)

Indicators	Control fish	Fish are infected with A. hydrophila
Length of fish, cm	21,5±0,26	20,7±0,30
Fulton fattening factor	2,30±0,04	2,22±0,04
Erythrocytes, million cells / mm^3	0,67±0,05	0,57±0,07
Leukocytes, thousand cells / mm^3	10,64±1,20	4,89±0,71
Lymphocytes, thousand cells / mm^3	9,81±1,09	4,31±0,60
T-lymphocytes, thousand cells / mm^3	2,62±0,37	1,74±0,26
B-lymphocytes, thousand cells / mm^3	1,54±0,21	0,82±0,13
T-lymphocytes, %	26,4±1,97	43,1±2,20
B-lymphocytes, %	16,7±1,51	20,0±1,50
BASB, %	48,19±4,00	50,50±4,04

During the experiment, the average values of antimicrobial properties (BASB) in both groups of fish did not change and were at the level of 48.19 and 50.50%.

With aeromonad infection without severe symptoms in fish, a significant increase in the percentage of low-activity T cells was observed (28.1%), compared with control (20.4%). They also increased 1.7 times in number. In diseased fish, low-activity T-lymphocytes amounted to 2.04 thousand cells. mm^3, while in their control was 1.17 thousand cells. mm^3. The percentage of highly active forms of T cells in diseased carp, on the contrary, decreased (5.9%), relative to control (14.9%), however, in quantitative terms did not differ from it (0.56 thousand cell mm^3 in both groups).

Analysis of the percentage of low-activity B cells in diseased fish showed a slight increase (15.0%) compared with the control group of fish (12.8%). In quantitative terms, the differences were much more significant. Due to the decrease in the total number of lymphocytes in the blood, significantly ($P < 0{,}01$) decreased the number of low-activity B cells (0.63 thousand cells mm^3), compared with the control group (1.20 thousand cells mm^3). The percentage of highly active B-lymphocytes in diseased carp was also higher (4.9%) than in control fish (4.0%), and the number of these cells is less (0.20 thousand cells mm^3) than control (0.35 thousand cells mm^3), however, no significant differences between them were noted.

Table 4

Functional activity of lymphocytes in carp in the experiment (n = 30)

Lymphocytes	Control fish	Fish are infected with A. hydrophila
Low-activity T-lymphocytes, %	20,4±1,32	28,1±1,06
Highly active T-lymphocytes, %	14,9±1,30	5,9±0,78
Low-active T-lymphocytes, thousand cells / mm^3	1,17±0,17	2,04±0,27
Highly active T-lymphocytes, thousand cells / mm^3	0,56±0,08	0,56±0,09
Low-activity B-lymphocytes, %	12,8±1,05	15,0±1,02
Highly active B-lymphocytes, %	4,0±0.60	4,9±0,58
Low-active B-lymphocytes, thousand cells / mm^3	1,20±0,15	0,63±0,10
Highly active B-lymphocytes, thousand cells / mm^3	0,35±0,06	0,20±0,04

We believe that the increase in the percentage of T- and B-lymphocytes was associated with the need to maintain the required number of immunocompetent cells for the body to function

5. Conclusion

Ecosystems are structural and functional elements of the biosphere. An ecosystem is a collection of living organisms, interconnected by biotic bonds through the flow of matter and energy, and inanimate components of their environment that are involved in metabolic processes. An ecosystem is a combination of living organisms and the environment, which are characterized by internal stability and have their own cycle of substances. Aquatic ecosystems combine abiotic and biota. Biota is a multicomponent complex of groups and populations of plants, animals and microorganisms.

Ecosystems are exposed to anthropogenic influences. The balance between species is disturbed due to interference with the aquatic environment. Due to environmental pollution, its effect on fish and other aquatic organisms changes. There is an impact on ecosystem connections. Changes in the environment provoke changes in the adaptive capacity of fish, leading to impaired health.

In natural hydrobiocenoses, fish, as well as pathogens of its diseases (aeromonads) are components of food chains formed by evolutionary literature.

In nature, such a disease as aeromonosis is an ecological concept. Violation of the ecological conditions of the species leads to stress and reduced immunity in fish. Then the disease of fish is possible.

At aeromonad infections with weak symptomatology at carp decrease in biological indicators was observed: growth, body weight, fatness. Survival of 57.1% is significantly lower than the control group (96.0%). The death of diseased fish throughout the experiment occurred gradually, without outbreaks.

Blood cell counts in diseased fish decreased. Especially important, leukocytes and lymphocytes. The number of erythrocytes in the blood of infected fish did not decrease significantly.

In immunological studies in the blood of carp-infected carp, the percentage of both T- and B-lymphocytes was higher than in uninfected. The introduction of the bacterium stimulated the immune response and the percentage of T-lymphocytes increased to 43.1%. The percentage of B cells increased not so much – up to 20.0%.

There was a decrease in the total number of lymphocytes in diseased carp more than twice, which led to a general decrease in T- and B-immunocytes in the blood of these fish. In diseased fish, the percentage and number of low-activity T-lymphocytes increased, which corresponded to the presence of an immune response to the bacterium.

During the experiment, the mean values of antimicrobial properties of serum (BASB) in both groups of fish did not change.

In our experiments, the influence of the temperature factor on the infectious process was determined, however, we cannot consider it absolute. Such a reaction on the part of fish is rather complex and is a ratio of both external environmental factors and internal mechanisms of stability of the fish themselves. This may be related, for example, to the degree of inbreeding of carp that occurs in fish farms, or to the natural resilience of fish populations in water bodies, which requires further research.

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