Biology of causative agents of crayfish mycoses in connection with environmental protection in crayfish producing reservoirs

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Abstract. In order to prevent outbreaks of fungal infections in reservoirs with crayfish we recommend to (1) performa bioassay on the disease of crayfish with aphanomycosis (crayfish plague); (2) determine the incidence of crayfish with signs of mycosis called burn-spot disease (BSD); (3) check for contamination of coarse grains with agents of mucoriosis; (4) oppose the ingress into the reservoir of waste water from cultivated fields carrying agents of fusariosis; (5) prevent over consolidation of crayfish population. These measures are taking into account the ability of pathogenic oomycetes to quickly infect crayfish with mobile zoospores, long-term viability of chlamydospores (gemmae) of Saprolegniaparasitica and oogoniaof Aphanomyces astaci. These factors support the infection in the reservoir and prevent the recovery of crayfish populations after outbreaks of mycoses.

1. Introduction

Crayfish belonging to subfamily Astacinae Latreille, 1802 (further astacins) of family Astacidae are native inhabitants of reservoirs of the Western Europe, the European part of the Russian Federation and some regions of Asia. They are considered tobe valuable objects of trade and cultivation. In the late 19th and early 20th centuries, significant reserves of astacins in the waters of Finland and North-West Russia provided annually up to a half of all crayfish products sold in the markets of Western Europe. Currently, due to the sharp decline in astacin reserves in Russian reservoirs, these crayfish are used for domestic consumption and they are no longer objects of export to Western Europe. One of the reasons for the reduction of Russian astacin stocks is the spread of mycoses in astacin populations, the causative agents of which are the pseudo - (or false) fungi of the class Oomycetes, and to true fungi of the generaMucor and Fusarium. A sign of a crayfish mycosis is the appearance of melanized spots of burn-spot disease (BSD) or yellowish color on the covers of his body and on the gills.

The oomycete Aphanomyces astaci Schikora 1906. This oomycete is the causative agent of crustacean plague (aphanomycosis) – affects European, Asian and Australian crayfish, settling mainly in tissues of ectodermal origin [1]. The appearance of this extremely dangerous oomycete in the waters of Europe is caused by the introduction of North American crayfish, which has been repeatedly carried out in Sweden and other European countries. The disease caused by this oomycete is epizootic, and

ends with the death of the population of astacins and other autochthonous crayfish. Germination of cysts of the pathogen settled on the covers or the gills of an astacin, forming a mycelium penetrating into the body of the crayfish, initiating the launch of a cascade of immune reactions peculiar to crustaceans. As a result, melanin (the final product of the oxidation of prophenoloxidase) is produced, which is delivered to the sites of introduction of A. astaci into the body of the crayfish and to its mycelium, causing melanization. Such immune response to infection is most effective in North American crayfish, which, when infected with A.astaci, and remaining quite viable, become its carriers. This is the reason for the rapid spread of the plague in contact with them to crayfish that arenon-immune to crayfish aphanomycosis [1]. This obligate parasite of European astacins was first described by a Czech researcher in 1903, but it was not recognized as an agent of crustacean plague was recognized until 1934. Currently, four foci of aphanomycosis are known, of which three are in Northern Europe, and one in Spain [1]. In the republics of the former USSR, the introduction of signal crayfish into natural reservoirs was carried out in Lithuania and Latvia in 1970-1980, but not received large scale, since it was found that in the waters of Europe, contacts of native astacins with the North American imported speciesoften end with outbreaks of aphanomycosis of crayfish.

If the study of aphanomycosis pays great attention to the aspect of its extreme danger to astacins, mycosis with chronic manifestation, combined under the common name burn-spot disease (BSD), remain poorly studied. It has been established that BSD occurs in the presence of injuries, with a high density of the crayfish populations, with pollution of water bodies, etc. The disease can be temporary and disappear with the improvement of the quality of the environment in the reservoir.

Burn-spot disease (BSD) of crayfish and its pathogens. In Russia the first report about BSD of crayfish appeared in 1875; in Europe about observation spotting on covers of crayfish with"disease of burn spots" (burn-spot disease, BSD) has not been mentioned till 1900. Insignificant occurrence of BSD is noted almost in every population of crayfish. The increase in the incidence of BSD in crayfish populations affects the quality of crayfish and harms the trade in these products: in countries of Europe, crayfish with lesions of BSD are not accepted for sale [2].

Little is known about the BSD of crayfish caused by the oomycete Saprolegnia. parasitica. It is believed that melanin produced by the immune system of long-fingered crayfish (Pontastacusleptodactylus = Astacusleptodactylus) can inhibit the growth of Saprolegnia. Spanish astacologists determined the possibility of infection of European broad-toed crayfish (Astacusastacus) and two North American species – signal crayfish (Pacifastacusleniusculus) and red marsh crayfish (Procambarusclarkii) – with Saprolegnia spores isolated from long-toed crayfish [3]. The infection begins with the deposition on the skin a crayfish Saprolegnia zoospores and mycelium germination from cysts into the body of crayfish through the cuticle. After 4 weeks after the beginning of the experiment, 20% of healthy crayfish became infected and died. When the abdominal cuticle of crayfish was damaged by rubbing it with an abrasive material, mortality increased approximately 3-fold and occurred within the first week after insemination. The mycelium of Saprolegnia has been found in necrotic tissue under the cuticle, and in some crayfish, hyphae have also been found in nerve traction. Saprolegnia is considered to be a possible cause of death of Atlantoastacuspallipes (=Austropotamobiuspallipes) populations in Spain. However, the question of the ability of S. parasitica to cause crayfish disease with increased mortality in natural conditions like in the case of A. astaci, currently remains open.

Pathogenic effects of oomycetes and true fungi on the body of crayfish are different. Thus, if motile zoospores of Saprolegnia, settling on the crayfish integuments, incist and form a mycelium that damages the body a crayfish with its penetration into its body, the mycelium of true fungi after infiltrating the body of a crayfish, exudes toxins dangerous for its life that reduce the activity of metabolic processes and can lead to death of the crayfish. The rapid death of the "host" in these cases may indicate the facultative nature of parasitism of these fungi. Hence, the need for an accurate diagnosis of pathogens of crayfish is necessary, which should be based on determining the species of the causative agent of the disease.

As a result of the study of European astacin mycoses in the pre-war and post-war periods, 16 species of pathogens of these diseases belonging to false and true fungi are described. However, while conducting an audit of the system of true fungi, Walter Gams [4], Alderman [5] and other mycologists encountered difficulties in diagnosing some previously described pathogens of crustacean mycoses, as well as the fact that not all of them could be detected. A review of the current systematic position of these pathogens on the databases of nomenclature data (MycoBank, Index Fungorum, etc.) showed that at present the so-called "good species" include 4 species of oomycetes, and among these fungi - 2 species from the genus Mucor and 5 species from the genus Fusarium. The names of the other five previously described pathogenic species of fungi are recognized as doubtful or having an uncertain systematic status [6]. In nomenclature databases, the names of these species of fungi are marked respectively by the terms nomenconfusum or nomeninvalidum. The type material for these species is currently unknown. K. L. Tarasov's comments on their possible systematic position are made according to descriptions and drawings from publications of European and Russian authors [6].

The purpose of these studies is to outline measures to protect the healthy environment in crayfish reservoirs, based on the biological characteristics of pathogens of mycoses of crayfish.

2. Material and methods

We used data on the spread of burn-spot disease (BSD) of crayfish in their natural populations in the reservoirs of the Middle Volga, the basins of the Msta and Velikaya Rivers, as well as in cases of cultivation crayfish in fish farms and aquariums. The distribution of BSD in reservoirs was judged by the incidence of crayfish with dark spots in samples of 100 to 200 individuals from the population. For identification of a species of the causative agent of a mycosis, clarified scrapings from the tissue located near the dark spots of the BSD were microscopically studied. Signs of mycelium, sporangiophores, chlamydospores and other structures of the pathogen were compared with their descriptions according to the diagnostic key compiled by K. L. Tarasov [7]. Mycelia of Saprolegnia and Mucor, found on tissue scrapings near BSD spots, were grown on microbiological media. In these cases, the identification of pathogens was carried out on colored preparations, which were made in a veterinary laboratory. Information about the biological characteristics of astacin pathogens was collected from publications on Mycology and analyzed.

3. Results

Of the 16 species of causative agents ofastacinmycoses presented indescriptions, the so-called false fungi (Kingdom Straminopila, Department Oomycota) belong to 4 species, namely Aphanomyces astaci (family Leptolegniaceae), Saprolegniaparasitica, S. australis and Scoliolegniaasterophora (all three familySaprolegniaceae). In terms of taxonomy these pathogens are referred as "good" species. The latter two species of the family Saprolegniaceaeare identified recently by the method of genosystematics [2]. If the pathogenicity of A. astaci and S. parasitica is confirmed by numerous studies, information about the properties of S. australis and Scoliolegniaasterophora as agents of BSD inastacinsis absent yet.

The main infectious units of pathogenic oomycetes are mobile zoospores of vegetative origin, which settle on the covers of crayfish, incist and form a mycelium that penetrates into the body of the crayfish. Floating zoospores of A. astacipossess chemotaxis, adhesion to the substrate, in particular, to the epicuticula of crayfish and have a great ability to spread. They have a great ability to spread. Crayfish infected by A. astaci before and after death are sources of a large number of zoospores floating and seeking new hosts. The problem, which is significant not only for the species identification of pathogens, but also for the justification of preventive measures to restore crayfish populations after epizootics, is the question of the existence of spores of sexual reproduction in parasitic oomycetes, capable of long-term survival in the reservoir. Proponents of the lack of oospores capable of long-term survival in parasitic oomycetes suggest that the reintegration of crayfish populations in the affected with aphanomycosis waters should be carried out in a short time, based on the short survival of A. astaci zoospores [1]. However, when determining the timing of measures for

reintegration of populations in the reservoir, in which there was an outbreak of crayfish plague, it is necessary to take into account the data of Rennerfelt [8], as well as of Latvian mycologists. The latter ones grew colonies of an oomycete from the material from samples taken in adysfunctional reservoir, which resemblein morphology and in destructive effects on healthy crayfisha culture of A. astaci [9].

Currently the following measures to combat the epizootics of aphanomycosis in the waters of a preventive nature are recognized:

- a ban on the use of fishing gear used in mycosis-infected waters;
- carrying out urgent catch of crayfish and withholding of dead specimens when outbreak of aphanomycosis is suspected;
- staging of a series of bioassays on aphanomycosis in the waters that are planned to use for crayfish culture. For this purpose, healthy crayfish are placed in cages in which they are kept for 2-3 months, creating normal living conditions for them (feeding, providing shelters, provision of density of not more than 6 copies/m^2). Cages should be placed in different places of the reservoir and checked regularly. Negative results of bioassays evidence in favor of the suitability of the pond for crayfish culture. The introduction of the planting material of crayfish into an unfavorable reservoir should be prohibited for a period of about 5 years.

Native oomycete of Eurasian reservoirs S. parasitica is known as a fish parasite. Saprolegniosis of crayfish occurs in the case of an increased density of their population in pools, ponds, aquariums, with pollution of the aquatic environment. Especially dangerous is the disease during the molting of crayfish. The high pathogenicity of Saprolegnia is caused by its ability to produce several generations of zoospores during the growing season and to form wintering chlamydospores (gemmae) under unfavorable conditions.

Measures to combat saprolegniosis:

- the cultivation of crawfish in monoculture (without fish);
- creating obstacles to the penetration of wild fish into the pond;
- drying and sanitary treatment of ponds and tanks after their use for the content of crayfish;
- monitoring of the crayfish population in order to take measures to counteract its overpopulation. (Methods for determining the number of the sexually mature part of the population are given in the relevant reference books concerning crayfish cultivation).

There is some information concerning the occurrence of mucoriasis in crayfish in cultural conditions, where the source of the disease can be used as a feed grain contaminated with Mucor racemosus [5].

True fungi as crayfish pathogens. At least 12 species of true fungi (Kingdom Fungi) are also considered to be causative agents of RPB in astacin, of which 2 species of fungi belong to the genus Mucor (Division Zygomycota, class Zygomycetes, or Mucoromycetes), and 10 species of fungi belong to different classes of the Division Ascomycota (ascus fungi).

2 species of pathogenic fungi from the genus Mucor are recognized as "good" species. M.racemosus was found in samples from melanized spots on the body of a broad-toed crayfish (A. astacus) from the reservoirs of the Pskov region and from the reservoirs of Saaremaa [2]. M. hiemalis was identified by genosystematics in samples from A. astacus from reservoirs of Saaremaa [2]. There is evidence of the occurrence of mucoriasis in crayfish in cultural conditions, where the source of the disease may be the use as food of grain infected with Mucor racemosus[2].

The remaining 10 species of these fungi-pathogens belong to different classes of the Division Ascomycota. Of these, 9 species are registered in Mycobank, and one species – Septocylindiumastaci Udalov 1973 – does not appear in the nomenclature databases.

From 9 registered in nomenclature bases of ascomycetes 6 species are recognized as “valuable”, and 3 as “invalid”). Of the Ascomycetes 5 species are considered as “good” species. They belong to

the anamorphic genus Fusarium. Only F. tabacinum has been reliably identified by Dr. C. Booth. 4 other species of Fusarium, namely F. melanochlorum, F. oxysporum, F. semitectum (F. roseum var.culmorum), F. solani – are only mentioned in the literature as possible causative agents of mycoses of astacins, but information about them in this capacity is unknown.

BSD, caused by Ascomycetes, occurs in the presence of injuries in crayfish, with a high density of the crayfish population, with pollution of reservoirs. The disease can be temporary and completely disappear with the improvement of the quality of the environment in the reservoir.

The role of true fungi in the initiation of mycoses is less known due to the lack of microbiological studies and the difficulty of identifying these pathogens of crayfish, in particular, Fusarium spp. It is known about the occurrence of mucoriosis of crayfish in cultural conditions, where the source of the disease can be the use of grain infected with Mucor racemosus as food [7]. Fusarioses occur in crayfish that have been injured in reservoirs in which net fishing is conducted, and where runoff from agricultural fields containing chlamydospores and other Fusarium structures, the main habitat of which is soil, enter.

Mucoriosis and fusariosis control measures are:

- not allowing net fishing in crayfish reservoirs due to injury of crayfish when they are removed from fishing nets;
- prevention of effluents from agricultural fields containing chlamydospores and other Fusarium structures in crayfish reservoirs;
- heat treatment of feed grain.

4. Conclusion

The study of pathogenic properties of causative agents of crayfish mycoses in order to determine measures to counteract these diseases, which cause great harm to valuable stocks of native Eurasian crayfish of the subfamily Astacinae, as well as for environmental reasons, showed the need for further studies of the biology of pathogenic false and true fungi, including improving methods of their taxonomic identification.

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