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The Structure of the Natural Microbiome of Sturgeons

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***Abstract*: *In order to determine the structure of the natural microbiome of sturgeons grown in a recirculating aquaculture system, samples from the surfaces of the fish body and systems of organs that have the most frequent contacts with external environment (surface of the skin, respiratory and digestive organs of fish) were studied. Pieces of fins, fragments of gill filaments and contents of the intestine were taken from ten conditionally healthy spiny sturgeons (Acipenser nudiventris) kept in two nursery pools and were used as the material for research. To conduct the study of the metagenome of sturgeon fish, the following was performed: extraction of DNA samples in accordance with the kit manufacturer instructions; analysis of the nucleotide sequence of the fragments; processing of the obtained sequences using conventional methods. The level of community diversity was assessed using the following environmental indicators: the Simpson (evenness), Chao (richness) and Shannon coefficients. Cluster analysis was used to assess diversity between communities (beta diversity). The Dice coincidence index, which accounts only for the presence or absence of a taxon, was used as a similarity measure. It was found that the highest values for all three estimates were characteristic of communities obtained from the surface of the gills and the lowest values were observed in communities obtained from intestinal scrapings. The results of cluster analysis with the use of the principal component method showed that the intestinal microbiomes of the two pools had the greatest difference and the microbiomes of the fin surfaces had the smallest difference. Thus, the dependence of the degree of differences between microbiomes on the pool they were obtained from increased in the following order: fin surface communities – gill surface communities – intestinal communities. Microbiomes obtained from the surface organs of fish were more similar to each other and intestinal microbiomes were less.*

Keywords* : *Sturgeon, Microbiome, Diversity, Metagenomics, Recirculating Aquaculture System.

I. INTRODUCTION

Preservation and restoration of sturgeon stocks in natural reservoirs by reducing fishing pressure on their populations is the main trend in world sturgeon breeding.

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It is achieved through the development of industrial aquaculture using recirculating aquaculture systems (RAS) and innovative technologies that can solve food safety problems [1-6].

Unfortunately, the prevailing stereotype that fish bred in controlled conditions is free from diseases does not reflect the actual situation [7]. Moreover, the susceptibility of sturgeons to diseases has clearly increased with the increase of aquaculture production volume. Diseases in sturgeons are caused by violations of veterinary-sanitary and zoohygienic guidelines for keeping and feeding fish, the absence of quarantine measures for new fish imported for reproduction, as well as genetic and other factors. In addition, an analysis of literary sources and our own experience shows that diseases, which are caused by opportunistic pathogenic microorganisms that are part of the natural microflora of water, such as aeromonosis and pseudomonosis, are often recorded in sturgeons bred in RAS. The pathogenesis of these diseases has a pronounced staging and occurs in the setting of a decrease in the overall resistance of the fish organism. These diseases begin with acute forms and then turn into chronic generalized forms, which subsequently lead to the death of fish, inflicting significant economic damage on sturgeon breeding enterprises [8-13].

Consequently, the study of the microbial structure of conditionally healthy sturgeons and RAS objects plays a very important role. Of the same importance is constant microbiological monitoring using not only classical methods, but completely new approaches, such as a detailed study of the structural features of the microorganism community in a RAS using the latest molecular methods. The “Everything is everywhere the environment selects” hypothesis in the case of microbiological monitoring and prediction of the physiological state of sturgeon fish grown in RAS can be reformulated as follows: the taxonomic structure of the entire microbial community of the RAS is an extremely sensitive indicator of fish health. Moreover, even the slightest change in the structure of the microbial community, chemical and physical indicators, etc. immediately affects the physiological status of fish [14-19].

The aim of the research was the study of the structure of the microbial communities of the parts of the body and systems of organs of sturgeons grown in RAS that have the most frequent contacts with external environment (the surface of the skin, organs of the respiratory and digestive systems), which allowed us to draw conclusions about possible pathologies. To achieve this goal, we performed the following tasks:

– Analysis of the nucleotide sequence of fragments;

– Determination of the level of community diversity;
– Assessment of the diversity between communities (beta diversity) using cluster analysis.

II. PROPOSED METHODOLOGY

A. General description

The research was carried out at the aquaculture complex of the Zhangir Khan West Kazakhstan Agrarian and Technical University. The objects of the research were ten conditionally healthy spiny sturgeons (Acipenser nudiventris) 4-5 years of age kept in two nursery pools (five in each). Biological samples for the analysis were obtained using intra vitam sampling, without the use of the decapitation technique. Pieces of fins, fragments of gill filaments and contents of the intestine taken from the spiny sturgeons kept in two nursery pools were used as the material for research.

B. Algorithm

Extraction of the DNA samples was carried out according to the kit manufacturer instructions (MACHEREY-NAGEL NucleoSpin Soil, MACHEREY-NAGEL, Germany). Amplicon libraries for the variable region of the 16SrRNAv3-v4 gene (GTGCCAGCMGCCGCGGTAA/GGACTACVSGGGTATCTAAT) were obtained using universal primers F515/R806. The analysis of the nucleotide sequence of the fragments was performed using Illumina technology on an "Illumina MiSeq" instrument (USA) with the use of the MiSeq® ReagentKit v3 (600 cycle) reagent kit with double-sided reading (2 * 300 n). Processing of the obtained sequences was performed using Illumina software with Trimmomatic (Bolger et al., 2014) and QIIME packages.

III. RESULTS ANALYSIS

The taxonomic analysis of the obtained libraries revealed the presence of 423 taxonomic units (TU). In the microbiome of the respiratory system, the most abundant were the representatives of the following families: Pseudomonadaceae, Chitinophagaceae, Moraxellaceae, Fusobacteriaceae, Clostridiaceae, Oxalobacteraceae, Sphingomonadaceae, Leuconostocaceae, Comamonadaceae, Nocardiaceae, Streptococcaceae, Deinococcaceae, Micrococcaceae, Staphylococcaceae and Microbacteriaceae (Figure 1).

The representatives of the families Pseudomonadaceae, Deinococcaceae, Moraxellaceae, Micrococcaceae, Exiguobacteraceae, Comamonadaceae, Staphylococcaceae, Nocardiaceae, Sphingomonadaceae, Xanthomonadaceae, Weeksellaceae, Microbacteriaceae and a group of non-attributed organisms were prevalent in the microbiome of the sturgeons' skin surface. The prevalence of Pseudomonas bacteria in the skin can be explained by their participation in the process of nitrification and self-purification of water. However, it should be remembered that a sharp increase in the concentration of these bacteria contributes to the onset of the disease [7].

The intestinal microflora included prokaryotes from the families: Fusobacteriaceae, Bacteroidaceae, Streptococcaceae, Verrucomicrobiaceae, Clostridiaceae, Porphyromonadaceae, Peptostreptococcaceae, Enterobacteriaceae, Leuconostocaceae, Lactobacillaceae, Deinococcaceae, Moraxellaceae, Enterococcaceae, Micrococcaceae. Additionally; a significant proportion of the organisms could not be identified.

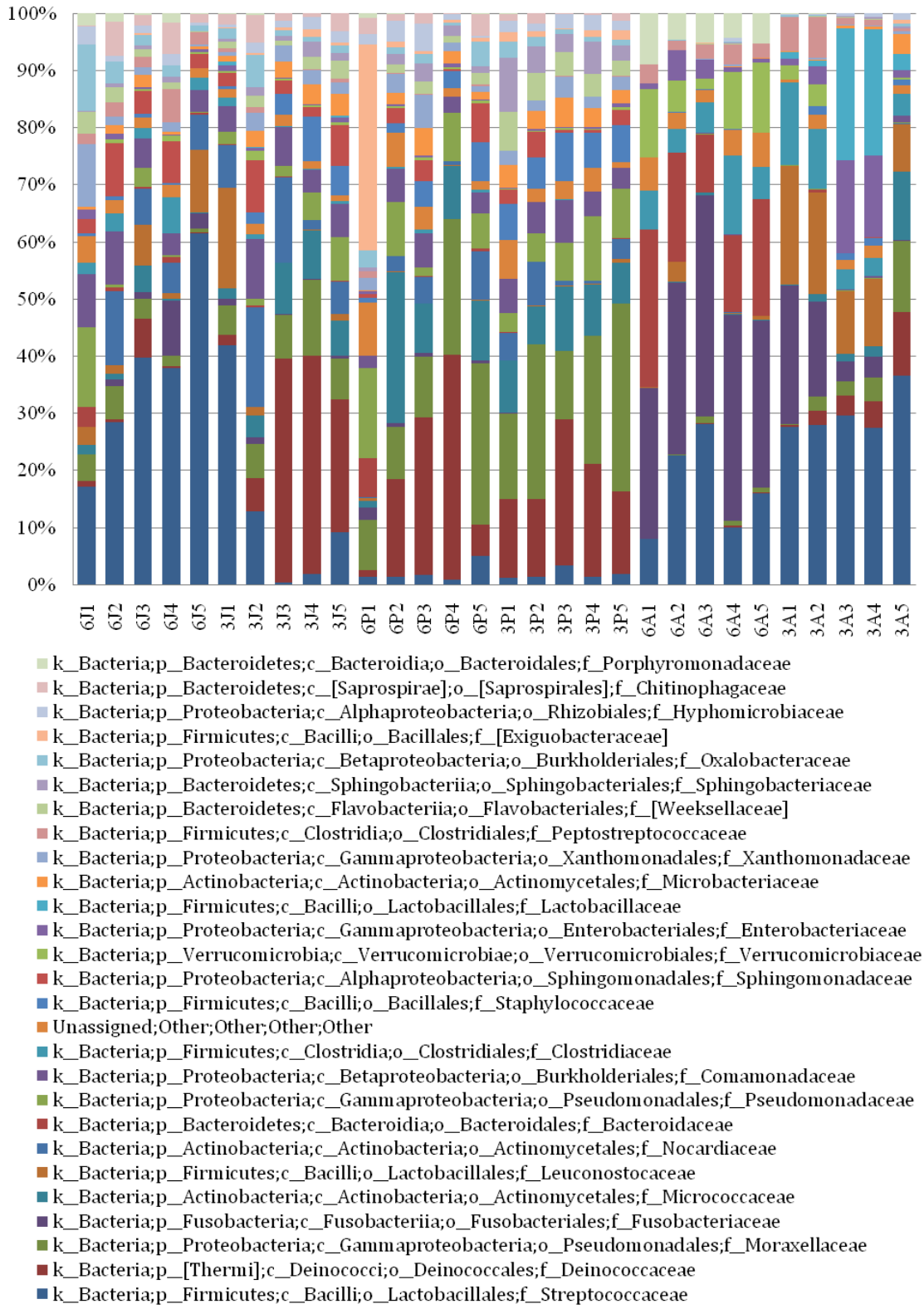

Fig. 1. The main taxonomic groups in the samples (A – communities of the intestinal microbiome; J – communities of the gill surface, P – communities of the skin)

The following environmental indicators were used in the assessment of the level of community diversity: the Simpson index (evenness), which demonstrates the evenness of the community, the Chao index (richness), which reflects species richness, and the Shannon index, which is an intermediate indicator. The highest values for all three estimates were recorded for the communities of the gill surface. The lowest values were observed in communities obtained from the intestinal scrapings (Table 1).

Table 1. Values of epy Simpson, Chao and Shannon indices for the samples

Communities	Sample number	Simpson index	Shannon index	Chao index
Intestinal microbiome	3A1	0.82	2.05	85.00
	3A2	0.85	2.37	95.00
	3A3	0.85	2.30	105.00
	3A4	0.86	2.43	101.00
	3A5	0.84	2.52	157.00
	6A1	0.85	2.31	73.00
	6A2	0.83	2.21	90.00
	6A3	0.79	2.11	110.00
	6A4	0.85	2.45	132.00
	6A5	0.86	2.34	85.00
Gill surface microbiome	3J1	0.82	2.70	178.00
	3J2	0.95	3.69	173.00
	3J3	0.84	2.67	136.00
	3J4	0.85	2.80	154.00
	3J5	0.93	3.41	175.00
	6J1	0.97	3.94	109.00
	6J2	0.94	3.73	191.00
	6J3	0.87	3.13	189.00
	6J4	0.90	3.37	182.00
	6J5	0.71	2.36	187.00
Skin surface microbiome	3P1	0.94	3.24	148.00
	3P2	0.91	3.07	140.00
	3P3	0.90	2.85	181.00
	3P4	0.90	2.87	159.00
	3P5	0.88	2.93	152.00
	6P1	0.92	3.46	97.00
	6P2	0.91	3.14	197.00
	6P3	0.92	3.30	168.00
	6P4	0.80	2.29	150.00
	6P5	0.91	3.18	138.00

To assess the diversity between communities (beta diversity), a cluster analysis was conducted. As a result of the analysis using the Dice coincidence index (which accounts only for the presence or absence of a taxon) as a similarity measure, all samples were divided into groups depending on the part of the body they were taken from (Figure 2).

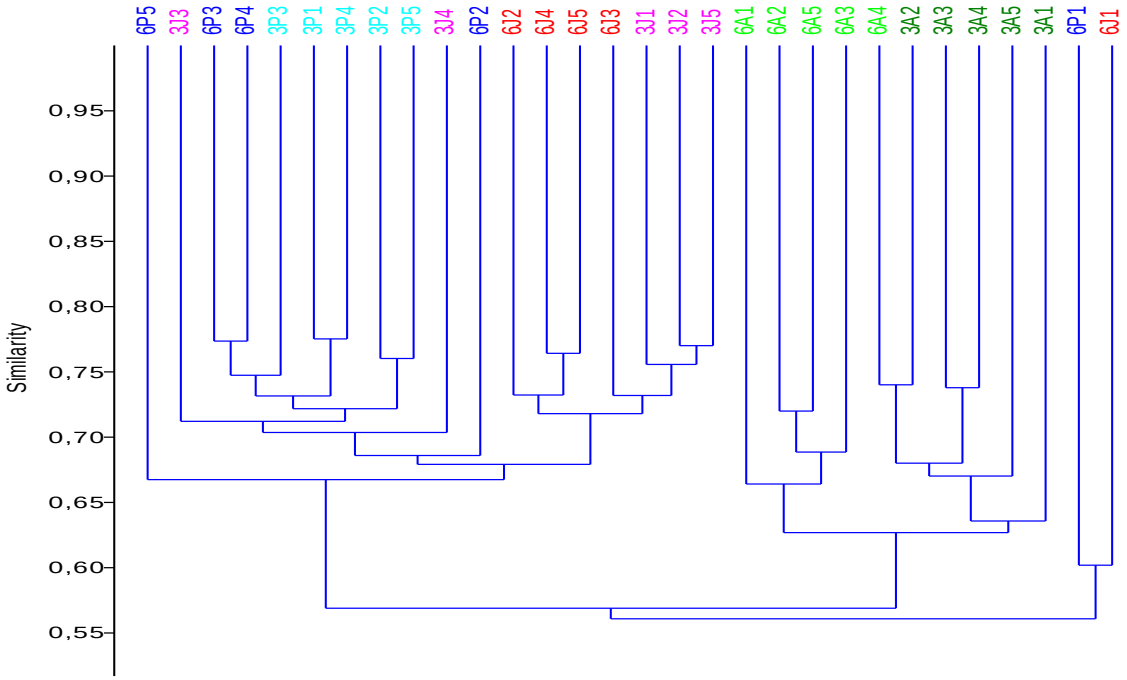

Fig. 2 Cluster analysis with the use of the Dice index as a similarity measure

As a result of the analysis using the Bray-Curtis index (which estimates not only the presence of a taxon, but also its proportion in the community) as a similarity measure, fin and intestinal microbiome samples were divided into two separate groups, while gill samples were dispersed between the intestinal and fin sample clusters (Figure 3). This can be an indication that the gill microbiome was the most susceptible to external influences, which might be due to the lower "immunity" of the gills to colonization by taxa from the environment.

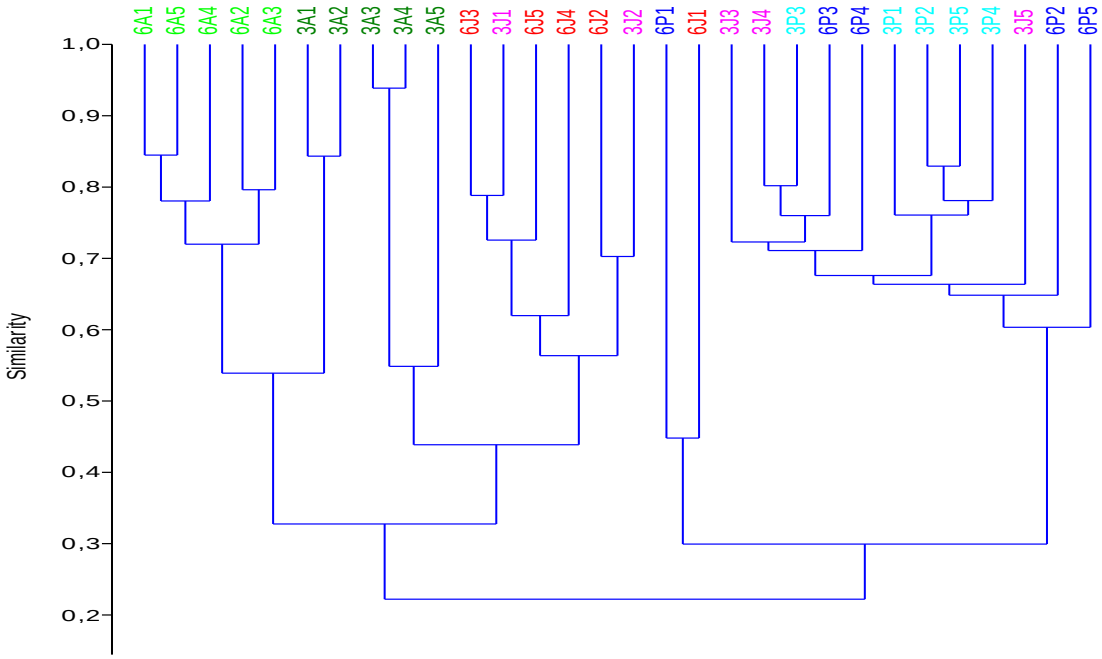

Fig. 3 Cluster analysis with the use of the Bray-Curtis similarity measure

The results of cluster analysis with the use of the principal component method (PCA, scatter-plot) showed the following trend: the intestinal microbiomes of the two pools had the greatest difference and the microbiomes of the fin surfaces had the smallest difference (Figure 4). Thus, the dependence of the degree of differences between microbiomes on the pool they were obtained from increased in the following order: fin surface communities – gill surface communities – intestinal communities.

A. Fin microbiomes were the least dependent on environmental conditions. The reasons for the pronounced differences between the intestinal microbiomes obtained from different pools remain unclear: they were not related to the species of fish (all of the studied fish belonged to the same species) and they were not related to the ration (it was identical for all fish). The only assumption that can be put forward at this stage of research is that the "average" physiology of the population of each pool had specific properties. Other possible reasons might become clear during the next year's research, when the main object will be aquatic microbiomes, including sediment, filtrates and biofilters.

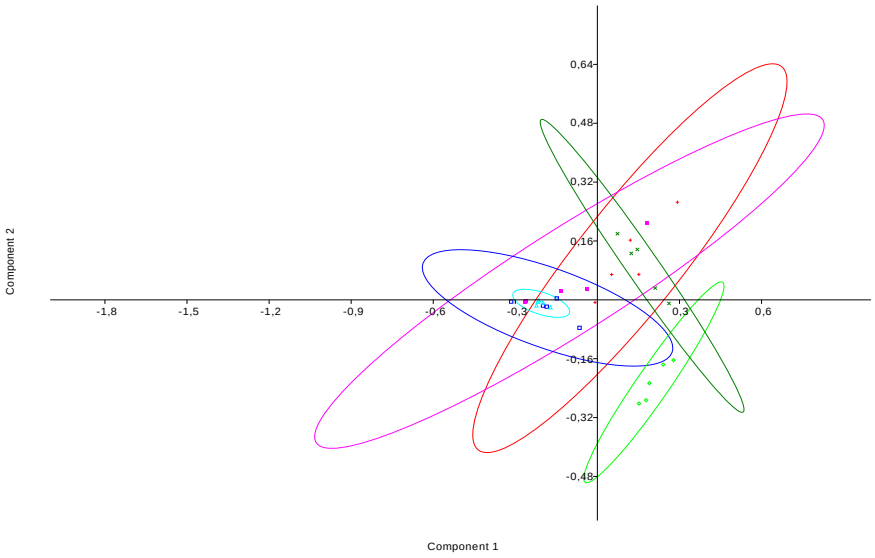

Fig. 4 Differences in communities in samples from different pools. Blue and green correspond to fin surface microbiomes, pink and red correspond to gill microbiomes and lime and green correspond to intestinal microbiomes from pools No. 3 and No. 6, respectively.

Identification of taxa that reflect the specifics of each type of microbiome (fin, gill, intestinal) was performed by comparing the fin with intestinal microbiomes, fin with gill microbiomes and gill with intestinal microbiomes. We registered the following: taxa, abundance of which in different communities differed by ten or more times; taxa, which had different proportions in different communities; taxa, abundance of which in different communities was almost the same; the number of taxa that were present in both microbiomes of a certain pair; the participation of numerous taxa with proportion in a community of more than 0.1% in each of the identified categories.

We identified 252 taxa to be present in both the fin and intestinal microbiomes (P/A). The abundance of 21 taxa was ten or more times higher in the fin surface community than in the intestinal community. These included the representatives of the families Coriobacteriaceae, Lactobacillaceae, Fusobacteriaceae, Mogibacteriaceae, Leuconostocaceae, Clostridiaceae, Porphyromonadaceae, Turicibacteraceae, Bacteroidaceae, Peptostreptococcaceae, Verrucomicrobiaceae, Enterobacteriaceae, Erysipelotrichaceae, Streptococcaceae, Neisseriaceae, Halomonadaceae; non-attributed representatives of the orders Bacteroidales, Clostridiales and Lactobacillales and of the class Bacilli, as well as unidentified bacteria. Moreover, in the fin surface microbiome, the abundance of the representatives of the order Bacteroidales was about 700 times lower than in the intestinal microbiome. Streptococcaceae and Fusobacteriaceae families were prevailing in the community, the proportion of these groups was 13 and 60 times lower, respectively, compared to the intestinal microbiome. The abundance of 15 TU was approximately the same and there were no groups of bacteria that dominated in the community among them. The proportion of 84 taxa was higher in the intestinal microbiome compared to the fin surface microbiome. These included the representatives of the families Alteromonadaceae, Legionellaceae, Rhodobacteraceae, Oxalobacteraceae, Koribacteraceae, Dermabacteraceae, Propionibacteriaceae, Rhabdochlamydiaceae, Patulibacteraceae, Xanthomonadaceae, Burkholderiaceae, Chthoniobacteraceae, Nitrospiraceae, Comamonadaceae, Brucellaceae, Nocardiopsaceae, Acetobacteraceae, Bacteriovoracaceae, Beutenbergiaceae, Cryomorphaceae, Rhodospirillaceae, Cytophagaceae, Caulobacteraceae, Erythrobacteraceae, Carnobacteriaceae, Pseudonocardiaceae, Listeriaceae, Microthrixaceae, Flavobacteriaceae, Bifidobacteriaceae, Kouleothrixaceae, Aerococcaceae, Exiguobacteraceae, Isosphaeraceae, Rubrobacteraceae, Sphingobacteriaceae, Weeksellaceae, Phyllobacteriaceae, Xenococcaceae, Nitrososphaeraceae, Sinobacteraceae, Gaiellaceae, Rhodocyclaceae, Pseudomonadaceae, Paenibacillaceae, Solibacteraceae, Nocardiaceae, Chitinophagaceae, Sphingomonadaceae, Piscirickettsiaceae, Solirubrobacteraceae, Prevotellaceae; unidentified representatives of the orders Rhizobiales, Acidimicrobiales, Sphingomonadales, Burkholderiales, Bacillales, Alteromonadales, Gemmatimonadales, Acidithiobacillales, Solirubrobacterales, Sphingobacteriales, WD2101, MIZ46, N1423WL, DS-18, CCU21, NB1-j, 0319-7L14, SBR1031; representatives of the classes Acidobacteria, Chloracidobacteria, Gammaproteobacteria, Anaerolineae, TM7-3, S085, ML635J-21, Gitt-GS-136, Gemm-1, OPB56; prokaryotes of the phylum FBP. The proportion of the representatives of the OPB56 class was about 670 times higher.

We found 351 taxa to be present in both fin and gill microbiomes (P/J). Thirty groups had lower abundance in the gill microbiome compared to the fin microbiome. These included the representatives of the families Caldilineaceae, auto67_4W, Thiotrichaceae, Turicibacteraceae, Opitutaceae, Leuconostocaceae, Coxiellaceae, Mogibacteriaceae, Halomonadaceae, Rhabdochlamydiaceae, HTCC2089, Haliangiaceae, Planctomycetaceae, Ellin515, OM60, Pirellulaceae, Streptococcaceae, Thermogemmatisporaceae, Geobacteraceae; representatives of the orders Phycisphaerales, Bacteroidales, Acidithiobacillales, Chlamydiales, Spirobacillales, Caulobacterales, Ellin6513, SC-I-84; representatives of the classes C0119 and Bacilli; prokaryotes identified as the representatives of the phylum Chloroflexi. The abundance of bacteria from the Caldilineaceae family was 83 times lower. The proportion of the representatives of the Streptococcaceae family, which was one of the abundant taxa in the fin microbiome, was 12 times lower. No noticeable changes in abundance were observed for 51 taxa, one of which accounted for a significant proportion in the structure of both communities. Only seven taxa had a significantly higher proportion in the gill microbiome than in the fin surface microbiome, among which were the representatives of the families Listeriaceae, Exiguobacteraceae, Dermabacteraceae, Bifidobacteriaceae, Piscirickettsiaceae, the order Sva0725 and the phylum FBP. The difference in abundance was less significant than in the comparison of the “surface” microbiomes with intestinal microbiomes – the most noticeable difference in the proportion (by 26 times) was observed for the Piscirickettsiaceae family.

As in the case of the fin surface microbiome, the number of taxa that were found in both gill and intestinal microbiome was 252. The proportion of the representatives of eight taxonomic groups was significantly lower in the intestinal microbiome than in the microbial community of the gill surface (J/A). These included the families Coriobacteriaceae, Lactobacillaceae, Bacteroidaceae, Fusobacteriaceae, Verrucomicrobiaceae, the orders Bacteroidales and Lactobacillales and unidentified bacteria. At the same time, the greatest difference was observed for the representatives of the Coriobacteriaceae family – the abundance of this group in the intestinal microbiome was 133 times lower than in the gill microbiome. The proportion of the representatives of the Fusobacteriaceae family, which were abundant in the intestinal microbiome, was 14 times lower. In 17 taxonomic groups, there was no noticeable difference in the abundance and one taxon among these accounted for a significant proportion in the community. The abundance of 101 taxa was more than ten times higher in the gill microbiome than in the intestinal microbiome. These included the representatives of the families: Bacteriovoracaceae, Xanthomonadaceae, Flavobacteriaceae, Isosphaeraceae, Alicyclobacillaceae,

Caulobacteraceae, Oxalobacteraceae, Rhodobacteraceae, Koribacteraceae, Methylophilaceae, Intrasporangiaceae, Propionibacteriaceae, Carnobacteriaceae, Cytophagaceae, Syntrophobacteraceae, Weeksellaceae, Chthoniobacteraceae, Cryomorphaceae, Comamonadaceae, Desulfovibrionaceae, Bradyrhizobiaceae, Bradyrhizobiaceae, Ellin6075, Mycobacteriaceae, Brucellaceae, Pseudomonadaceae, Caldilineaceae, Legionellaceae, Sinobacteraceae, Acetobacteraceae, EB1017, Pirellulaceae, Coxiellaceae, Pseudonocardiaceae, A4b, Parachlamydiaceae, Aurantimonadaceae, Gaiellaceae, Gemmataceae, Haliangiaceae, Microthrixaceae, Paenibacillaceae, Solibacteraceae, Planctomycetaceae, auto67_4W, Erythrobacteraceae, Solirubrobacteraceae, Rhodospirillaceae, Rhodocyclaceae, Phyllobacteriaceae, C111, Nitrospiraceae, Thiotrichaceae, Nitrososphaeraceae, Opitutaceae, Chitinophagaceae, Sphingomonadaceae, Nocardiaceae, OM60, Rubrobacteraceae, Prevotellaceae; unidentified representatives of the orders B97, Myxococcales, SBla14, Saprospirales, DS-18, d113, Burkholderiales, Tremblayales, CCU21, Pedosphaerales, WD2101, Gaiellales, Sphingomonadales, Solirubrobacterales, Rhizobiales, Legionellales, Acidimicrobiales, Actinomycetales, Chlamydiales, Ellin6513, Gemmatimonadales, Sphingobacteriales, SC-I-84, 0319-7L14, Caulobacterales, Rhodospirillales, NB1-j, N1423WL, RB41, MIZ46, Acidithiobacillales; unidentified representatives of the classes TM7-1, SJA-4, Ellin6529, ML635J-21, Chlamydiia, OPB56 and organisms that belong to the Chloroflexi phylum. For many taxa, we observed significantly higher abundance in the gill microbiome compared to the intestinal microbiome: the proportion of the unidentified representatives of the OPB56 class was about 1,300 times higher, the abundance of 19 taxa was more than 100 times higher. For comparison, in the pair intestinal microbiome – fin surface microbiome, only eight TU were characterized by such a difference in the proportion in the community.

The listed taxa were a discriminant mark of intestinal, gill and fin surface microbiomes. It is important to note that most of these taxa were not dominant in abundance. In the future studies, we will compare the obtained data with the results of the analysis of the microbiomes of water and biofilters and the probable origin of these taxa might be clarified and the prospects for using this data to organize microbiological monitoring of aquaculture will be studied (Figure 5).

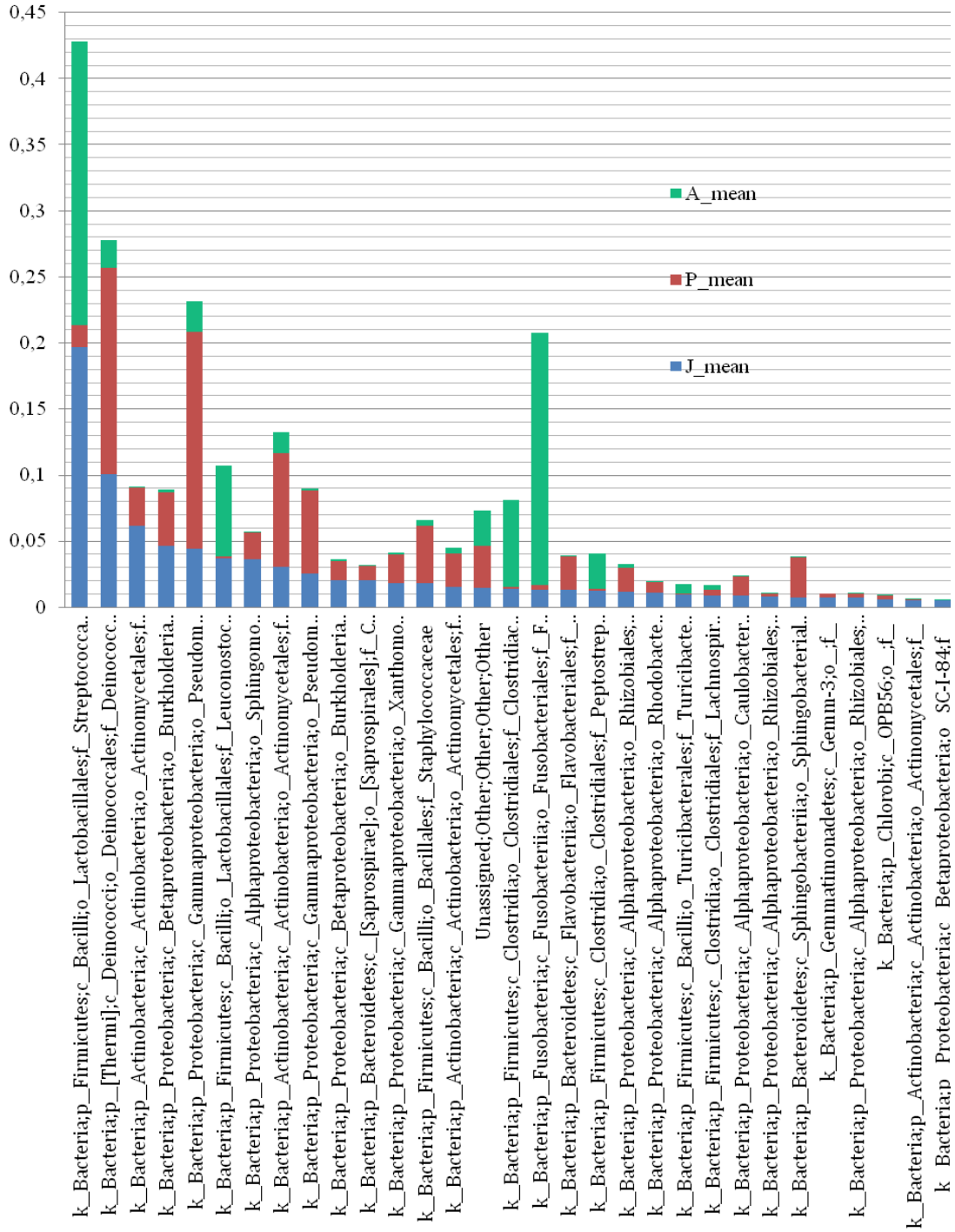

Fig. 5. Distribution of taxa by different types of samples.

Overall, our results showed that microbiomes obtained from the surface organs of fish were more similar to each other less similar to the intestinal microbiome. Comparison of the different types of samples shows that the gill and fin surface microbiomes were characterized by a more even distribution of taxa in the sample, while the taxonomic composition of the intestinal microbiome was more specific.

IV. CONCLUSION

The highest values of the diversity indicators – the Simpson index (evenness), which demonstrates the evenness of the community, the Chao index (richness), which reflects species richness, and the Shannon index, which is an intermediate indicator, – were recorded for the communities obtained from the surface of the gills. The intestinal microbiomes of the two pools had the greatest difference and the microbiomes of the fin surfaces had the smallest difference. The dependence of the degree of differences between microbiomes on the pool they were obtained from increased in the following order:

fin surface communities – gill surface communities – intestinal communities. Fin microbiomes were the least dependent on environmental conditions.

Therefore, the data obtained during the metagenomic analysis of the natural microbiome of sturgeons allows one to estimate the level of risk of the occurrence of infectious diseases caused by opportunistic pathogenic microflora and to develop and correct therapeutic and preventive measures.

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