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Examination of the Microbiome of Bastard Sturgeon Cultivated in the Conditions of Recirculated Water

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Abstract*: The research was aimed at analyzing the microbiome of the skin, the gill lamellae, and the intestines of sturgeon fish. After examining the structure of the microbial community of bastard sturgeon bred in the conditions of closed-circuit water supply, a conclusion can be made both about the physiological state of the fish and about possible pathologies. For making a collection, the following samples were taken from five bions in pool No. 3 and five bions in pool No. 6: the surface of the skin, namely, pieces of fins of sturgeon fish of Bastard Sturgeon species, and gill lamellae (by slicing the gills), and from the intestines (by introducing a swab through the cloaca). The samples were fixed in 96 % ethanol at the material collection locations. Each sample was assigned an identification number. DNA was isolated from the tissues of sturgeon using the set of reagent (MACHEREY-NAGEL NucleoSpin Soil) made by MACHEREY-NAGEL (Germany). The obtained sequences were processed using the Trimmomatic Fastq-Join toolkits, and OTU-picking was performed using the QIIME package. The studies showed that the greatest differences between the pools were in the microbiomes of the intestinal hole, and the least — in the microbiomes of the fin surface, i.e., the effect of a pool on the expression of the differences between the microbiomes increased in the following row: fins – gills – intestinal communities.

***Keywords*: sturgeon, closed-circuit water supply installation, metagenomics, microbiome, sequencing.**

I. INTRODUCTION

The world's oldest representatives of ichthyofauna are sturgeon fish, the main reserves of which for many centuries have been concentrated in the Caspian Sea. To date, the official catch of sturgeons sharply decreased and in general amounted for the Caspian Sea to less than one thousand tons. Many fish species are on the verge of extinction; the gene pool of separate races and populations of sturgeon fish has been lost. Despite this problem, the demand for products of sturgeon breeding (black caviar and marketable sturgeon) is not decreasing.

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On this background, sturgeon breeding farms with innovative technologies become relevant, where the industrial aquaculture is considered a dynamically developing area, which can resolve the problem of food security [1-6]. One of the ways of preserving the natural populations and increasing the stock of sturgeon species is associated with breeding in the conditions of recirculation aquaculture systems (RAS); however, even in these carefully controlled conditions, various pathologies of noncommunicable, infectious and parasitic etiology are registered. The reason for diseases in sturgeons are violations of the veterinary-sanitary and zoohygienic rules of keeping (pollution of the aquatic habitat, changes in the temperature and changes in the chemical composition of water, increased density of aquatic organisms in restricted areas), fish feeding, failure to take quarantine measures for imported new species of fish for reproduction, and other factors. Despite the circularity of the recirculated aquaculture fish farming system, there is still active contact of the fish with various environmental factors: feeds and ingredients thereof, fish care items and equipment, clothing of the personnel, which can contribute to the ingress of pathological elements from the outside and become the etiology of diseases. Besides, in the conditions of RAS, many diseases in sturgeon occur upon reduction of the overall resistance of the fish organism under the influence of various factors, when the opportunistic pathogenic microflora, which is part of the natural microflora of the water, starts showing the pathogenic actions and increases the risk of acute diseases, for example, aeromonosis and pseudomonosis that pass into the chronic forms, subsequently resulting in fish kill, thereby causing significant economic damage to fish breeding companies, which includes the loss of productivity, growth slowdown, deterioration of the marketable appearance, and fish kill [7-10]. In this regard, the role of an accurate analysis of the microbial background of healthy sturgeons and units of RAS, as well as the role of regular microbiological monitoring increase. In our studies, we suggest a new approach, which consists in detailed studying of the structural features of the microbial community in RAS using modern molecular methods, and in analyzing the relationship between the structure of microbial communities and the resistant status of sturgeon species. The "Everything is everywhere the environment selects" hypothesis formulated in 1934 under the influence of Beyerinck's ideas involved a vast research program, which has not lost its relevance today.

In respect to the problem of microbiological monitoring and predicting the physiological state of sturgeons grown in RAS, this hypothesis can be reformulated in the following way: the taxonomic structure of the overall microbial community in RAS is an extremely sensitive indicator of fish health. With that, even minor changes in the structure of the microbial community, chemical and physical indicators, etc. immediately affect the physiological status of the fish [11-15].

Thus, studying the structure of the microbial community in RAS, one can make a conclusion both about the physiological state of the fish and about possible pathologies. Surely, all these conclusions can only be made based on comprehensive preliminary studies. The main objective of the research was developing a complex of advanced molecular-genetic approaches, which for the first time would enable rapid and efficient analysis of the microbial communities in RAS [16, 17].

II. METHODS

A. General description

The objects of the study were five bastard sturgeons (Acipenser nudiventris) from pool No. 3 and five bastard sturgeons from pool No. 6 at the age of four to five years. Samples of the test material were taken using the method for taking in vivo biological samples, without decapitation of the sturgeons.

B. Algorithm

For studying the metagenome of bastard sturgeons, samples of DNA were isolated with the set of reagents (MACHEREY-NAGEL NucleoSpin Soil) made by MACHEREY-NAGEL (Germany) according to the manufacturer's instructions.

The amplicon libraries for the variable segment of gene 16SpPHKv3-v4 (GTGCCAGCMGCCGCGGTAA /GGACTACVSGGGTATCTAAT) were obtained with universal primers F515/R806.

The nucleotide sequence of the fragments was analyzed according to the technology of the Illumina company using apparatus Illumina MiSeq (USA) with a set of reagents MiSeq® ReagentKit v3 (600 cycle) with double-sided reading (2*300 n). The obtained sequences were processed using Illumina software, the Trimmomatic and the QIIME software suite [18].

III. RESULTS

For studying the metagenome of bastard sturgeons, DNA was isolated from the fragments of fins and gills. The samples were preliminarily prepared by mechanical destruction of the material. About 0.2 g of the fragment of fins and gills were crushed with a surgical blade into fragments with a size of 0.5 – 1 mm^2. 0.3 g of glass beads with a size of 0.5 mm, and 0.3 g — with a size of 0.1 mm were added to the fragmented samples along with 0.5 ml of the lysing solution from the kit for DNA isolation MACHEREY-NAGEL NucleoSpin Soil made by MACHEREY-NAGEL (Germany). The vials were shaken on a vortex in the horizontal position for five minutes at the maximum speed. Further isolation was performed following the manufacturer's instructions. During DNA isolation from the contents of the intestines, the swab was also crushed and 0.3 g of glass beads with the size of 0.5 mm and 0.3 g with the size of 0.1 mm were added to it, along with 0.5 ml of the lysing solution from the kit for DNA isolation MACHEREY-NAGEL NucleoSpin Soil (Figure 1).

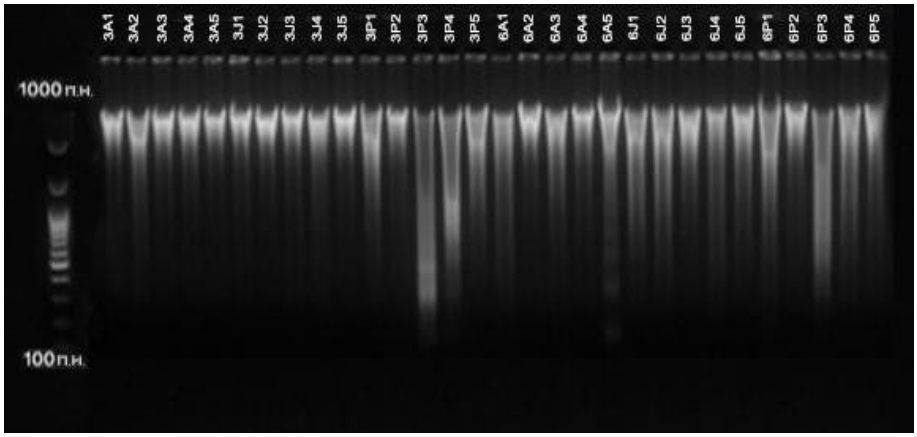

Fig. 1: Electrophoresis of the DNA isolated from fins (A), the gill plates (J) and the intestines (P) of bastard sturgeon

The DNA yield was 50 µl with the concentration of 20 – 50 ng/µl

By the results of the taxonomic analysis of the obtained libraries, a total of 423 taxonomic units (TU) were found. The most numerous for the microbiome from the surface of the gills were the representatives of the following families: Pseudomonadaceae, Chitinophagaceae, Moraxellaceae, Fusobacteriaceae, Clostridiaceae, Oxalobacteraceae, Sphingomonadaceae, Leuconostocaceae, Comamonadaceae, Nocardiaceae, Streptococcaceae, Deinococcaceae, Micrococcaceae, Staphylococcaceae, and Microbacteriaceae (Figure 2).

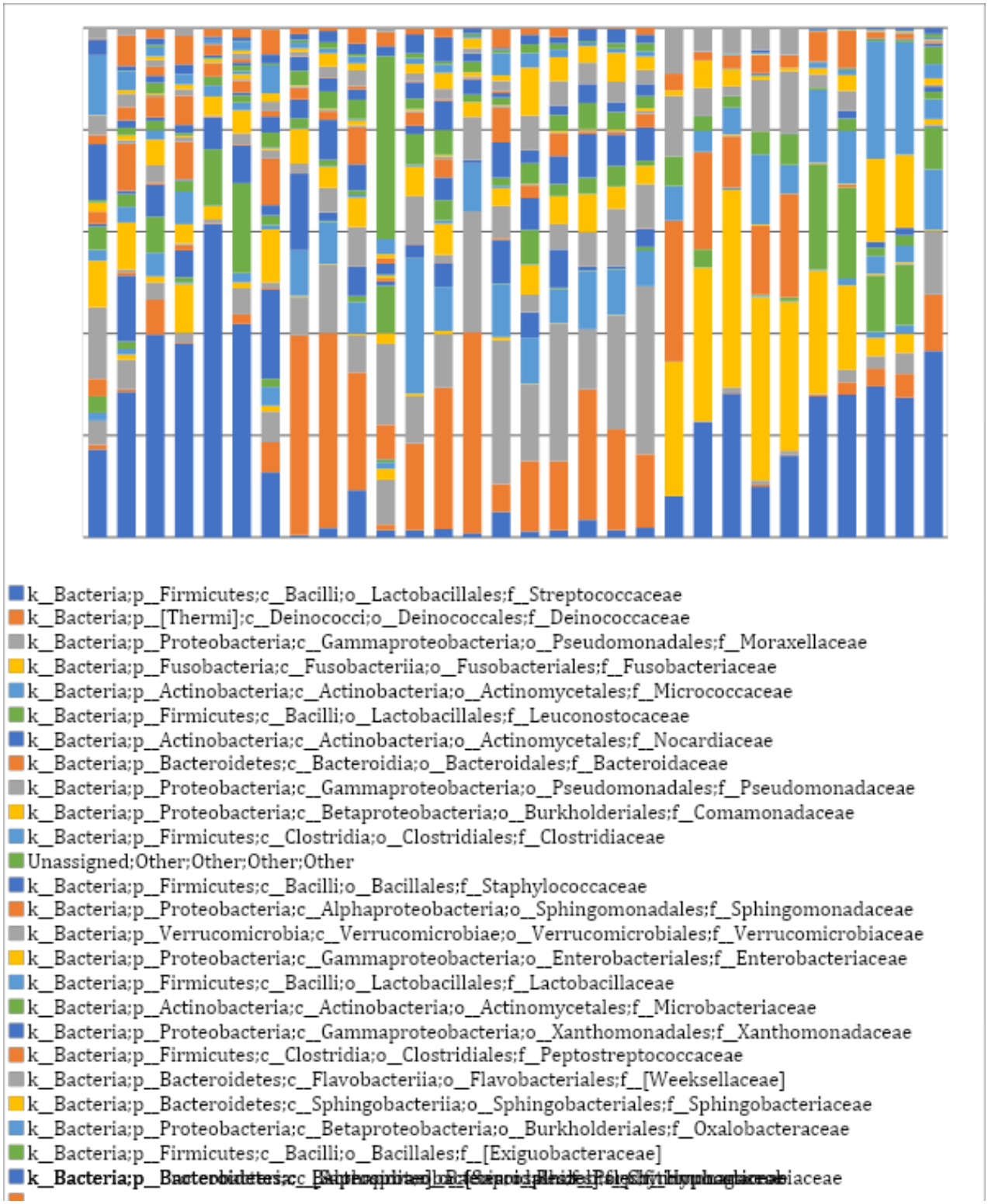

Fig. 2: Distribution of the main taxonomic groups by samples

In the microbiome characteristic of the surface of the skin, the representatives of the following families dominated: *Deinococcaceae, Moraxellaceae, Micrococcaceae, Pseudomonadaceae, Exiguobacteraceae, Comamonadaceae, Staphylococcaceae, Nocardiaceae, Sphingomonadaceae, Xanthomonadaceae, Weeksellaceae, Microbacteriaceae*, as well as a non-attributed group of organisms.

For the scrape taken from the rectum, the most typical were prokaryotes of the following families: *Fusobacteriaceae, Bacteroidaceae, Streptococcaceae, Verrucomicrobiaceae, Clostridiaceae, Porphyromonadaceae, Peptostreptococcaceae, Enterobacteriaceae, Leuconostocaceae, Lactobacillaceae, Deinococcaceae, Moraxellaceae, Enterococcaceae, and Micrococcaceae*. Also, a noticeable share is taken by a group of unidentified organisms.

The level of diversity of the communities was evaluated by the following environmental indicators: the overlap coefficient (evenness) that characterized the uniformity of the community, the Chao's coefficient (richness), which reflected the species richness, and Shannon's coefficient, which was an intermediate indicator. The greatest values for all three estimates were characteristic of the communities obtained from the surface of the gills. The least values were observed in the communities obtained from intestinal scrapes (Table 1).

Table 1: The values of the overlap coefficient, Shannon's coefficient, and Chao's coefficient by samples

Sample_name	Simpson_1-D	Shannon_H	Chao-1
1	2	3	4
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
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
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
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
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
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

The taxa that reflected the specific features of the microbiomes of each type (fin, gill, and intestines) were identified by comparing the fin microbiome to the intestinal one, fin microbiome to the gill one, and the gill microbiome to the intestinal one. This identified the taxa that changed their number upon comparison of the communities 10 or more times; the taxa that changed their shares the most; the taxa that retained their number almost unchanged; the number of taxa common to both microbiomes in the compared pair; and participation of numerous taxa with the share in the community of more than 0.1 % in each of the identified categories.

A comparison of the fin and intestinal microbiomes (P/A) revealed 252 common taxa. Upon transition from the community characteristic of the surface of the fins to the community from the rectum, 21 taxa showed a reduction in their number more than 10 times. These were the representatives of the following families: *Coriobacteriaceae, Lactobacillaceae, Fusobacteriaceae, Mogibacteriaceae, Leuconostocaceae, Clostridiaceae, Porphyromonadaceae, Turicibacteraceae, Bacteroidaceae, Peptostreptococcaceae, Verrucomicrobiaceae, Enterobacteriaceae, Erysipelotrichaceae, Streptococcaceae, Neisseriaceae, Halomonadaceae*; not attributed representatives of orders *Bacteroidales, Clostridiales*, and *Lactobacillales*, class *Bacilli*, as well as unidentified organisms from the Bacteria kingdom. With that, in the microbiome from the surface of the fins, the representatives of the *Bacteroidales* order reduced their number about 700 times, and the number of the taxa prevailing in the community included families *Streptococcaceae* and *Fusobacteriaceae*, the share of which reduced 13 and 60 times, respectively, compared to the intestinal microbiome. The quantity of 15 TU remained the same, and among them, groups of bacteria that dominant in the community were not found. Eighty four taxa were characterized by increasing their shares in the intestinal microbiome, compared to the fin microbiome. These included: the representatives of families *Alteromonadaceae, Legionellaceae, Rhodobacteraceae, Oxalobacteraceae, Koribacteraceae, Dermabacteraceae, Propionibacteriaceae, Rhabdochlamydiaceae, Patulibacteraceae, Xanthomonadaceae, Burkholderiaceae, Chthoniobacteraceae, Nitrospiraceae, Comamonadaceae, Brucellaceae, Nocardiopsaceae, Acetobacteraceae, Bacteriovoracaceae, Beutenbergiacae, 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 to families of the 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; the representatives of classes *Acidobacteria, Chloracidobacteria, Gammaproteobacteria, Anaerolineae*, TM7-3, S085, ML635J-21, Gitt-GS-136, Gemm-1, OPB56; and prokaryotes that belonged to phylum FBP.

The representatives of class OPB56 increased their share in the community approximately 670 times.

For the microbiomes of fins and gills (P/J), 351 taxa were common. A decrease in number upon transition from the fin microbiome to the gill one was observed in 30 groups. These were the representatives of families *Caldilineaceae*, auto67_4W, *Thiotrichaceae*, *Turicibacteraceae*, *Opitutaceae*, *Leuconostocaceae*, *Coxiellaceae*, *Mogibacteriaceae*, *Halomonadaceae*, *Rhabdochlamydiaceae*, HTCC2089, *Haliangiaceae*, *Planctomycetaceae*, Ellin515, OM60, *Pirellulaceae*, *Streptococcaceae*, *Thermogemmatisporaceae*, *Geobacteraceae*; representatives attributed to the level of *Phycisphaerales*, *Bacteroidales*, *Acidithiobacillales*, *Chlamydiales*, *Spirobacillales*, *Caulobacterales*, Ellin6513, SC-I-84; the representatives of classes C0119, *Bacilli*; and prokaryotes identified as the representatives of phylum *Chloroflexi*. The number of bacteria of family *Caldilineaceae* decreased 83 times, and the number of family Streptococcaceae, which was one of the numerous taxa in the fin microbiome, decreased 12 times. No notable change in the number was observed in 51 taxa which were a significant part of the structure of both communities. An increased share in the transition from the fin microbiome to the gill one was largely observed only for seven taxa, including families *Listeriaceae*, *Exiguobacteraceae*, *Dermabacteraceae*, *Bifidobacteriaceae*, *Piscirickettsiaceae*, order Sva0725, and phylum FBP. A change in the number was less significant than in the case of comparing the "surface" microbiomes to the intestinal one – the most noticeable increase in the share was observed for family *Piscirickettsiaceae* (26 times).

Like in the case with the fin microbiome, the number of common taxa at the gill and the intestinal microbiomes was 252 TU. Upon the transition from the microbial community of the surface of the gills to the intestinal microbiome (J/A), a significant decrease in the share in the community was observed for eight taxonomic groups. These included the representatives of families *Coriobacteriaceae*, *Lactobacillaceae*, *Bacteroidaceae*, *Fusobacteriaceae*, *Verrucomicrobiaceae*, bacteria belonging to orders *Bacteroidales* and *Lactobacillales*, and unattributed representatives of kingdom *Bacteria*. With that, the greatest change was observed in the number of the representatives of family *Coriobacteriaceae* (the number in the intestinal microbiome reduced 133 times, compared to the gill microbiome). The share of the representatives of family *Fusobacteriaceae*, which was quite numerous in the intestinal microbiome, decreased 14 times. Seventeen taxonomic groups did not show any significant changes in the number; they included the taxon with a significant share in the community. One hundred and one taxa increased their number 10 times in the gill microbiome, compared to the intestinal one. These included 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*; the representatives of B97 attributed to the level of the order, *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 classes TM7-1, SJA-4, Ellin6529, ML635J-21, *Chlamydiia*, OPB56, and organisms belonging to phylum *Chloroflexi*. For many taxa, a significant increase in the number of the gill microbiome was noted, compared to the intestinal microbiome: unidentified representatives of class OPB56 increased their share in the community about 1,300 times, and 19 taxa showed an increase in their numbers over 100 times. For comparison, upon transition from the intestinal microbiome to the fin one, only eight TU were characterized by such an increase in their shares in the community.

The listed taxa were the hallmark of the intestinal, the gill, and the fin microbiomes. It is important to note that most of these taxa were not dominant in their number (Figure 3).

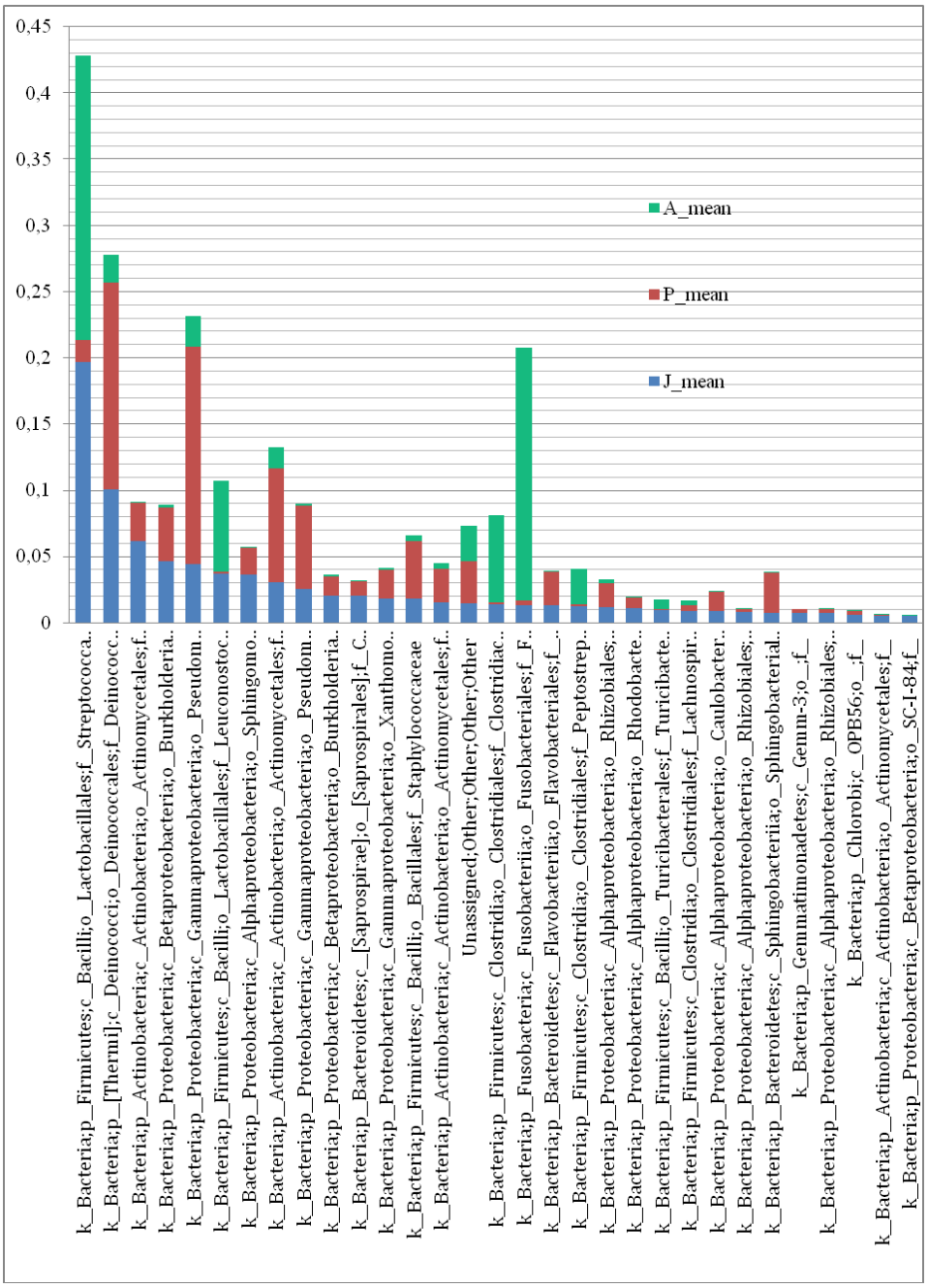

Fig. 3: Taxa distribution by various types of samples

The overall picture shows that the microbiomes obtained from the surface of fish organs are to a greater extent similar to each other, and to a lesser extent — to the intestinal microbiome. A comparison of the representativeness of the main TU in various types of samples also showed that gill and fin microbiomes were characterized by a more balanced distribution of the taxa in the entire sample, while the intestinal microbiome showed greater specificity in the taxonomic composition.

IV. CONCLUSION

The highly productive sequencing of 16spPHK gene in the obtained collection has shown that the most numerous for the microbiome from the surface of the gills are the representatives of families Pseudomonadaceae, Chitinophagaceae, Moraxellaceae, Fusobacteriaceae, Clostridiaceae, Oxalobacteraceae, Sphingomonadaceae, Leuconostocaceae, Comamonadaceae, Nocardiaceae, Streptococcaceae, Deinococcaceae, Micrococcaceae, Staphylococcaceae, and Microbacteriaceae. The natural microbiome of the skin surface is represented by the bacteria of families Deinococcaceae, Moraxellaceae, Micrococcaceae, Pseudomonadaceae, Exiguobacteraceae, Comamonadaceae, Staphylococcaceae, Nocardiaceae, Sphingomonadaceae, Xanthomonadaceae, Weeksellaceae, Microbacteriaceae, as well as an unattributed group of organisms. The rectum microbiome is represented by prokaryotes of families Fusobacteriaceae, Bacteroidaceae, Streptococcaceae, Verrucomicrobiaceae, Clostridiaceae, Porphyromonadaceae, Peptostreptococcaceae, Enterobacteriaceae,

Leuconostocaceae, Lactobacillaceae, Deinococcaceae, Moraxellaceae, Enterococcaceae, Micrococcaceae; a noticeable share belongs to a group of unidentified organisms.

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