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1 **Diet and other environmental factors shape the bacterial communities of fish**
2 **gut in an eutrophic lake**

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22 microbiota.

23 Running title: microbiota of fish gut and their prey items

24 **Abstract**

25 **Aims.** The aim of this work was to study the gut microbial diversity from eight species
26 of wild fish with different feeding habits, digestive physiology (gastric vs. agastric) and
27 provide comparative structural analysis of the microbial communities within their
28 environment (food items, water, sediments, and macrophytes).

29 **Methods and Results.** The microbiota of fish gut and their prey items were studied
30 using next generation high-throughput sequencing of the 16S ribosomal RNA genes. A scatter
31 plot based on PCoA scores demonstrated the microbiota formed three groups: 1) stomach and
32 intestinal mucosa, 2) stomach and intestinal content, and 3) prey and environment.
33 Comparisons using ANOSIM showed significant differences among intestinal content of
34 omnivorous, zoobenthivorous, zooplanktivorous-piscivorous fishes ($p \leq 0.1$). No significant
35 difference was detected for mucosa from the same groups ($p > 0.1$).

36 **Conclusions.** The interspecies differences in fish diet or their phylogenetic position
37 did not affect the microbiome of the intestinal mucosa, but diet might influence the
38 composition of the microbiota of the intestinal content.

39 **Significance and Impact of Study.** The data demonstrate that fish harbored specific
40 groups of bacteria that do not completely reflect the microbiota of the environment or prey.

41

42 **Introduction**

43 It is considered that the bacterial communities are the basis of a trophic pyramid that
44 is, on one side being utilized as a source of food by other animals, whereas from the other side
45 they hydrolyze the organic compounds in aquatic ecosystems (Ugolev 1985) thereby
46 modifying their surroundings. The metabolic plasticity of bacteria has allowed them to adapt
47 to different habitats and occupy various ecological niches (Hugenholtz *et al.* 1998; Fakruddin

48 and Mannan 2013). One such niche, the focus of this study, is the fish gut. The interior of the
49 the fish gut is an extension of the external environment and all the various members of the
50 microbial communities originating from different surrounding ecosystem compartments such
51 as the bottom sediments, water, food items, etc. The degree to which fish may accommodate
52 different bacterial communities should be reflected by differences in the anatomy of the
53 digestive system; while some fish have a properly defined stomach with an acidic pH other
54 species are agastric.

55 Previous studies revealed that the structure of the bacterial community within the gut
56 of freshwater fish is dominated by Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria
57 and Fusobacteria (Roeselers *et al.* 2011; van Kessel *et al.* 2011; Ni *et al.* 2012; Li *et al.* 2013)
58 and are likely to be significantly different from other bacterial communities associated with
59 their immediate environment (bottom sediments, water, surface of hydrobionts and
60 macrophytes, etc) (Romero and Navarrete 2006; Han *et al.* 2010). The aquatic habitats
61 beneficial for fish typically are not eutrophic, and have moderate to low abundance and
62 diversity of microbes. In contrast, the fish gut has a constant influx of carbon-rich nutrients
63 and some degree of protection from eukaryotic microbial predators thereby enhancing
64 microbial abundance and diversity within the gut (Giatsis *et al.* 2015). The abundance and
65 diversity of the gut microbial communities is due to the complex direct and indirect
66 interactions of many external and internal factors such as, age, diet and regime of feeding of
67 the host fish, the section of gut being examined, antimicrobial peptides (AMPs) secreted by
68 the host's eosinophylic granular cells (EGCs), season of the year, chemistry and temperature
69 of the water (Campbell and Buswell 1983; Šyvokienė 1991; Grisez *et al.* 1997; Ringø and
70 Gatesoupe 1998; Šyvokienė *et al.* 1999; Austin 2002; Sullam *et al.* 2012; Ostaff *et al.* 2013;
71 Clements *et al.* 2014). One of the key ecological factors, that is intensively studied and is able

72 to influence the qualitative (taxonomic composition) and quantitative (relative abundance of
73 each taxa) characteristics of the gut microbiota is the fish diet (Tanaka *et al.* 1996; Ringø *et al.*
74 2006; Uchii *et al.* 2006; Yang *et al.* 2007; Ward *et al.* 2009; Wu *et al.* 2010; Sullam *et al.*
75 2012; Bolnick *et al.* 2014; Larsen *et al.* 2014; Li *et al.* 2014; Tietjen 2014; Miyake *et al.* 2015;
76 Kashinskaya *et al.* 2015; Liu *et al.* 2016). However, a large number of these studies are
77 associated with fish species that are grown for aquaculture under specific controlled
78 conditions (Desai *et al.* 2012; Carda-Diéguez *et al.* 2013; Wu *et al.* 2013). Under these
79 controlled conditions of cultured fish, specific information has been obtained regarding the
80 influence of a broad range of dietary components on the microbiome of fish gut (Ringø *et al.*
81 2016). In contrast, fish from natural water bodies, or in open pond-type aquaculture where the
82 fish are partially or completely feeding on natural food items, the interpolation of such
83 information is difficult due to poorly described or unknown proximate composition of food
84 items. In such studies the fish species being examined are normally classified as, for example,
85 detritivorous, herbivorous, carnivorous, and omnivorous according to the dominant food
86 items in their diets. This approach makes the task of determining the relationships between the
87 structure of the gut bacterial community and fish diets much simpler due to the different taxa
88 of food items that can be classified within the same group (benthos, zooplankton, etc.), yet
89 could lead to erroneous conclusions. Hence, the study of many different species of fish with
90 different feeding habits associated to various relevant species-specific factors allows for a
91 more holistic determination of relationships between the compound composition of natural
92 fish diets and the structure of their gut microbiota. It also should be mentioned that in studies
93 where the microbiota of the gut from fish in natural water bodies were examined, the
94 researchers provide information about feeding habits or trophic positions that may be based
95 on previously obtained data, without collecting stomach and gut content of the studied fish for

96 compositional analysis (Sullam *et al.* 2012; Liu *et al.* 2013; Li *et al.* 2014; Baldo *et al.* 2015).
97 There are only a few works that present such data on the actual gut content of sampled fish
98 (Uchii *et al.* 2006). The first meta-analysis of the correlation between different factors,
99 including the type of source for bacterial DNA (intestinal content, complete gut, feces, etc.),
100 and the structure of bacterial communities revealed that most of the analyzed factors were
101 significant (Sullam *et al.* 2012). Most studies of this topic focus on the bacterial communities
102 of the gut content or the entire gut, while in only a few studies has the microbiota been
103 divided into separate mucosal and content components of the gut. Moreover, extrinsic factors
104 from the methodology of data acquisition restrict correct interpretation of results
105 (Kashinskaya *et al.* 2017).

106 The main aim of the present work was to study the structure of the communities of the
107 gut microbiota of sympatric fish species with different feeding habits, digestive physiology
108 (gastric vs. agastric) and provide comparative structural analysis of the microbial
109 communities within their environment (food items, water, sediments, and macrophytes). We
110 propose the hypothesis that the microbial communities of the gut mucus and gut content have
111 different correlations with fish diets, which are related to anatomical and physiological
112 differences among the fish as determined by evolution.

113

114 **Materials and methods**

115 ***Study area and sampling.*** Fish were collected in the middle of summer (June-July),
116 2012 in the estuarine area of the Chany Lake – Kargat River (hereinafter Chany Lake), which
117 is a shallow, eutrophic lake in Western Siberia (Russia, 54⁰36'56.3"N, 78⁰12'5.9"E). The
118 basin area is about 30 thousand km², with the lake having a surface area of (2004) 1500 km²;
119 and depths that fluctuate from 1.4–1.9 m to 4.8–8.5 m (Vasilyev *et al.* 2005). The collection

120 site is near to a canal that empties from the surrounding steppe into the main body of Manye
121 Chany Lake. For comparative analysis of gastrointestinal microbiota we used 51 individuals
122 of eight wild fish species, each with a different dietary regime: Prussian carp *Carassius*
123 *gibelio* (Linnaeus, 1758) (n = 5, total length (TL) = 222.1 ± 3.8 mm); Crucian carp *Carassius*
124 *carassius* (Linnaeus, 1758) (n = 4, TL = 193.8 ± 13.4 mm); Common carp *Cyprinus carpio*
125 (Linnaeus, 1759) (n = 13, TL = 341.2 ± 22.7 mm); roach *Rutilus rutilus* (Linnaeus, 1758) (n =
126 5, TL = 178.0 ± 3.9 mm); dace *Leuciscus leuciscus* (Linnaeus, 1758) (n = 5, TL = 174.6 ± 4.9
127 mm); ide *Leuciscus idus* (Linnaeus, 1758) (n = 7, TL = 282.7 ± 20.2 mm); perch *Perca*
128 *fluviatilis* (Linnaeus, 1758) (n = 8, TL = 160.3 ± 14.7 mm); pike-perch *Sander lucioperca*
129 (Linnaeus, 1758) (n = 4, TL = 277.6 ± 18.9 mm).

130 All fish were captured using gill-nets (mesh sizes 25 to 65 mm) and transported alive
131 to the laboratory in plastic containers (duration approximately 1 h). All fish were sacrificed
132 and mucosa and gut content samples collected aseptically as previously described
133 (Kashinskaya *et al.* 2015). For all individuals total DNA was extracted from 100 mg of each
134 subsample of intestinal mucosa (IM), intestinal content (IC), stomach mucosa (SM), and
135 stomach content (SC).

136 In addition, water, sediment and common reed (*Phragmites australis*) samples were
137 collected nearby the fish capture sites. Water was sampled from the upper 0.5 m of the water
138 column and pooled together from three locations in a sterile 3 L glass bottle. Microorganisms
139 from the water were collected by filtration of 100 mL of water onto 0.22 mm pore size
140 polyethersulfone membrane filter (22 mm diameter, Millipore, EXPRESS PLUS™).
141 Sediment samples were collected in a total mass of 5 g using a Petersen grab. The samples of
142 sediment from three locations were mixed and 0.1 g was used to extract DNA. Scrapings from
143 the underwater parts of 2 - 3 trunks of common reed were sampled with a spatula from an

144 approximate depth of 0.3 - 0.5 m and collected and pooled together into sterile tubes.
145 Approximate mass for DNA extraction was 0.1 g of wet plant material. The choice of
146 common reed as one of the environmental contributors to the fish gut microbiota was based
147 on the dominance of these plants in the surrounding water body (Vasilyev *et al.* 2005).

148 To better understand the environmental factors that influence the microbiota of the fish
149 gut, 28 individuals of invertebrates from 9 different taxa were also collected. The choice of
150 invertebrates was based on the dominant taxa of food objects analyzed in fish gut contents.
151 Invertebrates were collected at the same site of fish capture. The microbiota from the whole
152 body of the studied invertebrates was analyzed. Before DNA extraction the food objects were
153 rinsed in sterile distilled water three times. For additional details about sample collection see
154 Table 1.

155 ***Identification of fish feeding habits according to primary diet.*** Identification of the
156 prey organisms and determination of the importance of each prey in the fish dietary regime
157 was previously described in Solovyev *et al.* (2014). The degree of similarity of diet between
158 fish with different feeding habits was analyzed by Morista index which was carried out using
159 PAST, v. 3.16 (Hammer *et al.* 2011) and cluster analysis (Euclidean distance) using Statistica
160 6 (StatSoft; www.statsoft.com).

161 ***Sample preparation and DNA extraction.*** Before the DNA extraction, all 163 samples
162 (126 from all fish; 9 from environment microbiota; and 28 from invertebrates) were collected
163 into sterile microcentrifuge tubes with lysis buffer for DNA isolation and mechanically
164 homogenized by pestle for 1 min using a hand-held homogenizer. All samples were processed
165 to extract DNA following the DNA-sorb B kit manufacturer's protocols (kit for DNA
166 extraction, Central Research Institute of Epidemiology, Moscow, Russia).

167 Equimolar concentrations of total DNA extracted from each fish sample originating
168 from the same species, were pooled together to avoid erroneous conclusions that might occur
169 from high individual variation likely to be found in wild caught fish, as opposed to
170 commercially raised fish grown under highly uniform conditions (Ringø *et al.* 1995; Han *et*
171 *al.* 2010; Spanggaard *et al.* 2000; Roeselers *et al.* 2011; Sullam *et al.* 2012; Zarkasi *et al.*
172 2014; Kashinskaya *et al.* 2015).

173 ***16S rDNA library sequencing.***

174 All samples were analyzed and sequenced on MiSeq Illumina sequencer at the SB
175 RAS Genomics Core Facility (ICBFM SB RAS) as previously described (Kashinskaya *et al.*
176 2015), except samples from spiny water flea, diving beetle and water mite that were sent to a
177 commercial subcontractor (Envrogen, Moscow) and sequenced using the primer pair 5'-
178 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and
179 5'-
180 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-
181 3' that also target the same region of the 16S rDNA (Klindworth *et al.* 2013). Forward and
182 reverse read pairs were merged and quality filtered with Mothur 1.31.2 (Schloss *et al.* 2009).
183 Any reads with ambiguous sites and homopolymers of more than eight bp were removed, as
184 well as sequences shorter than 350 or greater than 500 bp. QIIME 1.9.1 (Caporaso *et al.* 2010)
185 was used for further processing of the sequences. *De novo* (abundance based) chimera
186 detection using USEARCH 6.1 (Edgar 2010) was applied to identify possible chimeric
187 sequences ('identify_chimeric_seqs.py' with an option '-m usearch61' in QIIME). After
188 chimera filtering, the QIIME script 'pick_open_reference_otus.py' with default options was
189 used to perform open-reference OTU picking by UCLAST (Edgar 2010), taxonomy
190 assignment (UCLAST, with a 0.80 confidence threshold), sequence alignment (PyNAST

191 1.2.2; Caporaso *et al.* 2010) and tree-building (FastTree 2.1.3; Price *et al.* 2010). This
192 algorithm involves several steps of both closed-reference and open-reference OTU picking
193 followed by taxonomy assignment, where the Greengenes core reference alignment (release
194 ‘gg_13_8’; DeSantis *et al.* 2006) was used as a reference. Chloroplast, mitochondria and non-
195 bacterial sequences were removed from further analysis. Raw reads were deposited in the
196 Sequence Read Archive (NCBI), accession numbers: SRP056565, SRP065371, SRP065460,
197 SRP065458, SRP065250, SRP065362, SRP056759, SRP125534.

198

199 ***Analysis of alpha and beta diversity.*** The samples were rarified to the lowest
200 sequencing effort (4863 sequences) using QIIME. The richness (number of OTU’s and Chao1
201 index) and diversity estimates (Shannon and Simpson index) per sample were calculated
202 using the same program. For estimating the differences between the richness and diversity
203 estimates NPMANOVA at $p \leq 0.05$ using PAST, v. 3.16 (Hammer *et al.* 2011). A weighted
204 UniFrac dissimilarity matrix (Lozupone and Knight 2005) was calculated and used for
205 downstream analyses. The matrix was used to perform principle coordinates analysis (PCoA)
206 to visualize differences among groups of samples (stomach mucosa, stomach content,
207 intestinal mucosa, intestinal content, prey, and environmental microbiota). To test the effect of
208 various explanatory variables: type of tissue (mucosa, content), trophic groups of fish
209 (omnivorous, zoobenthivorous-zooplanktivorous and piscivorous), fish physiology (agastric,
210 gastric), environmental compartments (prey, water, sediment and reed), on the groupings of
211 bacterial communities, the analysis of similarities (ANOSIM) on the distance matrix were
212 used as implemented in QIIME. Significance was determined by 10,000 permutations.

213 ***Testing correlations between fish diet and gut microbiome.*** The simple and partial
214 Mantel tests were used to test the hypothesis that structure of microbial communities of fish

215 gut content and/or gut mucosa is associated with fish diet. To this aim, dissimilarity matrices
216 of fish diet (Morista) and microbial communities of gut content and gut mucosa (weighted
217 UniFrac) were used. The genetic distance matrix between fish species were created and used
218 in a partial Mantel test to control for the effect of phylogenetic relationships. The partial
219 cytochrome oxidase subunit 1 (COI) sequences (652 bp long) representing each fish species
220 were mined from GenBank (*C. gibelio*: HM392057; *C. carassius*: HQ960716; *C. carpio*:
221 HM392076; *R. rutilus*: HM392103; *L. leuciscus*: HM902153; *L. idus*: HM902149; *P.*
222 *fluviatilis*: HM902175; *S. lucioperca*: HQ960674). The genetic K2P distances were calculated
223 in MEGA 6.0 (Tamura *et al.* 2013). The Mantel test was carried out using zt software (Bonnet
224 and Van de Peer 2002) with significance testing by 10,000 permutations.

225 **Results**

226 ***1. Diets of fish in Chany Lake.***

227 Intestinal content analysis identified detritus and chironomid larvae (*Chironomidae*
228 sp.) as the dominant food of adult Prussian, Crucian and Common carp (frequency of
229 occurrence is 100.0, 66.7 and 100.0%, respectively).

230 The diets of dace, roach and ide were dominated by the zooplanktonic spiny water flea
231 *B. longimanus* (frequency of occurrence is 54.5, 65.4. and 100.0%, respectively).

232 The stomach and intestinal contents of pike-perch is made up essentially of fry stage
233 fish from the Cyprinid family (100.0%), another small part of the diet of pike-perch was
234 provided by chironomid larvae and *B. longimanus* (frequency of occurrence is 100.0, 66.7 and
235 100.0%, respectively). The perch's diet is based largely on three groups of organisms: fish fry
236 from the Cyprinid family (up to 71.4%), benthic organisms (amphipods, larvae of
237 trichopterans, chironomid larvae and pupa's, molluscs) and zooplanktonic organisms (*B.*

238 *longimanus*). The components of secondary importance to the diet of these fish were more
239 species-specific (Fig. 1).

240 The Morista index was calculated to analyze the degree of similarity of diets among
241 studied fish species with different feeding habits (Table 2). Results from cluster analysis using
242 the Morista index values (not shown) identified three groups of fish: the first group
243 (omnivorous) includes Prussian carp, Crucian carp and Common carp ($0.89 < M_i < 0.92$); the
244 second group (zoobenthivorous-zooplanktivorous) is formed from roach, dace, and ide (0.81
245 $< M_i < 0.88$), and the third one (piscivorous) is presented by perch and pike-perch ($M_i = 0.71$).

246

247 ***2. Sequencing data and diversity analysis of the intestinal microbiota of fish and***
248 ***associated microbiota of environmental compartment.***

249 After rarification to the lowest sequencing effort samples contained from 106 to 1238
250 OTUs (Table 3). The rarefaction curves for all studied groups of samples reached a plateau
251 (not shown).

252 In the mucosa the highest species richness (number of OTU's and Chao1 value) was
253 observed in the perch and dace microbial communities, while the lowest one was detected in
254 the Crucian carp community (151 and 269.33, respectively). In the gut content the highest
255 species richness was detected in Common carp (423 and 834.48 for OTU's and Chao1,
256 respectively), while the lowest was observed in perch (106) and pike (288.48) for observed
257 number of OTUs and Chao1 index, respectively. The Shannon diversity index in both mucosa
258 and gut content ranged between 1.31 and 4.72, with the lowest and the highest ones in
259 stomach content and mucosa of perch. The Simpson index was at the same level (0.8 ± 0.03)
260 except for the stomach content of perch. All alpha diversity statistics are detailed in table 3.

261 No significant differences (Table 4) were observed for Shannon index values among
262 fish from different digestive morphology groups (One-way NPMANOVA, $p > 0.05$), but the
263 number of observed OTU's and Chao1 values between mucosa and intestinal content of
264 agastric fish were significantly different (OTU's: $p = 0.01$; Chao1: $p = 0.006$). A significant
265 difference was also observed for the Simpson index between mucosa of agastric fish and
266 content of gastric fish ($p = 0.02$). No significant differences were observed for both richness
267 and diversity estimates (Table 5) among trophic groups of fish ($p > 0.05$). Significant
268 differences were only observed for Chao1, number of OTU's and Shannon index between
269 microbiota associated with environment (water, sediment, and reed) and prey ($p \leq 0.05$) and
270 for Shannon and Simpson index values between prey and intestinal content of piscivorous fish
271 (Table 5).

272 The highest species richness in the bacterial community from prey was observed in the
273 diving beetle (590 OTU's; Chao index is 1214.84), while the lowest one was detected in the
274 water mite community 126 OTU's; Chao index is 219.88). The results of diversity estimates
275 showed that microbiota of *Gammarus* sp. were more diverse than microbiota of other preys.
276 Similarly, the highest richness and diversity estimates were observed in the sediment
277 community, while the lowest one was detected in the water community (Table 3).

278

279

3. Microbiota composition of gut mucosa and content of fish species.

280 Twenty four bacterial phyla were identified from the mucosa and content of fish. The
281 results of 16S rDNA sequencing showed that Proteobacteria and Bacteroidetes were the most
282 dominant phyla in all fish species, except pike-perch (Fig. 2a). Microbiota composition of gut
283 mucosa and content of analyzed fish species was significantly different (ANOSIM: $R = 0.86$,
284 $p = 0.01$). In all analyzed fish, except for Prussian carp, Common carp and roach, the phylum
285 Bacteroidetes was more abundant (varying from 47.5 to 66.7 %) in the mucosa than in

286 intestinal content (NPMANOVA, $p \leq 0.001$) (Fig. 2b). In contrast, the intestinal content was
287 dominated by Proteobacteria (from 36.8 to 98.4%, NPMANOVA, $p \leq 0.01$) except in pike-
288 perch which were dominated by Fusobacteria (70.0%).

289 As shown in figure 3 a and b at the family level, the microbiota of fish were also very
290 different between mucosa and content. The most abundant OTUs with 5% abundance
291 threshold associated with the intestinal mucosa (Fig. 3a) were *Chitinophagaceae* (from 28.9
292 to 66.1%) and *Sphingomonadaceae* (from 7.5 to 16.6%).

293 At the family level, the microbiota of the intestinal content of fish was very different
294 and the dominants that are shared among all fish species were not as clearly detected as with
295 the mucosa samples (Fig. 3b). Similarity of microbiota at this level was found among the
296 feeding habits of fish: omnivorous, zoobenthivorous-zooplanktivorous and piscivorous.

297 Results of the ANOSIM test showed significant influence of the trophic group
298 (omnivorous, zoobenthivorous, zooplanktivorous-piscivorous) on the microbiota of intestinal
299 content ($p = 0.01$), while significant differences for mucosa of the same groups were absent (p
300 = 0.693) (Table 6).

301 There were significant differences in microbiota composition (not shown) between
302 intestinal mucosa and intestinal content in agastric fish (ANOSIM $R = 0.84$, $p = 0.01$).
303 Intestinal mucosa and intestinal content in gastric fish were not different (ANOSIM: $R = 1.0$,
304 $p = 0.28$), but it should be noted that the R value was very high ($R = 1$) meaning that there did
305 exist an effect of this factor as for the comparison pair SM vs. SC ($R = 1$).

306

307 ***4. Fish diet vs fish phylogenetic relationship influence the gut microbiome.***

308 A strong positive correlation was found between the microbiome of the fish diet and
309 intestinal content of various fish species in a simple Mantel test ($r = 0.74$, $p < 0.001$), while no

310 correlation was found between feeding habits of fish and the microbiome of the intestinal
311 mucosa ($r = -0.13$, $p > 0.10$). A strong positive correlation was also discovered between the
312 microbiome of intestinal content and phylogenetic distances ($r = 0.71$, $p < 0.001$). However,
313 when controlled for diet this relationship became small and non-significant (partial Mantel
314 test: $r = 0.27$, $p > 0.10$). On the other hand, a positive correlation between the microbiome of
315 fish diet and intestinal content remained significant when controlled for phylogeny ($r = 0.41$,
316 $p < 0.05$). Hence, the phylogenetic relationships are not a confounding factor for the
317 correlation between the microbiome of diet and intestinal content and this correlation
318 probably represents a causal relationship. There was no correlation between the microbiome
319 of intestinal mucosa and genetic distances (simple Mantel test: $r = -0.14$, $p > 0.10$). Thus, the
320 interspecies differences in fish diet or their phylogenetic position do not affect the
321 microbiome of the intestinal mucosa, but diet might influence the composition of the
322 microbial communities of the intestinal content.

323 ***5. Microbiota associated with prey of fish and environmental compartments.***

324 ***5.1. Microbiota of prey.*** Thirty bacterial phyla were identified from the associated
325 microbiota of prey (aquatic invertebrates) (Fig. 4). From each prey, the phylum Proteobacteria
326 made up the majority of all sequences, except *Gammarus* sp., varying among different prey
327 from 48.4 to 96.7%. Bacteroidetes was the second most common phylum, varying in
328 abundance from 2.5 to 43.7% among prey (Fig. 4a). As opposed to all other prey microbiota,
329 the associated microbiota of *Gammarus* sp. was dominated by Firmicutes (38.7%),
330 Bacteroidetes (31.7%), and Proteobacteria (24.3%).

331 At the family level the most abundant OTUs associated with prey which had a 5%
332 abundance threshold, varied among the different samples and each prey had their specific
333 microbiota (Fig. 4b). For example, only the water cricket, backswimmer and water mite

334 contained the genus *Wolbachia* from the family *Rickettsiaceae* (41.7, 25.1 and 35.9%,
335 respectively), while *Gammarus* sp. contained the families *Lachnospiraceae* (20.7%) and
336 *Prevotellaceae* (11.0%). The associated microbiota of *B. longimanus* was also very different
337 in contrast to other types of prey and consisted of *Aeromonadaceae* (42.2%), *Shewanellaceae*
338 (24.3%) and family *Weeksellaceae* (9.9%).

339 **5.2. Environmental microbiota.** The maximum number of phyla (41) was identified
340 from the associated microbiota of water, sediment, and common reed. At the phylum level the
341 bacterial community of environmental compartments (water, sediment, and reed) was quite
342 similar to fish gut and prey. Proteobacteria and Bacteroidetes were the most important groups,
343 varying from 39.5 to 69.5% and from 19.9 to 39.4%, respectively. Microbiota of water was
344 mainly composed of bacteria from the families *Chitinophagaceae* (21.1%) and
345 *Pelagibacteraceae* (17.2%); microbiota of sediment: *Chitinophagaceae* (11.5%) and
346 *Saprospiraceae* (7.3%); reed: *Comamonadaceae* (25.3%) and *Rhodobacteraceae* (19.7%)
347 (Fig. 5b). However, a significant proportion of sequences in the environmental microbiota
348 consisted of numerous groups of bacteria of low abundance that varied from 0.01 to 5% (Fig.
349 5a). A large number of these sequences with low abundance belonged to the unknown group
350 and within that group their abundances of the total reads for water, sediment and reed were
351 42.1, 74.1 and 49.1%, respectively.

352

353 **6. Comparison between gastrointestinal microbiota of fish and associated microbiota of** 354 **environmental compartments.**

355 A scatter plot based on PCoA scores showed a grouping of the microbiota into 3
356 groups: 1) stomach and intestinal mucosa, 2) stomach and intestinal content, 3) prey and
357 environment. The microbial community of fish gut is divided in two groups that were

358 associated with either content or mucosa for all studied fish regardless of the gut organization
359 (gastric/agastric) and feeding habits (Fig. 6). Comparisons among these groups also showed
360 significant differences in analyzed microbiota (Table 7).

361

362 **Discussion**

363 ***Dominant microbiota of fish.***

364 The dominant bacterial phyla in both gut content and mucosa of the studied fish were
365 Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria and formed the core gut
366 microbiota communities at the phylum level. This result has been confirmed by other studies
367 of many freshwater fishes where Proteobacteria and Firmicutes were the most abundant phyla
368 (Uchii *et al.* 2006; Skrodenyte-Arbaciauskiene *et al.* 2008; Ward *et al.* 2009; Wu *et al.* 2010;
369 Sullam *et al.* 2012; Li *et al.* 2014; Silva *et al.* 2014; Ye *et al.* 2014; Baldo *et al.* 2015;
370 Kashinskaya *et al.* 2015; Liu *et al.* 2016). In other studies however, Fusobacteria has also
371 been found as one of the abundant phyla in intestinal content of fish from different families:
372 Cyprinidae, Ictalurus, Centrarchidae, Cichlidae, and Percichthyidae (van Kessel *et al.* 2011;
373 Larsen *et al.* 2014; Baldo *et al.* 2015; Liu *et al.* 2016).

374 In previous results (Kashinskaya *et al.* 2015; Kashinskaya *et al.* 2017), and herein, we
375 have shown that separation of intestinal microbiota of mucosa and content is a critical point
376 when studying the gut microbial communities. This is a distinct methodological difference as
377 compared to many previous studies where only intestinal content (Moran *et al.* 2005;
378 Skrodenyte-Arbaciauskiene *et al.* 2006; Uchii *et al.* 2006; Han *et al.* 2010; Smriga *et al.* 2010;
379 Silva *et al.* 2011; Navarrete *et al.* 2012; Wu *et al.* 2013), or whole gastrointestinal tract were
380 examined (Mac Cormack and Fraile 1990; Romero and Navarrete 2006; Lan and Love 2012;
381 McDonald *et al.* 2012; Li *et al.* 2013). Such differences in the type of sampling might lead to

382 biases when comparing results obtained by different researchers. Unfortunately, there are
383 only few available studies that focus on the structure of microbial communities associated
384 with gut mucosa and content in wild freshwater (Kim *et al.* 2007; Wu *et al.* 2010; Wu *et al.*
385 2012; Kashinskaya *et al.* 2015; Kashinskaya *et al.* 2017), and marine aquaculture fishes
386 (Carda-Diéguez *et al.* 2013; Xing *et al.* 2013). Thus, in order to avoid any erroneous
387 conclusions regarding associations among the samples analyzed, only studies in which gut
388 content and mucosa have been included for comparison in the discussion, whereas the data
389 obtained from whole gut (content and mucosa together) was not considered. From previous
390 studies, intestinal content from members of the Cyprinidae (*C. auratus*, *C. gibelio*
391 *Ctenopharyngodon idella*, *C. carpio*, *Hypophthalmichthys molitrix*, *H. nobilis*, *Megalobrama*
392 *amblycephala*) showed the dominant microbiota was represented by bacteria from the
393 families *Caulobacteraceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Veillonellaceae*,
394 *Micrococcaceae*, *Lachnospiraceae*, *Fusobacteriaceae* and *Halomonadaceae* (Wu *et al.* 2013;
395 Li *et al.* 2014; Liu *et al.* 2016). In the present work, the dominant microbial families of
396 intestinal content from fish was different and shared only the bacterial family
397 *Fusobacteriaceae* as a dominant for the Percidae examined.

398 While many studies have focused on intestinal content there are few which have
399 focused on the microbiota of the mucosa. The dominant families in mucosa of Cyprinidae and
400 Percidae were completely different from intestinal content and represented by
401 *Chitinophagaceae*, *Sphingomonadaceae* and *Caulobacteraceae*. These dominant bacterial
402 families observed from the mucosa of Cyprinidae are also significantly different from data
403 obtained for mucosa for other species: sea bass *Dicentrarchus labrax*, (Carda-Diéguez *et al.*
404 2013), *C. idella* (Tran *et al.* 2017), *Salmo salar* and *Oncorhynchus mykiss* (Kim *et al.* 2007;
405 Gajardo *et al.* 2016), and *P. fulvidraco* (Wu *et al.* 2010). When bacteria were classified at a

406 finer taxonomic resolution, a strong difference was revealed between fish species and
407 indicated that specific factors including gut compartment analyzed, fish trophic levels,
408 morphology of the gut, and other host genetic and environmental factors can influence the
409 composition of the fish gut microbiota.

410 It has been established that bacteria from the mucosa metabolize mucin proteins as
411 well as the O-linked glycans modifying mucin proteins (Koropatkin *et al.* 2012). This is a
412 character that sets bacteria inhabiting the mucus layer apart from other bacterial taxa from the
413 intestinal content. This imposes a selective pressure from the host gut on the bacterial
414 composition of the mucus layer and we can expect to find some significant differences in the
415 microbiota of the mucus layer among fish. However, the reverse is also true that the bacteria
416 inhabiting the gut shape the mucus layer (Ostaff *et al.* 2013; Jakobsson *et al.* 2015), thus it is a
417 complex relation with forces working in both directions. This is an area for future
418 investigation.

419

420 **Factors affecting composition of microbial communities.**

421 It has been demonstrated in several studies that phylogenetic relationships of the hosts
422 underlie the variation in gut microbiota of fish (Macfarlane and Macfarlane, 2009; Benson *et*
423 *al.* 2010; Bolnick *et al.* 2014a). Our results indicate that the diet is a primary factor affecting
424 composition of the microbiota of the gut content, but was not deterministic for the microbiota
425 of intestinal mucosa. The diets of fish from each feeding habits group showed only a minor
426 overlap in their primary food items (Fig. 1), while the composition of the microbiota from the
427 gut mucosa was quite similar among all fish species regardless of feeding habits, thus
428 suggesting that diet is imposing little selective pressure on the resident bacteria from the
429 mucosa.

430 In regard to the intestinal content, the microbiota of piscivorous species were
431 dominated by *Fusobacteriaceae*, *Rhodospirillaceae*, and *Enterobacteriaceae* at the family
432 level, and thus significantly different if compared with omnivorous and zoobenthivorous-
433 zooplanktivorous fish species. Differences such as these were also noted by other researchers
434 in the microbiota of the gut content of freshwater fish with different diets (Larsen *et al.*
435 2014; Liu *et al.* 2016; Li *et al.* 2014). This suggests that the piscivorous diet, high in protein
436 and/or fish oils, may alter the microenvironment in a way that facilitates habituation of these
437 bacterial families to the gut of piscivorous fish, while the omnivores, which may also include
438 significant invertebrate organisms in their diet, have families such as *Chitinophagaceae* in
439 their gut that may facilitate digestion of exoskeleton material (Glavina *et al.* 2010).
440 Hydrolysis of cellulose has also been ascribed to members of *Chitinophagaceae* (Chung *et al.*
441 2012) which would facilitate digestion of food intake from omnivores or herbivores. Thus,
442 trait-specific resource acquisition may impose deterministic influence on the microbial
443 diversity of the intestinal content; inversely, the ability to facilitate digestion of specific
444 dietary components, like cellulose or chitin, may be a trait that contributes to determining
445 resource acquisition.

446 In most studies of fish in which diet and microbiota are compared it has also been
447 supposed that fish with more generalized diets carry more diverse microbes than of specialist
448 fish species. Several studies have shown that the diversity of the microbiota of intestinal
449 content of omnivorous fish was higher than those of carnivorous ones (Ward *et al.* 2009;
450 Larsen *et al.* 2014). In our study no significant differences were observed for both richness
451 and diversity estimates among different trophic groups of fish (NPMANOVA, $p > 0.05$).
452 Moreover, multiple diet components for fish can interact non-additively to influence gut
453 microbial diversity. Thus in a study of stickleback and perch, diet manipulations with mixed

454 diets demonstrated a statistically significant lower diversity of intestinal microbiota when
455 compared to the microbiota of specialist fish species in two natural populations, and also in
456 the laboratory (Bolnick *et al.* 2014).

457 Fish gut microbial diversity could also be connected with the differences of the
458 digestive structure in terms of the presence/absence of a stomach (*i.e.* gastric and agastric
459 fish). In gastric fish species studied herein (perch and pike-perch), food passes through the
460 stomach before it enters the intestine. Within the stomach the bacteria associated with food
461 will be subjected to low pH levels (HCl) in the stomach with values of 1.5-2 (Solovyev *et al.*
462 2015; Solovyev *et al.* 2017) that could cause bacterial cell lysis and DNA degradation. Thus,
463 this could be an insuperable barrier for some groups of bacteria. In contrast, with agastric fish
464 (Prussian carp, Crucian carp, Common carp, dace, ide and roach) food goes directly into the
465 intestine. In our data no significant differences were observed between agastric and gastric
466 fish. On the other hand, Li and coworkers (Li *et al.* 2014) did find some differences along the
467 length of the gut in a gastric carnivorous species *S. chuatsi* that may reflect the influence of a
468 pH gradient created by stomach acid emptying into the intestine. Future studies are needed to
469 further understand this effect.

470 According to our results, families such as *Pseudoalteromonadaceae* and
471 *Aeromonadaceae* were the dominant microbiota of Prussian carp and Common carp, while in
472 other studies with these same fish species the microbiota was found to be dominated by
473 *Caulobacteraceae*, *Oxalobacteraceae*, and *Comamonadaceae* (Li *et al.* 2014), or by
474 Fusobacteria and *Halomonadaceae* (Liu *et al.* 2016). From these results we see that the same
475 fish species inhabiting different water bodies have gut microbial communities of different
476 composition. These differences may be due to a variety of deterministic aspects of the sample
477 collection, water quality or may be due to methodological differences as well, as described

478 previously (Kashinskaya *et al.* 2017), since the DNA extraction and sequencing platforms
479 used in each of the studies is distinct (Li and co-authors used PowerFecal DNA Isolation Kit
480 [Mo Bio, Inc. Carlsbad, CA, USA] and 454 pyrosequencing; whereas Liu and co-authors used
481 QIAamp DNA Stool Mini Kit [Qiagen, Valencia, USA] and an Illumina MiSeq sequencing
482 platform).

483

484 **Associated microbiota of prey and environmental compartments.**

485 The gut microbial diversity at the genus level encountered in this study in insects
486 indicated *Wolbachia* was most prevalent (14.1%). Bacteria from this genus are commonly
487 found in 17 insect orders: Ephemeroptera, Megaloptera, Diptera, Lepidoptera, Hymenoptera,
488 Orthoptera, Neuroptera, and Dermaptera (Yun *et al.* 2014). Our data showed that *Wolbachia*
489 spp., were also found in Hemiptera (*Corixidae* sp. and *Notonectidae* sp.) as reported
490 previously (Chen *et al.* 1996; Jeyaprakash and Hoy 2000). Members of this genus are known
491 as insect pathogens and their infections can be a cause of disruption in sex ratios for insects
492 due to gender specific sterilization (Werren *et al.*, 2003; Mcmeniman, *et al.*, 2009), but the
493 relevance of this bacterial genus in fish digestive physiology is unknown.

494 As a potential prey of zoobenthivorous-zooplanktivorous and piscivorous fish, we also
495 analyzed the microbiota associated with two cladoceran species – *Daphniidae* sp., *B.*
496 *longimanus* and microbiota of amphipods from the genus *Gammarus*. While most studies of
497 *Daphnia* microbiota have been focused on ecto- and endo-parasites (Caceres *et al.* 2013; Ebert
498 2005), the non-parasitic bacteria of *Daphnia* are very poorly known. The majority of the
499 sequences obtained from *Daphniidae* sp. in previous studies were assigned to the
500 *Comamonadaceae* family (Qi *et al.* 2014; Callens 2016) followed by *Aeromonadaceae*,
501 *Arcicella*, *Flavobacteriaceae* (Callens 2016). Other studies have shown the microbiota of

502 *Daphniidae* sp. to be dominated by *Aeromonas* spp., whereas the occurrence of other taxa was
503 lower and more variable (Roeselers *et al.* 2011). Our results indicated that *Chitinophagaceae*,
504 *Comamonadaceae* and *Cualobacteraceae* were the most prevalent families in the microbiota
505 of *Daphnia*. Differences among these results may reflect differences among different *Daphnia*
506 species or be more related to environmental differences in which they were collected. As was
507 mentioned above the associated microbiota of *B. longimanus* were very different in contrast to
508 other prey, but available data for comparative purposes is absent. The associated microbiota of
509 aquatic invertebrates were varied and these differences could be due to differences in the
510 environmental habitat, diet, developmental stage, and/or phylogenetic and trophic position of
511 the insect hosts. This invertebrate microbiota is in some way additive to the fish gut
512 microbiota, but more research will be needed to understand how the significant physiological
513 differences that exist between vertebrates and invertebrates determine which specific taxa are
514 contributors to the associated intestinal microbiota of fish in a functional manner.

515 In the literature there are conflicting ideas about the formation of the intestinal
516 microbiota of fish. On the one hand, the intestinal microbiota is different from that found in
517 the food, water and sediment (Romero and Navarrete 2006; Han *et al.* 2010). On the other
518 hand, some authors suggest that the microbiota of the digestive tract of fish is similar to the
519 microbiota of water and food objects (Cahill 1990; Ringø and Olsen, 1999; Olafsen 2001;
520 Romero and Navarrete 2006). Thus, a microbiota of grass carp (*Ctenopharyngodon idellus*) is
521 closer to the microbiota of water and sediment (Wu *et al.* 2012). Similar results were obtained
522 by the same authors studying Prussian carp where the microbiota of intestinal contents was
523 more closely related to the microbial community of the sediment (Wu *et al.* 2013); and in
524 grass carp the intestinal microbiota was more associated with food than with water and
525 sediment (Han *et al.* 2010). In the present paper, results of comparisons using an ANOSIM

526 test showed that there are significant differences among microbiota from fish gut and the
527 environment that have no correlation to phylogenetic and anatomical differences among fish
528 from different trophic levels. Our data correspond with results obtained by Bolnick and
529 coworkers who showed that the gut microbiota of wild freshwater fish is not a subset of the
530 microbes of their prey and water (Bolnick *et al.* 2014), thereby demonstrating that fish
531 harbored specific groups of bacteria that did not reflect the microbiota seen from prey and
532 environmental contributions (water, sediment, reeds). This specific difference might be due to
533 features of the fish digestive tract and its functioning (nutrient composition, pH, concentration
534 of bile salts and digestive enzymes, the host's immune system, etc.) (Hansen and Olafsen
535 1999).

536 Our observations of microbiota of eight wild fish species have demonstrated that at a
537 high taxonomic level the microbial communities of the gut mucosa might be quite similar
538 across a broader range of fish species. More particularly, as the microbiota of the mucosal
539 layer is more a resident within the host than bacteria in the gut content that can be passing
540 through as part of the diet, and the composition of the mucosal microbiota is more similar
541 regardless of evolutionary history or different digestive physiology, this suggests this work
542 represents a near approximation towards identifying a “core microbiome” for the intestinal
543 mucosa of fish. The gut content by contrast is more influenced by food intake.

544 Moreover, when bacteria were classified at the family and genus level, a strong
545 difference was revealed between fish species and indicated that their trophic levels can affect
546 the composition of the fish gut microbiota. The bacterial communities of the gut content differ
547 distinctly among fish with different feeding habits reflecting the host trophic level and mode
548 of resource acquisition, thereby influencing the individual gut microbiome diversity,
549 regardless of whether the fish host is gastric or agastric.

550 The data demonstrate that fish harbored specific groups of bacteria that do not
 551 completely reflect the microbiota of prey or environmental microbiota (water, sediment, and
 552 reed). These other microbial sources are additive, but not completely correlative, and the final
 553 composition is likely due to features of morphological structure of the fish digestive tract and
 554 its functioning (nutrient composition, pH, concentration of bile salts and digestive enzymes,
 555 the host's immune system and etc.) (Hansen and Olafsen 1999).

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782 **Tables**

783

784 **Table 1** Sample information (type of sample and number of individuals and samples
785 analyzed)

Host	Number of individuals/samples	Type of sample analyzed*
Fish		
Prussian carp (<i>C. gibelio</i>)	5	IM, IC
Crucian carp (<i>C. carassius</i>)	4	IM, IC
Common carp (<i>C. carpio</i>)	13	IM, IC
Roach (<i>R. rutilus</i>)	5	IM, IC
Dace (<i>L. leuciscus</i>)	5	IM, IC
Ide (<i>L. idus</i>)	7	IM, IC
Perch (<i>P. fluviatilis</i>)	8	SM, SC, IM, IC
Pike-perch (<i>S. lucioperca</i>)	4	SM, SC, IM, IC
Environment		
Water	3	100 ml
Sediment	3	0.1 g
Common reed (<i>P. australis</i>)	3	Scrapings (0.1 g)
Invertebrates		
Chironomid larva (<i>Chironomidae</i> sp.)	8	Whole
Daphnia (<i>Daphniidae</i> sp.)	9	Whole
Watercricket (<i>Corixidae</i> sp.)	3	Whole
Backswimmer (<i>Notonectidae</i> sp.)	2	Whole
Amphipod (<i>Gammaridae</i> sp.)	1	Whole
Caddis fly larva (<i>Trichoptera</i> sp.)	2	Whole
Spiny water flea (<i>Bythotrephes longimanus</i>)	1	Whole
Divingbeetle (<i>Dytiscidae</i> sp.)	1	Whole
Water mite (<i>Hydrachnidae</i> sp.)	1	Whole

786 *IM – intestinal mucosa; IC – intestinal content; SM – stomach mucosa; SC – stomach
787 content.

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789 **Table 2** The similarity matrix (Morista index) of diet between studied fish in Chany Lake

	Prussian carp	Crucian carp	Common carp	Roach	Dace	Ide	Perch	Pike-perch
Prussian carp	1.00	0.92	0.91	0.20	0.51	0.23	0.33	0.06

Crucian carp	1.00	0.89	0.20	0.46	0.26	0.34	0.05
Common carp		1.00	0.30	0.54	0.29	0.40	0.05
Roach			1.00	0.82	0.88	0.30	0.08
Dace				1.00	0.81	0.46	0.09
Ide					1.00	0.28	0.10
Perch						1.00	0.71
Pike-perch							1.00

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Table 3 Diversity analysis of microbial community of fish gut, their prey and environmental compartments in Chany Lake

Source	Species	Richness estimates		Diversity estimates	
		Number of observed OTU's	Chao1	Shannon	Simpson
Intestinal mucosa	Prussian carp	182	281.39	3.81	0.86
	Crucian carp	151	269.33	3.59	0.82
	Common carp	177	302.14	4.18	0.88
	Dace	163	418.94	3.46	0.81
	Roach	210	328.83	4.33	0.91
	Ide	174	295.54	3.91	0.84
	Perch	219	333.49	4.14	0.84
	Pike-perch	159	292.00	3.80	0.84
	Mean±SE	179.37±8.49	315.20±16.70	3.90±0.10	0.85±0.01
Stomach mucosa	Perch	194	295.08	4.72	0.91
	Pike-perch	166	324.05	3.81	0.84
	Mean±SE	180.00±14.00	309.57±14.49	4.27±0.46	0.88±0.04
Intestinal content	Prussian carp	357	646.55	4.00	0.80
	Crucian carp	221	371.09	3.37	0.70
	Common carp	423	834.48	4.65	0.85
	Dace	329	589.82	4.62	0.88
	Roach	248	363.50	4.31	0.86
	Ide	263	440.16	4.21	0.84
	Perch	214	677.24	3.57	0.80
	Pike-perch	138	288.48	2.02	0.53
	Mean±SE	274.13±32.13	526.42±66.93	3.84±0.31	0.78±0.04
Stomach content	Perch	106	319.00	1.31	0.34
	Pike-perch	204	357.03	3.71	0.81
	Mean±SE	155.00±49.00	338.02±19.02	2.51±1.20	0.58±0.24
Prey	Daphnia	343	566.89	5.50	0.93
	Bythotrephes	234	452.50	3.70	0.80
	Chironomids	394	665.73	5.98	0.96
	Gammarus	262	303.25	6.38	0.98
	Watercricet	212	342.81	4.24	0.89
	Backswimmer	226	362.50	4.43	0.91
	Caddis fly	289	437.22	5.37	0.94
Water mite	126	219.88	3.36	0.80	

	Diving beetle	590	1214.84	5.06	0.81
	Mean±SE	297.33±44.74	507.29±99.26	4.89±0.34	0.89±0.02
Environment	Water	416	652.91	5.96	0.95
	Sediment	1238	1884.08	8.92	0.99
	Reed	851	1094.14	7.97	0.99
	Mean±SE	835.00±237.43	1210.38±360.13	7.62±0.87	0.98±0.01

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Table 4 Alpha diversity analysis of microbiota of gastric and agastric fish

Source	Analyzed group	Richness estimates		Diversity estimates	
		№ of observed OTU's	Chao1	Shannon	Simpson
Mucosa	Gastric fish	184.5±13.8 ^{AB}	311.2±10.4 ^{AB}	4.12±0.2	0.85±0.02 ^{AB}
	Agastric fish	176.2±8.1 ^A	316.0±22.2 ^A	3.88±0.1	0.85±0.02 ^A
Content	Gastric fish	165.5±26.0 ^{AB}	410.4±90.0 ^{AB}	2.65±0.6	0.62±0.10 ^B
	Agastric fish	306.8±31.2 ^B	540.9±75.3 ^B	4.19±0.2	0.82±0.03 ^{AB}

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Uppercase letters denote statistically significant differences among analyzed fish groups at $p \leq 0.05$.

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Table 5 Alpha diversity analysis of microbiota of fish with different feeding habits and associated microbiota of prey and environment (water, sediments, and common reed)

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Source	Analyzed group	Richness estimates		Diversity estimates	
		№ of observed OTU's	Chao1	Shannon	Simpson
<i>Trophic groups</i>					
Mucosa	OM intestine	170.0±9.6 ^{AB}	284.3±9.6 ^{AB}	3.86±0.2 ^{AB}	0.85±0.02 ^{AB}
	ZB-ZP intestine	182.3±14.2 ^{AB}	347.8±36.9 ^{AB}	3.90±0.3 ^{AB}	0.85±0.03 ^{AB}
	PS intestine	189.0±30.0 ^{AB}	312.7±20.7 ^{AB}	3.97±0.2 ^{AB}	0.84±0.00 ^{AB}
	PS stomach	180±14.0 ^{AB}	309.6±14.5 ^{AB}	4.27±0.5 ^{AB}	0.87±0.04 ^{AB}
Content	OM intestine	333.7±59.5 ^{AB}	617.4±134.6 ^{AB}	4.01±0.4 ^{AB}	0.78±0.04 ^{AB}
	ZB-ZP intestine	280.0±24.9 ^{AB}	464.5±66.5 ^{AB}	4.38±0.1 ^{AB}	0.86±0.01 ^{AB}
	PS intestine	176.0±38.0 ^{AB}	482.9±194.4 ^{AB}	2.79±0.8 ^{BC}	0.66±0.10 ^B
	PS stomach	155±49.0 ^{AB}	338.0±19.0 ^{AB}	2.51±1.2 ^{AB}	0.57±0.20 ^{AB}
<i>Environmental compartment groups</i>					
Prey	Total	297.3±44.7 ^A	507.3±99.3 ^A	4.89±0.3 ^A	0.89±0.02 ^A
Environment	Total	835.0±237.4 ^B	1210.4±360.1 ^B	7.62±0.9 ^B	0.97±0.01 ^{AB}

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OM – omnivorous; ZB-ZP – zoobenthivorous-zooplanktivorous; PS – piscivorous. Uppercase letters denote statistically significant differences among environment microbiota (water, sediment, and common reed) and gut microbiota of fish (intestinal mucosa, intestinal content, stomach mucosa and stomach content) and their prey at $p \leq 0.05$.

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Table 6 Comparison of microbiota (ANOSIM) in fish with different trophic groups

Factor/Comparison	Global R	p-value	Number of groups	Sample size
Source				

IM vs. IC	0.86	0.01*	2	16
IM vs. SM	-0.22	0.79	2	10
IM vs. SC	1.00	0.02*	2	10
SM vs. SC	1.00	0.33	2	4
SM vs. IC	0.88	0.04*	2	10
Intestinal mucosa				
Trophic group	-0.10	0.693	3	8
Intestinal content				
Trophic group	0.76	0.010*	3	8
OM vs. PS	0.75	0.128	2	5
OM vs. ZB-ZP	0.78	0.059**	2	6
ZB-ZP vs. PS	0.92	0.079**	2	5

806 * – indicates significant association ($p \leq 0.05$); ** – indicates significant association $p \leq 0.1$.
 807 IM – intestinal mucosa; IC – intestinal content; SM – stomach mucosa; SC – stomach
 808 content; OM omnivorous; ZB-ZP – Zoobenthic/zooplanktivorous; PS – piscivorous.
 809

810 **Table 7** Results of comparisons (ANOSIM) of fish gut and environmental microbiota

Factor (source)	Global R	p-value	Number of groups	Sample size
IM vs. PR	0.50	0.01*	2	17
IM vs. EN	0.94	0.02*	2	11
SM vs. PR	0.33	0.08**	2	11
SM vs. EN	0.42	0.22	2	5
IC vs. SC	0.30	0.09**	2	10
IC vs. PR	0.20	0.02*	2	17
IC vs. EN	0.78	0.01*	2	11
SC vs. PR	0.33	0.08**	2	11
SC vs. EN	1.00	0.10**	2	5
PR vs. EN	0.16	0.22	2	12

811 * – indicates significant association ($p \leq 0.05$); ** $p \leq 0.1$. IM – intestinal mucosa; IC – intestinal
 812 content; SM – stomach mucus; SC – stomach content; EN – environment; PR – prey.
 813

814 Figure legend

815
 816 Figure 1 Diets of fish with different feeding habits in Chany Lake (frequency of occurrence).
 817 1 – phytoplankton; 2 – macrophyte; 3 – Gammaridae sp.; 4 – *Chydorus* sp.; 5 – Ostracoda sp.;
 818 6 – *B. longimanus*; 7 – other zooplankton; 8 – Chironomidae sp. (larvae); 9 – Chironomidae
 819 sp. (pupa); 10 – Trichoptera sp. (larvae); 11 – Heteroptera sp. (larvae); 12 – Molluscs; 13 –
 820 detritus; 14 – fish fry. (■) Prussian carp; (■) Crucian carp; (■) Common carp; (■) Dace;
 821 (■) Roach; (■) Ide; (■) Perch; (■) Pike-perch.
 822

823 Figure 2 Phylum composition of microbiota from stomach and intestine of each fish studied
 824 and groups classified with different feeding habits in Chany Lake. a – mucosa; b – content.

825 (■) Actinobacteria; (■) Bacteroidetes; (■) Cyanobacteria; (■) Firmicutes; (■)
 826 Fusobacteria; (■) Proteobacteria; (■) Others.

827

828 Figure 3 Family ratios of microbiota from stomach and intestine of each fish studied and
 829 groups classified with different feeding habits in Chany Lake. a – mucosa; b – content. (■)
 830 Aeromonadaceae; (■) Bifidobacteriaceae; (■) Caulobacteraceae; (■) Chitinophagaceae;
 831 (■) Clostridiaceae; (■) Enterobacteriaceae; (■) Fusobacteriaceae; (■) Prevotellaceae; (■)
 832 Pseudoalteromonadaceae; (■) Rhodospirillaceae; (■) Sphingomonadaceae; (■) Unknown
 833 Spirobacillales; (■) Vibrionaceae; (■) Others.

834

835 Figure 4 The associated microbiota of fish prey in Chany Lake. a – at the phylum level; b – at
 836 the family level. **Phylum:** (■) Actinobacteria; (■) Bacteroidetes; (■) Cyanobacteria; (■)
 837 Firmicutes; (■) Fusobacteria; (■) Proteobacteria; (■) Tenericutes; (■) Verrucomicrobia;
 838 (■) Others. **Family:** (■) Aeromonadaceae; (■) Caulobacteraceae; (■) Chitinophagaceae;
 839 (■) Comamonadaceae; (■) Enterobacteriaceae; (■) Flavobacteriaceae; (■)
 840 Lachnospiraceae; (■) Moraxellaceae; (■) Prevotellaceae; (■) Pseudomonadaceae; (■)
 841 Rickettsiaceae; (■) Ruminococcaceae; (■) Shewanellaceae; (■) Sphingomonadaceae; (■)
 842 Staphylococcaceae; (■) Synechococcaceae; (■) Weeksellaceae; (■) Others.

843

844 Figure 5 The associated microbiota of environmental compartments in Chany Lake. a – at the
 845 phylum level; b – at the family level. **Phylum:** (■) Actinobacteria; (■) Bacteroidetes;
 846 (■)Chlorobi; (■) Chloroflexi; (■) Cyanobacteria; (■) Firmicutes; (■) Fusobacteria; (■)
 847 Proteobacteria; (■) Others. **Family:** (■) ACK-M1; (■) Unknown Bacteroidales; (■)
 848 Chitinophagaceae; (■) Comamonadaceae; (■) Cryomorphaceae; (■) Pelagibacteraceae; (■)
 849 Rhodobacteraceae; (■) Saprospiraceae; (■) Synechococcaceae; (■) Others.

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851 Figure 6 Principle coordinates analysis (PCoA) for microbiota associated with gut mucosa
 852 and content of fish and environmental microbiota. Stomach mucosa (yellow), stomach content
 853 (orange), intestinal mucosa (black), intestinal content (blue), prey (violet), and environmental
 854 microbiota (brown).

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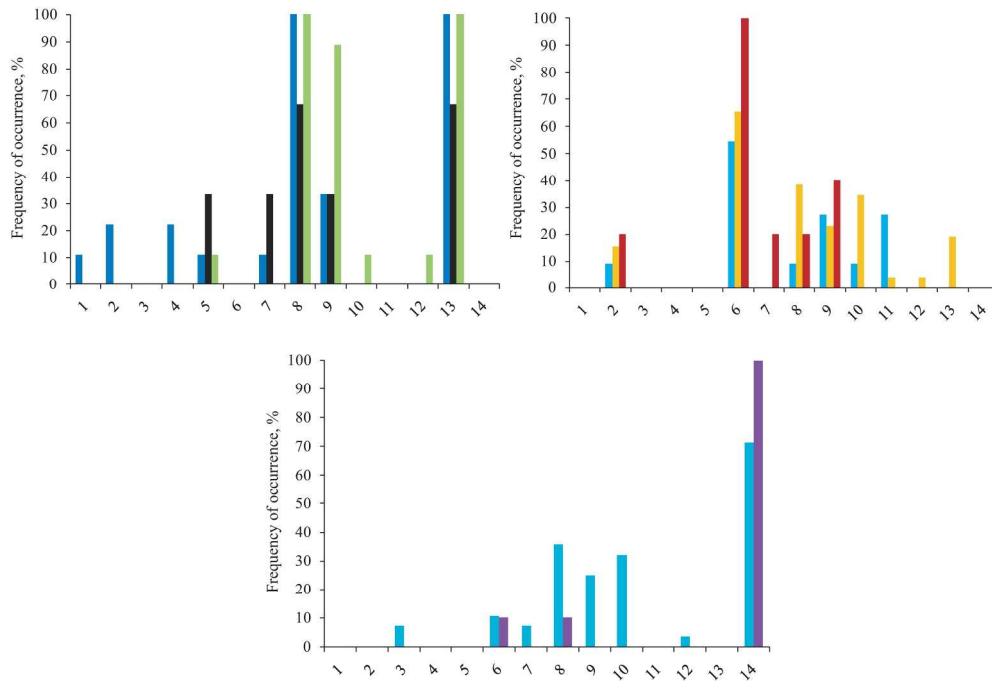


Figure 1 Diets of fish with different feeding habits in Chany Lake (frequency of occurrence). 1 – phytoplankton; 2 – macrophyte; 3 – Gammaridae sp.; 4 – Chydorus sp.; 5 – Ostracoda sp.; 6 – B. longimanus; 7 – other zooplankton; 8 – Chironomidae sp. (larvae); 9 – Chironomidae sp. (pupa); 10 – Trichoptera sp. (larvae); 11 – Heteroptera sp. (larvae); 12 – Molluscs; 13 – detritus; 14 – fish fry.

221x160mm (300 x 300 DPI)

Review

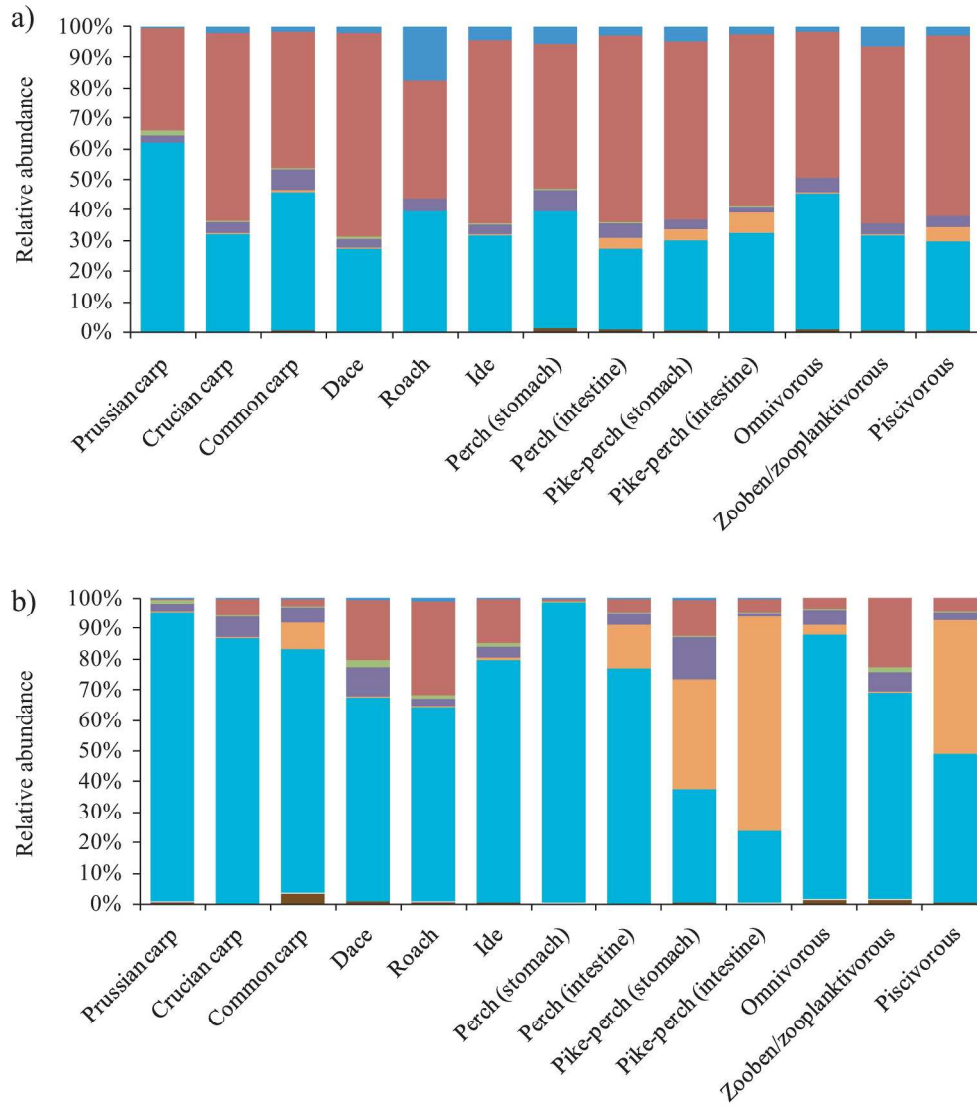


Figure 2 Phylum composition of microbiota from stomach and intestine of each fish studied and groups classified with different feeding habits in Chany Lake. a – mucosa; b – content.

232x260mm (300 x 300 DPI)

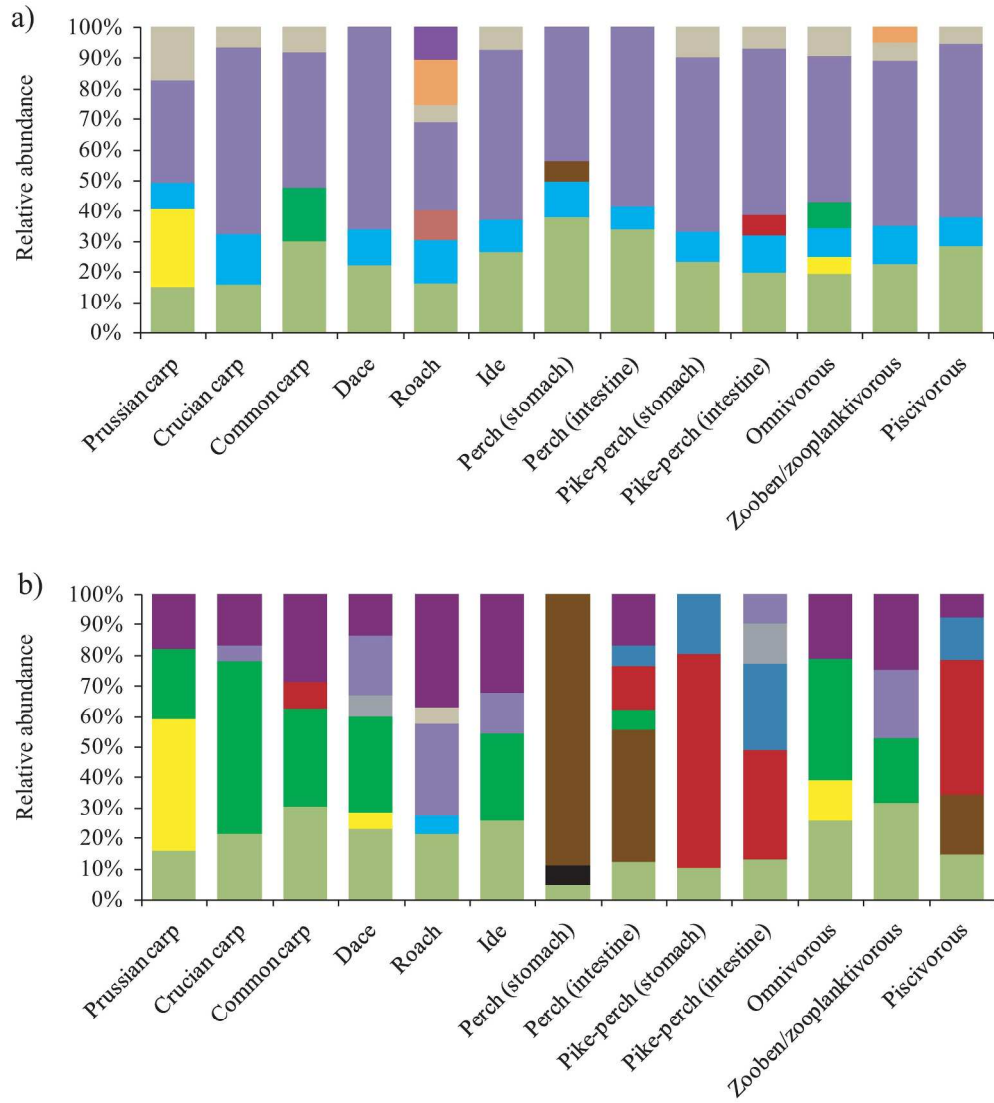


Figure 3 Family ratios of microbiota from stomach and intestine of each fish studied and groups classified with different feeding habits in Chany Lake. a – mucosa; b – content.

231x258mm (300 x 300 DPI)

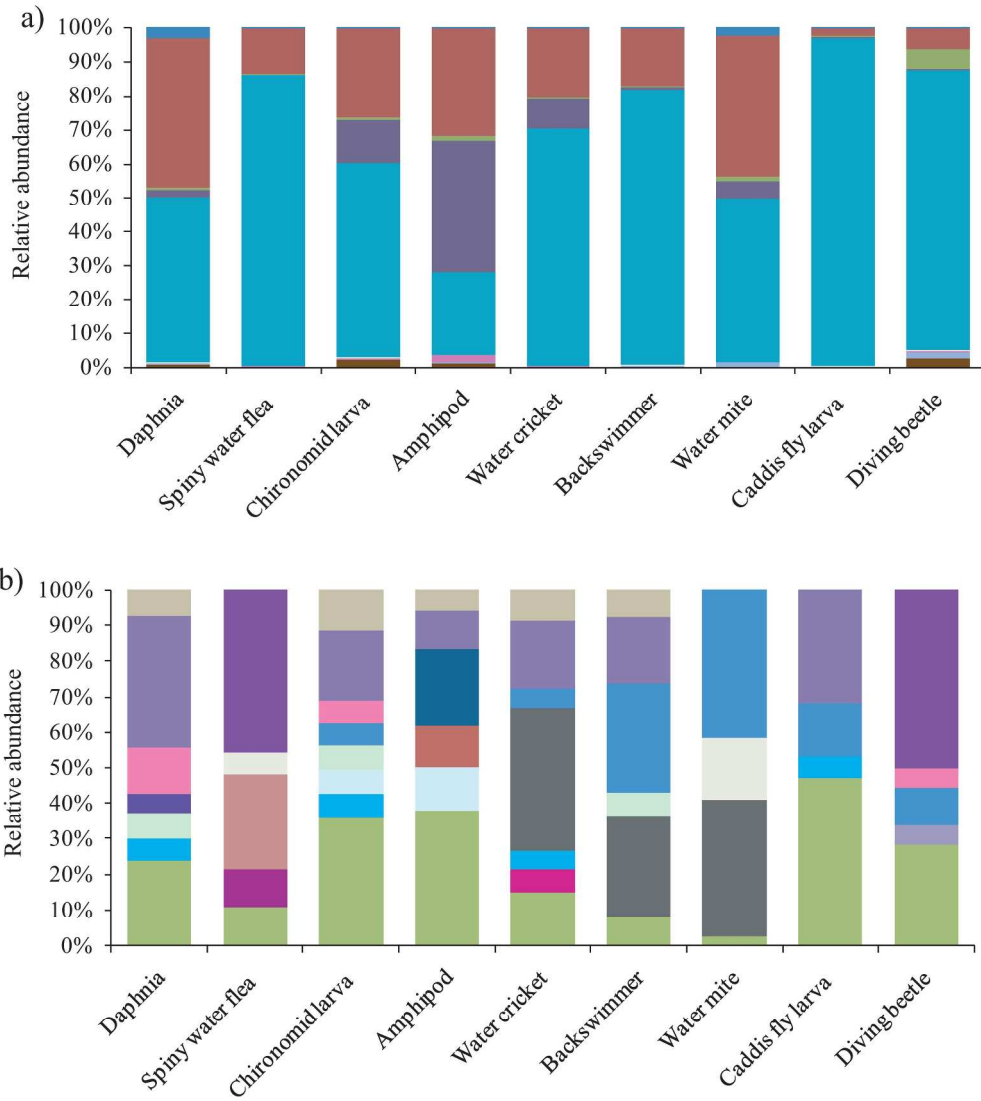


Figure 4 The associated microbiota of fish prey in Chany Lake. a – at the phylum level; b – at the family level.

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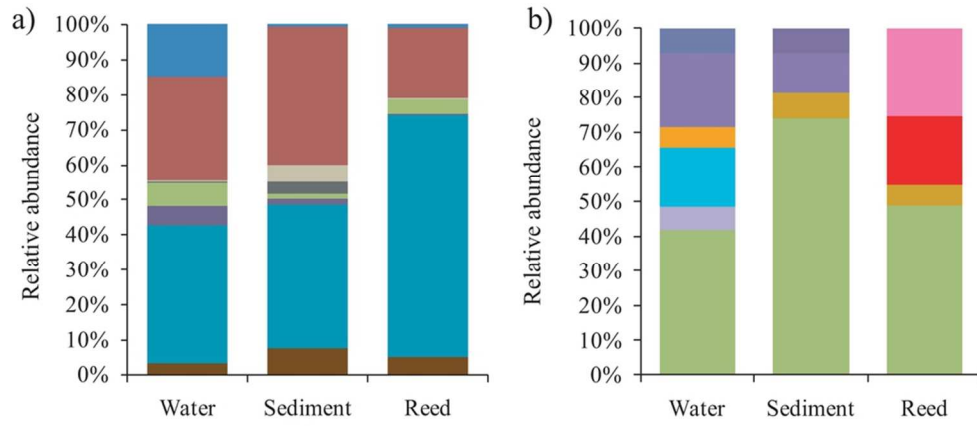


Figure 5 The associated microbiota of environmental compartments in Chany Lake. a – at the phylum level; b – at the family level.

89x38mm (300 x 300 DPI)

Peer Review

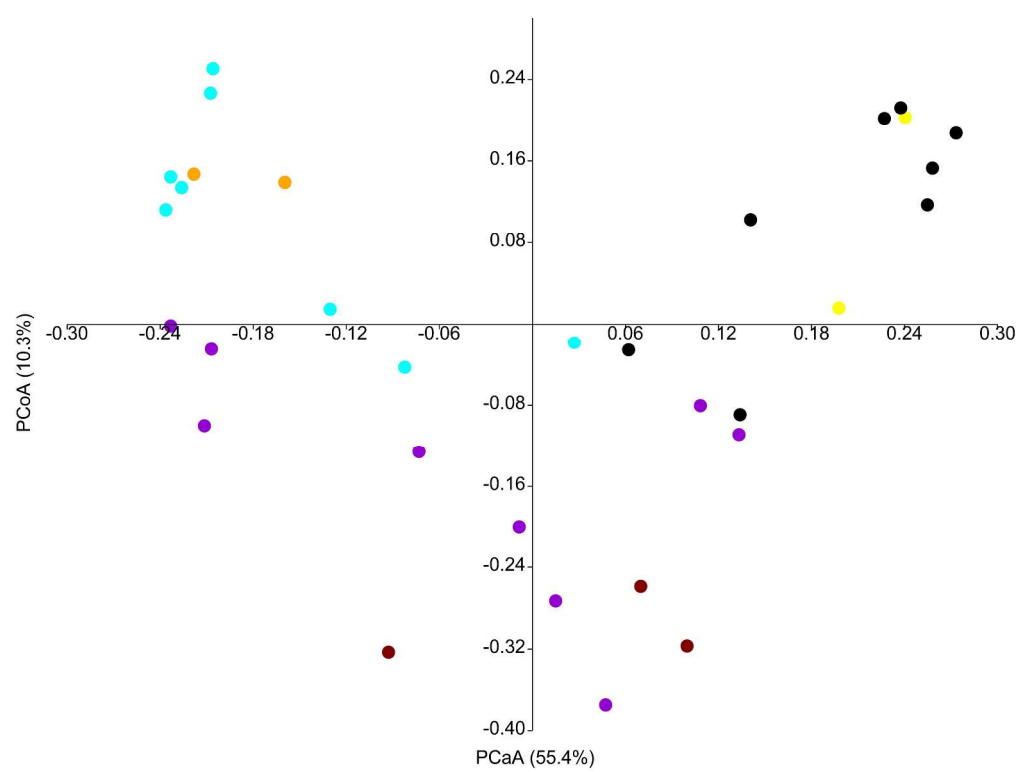


Figure 6 Principle coordinates analysis (PCoA) for microbiota associated with gut mucosa and content of fish and environmental microbiota. Stomach mucosa (yellow), stomach content (orange), intestinal mucosa (black), intestinal content (blue), prey (violet), and environmental microbiota (brown).

284x213mm (300 x 300 DPI)

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