

1 SECONDARY PRODUCTION OF HIGHLY UNSATURATED FATTY ACIDS BY
2 ZOOBENTHOS ACROSS RIVERS CONTRASTING IN TEMPERATURE

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15 **KEYWORDS** essential polyunsaturated fatty acids; river zoobenthos; secondary production;
16 biodiversity; water temperature; climate warming

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19 ABSTRACT

20

21 Highly unsaturated fatty acids (HUFA), namely eicosapentaenoic acid (20:5n-3, EPA) and
22 docosahexaenoic acid (22:6n-3, DHA), which are essential for many animals, including humans
23 are mainly produced in aquatic trophic webs. In fast-flowing rivers macrozoobenthos is the main
24 source of HUFA for fish and may be particularly vulnerable to thermal alterations associated
25 with climate change. We studied benthic communities in a unique natural ecosystem: the Yenisei
26 River downstream of the dam of Krasnoyarsk Hydroelectric Power Station with very low
27 temperature in summer because of discharge of cold water from deep in the reservoir, and its
28 tributaries with high summer temperature. This 'natural experiment' allowed to get rid of
29 confounding factors, such as differences in light, seasonality, geology (biogeochemistry) and
30 biogeography (regional species pools). As found, in spite of an increase of biodiversity and rates
31 of daily production in warm rivers compared to cold sites, DHA and partly EPA production of
32 zoobenthos decreased with increase of temperature because of changes in species composition.
33 Thus, in a climate warming context we can predict a decrease of production of these HUFA by
34 river zoobenthos and thereby a diminishing of their supply for fish and next to humans.

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36

37 INTRODUCTION

38

39 Impacts of global climate warming on the aquatic ecosystems are profound, and thereby
40 complex approaches to predicting their consequences are needed (Woodward et al., 2010).
41 Although many studies of the climate impacts are focused on structural attributes, such as
42 species richness and biodiversity (Woodward et al., 2010), functional responses of ecosystems as
43 sources or sinks in global carbon cycles evidently are of great importance for the complex
44 predicting of consequences of global warming (Perkins et al., 2012).

45 Besides fluxes of total carbon, production and transfer of particular compounds can be of
46 great importance for ecosystems' functioning. For instance, essential nutrients, highly
47 unsaturated fatty acids (HUFA), which are one of the factors controlling growth and
48 development of many animals, including humans, are produced primarily in aquatic ecosystems
49 by certain microalgae and then are transferred to terrestrial ecosystems through diverse food
50 chains (Gladyshev et al., 2009). Nowadays, the main source of essential HUFA in human's diet
51 is fish. Wild fish catch and aquaculture production [have](#) reached their upper limits, but still
52 cannot cover HUFA requirements of humankind (Gladyshev et al., 2013). HUFA content in
53 different fish species range around two orders of magnitude and strongly depend on HUFA
54 sources in their diet (Sargent et al., 1999; Gladyshev et al., 2013). Hence, high HUFA values in
55 biomass of planktonic and benthic invertebrates, as main food sources for fish, are required to
56 maintain proper HUFA content in wild fish catch.

57 The peculiar 'biosphere service' of aquatic ecosystems as the source of HUFA may be
58 threatened by the climate warming. Indeed, in pelagic ecosystems a decrease of HUFA contents
59 in the main conduit between microalgae and fish, zooplankton, under increased water
60 temperature was found recently (Maazouzi et al., 2008; Gladyshev et al., 2011). In rivers [or](#)
61 [rivers' stretches](#) with a high [current velocity](#), [zoobenthos is the main food source](#) for fish.
62 Nevertheless, there are practically no data on a possible effect of climate warming on HUFA
63 production by river zoobenthos. Note that among aquatic ecosystems, river communities are
64 expected to be particularly vulnerable to thermal alterations associated with climate change
65 (Isaak & Rieman, 2013).

66 There are several ways of studying the possible effects of the global warming on aquatic
67 ecosystems: i) mesocosm experiments (e.g., Strecker et al., 2004; Hines et al., 2013), ii) long-
68 term series of observations (e.g., Hampton et al., 2008; Floury et al., 2013) and iii) inferential
69 surveys that span large temperature gradients across differences in latitude and altitude (e.g.,
70 Jacobsen et al., 1997; Kattner & Hagen, 2009). However, all the above important methods have
71 certain evident shortcomings, namely decreased resemblance of mesocosms to natural

72 communities, insufficient availability of long-term series, and confounded effects of differences
73 in light, biogeochemistry and biogeography in latitude/altitude surveys (Woodward et al., 2010).
74 Recently some of these difficulties were overcome using a unique natural ecosystem: Icelandic
75 geothermal streams that lie 2 m - 2 km apart and have comparable physico-chemical properties
76 and an identical regional species pool, but strongly vary in temperature (Friberg et al., 2009).
77 This system represents a 'natural experiment' which allowed getting a set of new data on
78 possible effect of global warming on structure and functions of stream ecosystems (Woodward et
79 al., 2010; Perkins et al., 2012).

80 An opportunity to carry out similar 'natural experiment' also exists in middle reach of the
81 Yenisei River (Siberia, Russia). In summer, water temperature in the main channel of the river
82 about 100 km downstream of the dam of Krasnoyarsk Hydroelectric Power Station is ca. 6-12 °C
83 because of the cold water discharge from deep of the reservoir (Dubovskaya et al., 2004). In
84 contrast, tributaries of the river have usual summer temperatures, ca. 15-22 °C. Thus, we used
85 this unique ecosystem to study an effect of temperature on production of HUFA by river
86 zoobenthos as the main conduit between primary producers and fish, and thereby to estimate a
87 possible impact of climate warming on the 'biosphere service' of river ecosystems as providers
88 of these essential nutrients.

89 An increase of water temperature is known to potentially decrease HUFA content in
90 aquatic organisms via several mechanisms (Schlechtriem et al., 2006; Maazouzi et al., 2008;
91 Gladyshev et al., 2011), although it evidently accelerates aquatic production. Thus, the increase
92 of production of biomass may overcome a decrease of HUFA contents in this biomass, and
93 thereby HUFA production in a certain ecosystem may stay at the same level, or even increase
94 under climate warming. Thus, the general aim of our work was to clarify this uncertainty. We
95 focused on two most physiologically important and quantitatively prominent HUFA,
96 eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) and tested
97 following hypotheses: 1) in a cold river the same taxa have higher levels of EPA and DHA in
98 their biomass than those in a warm river; 2) in a cold river taxa with higher EPA and DHA
99 contents are more abundant; 3) production of EPA and DHA by zoobenthos in a cold river is
100 higher in spite of lower water temperature.

101

102 STUDY AREA

103

104 The study was carried out in the Yenisei River and two tributaries (Fig. 1). The Yenisei is
105 the longest river (4803 km) and has the greatest water discharge (636 km³ yr⁻¹) and discharge of
106 organic carbon among all the rivers flowing into the Arctic Ocean (Amon et al., 2012). Detailed

107 ecological characteristics of the river are given elsewhere (Gladyshev et al., 1993). In middle
108 reach the river is regulated by the dam of Krasnoyarsk Hydroelectric Power Station which passes
109 waters from 20-40 m depth of hypolimnion of the reservoir. The upstream reservoir is deep (ca.
110 110 m) and thermally stratified and had low phytoplankton biomass and organic carbon in
111 hypolimnetic waters near the dam (Ageev et al., 2008). This results in relatively low
112 phytoplankton biomass, on average 1-2 mg L⁻¹ (Ponomareva, 2015) and low particulate organic
113 carbon ranged from 0.13 to 0.25 mg L⁻¹ (Sushchik, unpublished data), in downstream river
114 section of about 50 km.

115 The Mana River, a right tributary of the Yenisei River, and the Kacha River, a left
116 tributary, (Fig. 1), have length 533 and 103 km and average annual discharge of 93 and 4.3 m³ s⁻¹,
117 correspondingly (Zapekina-Dulkeit & Dulkeit, 1961; Panov & Spitsyna, 2010).

118 We sampled four sites which located 15-30 km downstream of the dam: the left and right
119 banks of the Yenisei, the Mana and the Kacha (Fig. 1). All sampling sites are upstream of the
120 municipal area of Krasnoyarsk city (Fig. 1), and have similar hydrological characteristics:
121 current velocity 1-2 m s⁻¹ and stony beds.

122 Main primary producers in the rivers are epilithic microalgae and cyanobacteria. Diatom
123 and green taxa mainly comprise epilithic biofilms in all three rivers, while cyanobacteria
124 sporadically occur in the Yenisei and Kacha (Table 1). There are many common species in all
125 three studied rivers (Table 1).

126

127 MATERIAL AND METHODS

128

129 The sites were sampled monthly from May to September 2012 and 2013, about 5-10 m
130 from the shore (ca. 0.5-0.7 m of depth). To compare the two shallow rivers, Mana and Kacha,
131 with the large Yenisei River, in the latter we sampled only littoral sites. Water samples for
132 nutrient's analysis were collected with a bucket from the middle of water column. Zoobenthos
133 samples were collected using a Surber sampler (quadrant 40×35 cm, mesh size 0.25 mm). In
134 2012, one sample was taken from each site per sampling date, and in 2013, three replicates were
135 collected. The samples were transported fresh to the laboratory within 1-2 hours of collection.

136 In the laboratory, macrobenthic animals were removed from the sample using forceps and
137 preserved with 70% ethanol. The organisms were identified under a stereomicroscope and sorted
138 to species or genera. The organisms of various taxonomic groups were weighed after gently
139 blotting with filter paper to derive their wet weight (WW). Then, areal biomass concentration of
140 each taxonomic group, g m⁻², was obtained by dividing its wet mass by the area of the sampler
141 quadrat.

142 For fatty acid (FA) and organic carbon analyses, live individuals of particular taxa were
 143 withdrawn from the samples during the sorting, if the numbers were high enough to pool them
 144 and obtain an appropriate biomass (≥ 10 mg WW). Pooled samples for Chironomidae larvae and
 145 Oligochaeta could include several species. In the case of Ephemeroptera, Trichoptera, Hirudinea
 146 and Gastropoda most samples were comprised a single species or genus. Samples of Amphipoda
 147 were, as a rule, a mix of two species. The live animals were placed into beakers with filtered
 148 water of a temperature close to the field for 24 h to allow their gut contents to empty.

149 Then individuals of each sample were subdivided in two sub-samples for FA and carbon
 150 analyses. For FA analyses the animals were placed in chloroform-methanol mixture (2:1, v/v)
 151 and kept frozen (-20 °C) until analysis. Animals of the other sub-samples were wet weighed,
 152 dried until constant weight at 75 °C, and dry weighed to measure moisture content of a given
 153 taxa. The dried biomass was kept in a desiccator for further organic carbon analyzed.

154 Lipid extraction and preparation of fatty acid methyl esters (FAMES) were the same as in
 155 our previous works (e.g., Kalachova et al., 2011). A gas chromatograph equipped with a mass
 156 spectrometer detector (6890/5975C, “Agilent Technologies”, USA) and with a capillary HP-
 157 FFAP column was used. The instrument conditions were described in the above paper. Data
 158 were collected and analyzed using the GC ChemStation program (“Agilent Technologies”,
 159 USA). The FAMES were quantified according to peak area of the internal standard,
 160 nonadecanoic acid, which solutions were added to samples prior the lipid extraction. The
 161 samples for total organic carbon were analyzed with a Flash EA 1112 NC Soil/MAS 200
 162 elemental analyzer (ThermoQuest, Italy).

163 Water samples for soluble nutrients were filtered through pre-cleaned membrane filters
 164 with pore diameter of 0.45 μm (Vladypor, Russia) and analyzed on the day of sampling.
 165 Inorganic phosphorus was determined by the ammonium-molybdate method on a
 166 spectrophotometer at 680 nm. Ammonia nitrogen was determined by the nesslerization method,
 167 at 425 nm. Nitrate nitrogen was measured by reduction into nitrite form onto a cadmium column
 168 followed by the reaction with sulfanilamide and naphthylamine and spectrophotometry at 530
 169 nm.

170 To measure the between-river β -diversity in the macroinvertebrate assemblages the
 171 Sorensen similarity index was calculated like in similar studies (Woodward et al., 2010).

172 Daily production, P_D ($\text{g m}^{-2} \text{ day}^{-1}$), was calculated by the formula:

$$173 P_D = G B$$

174 where G (day^{-1}) is the daily instantaneous growth rate and B (g m^{-2}) is biomass (WW). Values of
 175 G for arthropods were calculated according to the following formula:

$$176 G = a e^{bT}$$

177 where a and b are taxon-specific constants (Table 2) and T is temperature, °C. Daily
 178 instantaneous growth rates for Oligochaeta, Hirudinea and Gastropoda were calculated as
 179 follows:

$$180 \quad G = G_{20} / 2^{0.1(20-T)}$$

181 where G_{20} is taxon-specific constant (Table 2).

182 Seasonal production for the each zoobenthos taxon, P_S , was calculated by trapezium
 183 method of integration:

$$184 \quad P_S = \sum_{i=1}^n \frac{P_{Di} + P_{Di+1}}{2} \cdot (t_{i+1} - t_i)$$

185 where t is time (days). Since the integrated time spans in 2012 and 2013 were 128 and 125 days,
 186 respectively, P_S of 2013 was recalculated for 128 days by multiplying by 128/125 ratio. P_S
 187 values were converted to carbon units using average taxon-specific moisture and carbon
 188 contents. To calculate EPA and DHA seasonal production, the secondary production in carbon
 189 units (g C m^{-2} 128 days) for each taxon was multiplied by the average taxon-specific HUFA/C
 190 ratio (mg g^{-1}). Then, secondary production of biomass of all taxa, as well as EPA and DHA
 191 taxon-specific production were summarized to obtain the total production values. Weighted
 192 average temperature and biomass were also calculated for the studied periods of 2012 and 2013
 193 by the trapezium method of integration.

194 Standard errors, one-way ANOVA and Tukey HSD *post-hoc* test (Brown, 2005) were
 195 calculated conventionally, using STATISTICA software, version 9.0 (StatSoft, Inc.).

196

197 RESULTS

198

199 Average water temperatures and concentrations of inorganic nutrients (ammonium and
 200 nitrate nitrogen, and inorganic phosphorus) are given in Table 1. The most profound difference
 201 in average seasonal temperatures, 6.1 °C, was between the Yenisei River (left bank) and the
 202 Mana River. The sampling site 2 of the Yenisei was affected by the Mana River discharge (Fig.
 203 1) and thereby had a bit higher values of water temperature than the left-bank site (Table 1). In
 204 general, the Kacha River appeared to be richer in inorganic nutrients compared to the other
 205 studied rivers.

206 The species richness in the two warm rivers was evidently higher, than that in the Yenisei
 207 River (Table 3). Concerning abundant taxa, Amphipoda were found only in the Yenisei River,
 208 while Hirudinea occurred only in the warm rivers, Mana and Kacha, and Chironomidae were the
 209 most species-rich group (Table 3).

210 In the Yenisei River two amphipod species had the highest frequencies (Table 4), and
211 they constituted most part of total biomass, up to 92.7%. In the Mana River Chironomidae larvae
212 *Thienemannimyia* sp. had the highest frequency, followed by Ephemeroptera larvae *Ephemera*
213 *orientalis* (Table 4). The latter taxa dominated in biomass of zoobenthos: 56.6% and 38.4% in
214 2012 and 2013, correspondingly (Fig.2). In the Kacha River Chironomidae larvae *Orthocladius*
215 *thienemanni* was the most frequently occurred species (Table 4), while Ephemeroptera larvae
216 were dominant in the total biomass (Fig.2).

217 Species overlap between the river communities (β -diversity) according to Sorensen
218 similarity index was the highest for the Yenisei sites (Table 4). The warm rivers, Mana and
219 Kacha, also had comparatively high similarity index, while they were weakly similar to the
220 Yenisei sites.

221 We used one-way ANOVA to estimate inter-site variation (effects) of EPA content in
222 each taxa. We excluded from the analysis the taxa occurred only in one or two habitats or those
223 that occurred sporadically. For Oligocheta, Ephemeroptera and Trichoptera, there was no
224 statistically significant effect of river habitat on the EPA content (Fig.3). Chironomids inhabited
225 sites of the Yenisei River, primarily *Prodiamesa olivacea* (Table 4), had significantly higher
226 EPA contents (Fig.3) than those from the Mana River and the Kacha River, primarily
227 *Microtendipes pedellus* and *Orthocladius thienemanni* (Table 4).

228 DHA contents per wet weight in most taxa, except Amphipoda, were negligibly small and
229 thereby were not included in the ANOVA. Contents of DHA in Amphipoda from site 1 and site
230 2 did not differ significantly: $F = 2.46$, $P = 0.1374$.

231 Since there were no statistically significant differences in HUFA contents of the same
232 taxa inhabited the studied rivers, we combined the biochemical data from all the sites and
233 calculated the averages of HUFA contents for all the studied taxa (Table 5). The only exception
234 was chironomids (Fig. 3), which were subdivided into two groups, Chironomidae 1-2 and
235 Chironomidae 3-4, according to the sampling site numbers (Fig.1). There were overall
236 statistically significant differences among taxa in HUFA per cents and contents according to one-
237 way ANOVA (Table 5). Chironomidae 1-2 had the highest EPA level and content, followed by
238 Limoniidae and Ephemeroptera, and Amphipoda had the highest DHA level and content (Table
239 5).

240 In the Yenisei River, left bank, there was the highest biomass and production in spite of
241 the lowest water temperature (Table 6). In turn, production in the Kacha River was higher than
242 that in the Yenisei River, right bank and in the Mana River in the both seasons, although values
243 of biomass at these sites were comparable (Table 6). Production of EPA and especially DHA at
244 the coldest site 1 also had the highest values in both seasons (Table 6). However, ratios of EPA

245 production to the biomass production, P_{EPA}/P_S , in all sites were comparable. In contrast, values
246 of P_{DHA}/P_S in the Yenisei River, at both sites, were evidently higher than those in the warm
247 rivers (Table 6).

248 Average values of EPA production for two seasons in the Yenisei River, site 1, were
249 significantly higher, than those at the other sites after ANOVA (Fig. 4). The average EPA
250 production in the Kacha River was significantly higher than that in the Yenisei River, site 2, and
251 the Mana River. The highest average DHA production occurred in the coldest site 1, and its
252 value at site 2 with the intermediate water temperature also was significantly higher, than those
253 in two warm rivers (Fig. 4).

254

255 DISCUSSION

256

257 Our ‘natural experiment’ confirmed that although the Yenisei has a big reservoir
258 upstream and is a substantially larger river than its tributaries, the Mana and Kacha, their
259 hydrochemical characteristics, e.g. inorganic nutrient contents, are rather similar. Moreover,
260 dominant primary producers in all the rivers, benthic (epilithic) microalgae, were represented by
261 the same taxonomic groups, diatoms and greens.

262 In contrast to hydrochemical characteristics, the summer temperatures strongly contrasted
263 in the Yenisei versus the Mana and the Kacha rivers, up to 12.2 °C. This contrast seems to be one
264 of the main causes of some differences of species composition of the diatom and green
265 microalgae, as well as in zoobenthos in the cold-water Yenisei and the warm-water Mana and
266 Kacha (Table 1, 3-4).

267 We assessed if differences in species composition of biofilms in the studied rivers could
268 affect food quality for benthic animals concerning levels of the omega-3 HUFA and their
269 precursor, α -linolenic acid (ALA). All the dominant genera of greens, *Ulothrix*, common for the
270 Yenisei River, both banks, and the Mana River, and *Cladophora*, common for the Yenisei River,
271 right bank, and the Kacha River, are known to have high levels of ALA (Torres-Ruiz et al.,
272 2007; Sushchik et al., 2010). Diatom species of the Yenisei River, for instance, *Didymosphenia*
273 *geminata*, are especially rich in EPA (Sushchik et al., 2010), and they also occurred in the Mana
274 River and the Kacha River. Moreover, periphyton communities where warm-water *Melosira*
275 *varians* dominated, like in the Mana and Kacha, also had high EPA levels (Huggins et al., 2004;
276 Hill et al., 2011). Hence, diatom-green epilithic communities occurred in the cold-water Yenisei
277 and in the warm-water Mana and Kacha rivers, appeared to be equally good sources of the
278 omega-3 fatty acids for zoobenthos.

279 The increase in species richness of zoobenthos (α -diversity) in the two warm rivers
280 relatively to the cold-water river, is in a good agreement with data of some other authors that
281 global warming will lead to higher local and regional richness of freshwater benthic
282 macroinvertebrate assemblages (Oertli et al., 2008; Floury et al., 2013). The studied warm and
283 cold communities had slight similarity (β -diversity), although they were within a relatively small
284 area and thereby had no biogeographical and dispersal constraints. In similar studies of Icelandic
285 geothermal streams clear changes in both macroinvertebrate community structure and diversity
286 with temperature were also found (Friberg et al., 2009).

287 Our first hypothesis, that in the cold river the same taxa have higher levels of EPA and
288 DHA than those in warm rivers, was confirmed only for Chironomidae. The other studied taxa
289 had similar HUFA contents, in spite of only partial overlapping of species in the habitats.
290 Different species of Hirudinea occurred in the Kacha and Mana, but had nearly identical HUFA
291 contents. The similarity of HUFA contents in spite of different habitats within the same taxa at
292 the level of orders (Ephemeroptera, Trichoptera) and even subclasses (Oligochaeta, Hirudinea)
293 appeared to be in good agreement with recent finding, that namely phylogenetic factor primarily
294 controls fatty acid composition of aquatic invertebrates (Makhutova et al., 2011; Lau et al.,
295 2012). However, we found the differences in EPA contents at the lower taxonomic level, namely
296 within Chironomidae family. The dominant chironomid of the Yenisei, *Prodiamesa olivacea*, is
297 evidently cold-water affiliate, like other Diamesinae (Woodward et al., 2010), and has the
298 highest EPA content. Thus, the question concerning phylogenetic vs environmental regulation of
299 fatty acid composition of aquatic invertebrates seems to be still on the table.

300 Our second hypothesis that in the cold river taxa with higher HUFA contents are more
301 abundant was confirmed for DHA only. Indeed, in both sites of the Yenisei River Amphipoda
302 with the highest DHA content were the dominant species. In the warm rivers, the taxa with the
303 lowest DHA contents, Ephemeroptera and Limoniidae, were abundant.

304 In general, contents of HUFA in zoobenthos in the studied rivers were close to those
305 reported in literature for the same freshwater taxa: insect larvae (Torres-Ruiz et al., 2007;
306 Makhutova et al., 2011; Sánchez-Muros et al., 2014), Amphipoda (Kolanowski et al., 2007;
307 Czesny et al., 2011), Oligochaeta (Goedkoop et al., 2000; Torres-Ruiz et al., 2007) and
308 Hirudinea (Lau et al., 2012). However, Lau et al. (2012) reported twice lower EPA percentages
309 for Ephemeroptera and Trichoptera, and Shipley et al. (2012) reported ~5-fold lower percentages
310 and ~9-fold lower contents per weight of this HUFA for Ephemeroptera. Like in our study all the
311 above authors found high DHA contents in Amphipoda and negligible amounts of this HUFA in
312 other zoobenthos taxa.

313 Our third hypothesis that production of HUFA by zoobenthos is higher in the cold river
314 appeared to **be true for DHA, and partly for EPA**. Indeed, EPA production in the warm Kacha
315 River was significantly higher, than that in colder site at right bank of the Yenisei River.
316 However, in the coldest site at the left bank of the Yenisei River the highest production of EPA
317 occurred.

318 EPA and especially DHA are known to be of key physiological importance for fish
319 growth and development (Sargent et al., 1999; Tocher, 2003). Indeed, DHA composes up to
320 ~30% of fish brain lipids (Shields et al., 1999), but capacity of most fish to synthesize this
321 HUFA from short-chain precursors is very limited (Tocher, 2003). Thus, shortage in food supply
322 of DHA significantly affects fish growth and development (Vizcaino-Ochoa et al., 2010; Zakeri
323 et al., 2011). According to our data, in the warm rivers, **standing stock and production of**
324 **zoobenthos, as the main food supply for fish, had significantly lower DHA content**.

325 In turn, fish are the main source of EPA and DHA for humans (Gladyshev et al., 2013).
326 To prevent cardiovascular diseases and neural disorders personal daily intakes of EPA+DHA ca.
327 0.5 – 1.0 g are necessary for most people (Kris-Etherton et al., 2009; Harris et al., 2009).
328 However, world wild fish catch and aquaculture production are limited, and at present
329 humankind faces with deficiency of EPA and DHA in diet (Gladyshev et al., 2009). If our results
330 are interpreted in a climate warming context we can predict a decrease of production of these
331 HUFA in river ecosystems and thereby a diminishing of their supply for humans through fish.
332 Indeed, there are indirect evidences that the loss of cold freshwater habitats may cause a decrease
333 of biochemically valuable fish production. In Europe climate warming stimulates a shift from
334 salmonids to percids, and from percids to cyprinids (Jeppesen et al., 2010). As generalized
335 recently, average EPA and DHA content in fish biomass decreased significantly from salmonids
336 to percids, and from percids to cyprinids (Gladyshev et al., 2013). Thus, fish of high nutritive
337 value for humans, salmonids, which preferred cold habitats are substituted by fish of lower
338 nutritive value. This tendency is explicit in the studied ecosystems: in the cold sites of Yenisei
339 River salmonid Siberian grayling *Thymallus arcticus* Pallas is the dominant species (Zuev et al.,
340 2011), while in the both warm rivers, Mana and Kacha, cyprinids Siberian dace *Leuciscus*
341 *leuciscus baicalensis* (Dybowski) and gudgeon *Gobio gobio cynocephalus* (Dybowski) are the
342 most abundant (Zapekina-Dulkeit & Dulkeit, 1961; Chugunova & Pronin, 2011).

343 According to our present data, the differences of EPA and DHA contents and production
344 in zoobenthos of the cold and warm rivers were caused by the differences of species
345 composition. This finding is in a good agreement with our previous data that water temperature
346 affected contents of these HUFA in lake zooplankton via changing of community taxonomic
347 structure (Gladyshev et al., 2011). Thus, the cold-adapted species, primarily Amphipoda, gave

348 higher standing stock biomass and higher production, which was more valuable concerning
349 HUFA contents for organisms of higher trophic levels. Both species of amphipods, *E. viridis* and
350 *G. fasciatus*, are invaders from the Baikal Lake, which is known by low annual temperatures
351 (Gladyshev & Moskvicheva, 2002).

352 Many studies reported changes of structural characteristics, species composition and
353 biodiversity caused by climate change. However, reliance on structure alone makes it difficult to
354 prescribe ecosystem management or restoration actions for a specific ecosystem service (Palmer
355 & Febria, 2012). We tried to convert the changes of species composition of macrozoobenthos to
356 the specific service of aquatic ecosystems: production of HUFA. We found that increase of
357 biodiversity of river zoobenthos with the increase of water temperature, occurred like in other
358 studies, was not accompanied by increase of HUFA production. On the contrary, the less diverse
359 cold-water community gave the highest production of EPA and DHA. Thus, our 'natural
360 experiment' gave an evidence of the strong effect of temperature on HUFA production by river
361 zoobenthos, in the absence of the confounding effects (e.g. biogeography, seasonality and
362 physico-chemical conditions). Basing on the data obtained we can predict that a climate warming
363 can diminish DHA and partly EPA production by river zoobenthos with possible negative
364 consequences for organisms of progressively higher trophic levels.

365

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370

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552

553 **Table 1.** Physico-chemical parameters (means \pm standard errors, n=10, May-September 2012-
 554 2013) and abundance of epiphytic algal and cyanobacterial taxa in the studied sites. Biotic
 555 parameters were estimated according to literature data: the Yenisei River, left bank (Sushchik et
 556 al., 2010); the Yenisei River, right bank (Gaevsky et al., 2006); the Mana River (Anufrieva et al.,
 557 2008); the Kacha River (Gold et al., 2005).

Characteristics	Yenisei, left bank	Yenisei, right bank	Mana	Kacha
Temperature (°C)	8.4 \pm 0.8	11.7 \pm 0.8	14.5 \pm 1.6	14.2 \pm 1.7
Ammonium nitrogen (mg N L ⁻¹)	0.10 \pm 0.02	0.13 \pm 0.04	0.12 \pm 0.03	0.27 \pm 0.06
Nitrate nitrogen (mg N L ⁻¹)	0.14 \pm 0.04	0.05 \pm 0.01	0.10 \pm 0.03	0.25 \pm 0.05
Inorganic phosphorus (mg P L ⁻¹)	0.010 \pm 0.002	0.006 \pm 0.001	0.005 \pm 0.001	0.019 \pm 0.004
Bacillariophyta				
<i>Cocconeis placentula</i> Ehr.	++	++	++	
<i>Diatoma tenuis</i> (Lyngb) Ag.	++	++	++	+
<i>Diatoma vulgare</i> Bory				++
<i>Didymosphenia geminata</i> (Lyngb.) M.Schmidt	+++	+	++	+
<i>Melosira varians</i> C.Agardh			+	+
<i>Navicula</i> sp.	+	++	+	+
<i>Rhoicosphenia abbreviate</i> (Kütz.) Grun.	+++	+++	++	
<i>Synedra ulna</i> (Nitzsch) Ehr		+	++	+
genus <i>Cymbella</i>	++	++	+++	+++
genus <i>Fragilaria</i>	+	+	+	
genus <i>Gomphonema</i>	+++	+++	++	
Chlorophyta				
<i>Cladophora glomerata</i> (L) Kütz.		+	+	+++
<i>Spyrogyra</i> sp.			++	
<i>Ulothrix zonata</i> Kütz.	++	++	+	
Cyanophyta				
Oscillatoriaceae	+	+		+

558

559 +++ often occurred at high biomass

560 ++ often occurred at moderate biomass or sporadically occurred at high biomass

561 + often occurred at low biomass or sporadically occurred at moderate biomass

562 **Table 2.** Constants for calculation of daily instantaneous growth rate of freshwater zoobenthos.
 563

Taxon	<i>a</i>	<i>b</i>	G_{20}	Reference
Oligochaeta			0.030	Berezina, 2008
Hirudinea			0.034	Nikolaev, 1980
Amphipoda	0.0160	0.009		Berezina, 2008
Odonata	0.0075	0.080		Golubkov, 2000
Ephemeroptera	0.0130	0.099		Golubkov, 2000
Trichoptera	0.0130	0.054		Golubkov, 2000
Plecoptera	0.0166	0.028		Golubkov, 2000
Diptera*	0.0075	0.143		Golubkov, 2000
Chironomidae	0.0087	0.142		Golubkov, 2000
Gastropoda			0.032	Arakelova, 1999

564

565 *other, than Chironomidae

566

567 **Table 3.** Number of macrozoobenthos species at the sampling sites in May-September, 2012 and
 568 2013. Number of samples at each site, n = 20.

Taxa	Yenisei, left bank	Yenisei, right bank	Mana	Kacha
Plathelminthes				
Turbellaria	1	0	0	0
Nemathelminthes				
Nematoda	0	0	0	2
Annelida				
Oligochaeta	3	5	6	5
Hirudinea	0	0	3	2
Arthropoda				
Crustacea				
Amphipoda	2	2	0	0
Insecta				
Odonata	0	1	2	0
Ephemeroptera	5	7	22	10
Trichoptera	3	5	17	8
Plecoptera	0	2	10	0
<i>Diptera</i>				
Chironomidae	14	20	40	29
Ceratopogonidae	0	1	1	1
Limoniidae	0	1	2	3
Tabanidae	0	0	0	1
Empididae	0	0	1	1
Simuliidae	0	0	1	0
Blephariceridae	0	0	1	0
Tipulidae	0	0	1	0
Mollusca				
Bivalvia	0	1	1	0
Gastropoda	0	1	3	1
Total	28	46	111	63

569

570

571 **Table 4.** List of species with high frequencies (F, % of the all samples taken at a given site) and
 572 Sorensen similarity indexes for zoobenthos β -diversity between the sites. The frequency of a
 573 species was considered as high if $F \geq 50\%$ at least at one site. Number of samples at each site, n
 574 = 20, May-September, 2012 and 2013.

Species	Frequency, %			
	Yenisei, left bank	Yenisei, right bank	Mana	Kacha
Oligochaeta				
<i>Limnodrilus hoffmeisteri</i> Claparede			10	70
<i>Slavina appendiculata</i> d'Udekem	50	25		
<i>Stylodrilus herringianus</i> Claparede			90	
<i>Tubifex tubifex</i> O.F.Muller	15	40	5	65
Amphipoda				
<i>Eulimnogammarus viridis</i> Dybowski	100	65		
<i>Gmelinoides fasciatus</i> Stebbing	20	90		
Ephemeroptera				
<i>Baetis</i> gr. <i>fuscatus</i>			50	40
<i>Caenis pseudorivulorum</i> Keffermüller			10	55
<i>Choroterpes altioculus</i> Kluge			70	
<i>Ecdyonurus jornensis</i> Bengtsson				65
<i>Epeorus pellucidus</i> Brodsky	5	25	70	15
<i>Ephemera orientalis</i> McL.	5	55	95	55
<i>Ephemerella ignita</i> Poda			50	40
<i>Ephemerella lenokii</i> Tshernova			70	10
<i>Potamanthus luteus</i> L.	5	60	80	
<i>Rhithrogena lepnevae</i> Brodsky			65	
Trichoptera				
<i>Ceraclea exisa</i> Morton			55	35
<i>Lepidostoma hitrum</i> Fabricius			75	
<i>Psychomyia pusilla</i> Fabr.			15	75
<i>Sericostoma personatum</i> Kirby et Spense			55	
<i>Setodes</i> sp.			80	
Chironomidae				
<i>Cryptochironomus defectus</i> Kieffer			15	55
<i>Microtendipes pedellus</i> De Geer	10	35	80	75
<i>Orthocladius saxicola</i> Kieffer			30	60
<i>Orthocladius thienemanni</i> Kieffer			85	100
<i>Polypedilum bicrenatum</i> Kieffer	5	15	10	50
<i>Polypedilum scalaenum</i> Schrank			80	55
<i>Prodiamesa olivacea</i> Meigen	75	25		5
<i>Rheotanytarsus</i> sp.			10	80
<i>Stictochironomus crassiforceps</i> Kieffer		10	5	85
<i>Thienemannimyia</i> sp.			100	35
Sorensen similarity indexes				
Yenisei, right bank	0.62			
Mana	0.12	0.19		
Kacha	0.16	0.24	0.46	

Table 5 Average (\pm standard errors) moisture and organic carbon contents and percentages (of total fatty acids) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and their contents in biomass of zoobenthos taxa in the studied rivers in May-September, 2012 and 2013: n – number of samples for moisture and organic carbon analyses or fatty acid analyses (in parentheses) , F – Fisher’s test for categorical predictor in one-way ANOVA and P – statistical significance of ANOVA results. Means labeled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD *post-hoc* test.

Taxa	N	Moisture, % ww	C, % dw	EPA, %	DHA, %	EPA, mg g ⁻¹ ww	DHA, mg g ⁻¹ ww
Oligochaeta	7(15)	76.2 \pm 1.6	41.5 \pm 1.2	13.6 \pm 1.0 ^{AB}	1.1 \pm 0.2 ^A	1.48 \pm 0.14 ^A	0.12 \pm 0.02 ^A
Hirudinea	7(8)	80.5 \pm 1.3	47.9 \pm 0.6	15.7 \pm 1.9 ^{AB}	0.6 \pm 0.2 ^{ABD}	1.43 \pm 0.30 ^A	0.06 \pm 0.02 ^A
Amphipoda	19(17)	73.9 \pm 1.2	38.0 \pm 0.9	17.1 \pm 0.7 ^A	3.2 \pm 0.3 ^C	2.79 \pm 0.37 ^{AB}	0.47 \pm 0.05 ^B
Ephemeroptera	36(48)	78.0 \pm 0.7	47.3 \pm 0.8	16.8 \pm 0.7 ^A	0.1 \pm 0.0 ^D	3.64 \pm 0.26 ^{BC}	0.02 \pm 0.00 ^C
Trichoptera	14(23)	76.9 \pm 1.9	47.8 \pm 1.6	10.1 \pm 1.3 ^B	0.3 \pm 0.1 ^{BD}	3.03 \pm 0.49 ^A	0.07 \pm 0.01 ^{AC}
Plecoptera	6(8)	71.4 \pm 2.9	48.4 \pm 0.6	15.0 \pm 1.9 ^{AB}	0.2 \pm 0.1 ^{BD}	2.40 \pm 0.39 ^A	0.05 \pm 0.02 ^{AC}
Chironomidae 1-2	7(8)	76.9 \pm 2.6	48.7 \pm 1.4	19.4 \pm 1.1 ^A	0.4 \pm 0.1 ^{ABD}	5.21 \pm 0.45 ^C	0.10 \pm 0.02 ^{AC}
Chironomidae 3-4	10(13)	79.2 \pm 1.9	45.5 \pm 1.5	13.4 \pm 1.9 ^{AB}	0.3 \pm 0.1 ^{BD}	2.33 \pm 0.36 ^A	0.05 \pm 0.01 ^{AC}
Limoniidae	3(3)	83.4 \pm 2.4	50.3 \pm 0.2	19.6 \pm 3.6 ^{AB}	0.2 \pm 0.1 ^{ABD}	3.30 \pm 1.85 ^{AC}	0.04 \pm 0.02 ^{AC}
Gastropoda	4(5)	79.7 \pm 2.6	37.5 \pm 3.0	11.9 \pm 1.2 ^{AB}	0.9 \pm 0.3 ^{AB}	0.62 \pm 0.11 ^A	0.05 \pm 0.02 ^{AC}
F	-	-	-	4.79	67.34	6.03	46.80
P	-	-	-	0.00001	0.00000	0.00000	0.00000

Table 6. Weighted mean seasonal temperature (T , °C), weighted mean seasonal biomass of zoobenthos (B , g C m⁻²), seasonal production of zoobenthos (P_S , g C m⁻² 128 days), seasonal production of eicosapentaenoic acid (P_{EPA} , mg m⁻² 128 days) and docosahexaenoic acid (P_{DHA} , mg m⁻² 128 days) by zoobenthos and their ratios (mg g⁻¹ C) in the studied rivers: S1 – the Yenisei River, left bank; S2 – the Yenisei River, right bank; S3 – the Mana River; S4 – the Kacha River.

Site, year	T	B	P_S	P_{EPA}	P_{DHA}	P_{EPA}/P_S	P_{DHA}/P_S
S1 2012	9.0	3.12	7.67	244.6	30.7	31.9	4.0
S1 2013	8.0	4.08	9.08	270.6	40.7	29.8	4.5
S2 2012	12.7	0.98	2.39	66.8	9.7	27.9	4.1
S2 2013	12.0	1.12	3.62	117.7	8.8	32.5	2.4
S3 2012	17.1	0.26	2.12	65.0	0.7	30.6	0.3
S3 2013	14.4	0.51	2.28	60.5	0.8	26.6	0.4
S4 2012	15.8	0.64	3.97	106.2	1.7	26.8	0.4
S4 2013	15.3	0.43	4.49	152.1	1.2	33.9	0.3

Figure legends

Fig. 1. Map of the studied area. Dam – the dam of Krasnoyarsk Hydroelectric Power Station. Dashed area indicates Krasnoyarsk city. Sampling sites: 1 – the Yenisei River, left bank; 2- the Yenisei River, right bank; 3 – the Mana River; 4 – the Kacha River.

Fig. 2. Average seasonal percent biomass ($\text{g m}^{-2} \text{ ww}$) of quantitatively prominent zoobenthos taxa. Sampling site abbreviations: S1 – the Yenisei River, left bank; S2 – the Yenisei River, right bank; S3 – the Mana River; S4 – the Kacha River.

Fig. 3. Average content of eicosapentaenoic acid (EPA) in zoobentos taxa occurred in all the studied rivers, May-September, 2012 and 2013. Bars represent standard errors. Means labeled with the same letter are not significantly different from each other at $P < 0.05$ after Tukey HSD *post hoc* test; absence of the letter labels indicates that ANOVA is not significant.

Fig. 4. Average seasonal production (May-September, 2012 and 2013, 128 days) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by zoobenthos in the studied rivers: S1 – the Yenisei River, left bank; S2 – the Yenisei River, right bank; S3 – the Mana River; S4 – the Kacha River. Production values were calculated for single and triple zoobenthos biomass data in 2012 and 2013, respectively; then they were averaged, bars represent standard errors. Means labeled with the same letter are not significantly different from each other at $P < 0.05$ after Tukey HSD *post hoc* test.