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Fatty acid composition in the white muscle of Cottoidei fishes of Lake Baikal reflects their habitat depth --Manuscript Draft--

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Abstract:	<p>Lake Baikal is a unique freshwater environment with maximum depths over 1600m. The high water pressure at the lakebed strengthens the solidifying effect of low water temperature on animal tissue lipids, and thus the effective temperatures in the depths of the lake equal subzero temperatures in shallow waters. Cottoidei species has colonized the different water layers of the lake, and developed different ecology and physiology reflected in their tissue biochemistry. We studied by gas chromatography the composition of fatty acids (FAs), largely responsible for tissue lipid physical properties, in the white muscle tissue of 13 species of the Cottoidei fish; 5 benthic abyssal, 6 benthic eurybathic and 2 benthopelagic species. The FA profiles reflected habitat depth. The muscles of the deepest living species contained little polyunsaturated FAs (PUFAs) and were instead rich in monounsaturated FAs (MUFAs), which may be due to occasional weak food web links to the PUFA-rich primary producers of the photic water layer, high MUFA supply from their benthic diet, and conversion of saturated FAs (SFAs) to MUFAs in the tissues of the fish. Despite the MUFA percentage among the abyssal species reached even 50 % (by weight) of total FAs, the PUFA percentage still remained above 20% in every species. The muscle MUFA/SFA ratio correlated negatively with the PUFA content of the fish muscle, suggesting viscosity control integrating the fluidity contributions from the dietary PUFAs and potentially endogenous MUFAs.</p>	
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explained to us that Environmental Biology of Fishes does not allow any changes in authorship of manuscript after initial submission. Thus, as the only additional change to the manuscript, we were asked to remove the new author Melissa Westberg from the author list and include mention of assistance from her in the Acknowledgements. In this new version we have followed these instructions, and hope that our manuscript is now suitable for publication in the Journal. Since this is a small and easy-to-find change, we are submitting only the unmarked word-file of the manuscript texts and tables (with the previous Figure file).

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4 **Fatty acid composition in the white muscle of Cottoidei**

5 **fishes of Lake Baikal reflects their habitat depth**

6

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17 Temperature adaptation, Taxonomy, Trophic ecology, Viscosity homeostasis, White muscle

18 **Running head:** Fatty acids in Cottoidei fishes of Lake Baikal

19

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30 **Abstract** Lake Baikal is a unique freshwater environment with maximum depths over
31 1600m. The high water pressure at the lakebed strengthens the solidifying effect of low water
32 temperature on animal tissue lipids, and thus the effective temperatures in the depths of the
33 lake equal subzero temperatures in shallow waters. Cottoidei species has colonized the
34 different water layers of the lake, and developed different ecology and physiology reflected in
35 their tissue biochemistry. We studied by gas chromatography the composition of fatty acids
36 (FAs), largely responsible for tissue lipid physical properties, in the white muscle tissue of 13
37 species of the Cottoidei fish; 5 benthic abyssal, 6 benthic eurybathic and 2 benthopelagic
38 species. The FA profiles reflected habitat depth. The muscles of the deepest living species
39 contained little polyunsaturated FAs (PUFAs) and were instead rich in monounsaturated FAs
40 (MUFAs), which may be due to occasional weak food web links to the PUFA-rich primary
41 producers of the photic water layer, high MUFA supply from their benthic diet, and
42 conversion of saturated FAs (SFAs) to MUFAs in the tissues of the fish. Despite the MUFA
43 percentage among the abyssal species reached even 50 % (by weight) of total FAs, the PUFA
44 percentage still remained above 20% in every species. The muscle MUFA/SFA ratio
45 correlated negatively with the PUFA content of the fish muscle, suggesting viscosity control
46 integrating the fluidity contributions from the dietary PUFAs and potentially endogenous
47 MUFAs.

48 Introduction

1 49

2
3 50 Lake Baikal is a relatively pristine rift lake with the depth and temperatures common
4
5 51 to ocean deep-sea areas. Seasonal fluctuations of water temperature cease below 300 m and at
6
7 52 the max depth of ~1640 m, the pressure is 164 atmospheres and temperature close to 0°C. The
8
9 53 average annual temperature in the open lake is 3°C. Baikal is the world's most ancient
10
11 54 freshwater lake (formed 20-25 million years ago), and its Fauna including diverse fish is
12
13 55 largely endemic. The abyssal communities of fish and other organisms of the lake have been
14
15 56 adapted to the high pressure, low temperature and scattered dietary sources, but very little
16
17 57 comparative biochemistry has been conducted on them. During this century, a rapid climate
18
19 58 change is expected to happen in the Baikal region, and when the duration of ice cover and its
20
21 59 transparency are decreased the spring bloom of diatoms occurring under the ice is harmed
22
23 60 (Semovski et al. 2000; Moore et al. 2009). The spring ice is an essential seat sustaining this
24
25 61 rich bloom, which nourishes not only pelagic but also benthic animals. The rapidly sinking
26
27 62 diatom mass is an important food resource for benthic animal communities providing them
28
29 63 with different fatty acids (FAs) (Shishlyannikov et al. 2014). In addition, the increasing
30
31 64 eutrophication, starting from the shore line and deltas, will finally affect benthic community
32
33 65 structure and biomass of the deep parts of the lake (Chandra et al. 2005). At the same time,
34
35 66 vertical migrations of cold-water stenothermal fish are likely affected since these species
36
37 67 avoid entering the warming waters of the photic zone because of the physiological and
38
39 68 biochemical stress caused by elevated temperature. Thus the benthic fish communities of the
40
41 69 lake will be facing changes in the quantity and quality of food sources accessible for them. It
42
43 70 is of note that the Lake Baikal Fauna is land locked with limited possibilities to migrate and
44
45 71 find new habitats with optimal thermal conditions and suitable food resources, and therefore
46
47 72 the expected changes due to climate change in the fish communities of Lake Baikal may be
48
49 73 more pronounced and faster than the changes in the fish communities of polar sea areas.

50
51 74 Comparative and experimental works with deep-sea fish have shown that the tissues of
52
53 75 the deepest living fish have the lowest activities of metabolic enzymes and oxygen
54
55 76 consumption. To maintain some metabolism, they require compensatory adjustment of cell
56
57 77 membrane fluidity, which provides integral membrane proteins conformational freedom
58
59 78 needed for optimal function. Indeed, when the interspecies fluidity comparisons are carried
60
61 79 out at room temperature and normal pressure, the membranes of the deep-living fish are the
62
63 80 most fluid (Somero 1992; Sebert 2002). This is due to compensatory biochemical
64
65 81 mechanisms, and without them the high pressure and low temperature of the natural habitat

82 would make their lipid membranes too viscous. In near 0°C water, the additional membrane
83 solidifying effect due to the pressure of 160 atm is equal to decreasing the temperature further
84 by approximately 3°C (Macdonald 1984; Cossins and Macdonald 1989). Thus the challenge
85 equals to maintaining the lipid viscosity homeostasis in subzero surface water. Structural
86 modifications of membrane lipids are acknowledged to play a central role in the thermal
87 adaptation of poikilotherm tissues, and the physical properties of the lipids are largely
88 determined by their fatty acid (FA) composition (Hazel 1995; Käkälä et al. 2008). To achieve
89 enhanced membrane fluidity poikilotherms can utilize diet-derived unsaturated FAs or
90 increase the degree of their FA unsaturation by inserting new double bonds into the existing
91 acyl chains by using desaturase enzymes (Trueman et al. 2000; Hsieh et al. 2007). The FA
92 chain length also affects fluidity, but in lesser degree than the double bond content. The
93 dietary supply of different FAs to be used for tissue lipids differs when entering from the
94 pelagic photic water layers to the benthic abyssal zone, where also the abundance of food may
95 have large spatial and temporal variability (Bühning and Christiansen 2001). Unfortunately,
96 the Baikalian species studied for FAs are few, and information *e.g.* on true abyssal fish
97 species, which from the perspective of environmental adaptation are among the most
98 interesting ones, is mostly missing, giving this far little opportunities for ecophysiological
99 interpretations (Morris 1984; Kozlova and Khotimchenko 1993; Kozlova and Khotimchenko
100 2000; Grahl-Nielsen et al. 2011; Averina et al. 2011).

101 First, Morris (1984) determined FA composition of some of the most common
102 Baikalian species of copepods, gammarids and turbellarians and two endemic species of fish,
103 large and small golomyanka (*Comephorus baikalensis* and *C. dybowskii*). The FA
104 composition of these two fish species, having the habit of moving throughout the entire water
105 column, was also determined in later investigations (Kozlova and Khotimchenko 2000). In
106 addition, data on FA composition for *Cottocomephorus* (Kozlova and Khotimchenko 1993)
107 and *Coregonus* (Ju et al. 1997) species of fish and also for some sponges, mollusks and
108 amphipods are available (Dembitsky et al. 1994b,c; Bazarsadueva and Radnaeva 2013).
109 Previously we have determined the FA composition in the tissues of 13 species or ecoforms
110 of Lake Baikal fish (Grahl-Nielsen et al. 2011). The studies so far, focusing on the species
111 inhabiting shallow waters or the upper half of the water column, have shown that the fish of
112 the lake have species-specific FA compositions, which in general are characterized by high
113 proportions of n-3 polyunsaturated FAs (n-3 PUFAs) but have at the same time high levels of
114 n-6 PUFAs, resulting in a low n-3/n-6 ratio, characteristic for freshwater fish (Ackman 1967).

115 We got the opportunity to collect samples of several Cottoidei fishes by using deep-
116 sea trawling and manned submersibles (Table 1). These fish included 9 species from the

117 Abyssocottidae family, which belonged to 2 ecological groups - benthic abyssal and benthic
118 eurybathic fish, both groups mainly dwelling at deep depths, 400-1600 m Additional species
119 sampled belonged to the Cottidae family; one abyssal and one eurybathic species from the
120 genus of *Batrachocottus* and two benthopelagic species from the genus *Cottocomephorus*.
121 The origin and evolution of these Cottoidei fishes is connected with the geohistory of the
122 Baikal rift zone and global and regional climate change. About 2 million years ago,
123 decreasing temperatures of the Quaternary period led to the extinction of the Tertiary fauna
124 and created favorable conditions for cold water fishes. According to Dorogostaisky (1923)
125 and Taliev (1955), ancestral forms of Cottoidei fish of the North Pacific penetrated the early
126 Lake Baikal and began adaptive radiation, which led to the emergence of endemic species,
127 genera, and possibly families (Fig. 1). Alternative views of the Cottoidei fishes originating
128 from the Miocene period have also been presented (Berg 1922; Sideleva 1993). Since the
129 invasion of the lake the early Cottoidei fishes specified and inhabited a wide range of depths.

130 With increasing depth of the Baikal water column, there is a reduction in fish
131 diversity, number and biomass. The abyssal zone of the lake, with depths below 300m, is
132 inhabited only by endemic Cottoidei species, which have no air bladder. The highest species
133 richness, 12–15 species, is found at the depth of 500–700m. At the depths of over 1000m, the
134 number of species is 5–6, and at maximum depths usually only 3 species, namely *Cottinella*
135 *boulengeri*, *Abyssocottus gibbosus* and *Abyssocottus korotneffi*, are found. Fishes in the
136 abyssal zone are divided into truly abyssal species, which are exclusively dwelling at great
137 depths but during ice cover may visit upper waters, and eurybathic species, which frequently
138 migrate and feed over a wide range of depths (as examples abyssal *Batrachocottus* species
139 and eurybathic *Limnocottus griseus* in Fig. 2). During winter months the eurybathic species
140 are often found in the upper water but when ice melts and the temperature of the upper water
141 rises they also prefer staying in the deeper parts of their habitat. In general, gammarids and
142 debris are the base of the diet of true abyssal species. The importance of amphipods and
143 variability of the diet is larger in the diet of eurybathic species. The species that on daily basis
144 swim and feed at the bottom as well as in midwaters or even near the surface are called
145 benthopelagic. Benthopelagic species mainly consume planktonic animals (e.g. *Epishura*
146 *baicalensis* and *Macrohectopus branickii*). All abyssal Cottoidei fish are characterized by
147 elongated period of spawning at the constantly low water temperature. The spawning lasts
148 from 2 to 4 months, and depending on the species it occurs in late autumn, mid-winter or even
149 late spring but not reported in July-September. The males are guarding the clutches of eggs
150 during their development (Fig. 2C). The habitat, diet and spawning time, if known, are listed
151 by species in Table 2.

152 In this study we performed so far the most comprehensive comparison of the FA
153 composition in the muscle tissue of the Cottoidei fish of Lake Baikal. It was hypothesized that
154 the muscle FA composition may reflect the foraging ecology of the species *i.e.* supply of
155 different dietary FAs available in the habitats, but the composition may also be affected by
156 adaptive metabolic differences, *i.e.* the ability to endogenously synthesize low-melting FAs.
157 The metabolism of different FAs in fish tissues and the potential dietary sources of these FAs
158 are described in Table 3. The general view of cold-adapted ectothermic animals is that their
159 tissues contain high proportions of polyunsaturated FAs (PUFAs) in order to keep the lipid
160 membranes semifluid, which provides optimal microenvironment for membrane proteins to
161 function, and also to keep storage lipids fluid enough to be accessible for enzymes
162 hydrolyzing them for energy (Hazel 1995; Werbrouck et al. 2016). Thus we hypothesized that
163 if the true abyssal species during summer months (when they reside at the bottom and feed on
164 benthic items) have weak connection to the PUFA-rich food webs of the upper water, their
165 tissue lipids need to be maintained fluid by accumulating MUFAs, which can be dietary or
166 synthesized *de novo* by the fish. If so, how large proportion of such MUFAs are needed at the
167 bottom of Lake Baikal to compensate for the lack of PUFAs? Further if PUFAs are few, how
168 low their proportions can go in the tissues of the fish without risking essential biological
169 functions?

170 The species studied in this work had different habitat depths and represented a gradual
171 shift in their foraging ecology, from the fishes largely supported by the pelagic photic food
172 web to the fishes utilizing abyssal benthic food sources. This collection of fish studied
173 allowed us to examine whether the habitat depth is reflected in the FA biochemistry of the
174 fish living in this freshwater system with oceanic depths. If the benthic supply of PUFAs
175 relies largely on the food items of the upper water layers, we should be able to see a gradual
176 shift in tissue FA composition, from PUFA dominance in the benthopelagic species to MUFA
177 dominance in the true abyssal species. Alternatively, the particle rain from the photic water
178 layer and occasionally found carcasses or some invertebrates and vertebrates would provide
179 sufficient amounts of PUFAs to the abyssal species to maintain similar tissue FA composition
180 as found in the benthopelagic species. Finding a very large difference would mean that the
181 changes in duration of ice cover affecting the plankton bloom and vertical temperature
182 gradients limiting migration towards surface may alter the PUFA supply of the true abyssal
183 species, especially, and in future cause survival challenge for them.

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185
186

187 **Materials and methods**

188

189 **Field Sampling**

190

191 The 13 species of Cottoidei fish were collected in July-August, outside the reported
192 spawning season of the fish, from different locations of Lake Baikal by using either deep-sea
193 trawling or manned submersibles “Mir-1” and “Mir-2” (The International research expedition
194 “Mirs on Lake Baikal”, 2008-2010) (Table 1). Due to the tedious catching procedure, all the
195 individuals caught were included in this study (but a male *Batrachocottus nikolskii* still seen
196 to be guarding eggs was left unharmed). The order of the Federal Agency of Fisheries No 283
197 sets no restrictions for sampling the fish at Lake Baikal and thereby the sampling was
198 approved by the official authorities. The collected fish were stored at -25°C for less than 7
199 days prior to laboratory analyses.

200

201 **Sample derivatization**

202

203 In laboratory, weighed samples of white muscle (~30-40 mg, skin was removed and
204 subsamples of muscle were taken from both sides of the fish body and combined) were placed
205 into 15 ml glass tubes with 1 ml of anhydrous methanol containing 2 M HCl. The tubes were
206 filled with argon, then securely closed, and heated for 2 h at 90°C for complete methanolysis
207 (Meier et al. 2006). After cooling to room temperature, the tubes were opened and the
208 methanol was evaporated down to about 0.5 ml under a stream of nitrogen, and 0.5 ml
209 distilled water was added to reduce the solubility of the FA methyl esters (FAMES) formed,
210 which subsequently were extracted with 2 × 1 ml hexane.

211

212 **Fatty acid analysis**

213

214 One microliter of the combined hexane extracts were injected splitless (the split was
215 opened after 4 min) and chromatographed on a 25 m × 0.25 mm (i.d.) fused silica column
216 with polyethylene-glycol (PEG) as stationary phase with a thickness of 0.2 µm (CP-WAX
217 52CB Chrompack). Helium at 20 psi was used as the mobile phase. The column was mounted
218 in an Agilent 6890A gas chromatograph equipped with a mass-selective detector 5973N. The
219 oven was programmed as follows: 90°C for 4 min, 30°C min⁻¹ up to 165°C, then 3°C min⁻¹
220 up to 225°C, and kept isothermal at this final temperature for 10.5 min before cooling for the
221 next run.

222 The chromatographic peaks of the methyl ester derivatives were identified by retention
223 time and mass spectra (employing the spectra of the authentic standards and reference spectra
224 published in the library of Christie, <http://lipidlibrary.aocs.org/>). For quantification and
225 monitoring of the performance of the GC column, a standard mixture of 20 FAMES (GLC
226 reference standard 68D from Nu-Chek Prep, Elysian) was chromatographed for each tenth
227 sample. Empirical response factors relative to 18:0 were computed for the FAMES present in
228 known amounts in the standard mixture. The response factors for each of the FAMES not
229 present in the standard mixture were estimated by comparison with the standard FAMES
230 which resembled them most closely in terms of chain length and number of double bonds.
231 The areas of the FAME peaks were corrected with the response factors, and the relative
232 amount of each FA in a sample was expressed as a percent of the sum of all FAs in the
233 sample. These practices accounted for the differences in FID detector responses for different
234 FA structures (Ackman 1992) and additionally monitored for the potential random variation
235 in the detector responses of the equipment used. Altogether 36 FAs were detected, but many
236 were present in levels close to the detection limit, and therefore would contribute more noise
237 than real information to the data set. Thus, the comparisons of ecological groups of fish were
238 based on the 13 major FAs.

239

240 Statistical analyses

241

242 Principal component analysis (PCA) in the software package Sirius 8.5 (Pattern
243 Recognition Systems, Bergen, Norway) was used as multivariate statistical method to
244 describe the compositional relationships of the fishes. The FA data used as loadings were
245 arcsine transformed to improve normality, and subsequently standardized (deviations of each
246 variable equalized) to prevent the variables with the largest numbers from dominating the
247 analyses. Following the PCA, which described the compositional differences between the
248 fishes from the three ecological groups (benthic abyssal, benthic eurybathic and
249 benthopelagic), soft independent modeling of class analogy (SIMCA, available in Sirius 8.5)
250 was used for pair wise testing of the statistical significance of the separation between these
251 groups (Wold and Sjöström 1977) ($P < 0.05$ was regarded as significant). In addition, a
252 membership plot (available in Sirius 8.5) was created. This analysis indicated the probability
253 (0 – 1) each individual belonged to the PCA-based models of the three ecological groups
254 (weighting exponent 2 was used, details of the residual and distance calculations of this
255 analyses are found in Sirius 8.5). Univariate comparisons of the percentages of FA structural
256 categories (SFA, MUFA and PUFA), percentages of individual FAs, and specific FA ratios in

257 the fish ecological groups were performed with arcsine transformed data by using the
258 Kruskal–Wallis H test for multiple comparisons in which the significance levels were
259 adjusted by the Bonferroni correction to account for the inflation of type I error. Regression
260 analysis for arcsine transformed percentage data was used to study whether the MUFA/SFA
261 ratio (sum wt% of all MUFAs divided by sum wt% of all SFAs) of muscle lipids (which can
262 be adjusted by fish endogenously by desaturase enzyme) correlated with diet-derived PUFA
263 totals (*i.e.* exogenously acquired lipid fluidity). The regression plot however uses
264 untransformed data points to express in understandable way the different alternative FA
265 compositions meeting the criteria for functional tissue lipids of fish living at low temperature
266 and high pressure.

269 Results

271 The differences in the FA composition of the muscles of the studied ecological groups of fish
272 species were first visualized by PCA (Fig. 3a). The first principal component, PC1 explained
273 as much as 43% of the total variation in the data, and placed the benthic abyssal fishes with
274 high relative amounts of 16:1n-7, 18:1n-9, 18:2n-6, 14:0 and 18:1n-7 on the left side of the
275 plot. The benthic eurybathic and benthopelagic fishes tended to be located on the right with
276 their high relative amounts of 22:6n-3, 18:0, 16:0, 20:4n-6 and 20:5n-3. The PC2 axis (14%)
277 separated benthic eurybathic and benthopelagic fishes from each other. On this axis, the
278 muscle samples of the eurybathic fishes contained more 22:5n-6 and 20:4n-6, and those of the
279 benthopelagic fishes more 22:5n-3 and 22:6n-3 (Fig. 3a). Based on SIMCA, the fatty acid
280 compositions of the benthopelagic fishes differed statistically significantly ($P < 0.05$) from the
281 abyssal and eurybathic fishes (table insert of Fig. 3a). The FA compositions of the abyssal and
282 eurybathic groups of fish did not differ. Following the PCA, a membership plot was created,
283 which indicated the probability level each individual fish belonged to the PCA-based models
284 of the three ecological groups (Fig. 3b). A total of 58 individual samples per 62 showed a
285 probability (of being a member of its ecological group) higher than 0.5. However, one half of
286 the *Batrachocottus nikolskii* (1/2) and *Asprocottus abyssalis* (3/6) samples showed similar or
287 higher similarity with another model.

288 The benthic abyssal fishes had significantly lower relative SFA contents than the
289 benthic eurybathic or benthopelagic fishes (median 21% versus 25–26%, $H = 21.6$, $P < 0.001$)
290 (Fig. 4). The main individual SFA was 16:0, which was present in the abyssal species with a
291 median of 14% (Table 4) and species means ranging 12–14% (Table 5). The benthic

292 eurybathic and benthopelagic fishes contained slightly more of 16:0, their medians being 18%
293 and species means ranging 17–20% and 16–18%, respectively (Tables 4, 5). The relative
294 amounts of 18:0 were about 2% in the four benthic abyssal species (*Abyssocottus gibbosus*,
295 *Abyssocottus korotneffi*, *Cottinella bouleengeri* and *Batrachocottus nikolskii*) the habitat of
296 which reaches the depths of 1400–1600 m (Tables 4, 5). In the benthic eurybathic and
297 benthopelagic species, the relative amounts were higher, with medians 4% and large variation
298 (means 3–7%) among the species (Tables 4, 5). The benthic abyssal *Neocottus thermalis*,
299 living on hydrothermal field at the depth of only 400–480 m, also contained 4% of 18:0 (Table
300 5).

301 The benthic abyssal fishes contained significantly larger proportions of
302 monounsaturated FAs (MUFAs) than the benthic eurybathic and benthopelagic fishes (median
303 45% versus 23 and 14% in the eurybathic and benthopelagic fishes, respectively, $H = 31.0$, P
304 < 0.001) (Fig. 5a). The abyssal fishes contained 12% median level of 16:1n-7 while the
305 eurybathic and benthopelagic ones had only 4% (Table 4). In 18:1n-9, there was a decreasing
306 trend of the medians: 24, 13 and 6%, from the abyssal via eurybathic to benthopelagic fishes
307 (Table 4). When species means were compared, the variability in the levels of these two major
308 MUFAs was the largest in the eurybathic fish group (Table 5).

309 The major MUFAs of the fishes, 16:1n-7 and 18:1n-9 are products of $\Delta 9$ desaturase
310 enzyme, inserting the first double bond into the 9th carbon of 16:0 or 18:0, calculated from
311 carboxyl group (n-x calculated from methyl end). Thus calculating the ratios 16:1n-7/16:0 and
312 18:1n-9/18:0 gives indices, which describe the activity of this desaturation, which may have
313 happened in either the dietary organisms or in the tissues of the fish, or in both. For the
314 benthic abyssal fishes, the median of the ratio 16:1n-7/16:0 was 0.9 (wt% per wt%) while in
315 the benthic eurybathic and benthopelagic fishes it remained at 0.2 ($H = 24.3$, $P < 0.001$) (Fig.
316 5b). The ratio 18:1n-9/18:0 was 13 in the abyssal fishes but only 3 and 2 in the eurybathic and
317 benthopelagic species, respectively ($H = 33.7$, $P < 0.001$) (Fig. 5c).

318 The muscles of the benthic abyssal fishes contained significantly smaller proportions
319 of polyunsaturated FAs (PUFAs) than the benthic eurybathic and benthopelagic fishes
320 (median 32% versus 51% and 60% in the eurybathic and benthopelagic fishes, respectively, H
321 $= 28.5$, $P < 0.001$) (Fig. 6). This difference was largely due to the high proportions of 22:6n-3
322 in the eurybathic and benthopelagic fishes. In the abyssal fishes the median value of 22:6n-3
323 was only 7% while the eurybathic and benthopelagic fishes contained 19% in 33% (Table 4).
324 The abyssal species contained also less 20:4n-6 and 20:5n-3 than the eurybathic fishes (Table
325 4). The eurybathic species means of 20:4n-6 were consistently higher than in the species from
326 the other ecological groups (Table 5).

327 Next we studied the concept of PUFA levels regulating MUFA levels (Ntambi 1999)
328 in the muscle samples of the Cottoidei fishes. We tested this principle with our comparative
329 data by studying the regression of the PUFA totals and MUFA/SFA ratios (this ratio is
330 indicating whether 16:1n-7 and 18:1n-9 and other minor MUFAs had been produced from
331 their SFA precursors). Statistically significant negative correlation ($R^2 = 0.7996$, $P < 0.001$,
332 arcsine transformed data) was found (Fig. 7). The axis intersections of the fitted line ($y = -$
333 $0.0475x + 3.4367$, with no transformation) also gave suggestions for theoretical requirements
334 for the PUFA and MUFA contents in the muscle total lipids of these fish. The graph
335 suggested that if the muscle tissue contains approximately 70% PUFAs (x-axis intersection),
336 the remaining 30% can be comprised of SFAs with no need for MUFA production. In
337 addition, in the theoretical total absence of PUFA, the proportion of MUFAs should be more
338 than 3-fold the amount of SFAs (y-axis intersection ~ 3.5), thus meaning that about 75% of
339 MUFA and 25% SFA would be an alternative acceptable FA composition for the muscle total
340 lipids of these fish.

341 The benthic abyssal fishes not only contained significantly lower relative amounts of
342 PUFAs than the benthic eurybathic or benthopelagic fishes but the PUFAs present in their
343 tissues were structurally different. The abyssal fishes had low n-3PUFA/n-6PUFA (later n-
344 3/n-6) ratios (median 2.3), and these values showed an increasing trend via eurybathic (2.8) to
345 benthopelagic fishes (8.5), in which the n-3PUFAs clearly dominated (Fig. 8). The ratios of
346 the abyssal, eurybathic and benthopelagic fishes were all statistically significantly different (H
347 $= 34.4$, $P < 0.001$, in pair wise comparisons $P < 0.001$, except abyssal versus eurybathic $P =$
348 0.047).

350 Discussion

351
352 The multivariate analyses clearly indicated that the FA compositions in the muscle of the
353 Cottoidei fishes were associated with their assumed habitat and ecology (characterized in
354 Table 2). The abyssal fishes were rich in MUFAs, the eurybathic and benthopelagic fishes
355 contained high proportions of PUFAs, and the benthopelagic fishes were separated from the
356 eurybathic ones with their higher percentages of n-3 PUFAs. In addition, the membership
357 probabilities calculated for the individual samples suggested that the ecological groupings
358 were correct. Only two species out of the 13 studied ones were found to have strong
359 characteristics of two groups. In them, one half of the individuals belonged to the assumed
360 group and the other half to another group. For example the muscle FA profiles suggested that
361 the species *Asprocottus abyssalis* is a transitional form between the benthic abyssal and

362 benthic eurybathic groups (the two individuals of the species *Batrachocottus nikolskii* do not
363 allow making firm conclusions). It is of note that the muscle FA composition of *Cyphocottus*
364 species and *Cottomephorus grewinkii* could have been affected by their late spring
365 spawning (Table 2) presumably causing recent lipid catabolism and thus affecting muscle FA
366 composition in summer (Henderson et al. 1984). However the FA compositions of these
367 species were not exceptional in their ecological groups, which suggest that variation in
368 reproductive stage did not impair data interpretation.

369 The SFAs have high melting points and thus their concentrations are usually low in the
370 tissues of cold-water fish. In the Cottoidei fish of Lake Baikal the median percentages of
371 SFAs were 21% in the abyssal fishes and 25-26% in the eurybathic and benthopelagic fishes,
372 while temperate fish usually contain 35-42 % SFAs and in tropical fish the value can exceed
373 50% (Nair 1978; Tanakol et al. 1999). Inserting of the first double bond into a SFA
374 dramatically decreases the melting point of the FA and the lipids they are incorporated in
375 (Knothe and Dunn 2009). The responsible enzyme, $\Delta 9$ desaturase is ubiquitously expressed in
376 fish and all eukaryotic organisms and found activated in cold acclimation and adaptation
377 (Trueman et al. 2000 Castro et al. 2011). Thus a high proportion of MUFAs, such as found in
378 the benthic abyssal fish of this study (median 45%) meets the thermal requirements of the
379 habitat.

380 The high ratios of 16:1n-7/16:0 and 18:1n-9/18:0 in the benthic abyssal fish can in
381 large part be explained by the diet, *i.e.* temperature adaptation that has already occurred in the
382 lipids of dietary organisms, and if required the remaining temperature adaptation of tissue
383 lipid fluidity can be achieved by the $\Delta 9$ desaturation of SFAs in the tissues of the fish. The
384 benthic sediments provide the fish with bacteria, diatom-rich mass of sunk algae and
385 invertebrates, the lipids of which contain large percentages of both SFAs and MUFAs (Zink
386 et al. 2008; Kelly and Scheibling 2012; Shishlyannikov et al. 2014). Deep-sea bacteria are
387 known to exhibit large increases in MUFAs in response to elevated cultivation pressure
388 (Allen et al. 1999), which suggest that the deepest living bacterial mat may also contain
389 significant amounts of MUFAs. Baikal diatoms were reported to be rich in 16:1n-7, and their
390 ratios of 16:1/16:0 are comparable or even higher than those found in the muscle of abyssal
391 fish (Shishlyannikov et al. 2014). However, in the deep sea gammarids, mollusks and
392 crustaceans the ratio 16:1/16:0 is at highest about 0.5 and mostly much lower than in the
393 abyssal fish muscle (Morris 1984, Kozlova and Khotimchenko 1993, Dembitsky 1994a,b).
394 Instead of providing much 16:1n-7 (or any other 16:1 isomer), the deep sea gammarids and
395 mollusks contain very high proportion of 18:1n-9, comparable to the levels of the abyssal fish
396 muscle, and at least 3 times the levels found in the common crustaceans of Lake Baikal,

397 *Epishura baicalensis* and *Macrohectopus branickii* (Morris 1984, Kozlova and Khotimchenko
398 1993, Dembitsky 1994a,b). Thus a combined diet of diatom rich debris and gammarids or
399 other invertebrates could provide the abyssal fish with both high amounts of 16:1n-7 and
400 18:1n-9. Provided that the dietary supply of MUFA would not ensure proper tissue lipid
401 fluidity, then fish have the capacity to convert SFAs to MUFAs by the $\Delta 9$ -desaturase enzyme
402 (Trueman et al. 2000). Thus, we hypothesized that if the tissue PUFA content is low and the
403 MUFA/SFA ratio of the diet would not ensure proper membrane fluidity, the fish species
404 entering the deepest layers of the water column and being (due to the combined effect of low
405 temperature and high pressure) subject to the highest risk of solidifying cellular membranes,
406 are able to adjust their tissue lipid fluidity by converting SFAs to MUFAs. Thus this ratio
407 MUFA/SFA of their tissues would always be optimal.

408 Both PUFAs and MUFAs lower lipid melting point, and thus the proper fluidity of
409 tissue lipids can be obtained by adjusting their contents of MUFAs according to the supply of
410 dietary PUFAs. Apparently the main reasons for the MUFA dominance over PUFA in the
411 muscle of the Cottoidei species inhabiting the deepest layers of Lake Baikal is the lack of
412 PUFAs in the abyssal food web. There was a striking difference in the relative amounts of
413 MUFAs and PUFAs in the benthic abyssal fish versus the benthic eurybathic and
414 benthopelagic fishes (Fig. 5a, 6). In the abyssal fishes, MUFAs were present with 45% levels,
415 and thus they dominated over PUFAs. The MUFA levels of the benthic eurybathic and
416 benthopelagic fishes were one half or less of the abyssal values. Instead, the eurybathic and
417 benthopelagic fishes had very high PUFA levels, about 50-60%. The PUFA level of the
418 abyssal fishes was only about 30%. This suggests that the trophic links between the benthic
419 abyssal fish species and the organisms of the productive photic layers rich in plankton-derived
420 PUFAs are weak. Divergent muscle FA compositions have also been found among different
421 deep ocean fishes (Bakes et al. 1995; Lea et al. 2002; Økland et al. 2005; Drazen et al. 2009;
422 Penthybridge et al. 2010). The range reported in these previous studies for the total
423 proportions of MUFAs and PUFAs have varied between 20% and 65%. Even the relative
424 amounts of SFAs were found to vary between 15% and 40%. Thus different deep-sea fish
425 appear to tolerate a very different tissue FA composition, and low water temperature or the
426 high pressure as such do not necessitate replacing PUFAs by MUFAs. In addition to
427 modifying FA structures, mechanical strength against pressure can be achieved by increasing
428 tissue cholesterol levels (Kato and Hayashi 1999). In the different benthic fish of Lake Baikal,
429 however, this variability in the relative MUFA and PUFA contents was related to the habitat
430 depth, which suggest very different dietary supplies.

431 With the ideal comparative data of Lake Baikal Cottoidei fishes, we studied the
432 concept of PUFA levels regulating the MUFA levels in deep sea fish. It has been proposed
433 that high dietary PUFA levels regulate gene transcription and inhibit the production of the $\Delta 9$ -
434 desaturase enzyme, which results in lowered rate of MUFA production from the SFA
435 precursors (Ntambi 1999). This feedback system would integrate the contributions from the
436 dietary PUFA supply and endogenous MUFA production to membrane fluidity and ensure
437 accurate viscosity homeostasis. Naturally this compositional regulation may largely happen in
438 the dietary items of the fish already, but the MUFA levels are likely further modulated in the
439 tissues of the fish. The FAS of total lipids studied originate partly from structural polar lipids
440 and partly from neutral storage lipids but both types of lipids need to be in the proper
441 semiviscous physical state for optimal functions and to be accessible for energy production
442 (Hazel 1995; Werbrouck et al. 2016). The statistically significant negative correlation found
443 between the muscle PUFA percentage and the ratio MUFA/SFA (indicating the functioning of
444 $\Delta 9$ desaturase) suggest that the supply of PUFAs determines the degree of MUFA production
445 (Fig. 7). In addition this analysis suggested that if the muscle tissue contains 70% PUFAs, the
446 remaining 30% can be comprised of SFAs, or alternatively about 75% MUFAs and 25%
447 SFAs would at least in theory be equally acceptable FA composition for the muscle total
448 lipids of the Cottoidei fish. However, the lowest PUFA level recorded (*i.e.* minimum
449 requirement) was 24% (Fig. 7), which clearly points out that, despite potentially sufficient for
450 membrane fluidity control (Hazel 1995), the MUFAs cannot qualify for all the biological
451 functions that require the 22:6n-3, usually plentiful in fish, or other highly unsaturated FAs
452 (Stillwell and Wassall 2003).

453 The n-3/n-6 ratios were very high for the benthopelagic fishes and low in the benthic
454 abyssal and eurybathic fishes. The ratios found in the abyssal and eurybathic fishes of Lake
455 Baikal were similar to those reported for shallow-water freshwater fish consuming benthic
456 prey (Ahlgren et al. 1994), and earlier we found very similar values for several fish species
457 inhabiting shallow waters of lake Baikal and having benthic influence in their diets, *i.e.* the
458 ide *Leuciscus idus*, roach *Rutilus rutilus* and silver carp *Carassius auratus* (1.4-2.3) (Grahl-
459 Nielsen et al. 2011). In the same way, the n-3/n-6 ratios of the benthopelagic species studied
460 in this work resembled the ratios of previously studied Baikal fish species utilizing pelagic
461 prey, such as whitefish *Coregonus baikalensis* and grayling *Thymallus baikalensis* (values
462 close to 7) (Grahl-Nielsen et al. 2011). The rich supply of n-3 PUFAs from the primary
463 producers of the photic water layer is a common global phenomenon in aquatic ecosystems
464 (Brett and Müller-Navarra 1997), and also worldwide freshwater fish have in general smaller

465 n-3/n-6 ratios than pelagic Ocean fish (Ackman 1967; Steffens and Wirth 2005; Abouel-
466 Yazeed 2013).

467 To conclude, the muscles of the Cottoidei fishes of Lake Baikal showed a remarkable
468 flexibility in terms of FA composition. The abyssal fishes inhabiting the deepest layers of the
469 lake had clear MUFA dominance and the eurybathic and benthopelagic species had PUFA
470 dominance. In addition, the values for n-3/n-6 ratio decreased with increasing water depth,
471 which may reflect weak trophic links between the abyssal fish species and the primary
472 producers of the photic water layer rich n-3PUFA. Ahlgren et al. (2009) have summarized
473 data of temporal and tropical freshwater fishes from shallow waters and concluded that the
474 PUFA content and pattern in herbivorous-omnivorous fish are mainly controlled by the
475 supply and quality of food, whereas in carnivorous fish species the tissue PUFA pattern goes
476 through more extensive structural modifications. Our data of deep water freshwater fish of
477 Lake Baikal showed that the main determinant of their muscle fatty acid composition was
478 habitat depth, possibly involving more restricted choices of dietary items in the benthic
479 abyssal habitats of the lake than in the benthopelagic habitat supporting PUFA-rich dietary
480 items. The fact that the divergent MUFA/SFA ratio inversely correlated with the divergent
481 relative amount of PUFAs in the muscles of the Cottoidei fish suggests efficient viscosity
482 control integrating the MUFA and PUFA contents in the tissue.

483 The current work encourages conducting further studies to understand the role of
484 lipids and FAs in the biology of the Cottoidei fishes. Controlled feeding experiments with
485 abyssal species are not feasible, but detailed mass spectrometric studies on the molecular
486 structures of individual lipid species in each lipid class could further clarify the restrictions
487 and possibilities for the incorporation of different FAs into the tissues of fish species living at
488 extreme depths. Our work suggest that the true abyssal fishes of Lake Baikal required a 24%
489 minimum level of PUFAs in their lipids. Thus, it is worth considering whether the expected
490 environmental changes could cause such alterations in food web structure or accessibility of
491 PUFA-rich food items for the fish that such minimum PUFA requirement would not be filled
492 in the future. It is of note that the current study was conducted by sampling the fishes during
493 summer, and therefore the potential seasonal changes in the foraging ecology, physiology and
494 biochemistry of these deep living fishes remain to be studied further.

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660 Table 1. Examined fish species of the Cottidae/Cottocomephorinae and Abyssocottidae
 661 families, information on their collection, and species ecology.

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	Species	Catch location and time	Catch method*	Catch depth, m	Species ecology
Cottidae/ Cottocomephorinae	<i>Batrachocottus multiradiatus</i> (N = 2)	Frolikha bay, Aug 2009	m	409	benthic, eurybathic
	<i>Batrachocottus nikolskii</i> (N = 2)	Frolikha bay, Aug 2009	m	424	benthic, abyssal
	<i>Cottocomephorus grewingkii</i> (N = 4)	Selenga river delta, July 2010	t	200	benthopelagic
	<i>Cottocomephorus inermis</i> (N = 8)	Northern Baikal, July 2010	t	not known	benthopelagic
Abyssocottidae	<i>Abyssocottus gibbosus</i> (N = 6)	Izhimei, July 2009	t, m	1597	benthic, abyssal
	<i>Abyssocottus korotneffi</i> (N = 4)	mud volcano “Saint Petersburg”, July 2009	t, m	1396	benthic, abyssal
	<i>Asprocottus abyssalis</i> (N = 6)	Location not known, July 2010	t	not known	benthic, eurybathic
	<i>Cottinella boulengeri</i> (N = 2)	Northern Baikal, July 2010	t	690	benthic, abyssal
	<i>Cyphocottus</i> species (N = 8)	Northern Baikal, July 2010	t	not known	benthic, eurybathic
	<i>Limnocottus bergianus</i> (N = 2)	Frolikha bay, Aug 2009	m	400	benthic, eurybathic
	<i>Limnocottus griseus</i> (N = 8)	Chivyrkuisky bay, July 2010	t	400	benthic, eurybathic
	<i>Limnocottus pallidus</i> (N = 8)	Chivyrkuisky bay, July 2010	t	400	benthic, eurybathic
	<i>Neocottus thermalis</i> (N = 2)	Frolikha bay, Aug 2009	m	409	benthic, abyssal

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*t = deep-sea trawling; m = manned submersibles (Mir-1 or Mir-2)

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Table 2. Habitat, diet and spawning time of the examined fish species of the Cottidae/Cottocomephorinae and Abyssocottidae families arranged by species ecology.

Species ecology	Species	Habitat depth (m)	Preferred ground	Diet	Spawning time
benthic, abyssal	<i>Abyssocottus gibbosus</i>	400-1600	silty, silty-stony	bottom gammarids	Jan-Feb
	<i>Abyssocottus korotneffi</i>	200-1600	muddy	bottom gammarids	winter
	<i>Cottinella boulegeri</i>	400-1600	silty-calcareous	60% phytoplankton debris, 40% gammarids and other animals	late autumn-early winter
	<i>Batrachocottus nikolskii</i>	100-1400	silty-stony	golomyanka, also invertebrates	Apr-May
	<i>Neocottus thermalis</i>	400-480	hydrothermal field; silt, bacterial and debris mat	amphipods in bacterial mat	no record
benthic, eurybathic	<i>Asprocottus abyssalis</i>	150-1400	muddy	small gammarids	Sept-Feb
	<i>Limnocottus griseus</i>	200-1300	silty-stony	mollusks and gammarids	late autumn-early winter
	<i>Limnocottus bergianus</i>	100-1000	muddy	amphipods, including <i>Brachyuropus grewingkii</i>	spring
	<i>Limnocottus pallidus</i>	100-1000	muddy, silty, sandy	90% gammarids, 5% young Cottoidei fish	Feb-Mar
	<i>Batrachocottus multiradiatus</i>	50-900	clay	amphipods	Mar-May
	<i>Cyphocottus</i> species	30-600	muddy-stony	bottom gammarids, pelagic amphipods, oligochaetes and fish	Mar-June
benthopelagic	<i>Cottocomephorus inermis</i>	20-1200	silty, silty-sandy (part of the day in water column)	planktonic animals; <i>Macrohectopus branickii</i> , <i>Epishura baikalensis</i> , young <i>Cottocomephorus</i>	Feb
	<i>Cottocomephorus grewingkii</i>	0.5-400	silty, sandy, sandy-stony (part of the day in water column)	75% planktonic, especially <i>Epishura baicalensis</i> , and 25% benthic animals	Jan-Mar, May-June

Information from Taliev 1955; Sideleva and Fialkov (2015)

Table 3. The metabolic origin of the main FAs found in the muscle tissue of the Lake Baikal fish and their potential dietary sources

	Metabolism in fish	Potential dietary source
14:0	Main product of <i>de novo</i> synthesis 16:0 can be chain shortened to 14:0	Proteobacteria, diatoms (Kelly and Scheibling 2012); abundant in Baikal sediment bacteria (Zink et al. 2008) and diatoms (Shishlyannikov et al 2014).
16:0	Main product of fatty acid <i>de novo</i> synthesis	Ubiquitously in prokaryotes and eukaryotes
16:1n-9	<i>De novo</i> synthesized 16:0 can be elongated to 18:0, and then desaturated to 18:1n-9 and subsequently chain shortened to 16:1n-9 (the latter step is increasing lipid fluidity)	Moderate levels in Baikal deep-sea mollusks and gammarids (Dembitsky et al. 1994a,b)
16:1n-7	<i>De novo</i> synthesized 16:0 can be desaturated to 16:1n-7, which increases lipid fluidity	Diatoms, bacteria (Kelly and Scheibling 2012); abundant in Baikal diatoms (Shishlyannikov et al 2014) and sediment bacteria (Zink et al. 2008), and moderate levels in the deep-sea gammarids (Morris 1984).
18:0	<i>De novo</i> synthesized 16:0 can be elongated to 18:0, which decreases lipid fluidity	Various animal sources
18:1n-9	<i>De novo</i> synthesized 16:0 is elongated to 18:0, which is desaturated to 18:1n-9	Various sources; brown macroalgae, deep sea crustaceans and fishes (Kelly and Scheibling 2012); abundant in Baikal golomyanka <i>Comephorus baicalensis</i> and deep-sea gammarids (Morris 1984)
18:1n-7	<i>De novo</i> synthesized 16:0 is desaturated to 16:1n-7, which is elongated to 18:1n-7	Bacterial marker, which is also found in most animals but with a ratio 18:1n-7/18:1n-9 < 1 (Kelly and Scheibling 2012); abundant in Baikal deep-sea gammarid, <i>Brachyuropus grewingkii</i> (Dembitsky et al. 1994a).
18:2n-6	Fish do not <i>de novo</i> synthesize	Vascular plants, green macroalgae (Kelly and Scheibling 2012); moderate levels in Baikal deep-sea mollusks and gammarids (Dembitsky et al. 1994a,b).
20:4n-6	Fish do not <i>de novo</i> synthesize but can convert dietary 18:2n-6 to 20:4n-6	Protozoa, microeukaryotes, red algae (Kelly and Scheibling 2012); based on fish studies of Lake Baikal and North Atlantic, elevated level of 20:4n-6 and other C20-22 n-6 PUFAs was linked to benthic ecology (Grahnl-Nielsen et al 2011, Käkälä et al 2005).
20:5n-3	Fish do not <i>de novo</i> synthesize but can convert dietary n-3 precursors to 20:5n-3	Diatoms, brown and red macroalgae (Kelly and Scheibling 2012); abundant in Baikal diatoms (Shishlyannikov et al 2014), and crustacea <i>Epishura baicalensis</i> and <i>Macrohectopus branickii</i> (Kozlova and Khotimchenko 1993) but low in the deep-sea gammarids and mollusks (Dembitsky et al. 1994a,b)
22:5n-6	Fish do not <i>de novo</i> synthesize but can convert 18:2n-6 or 20:4n-6 to 22:5n-6	No specific abundant source but can be produced in fish from the other n-6 PUFAs
22:5n-3	Fish do not <i>de novo</i> synthesize but can elongate 20:5n-3 to 22:5n-3	No specific abundant source but can be produced in fish from the other n-3 PUFAs
22:6n-3	Fish do not <i>de novo</i> synthesize but can convert 20:5n-3 or 22:5n-3 to 22:6n-3	Dinoflagellates, pelagic zooplankton, pelagic fish (Kelly and Scheibling 2012); abundant in Baikal crustacea <i>Epishura baicalensis</i> and <i>Macrohectopus branickii</i> (Kozlova and Khotimchenko 1993) but low in the diatoms (Shishlyannikov et al 2014) and deep-sea gammarids and mollusks (Dembitsky et al. 1994a,b)

Table 4. Main FAs (wt%, median \pm median absolute deviation), in the muscle tissue of the Lake Baikal fish calculated by ecological groups: benthic abyssal (AB), benthic eurybathic (EB) and benthopelagic (BP) fishes. To study statistical differences in the relative amounts of the fatty acids in the different groups, the Kruskal–Wallis H test (df = 2) for multiple pairwise comparisons was performed with the significance adjusted by the Bonferroni correction.

	benthic abyssal (N=16)	benthic eurybathic (N=34)	benthopelagic (N=12)	H	P	AB vs EB	AB vs BP	EB vs BP
14:0	4.1 \pm 1.2	1.4 \pm 0.7	2.0 \pm 0.9	13.3	P = 0.01	P = 0.001	NS	NS
16:0	14.3 \pm 1.6	17.7 \pm 1.4	18.4 \pm 1.6	23.2	P < 0.001	P < 0.001	P < 0.001	NS
16:1n-9	0.4 \pm 0.3	0.1 \pm 0.1	0.02 \pm 0.02	6.8	P = 0.033	NS	P = 0.027	NS
16:1n-7	11.6 \pm 1.3	4.1 \pm 2.2	3.9 \pm 2.2	19.7	P < 0.001	P = 0.001	P < 0.001	NS
18:0	2.0 \pm 0.7	4.1 \pm 0.7	3.7 \pm 0.4	17.2	P < 0.001	P < 0.001	NS	NS
18:1n-9	24.4 \pm 4.6	13.3 \pm 3.8	6.3 \pm 2.6	41.5	P < 0.001	P < 0.001	P < 0.001	P = 0.001
18:1n-7	4.0 \pm 0.8	3.4 \pm 0.8	4.2 \pm 1.1	4.4	NS	–	–	–
18:2n-6	4.2 \pm 1.4	1.7 \pm 0.6	1.0 \pm 0.2	16.0	P < 0.001	P = 0.024	P < 0.001	NS
20:4n-6	4.4 \pm 0.9	7.9 \pm 2.1	3.6 \pm 0.8	35.7	P < 0.001	P < 0.001	NS	P < 0.001
20:5n-3	9.7 \pm 1.1	14.3 \pm 2.3	12.2 \pm 1.3	16.0	P < 0.001	P < 0.001	NS	NS
22:5n-6	1.0 \pm 0.3	1.3 \pm 0.8	0.6 \pm 0.4	9.4	P = 0.009	NS	NS	P = 0.007
22:5n-3	1.3 \pm 0.7	2.1 \pm 0.6	2.4 \pm 0.5	7.7	P = 0.022	NS	P = 0.017	NS
22:6n-3	6.6 \pm 1.8	18.9 \pm 6.0	32.7 \pm 4.3	40.9	P < 0.001	P < 0.001	P < 0.001	P = 0.002

Table 5. Main FAs (wt%, mean \pm SD, only significant decimals shown) in the muscle tissue of the studied Lake Baikal fish calculated by species, which were arranged from benthic abyssal (on left) via benthic eurybathic to benthopelagic species (on right).

Species (sample number)	<i>Abyssocottus gibbosus</i> (N=6)	<i>Abyssocottus koromeffi</i> (N=4)	<i>Cottinella boulengeri</i> (N=2)	<i>Batrachocottus nikolskii</i> (N=2)	<i>Neocottus thermalis</i> (N=2)	<i>Asprocottus abyssalis</i> (N=6)	<i>Limnocottus griseus</i> (N=8)	<i>Limnocottus bergianus</i> (N=2)	<i>Limnocottus pallidus</i> (N=8)	<i>Batrachocottus multiradiatus</i> (N=2)	<i>Cyphocottus species</i> (N=8)	<i>Cottocomephorus inermis</i> (N=8)	<i>Cottocomephorus grewingkii</i> (N=4)
14:0	4 \pm 1	3 \pm 2	4.5	4.0	0.9	1 \pm 1	1.6 \pm 0.7	0.8	2 \pm 1	2.3	0.9 \pm 0.3	1.5 \pm 0.9	3 \pm 1
16:0	14 \pm 2	14 \pm 1	12.9	12.2	14.0	17 \pm 1	17 \pm 2	20.3	18 \pm 2	18	17 \pm 2	18 \pm 1	16 \pm 2
16:1n-9	0.13 \pm 0.02	3 \pm 2	0.3	0.5	6.7	4 \pm 2	0.9 \pm 0.4	0.4	0.2 \pm 0.2	1.4	0.04 \pm 0.02	0.09 \pm 0.02	0 \pm 0
16:1n-7	9 \pm 3	11 \pm 1	12.0	15	15.3	11 \pm 6	5 \pm 2	1.7	4 \pm 2	6.3	1.8 \pm 0.9	5 \pm 4	4 \pm 1
18:0	2 \pm 1	2 \pm 1	1.9	1.6	3.9	4 \pm 1	3.3 \pm 0.5	7.3	3.4 \pm 0.9	4.2	5.5 \pm 0.9	3.8 \pm 0.8	3.4 \pm 0.8
18:1n-9	26 \pm 5	24.3 \pm 0.7	31.8	22.5	15.5	17 \pm 3	13 \pm 3	11.1	16 \pm 3	14.6	9 \pm 1	8 \pm 1	3.1 \pm 0.9
18:1n-7	3.7 \pm 0.6	2.7 \pm 0.4	5.2	6.7	4.8	3.7 \pm 0.7	3 \pm 1	3.1	3 \pm 1	3.3	2 \pm 1	4 \pm 1	2.7 \pm 0.7
18:2n-6	4 \pm 1	2 \pm 2	5.0	4.2	1.5	1.7 \pm 0.7	2.0 \pm 0.7	0.8	2 \pm 1	2.6	1.1 \pm 0.2	1.0 \pm 0.2	1.5 \pm 0.6
20:4n-6	5 \pm 2	4 \pm 1	3.3	3.0	4.8	8 \pm 3	8 \pm 1	11.0	7 \pm 3	7.7	10 \pm 1	4 \pm 1	3.2 \pm 0.7
20:5n-3	11 \pm 2	10 \pm 1	8.2	6	9.4	10 \pm 3	20 \pm 5	9.6	14 \pm 2	12.7	14 \pm 2	11.2 \pm 0.8	15 \pm 2
22:5n-3	1.2 \pm 0.7	3 \pm 2	0.3	1.9	0.6	1.7 \pm 0.7	1 \pm 1	1.5	2.1 \pm 0.6	2.5	2 \pm 1	2.3 \pm 0.8	8 \pm 6
22:5n-6	0.9 \pm 0.4	0.8 \pm 0.2	0.4	0.8	1.4	1.0 \pm 0.9	1 \pm 1	0.9	1.1 \pm 0.8	1.7	1 \pm 1	0.5 \pm 0.3	0.2 \pm 0.1
22:6n-3	9 \pm 4	8 \pm 2	4.7	6	6.1	13 \pm 5	14 \pm 4	23.4	19 \pm 4	17.7	27 \pm 4	33 \pm 7	31 \pm 3

Figure Legend

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4 Fig. 1. Taxonomic relationships of Lake Baikal Cottoidei fish species according to V.G. Sideleva
5 (Sideleva, 1982; 2001). The classification according to D.N. Taliev is on the right side of the
6 graph (Taliev, 1955). The chart was redrawn from Kontula et al. (2003) and the species studied
7 here for FA composition are marked with bold text.
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13 Fig. 2. A,B) Abyssal fishes from the genus *Batrachocottus* on the bottom of Lake Baikal, C)
14 *Batrachocottus nikolskii* male is guarding clutch of eggs, D) Caught *Limnocottus griseus*. Photos
15 by Selmeg Bazarsadueva (A, B, D) and Oleg Khlystov (C).
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21 Fig. 3. A) PCA bilot plot (of scores and loadings) describing the compositional similarities and
22 differences between the ecological groups of the studied Cottoidei fishes. Results of the pair wise
23 SIMCA analyses testing the statistical significance (at $P < 0.05$ level) of the separations by PCA
24 are shown as a table insert. The samples of benthic abyssal species were circled with black
25 outline, samples of benthic eurybathic species with light grey outline, and those of benthopelagic
26 species with dark grey outline. B) Membership plot indicating the probability (0 – 1) each
27 sample belonged to the PCA-based models of the three ecological groups. Species abbreviations:
28 *Ag*, *Abyssocottus gibbosus*; *Ak*, *Abyssocottus korotneffi*; *Cb*, *Cottinella bouleengeri*; *Bn*,
29 *Batrachocottus nikolskii*; *Nt*, *Neocottus thermalis*; *Aa*, *Asprocottus abyssalis*; *Lg*, *Limnocottus*
30 *griseus*; *Lb*, *Limnocottus bergianus*; *Lp*, *Limnocottus pallidus*; *Bm*, *Batrachocottus*
31 *multiradiatus*; *C*, *Cyphocottus* species; *Ci*, *Cottocomephorus inermis*; *Cg*, *Cottocomephorus*
32 *grewingkii*.
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44 Fig. 4. Differences in the total proportions of saturated FAs (SFA, $H = 21.6$, $P < 0.001$) in the
45 muscle tissue of the benthic abyssal, benthic eurybathic and benthopelagic fishes indicated by
46 boxplots showing (from top), the maximum value, third quartile, median, first quartile and
47 minimum value (after the procedure removed a single outlier if detected). The values of
48 ecological groups marked with different letter (the group with largest median receiving “a”)
49 differed according to the Kruskal–Wallis H test ($df = 2$) of multiple comparisons using arcsine
50 transformed data and significance levels adjusted by the Bonferroni correction (all significances
51 marked by the different letters were $P < 0.001$). Similar plots for the individual species are shown
52 on right with no statistics (due to the low sample number in many species). Species abbreviations
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1 Fig. 5. Differences in the total proportions of monounsaturated FAs (MUFA, $H = 31.0$, $P <$
2 0.001), and ratios of B) 16:1n-7/16:0 ($H = 24.3$, $P < 0.001$), and C) 18:1n-9/18:0 ($H = 33.7$, $P <$
3 0.001) in the muscle tissue of the benthic abyssal, benthic eurybathic and benthopelagic fish
4 indicated by boxplots showing (from top), the maximum value, third quartile, median, first
5 quartile and minimum value (after the procedure removed a single outlier if detected). The
6 values of ecological groups with different letter (the group with largest median receiving “a”)
7 differed according to the Kruskal–Wallis H test ($df = 2$) of multiple comparisons using arcsine
8 transformed data and significance levels adjusted by the Bonferroni correction (all significances
9 marked by the different letters were $P < 0.001$, except for 18:1n-9/18:0, benthic eurybathic versus
10 benthopelagic, $P < 0.032$). Similar plots for the individual species are shown on right with no
11 statistics (due to the low sample number in many species). Species abbreviations as in Fig 3.
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22 Fig. 6. Differences in the total proportions of polyunsaturated FAs (PUFA, $H = 28.5$, $P < 0.001$)
23 in the muscle tissue of the benthic abyssal, benthic eurybathic and benthopelagic fish indicated
24 by boxplots showing (from top), the maximum value, third quartile, median, first quartile and
25 minimum value (after the procedure removed a single outlier if detected). The values of
26 ecological groups with different letter (the group with largest median receiving “a”) differed
27 according to the Kruskal–Wallis H test ($df = 2$) of multiple comparisons using arcsine
28 transformed data and significance levels adjusted by the Bonferroni correction (all significances
29 marked by the different letters were $P < 0.001$). Similar plots for the individual species are shown
30 on right with no statistics (due to the low sample number in many species). Species abbreviations
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42 Fig. 7. Regression analysis of the interdependence between the muscle polyunsaturated FA
43 (PUFA) totals and the MUFA/SFA ratio (integrating dietary supply of MUFAs and SFAs and
44 potential conversion of SFA to MUFA in the fish) among the individual muscle samples
45 showing a statistically significant negative correlation ($R^2 = 0.80$, $P < 0.001$ using arcsine
46 transformed data; $y = -0.0475X + 3.4367$ with untransformed data to allow easy interpretation;
47 see Results). The data points for the individuals of benthic abyssal fish species are marked with
48 black diamonds, the points of benthic eurybathic fishes with light grey diamonds and the points
49 of benthopelagic fishes with dark grey diamonds.
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Fig. 8. Differences in the ratio of n-3 PUFA total to n-6 PUFA total ($n-3/n-6$, $H = 34.4$, $P < 0.001$) in the muscle tissue of the benthic abyssal, benthic eurybathic and benthopelagic fish indicated by boxplots showing (from top), the maximum value, third quartile, median, first quartile and minimum value (after the procedure removed a single outlier if detected). The values of ecological groups with different letter (the group with largest median receiving “a”) differed according to the Kruskal–Wallis H test ($df = 2$) of multiple comparisons using arcsine transformed data and significance levels adjusted by the Bonferroni correction (all significances marked by the different letters were $P < 0.001$, except for benthic abyssal versus benthic eurybathic, $P < 0.047$). Similar plots for the individual species are shown on right with no statistics (due to the low sample number in many species). Species abbreviations as in Fig 3.

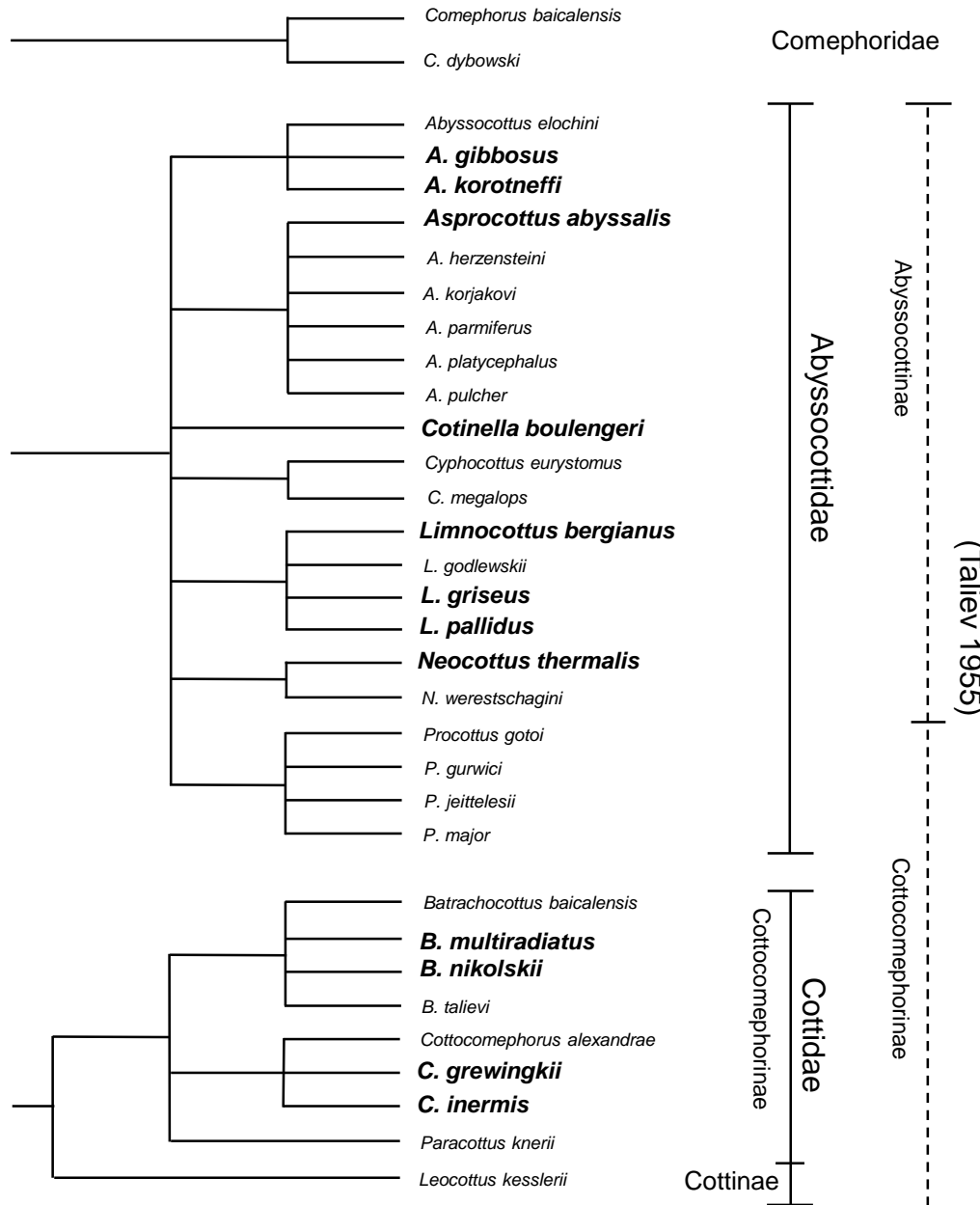


Fig. 1

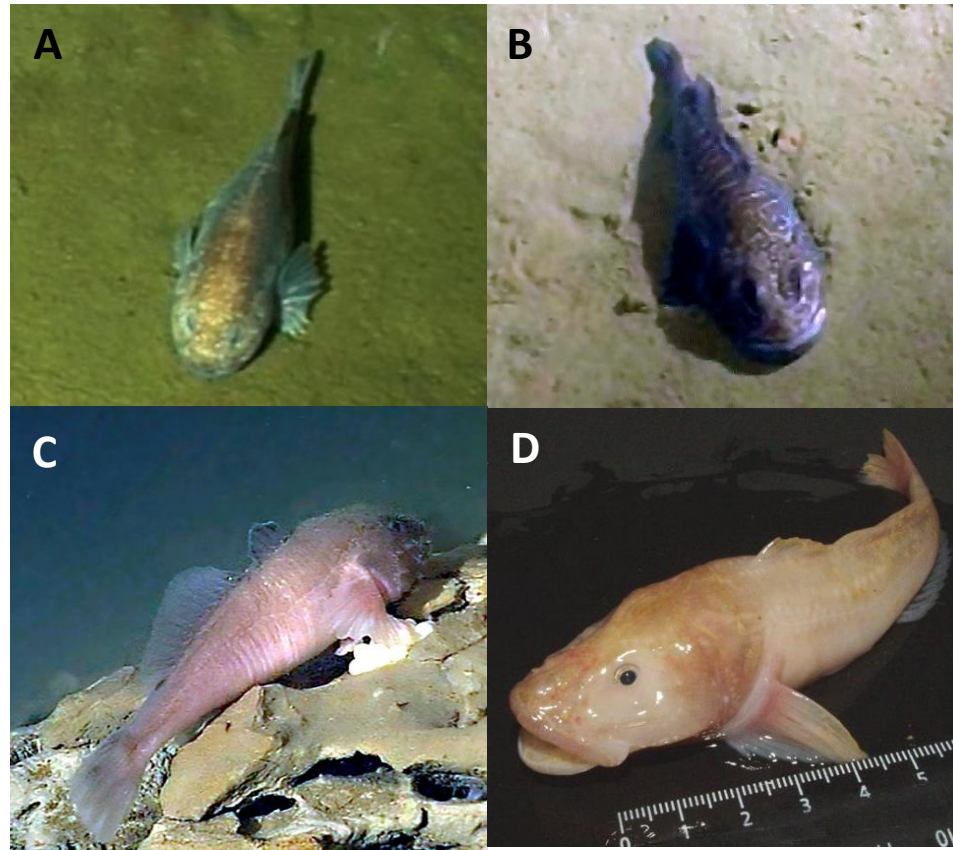


Fig. 2

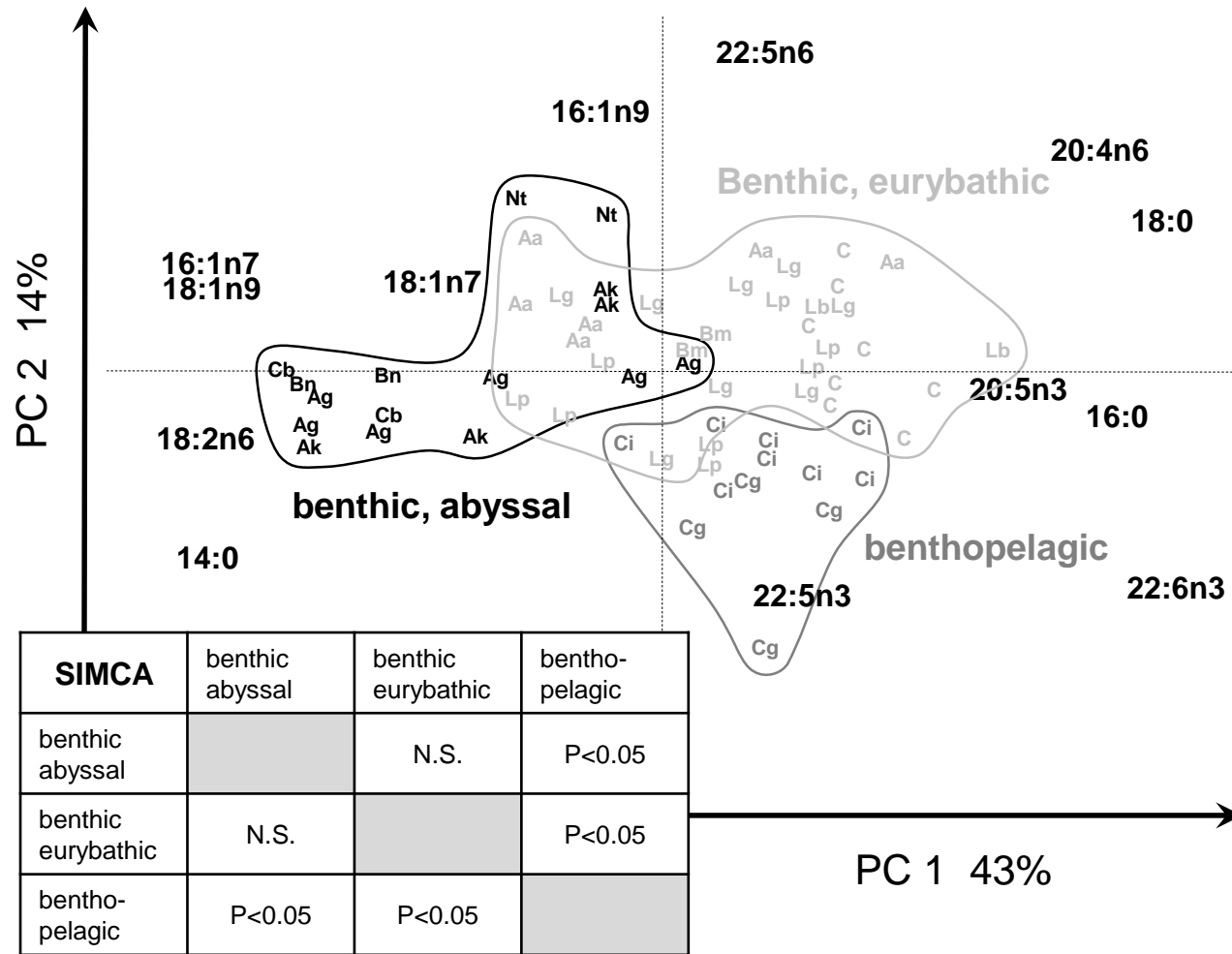


Fig. 3A

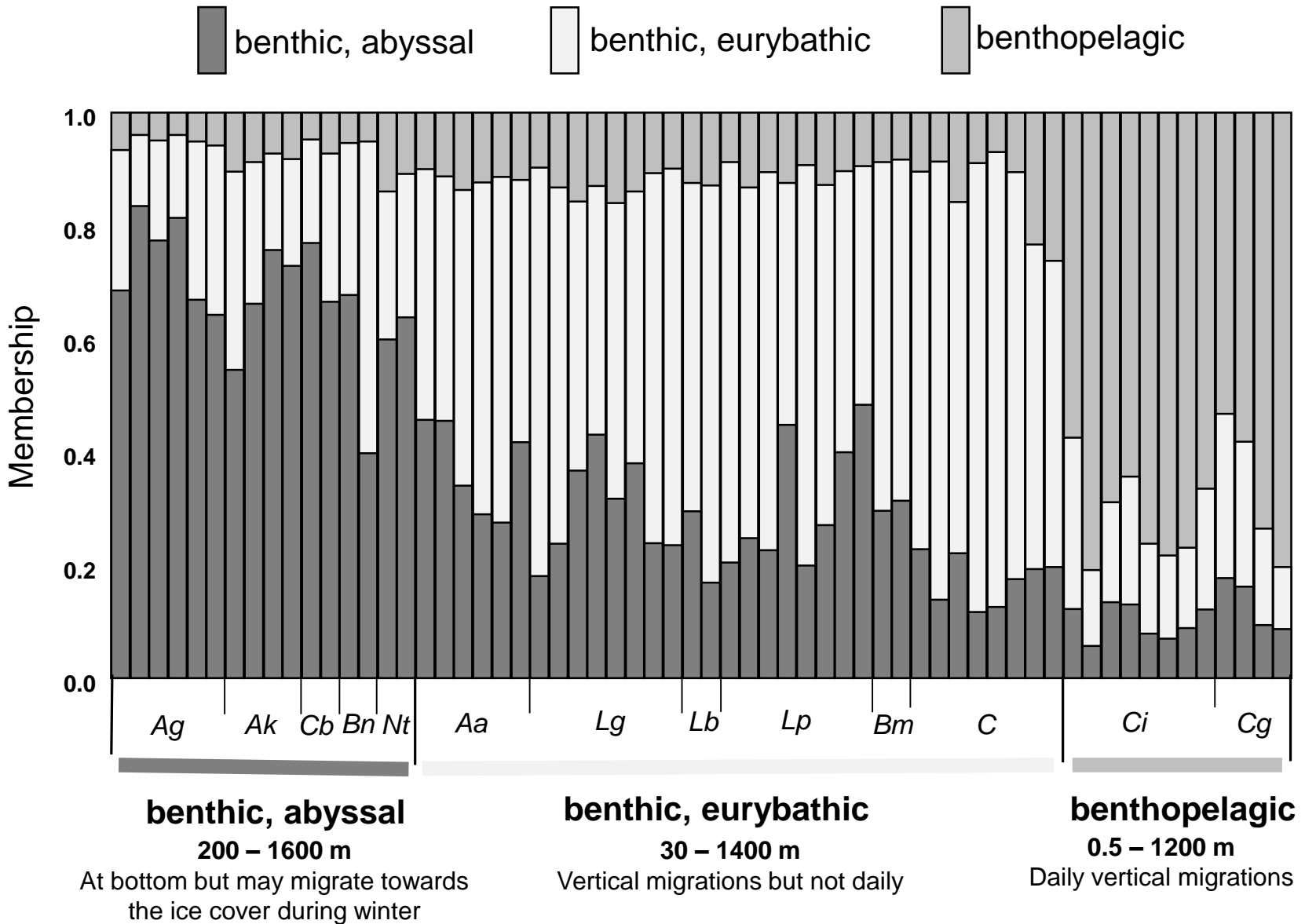


Fig. 3B

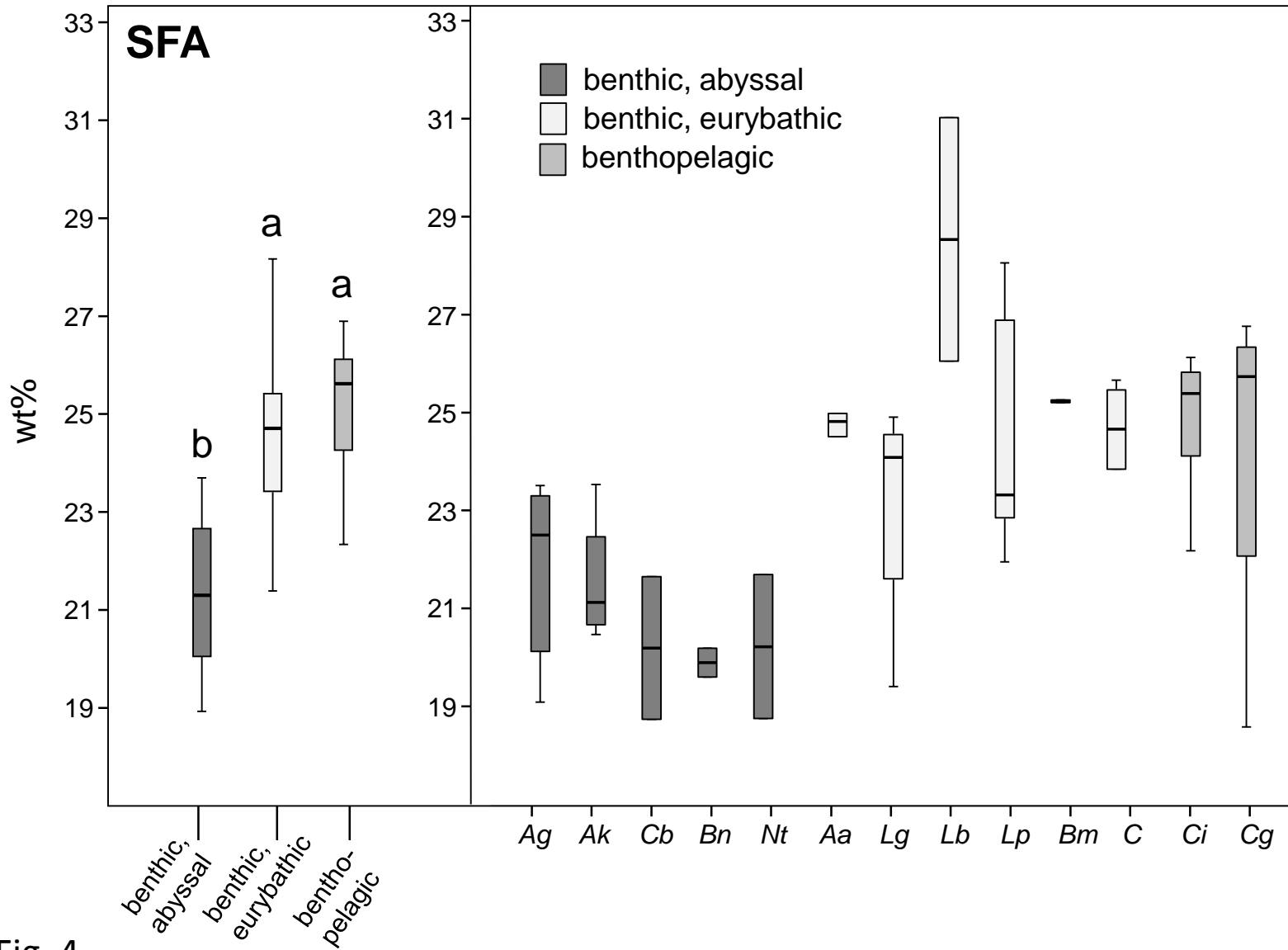
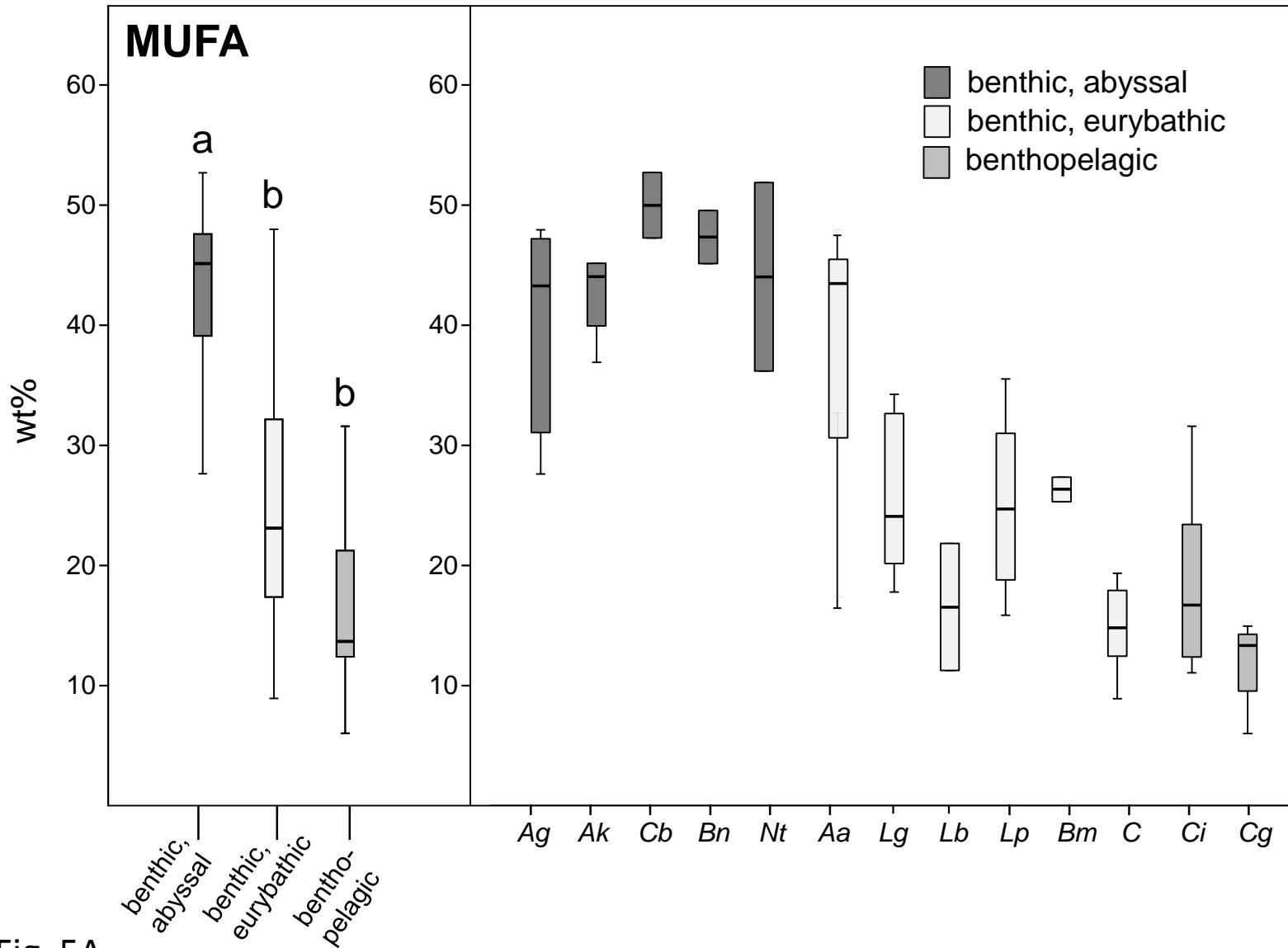


Fig. 4



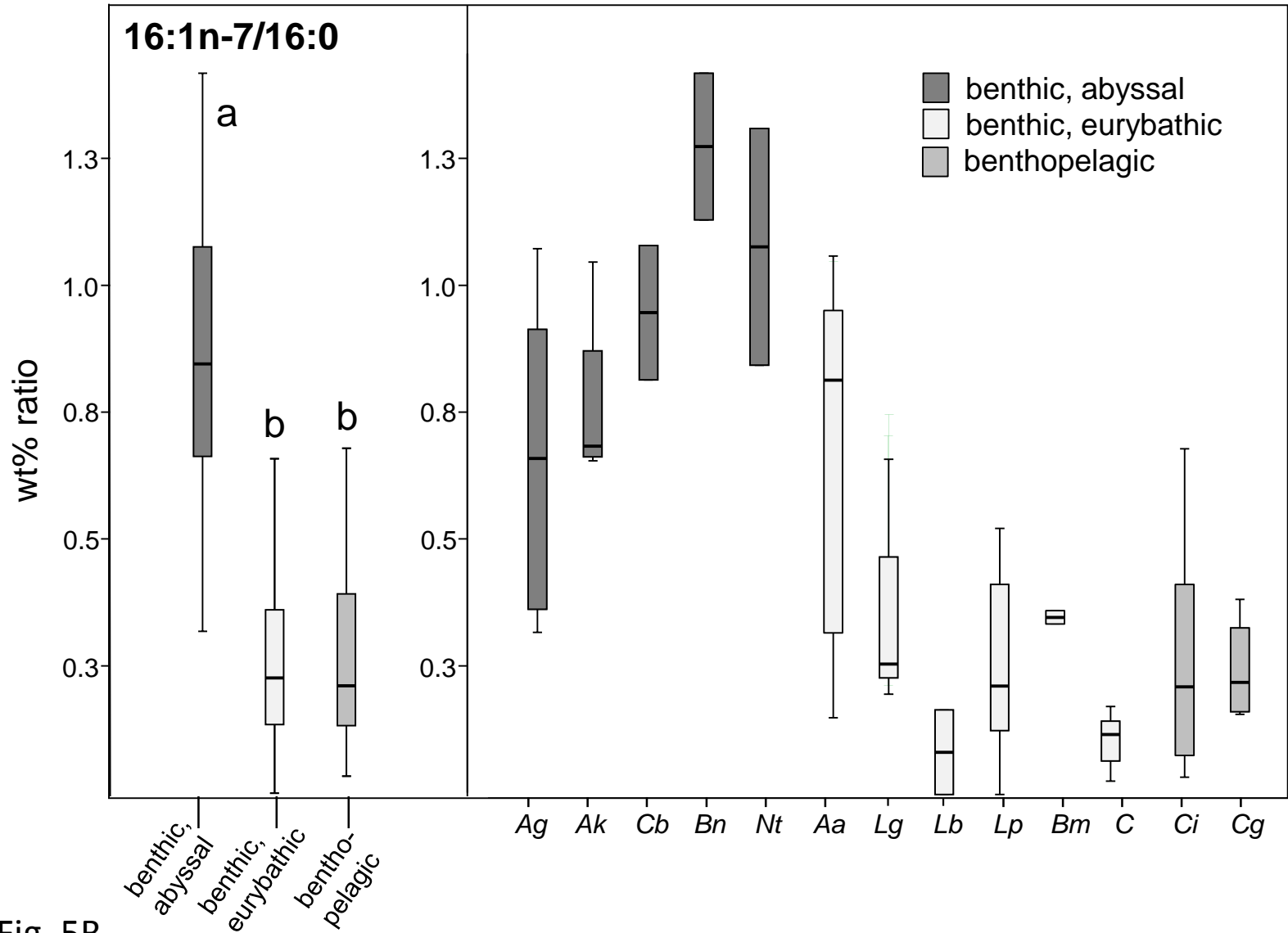


Fig. 5B

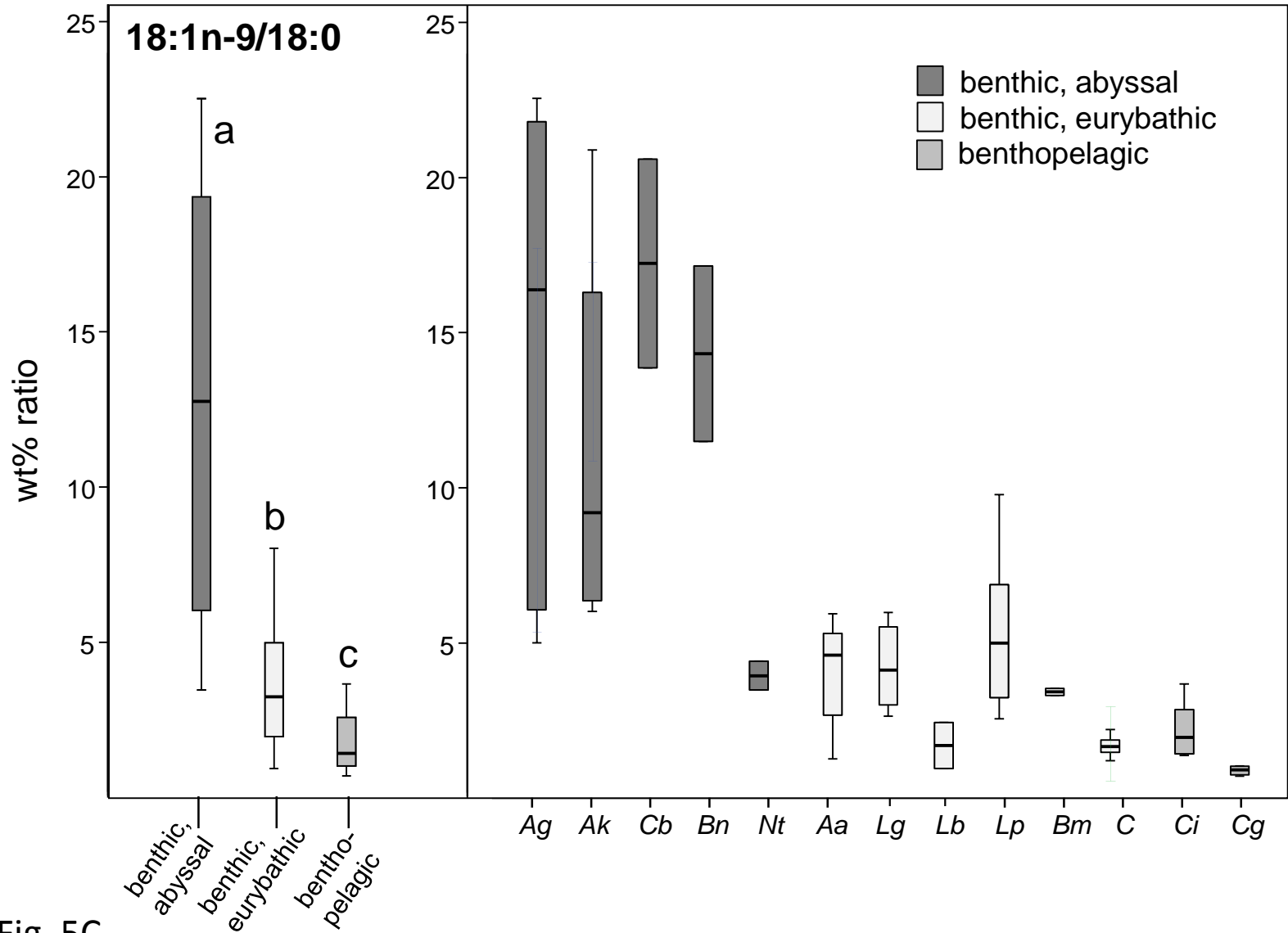


Fig. 5C

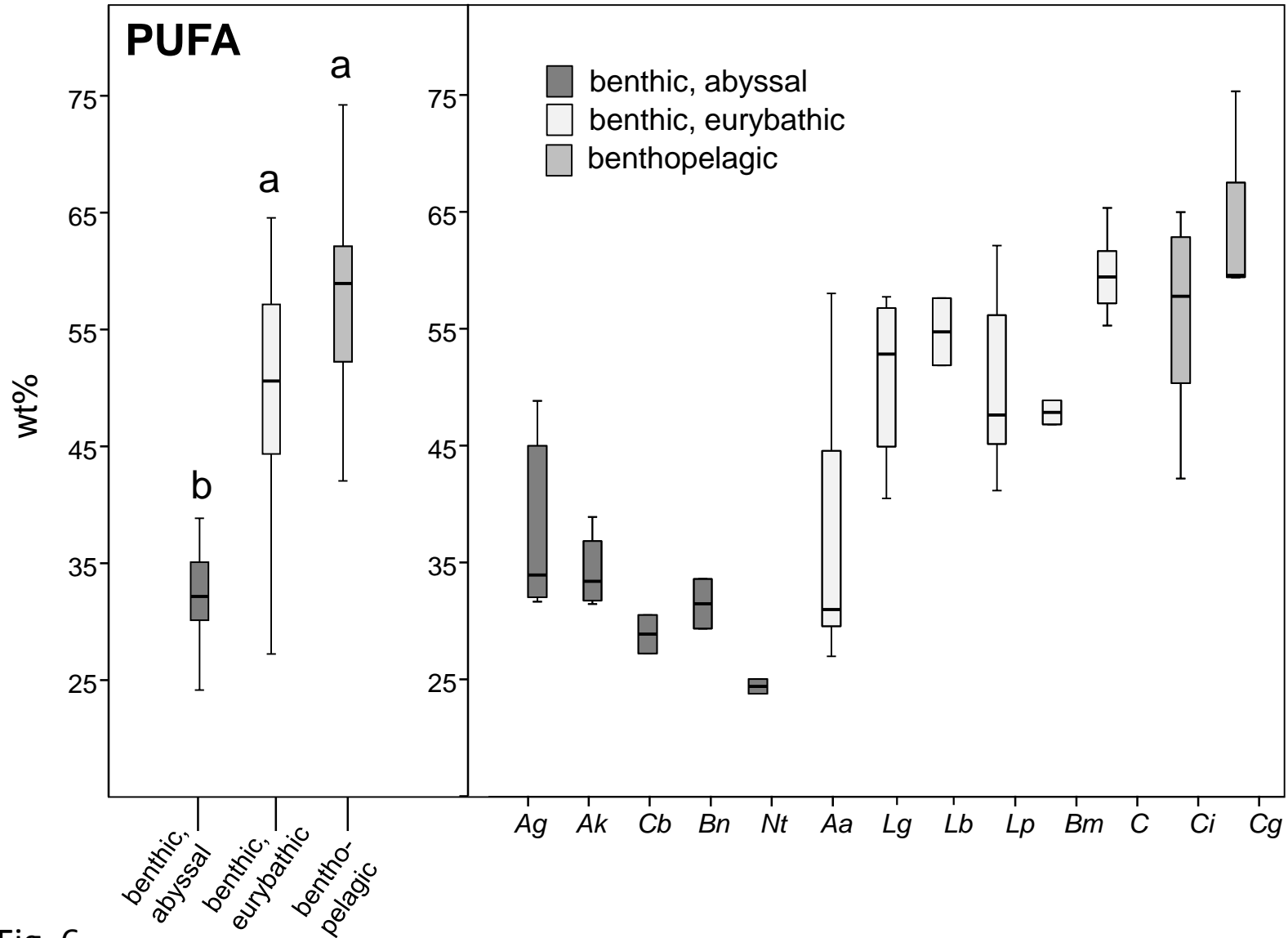


Fig. 6

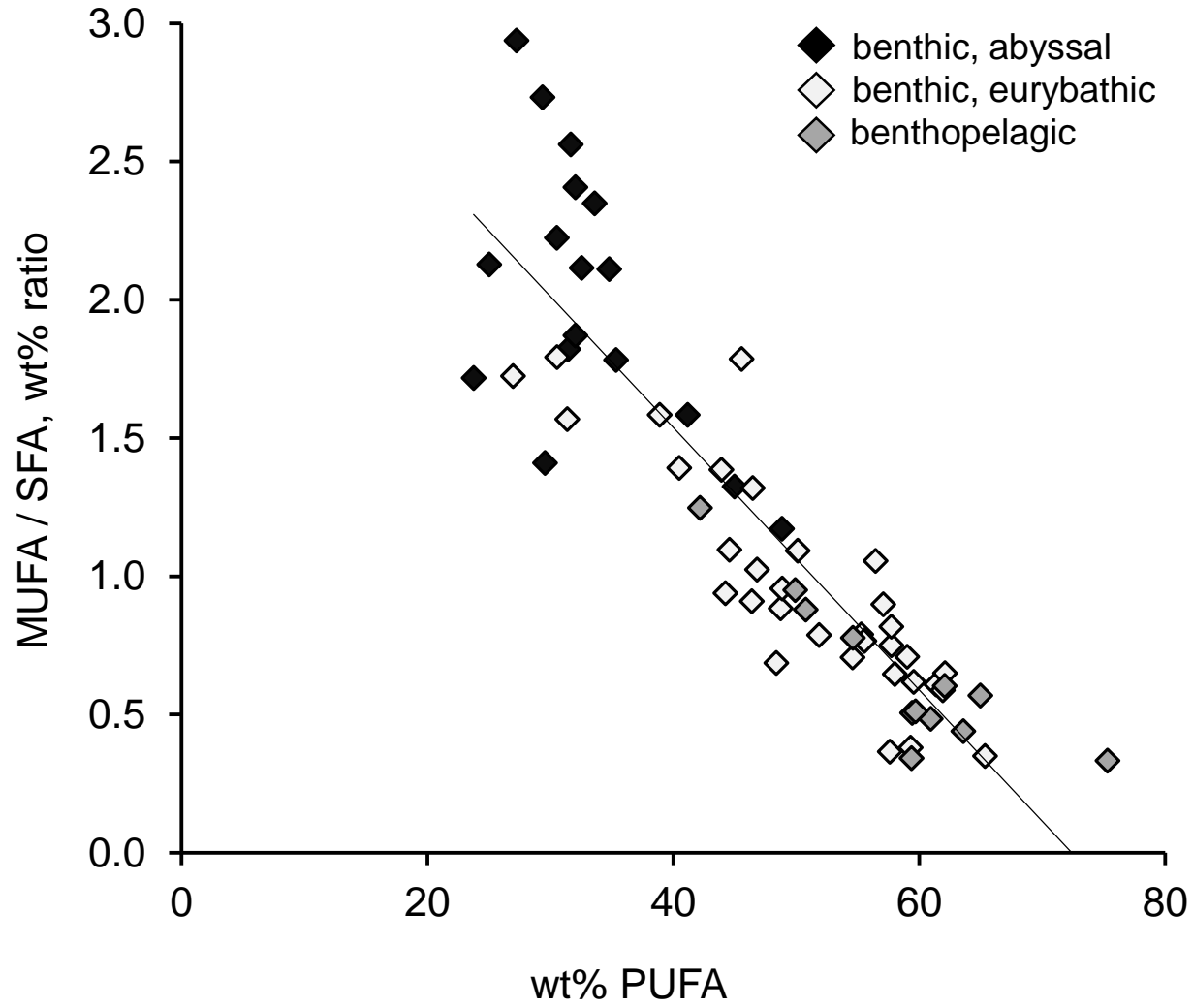


Fig. 7

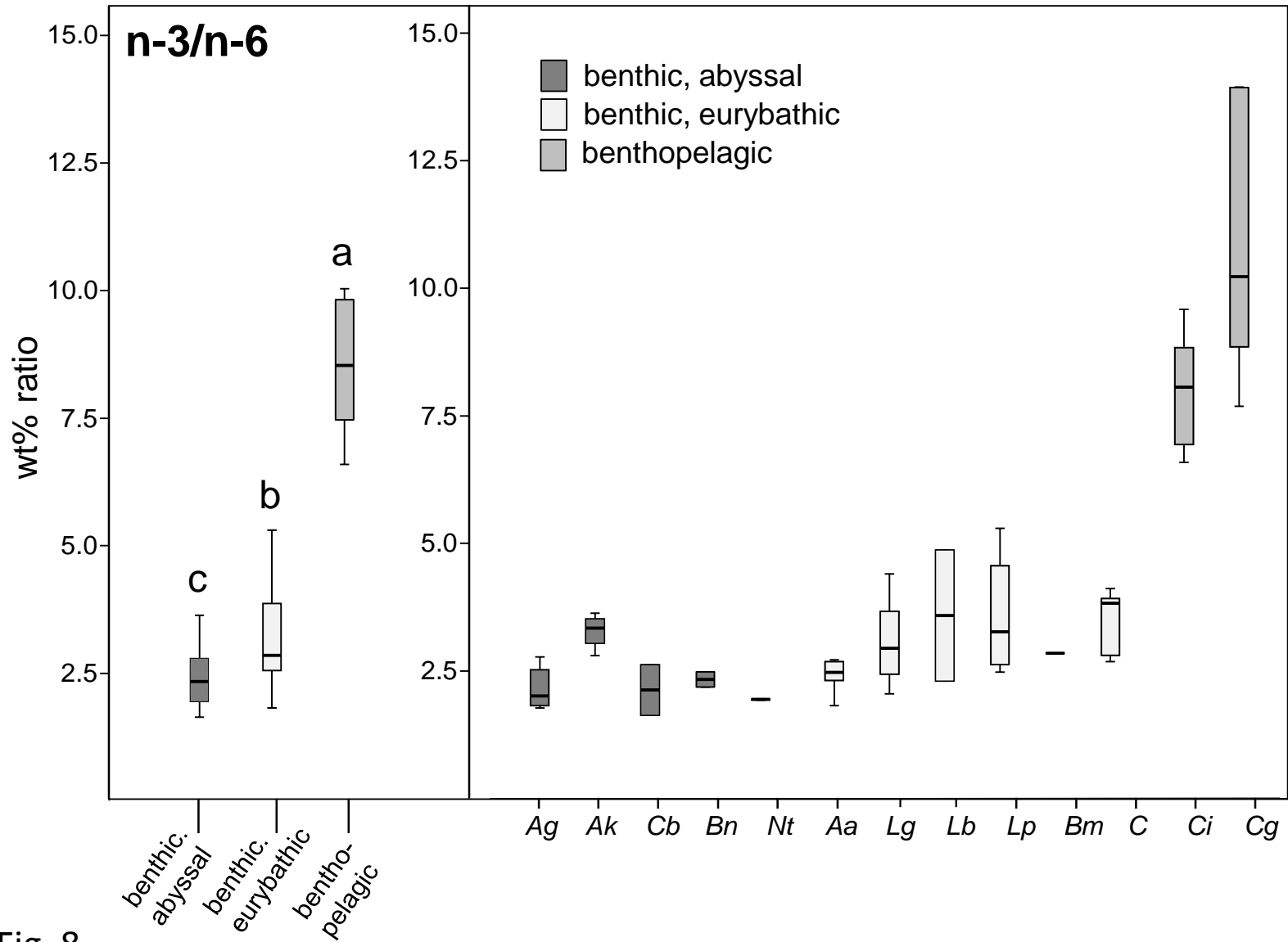


Fig. 8



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