

1 Waterbugs (Heteroptera: Nepomorpha, **Gerromorpha**) as sources of essential n-3
2 polyunsaturated fatty acids in Central Siberian ecoregions

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23 Summary

- 24 1. Aquatic systems are considered a main source of essential long-chain n-3 polyunsaturated
25 fatty acids (PUFA), which are preferentially synthesized by microalgae and transferred
26 along food chains to terrestrial consumers. Emerging aquatic insects comprise a
27 significant part of this transfer of the essential PUFA from water to land. Quantitative
28 data on PUFA content and composition are available mainly for rheophilic insects while
29 taxa that are characteristic of wetlands and stagnant water bodies, such as aquatic
30 Heteroptera, remain relatively unstudied.
- 31 2. We investigated the role of various waterbug taxa (Heteroptera: Nepomorpha) inhabiting
32 different ecoregions in temperate Central Siberia (Russia) as potential sources of PUFA.
33 The ecoregions were steppe, forest-steppe, hemiboreal forest and montane coniferous
34 forest. Although these waterbug insects are aquatic in both larval and adult stages, they
35 can disperse through the landscape and transfer essential PUFAs from water to land so
36 making them potentially available to terrestrial consumers.
- 37 3. Species of Naucoridae, Notonectidae and Corixidae were generally dominant in all
38 ecoregions, attaining maximum biomass in the steppe. We showed that habitat or
39 ecoregion played a major role in the variability of fatty acid composition of Notonectidae
40 and Gerridae but not Corixidae. In contrast, the biochemical composition of the only
41 naucoridae, *Ilyocoris cimicoides*, was largely affected by life stage.
- 42 4. Both the dominant families and species within them differed with respect to their mass-
43 specific contents of essential long-chain PUFA of the n-3 family, namely
44 eicosapentaenoic and docosahexaenoic acids. Corixid species had the highest content of
45 these two essential PUFAs among the waterbug studies, and relative to literature reports
46 for other aquatic insects. Corixids thus appear to be a potentially important vector for
47 transfer of the essential biochemical compounds from water to land, especially in steppe
48 ecoregions with numerous ephemeral water bodies.
49

50 **Introduction**

51

52 The magnitude and importance of resource transfers across the interface between land and water
53 is increasingly recognized (e.g., Baxter, Fausch & Saunders, 2005; Richardson, Zhang &
54 Marczak, 2010). Adult aquatic insects flying from streams and lakes to adjacent terrestrial
55 systems represent the most prominent flux of organic matter and nutrients originating from fresh
56 waters (Richardson *et al.*, 2010, but see Schriever, Cadotte & Williams, 2014). These aquatic
57 insects are important food sources to a wide range of riparian predators, such as arthropods, birds,
58 bats and amphibians (Sabo & Power, 2002; Iwata *et al.*, 2010; Reimer, Baerwald & Barglay,
59 2010; Stenroth *et al.*, 2015). Moreover, subsidies provided by aquatic insects could influence
60 recipient food webs, indirectly affecting predators, decomposers, and even herbivores and plants
61 (Schriever *et al.*, 2014).

62 Although fluxes from water to land have generally been reported to be much less than
63 reciprocal (land to water) fluxes, responses of recipient ecosystems are largely governed by the
64 quality of the subsidies (Bartels *et al.*, 2012). Marcarelli *et al.* (2011) argued that low-flux, high-
65 quality subsidies may disproportionately affect food webs and ecosystem processes (e.g.,
66 secondary production and trophic cascades). Water to land fluxes of emerging aquatic insects are
67 of high nutritional quality for terrestrial consumers, due to high concentrations of nutrients
68 (nitrogen and phosphorus) they contain (Bartels *et al.*, 2012). However, along with inorganic
69 nutrients, emerging insects may also contain essential polyunsaturated fatty acids (PUFA) that
70 can limiting the growth and development of terrestrial consumers (Tocher, 2003; Gladyshev,
71 Sushchik & Makhutova, 2013).

72 Long-chain PUFAs of the n-3 family, namely eicosapentaenoic acid (20:5n-3, EPA) and
73 docosahexaenoic acid (22:6n-3, DHA), are essential for many omnivorous consumers, including
74 humans, as they cannot be effectively synthesized *de novo* but are required for physiological
75 functioning (Lands, 2009). These PUFA are synthesized by algae, such as diatoms, cryptophytes,

76 euglenoids and dinoflagellates, and transferred through food webs and accumulated in biomass
77 of aquatic consumers (Gladyshev *et al.*, 2013; Hixson *et al.*, 2015; Twining *et al.*, 2015), with
78 the result that aquatic ecosystems serve as the principal dietary source of n-3 PUFA for terrestrial
79 animals.

80 The majority of studies that have measured insect production and fluxes from water to
81 land have been focused on lotic systems (Muehlbauer *et al.*, 2014; Schriever *et al.*, 2014),
82 resulting in accumulation of fatty-acid composition and PUFA content of primarily rheophilic
83 insects, mainly Ephemeroptera, Plecoptera and Trichoptera as well as some Chironomidae
84 (Descroix *et al.*, 2010; Torres-Ruiz, Wehr & Perrone, 2010; Lau *et al.*, 2012, Volk & Kiffney,
85 2012; Sauvanet *et al.*, 2013). These taxa do not, however, constitute the main macroinvertebrate
86 fauna of permanent and temporary ponds and other wetlands, which are often dominated by
87 cyclic colonizers such as dysticid beetles and corixid waterbugs (Batzer & Ruhi, 2013).
88 Biochemical data for such wetlands insects are scarce, with the exception of Culicidae
89 (Kominkova *et al.*, 2012; Sushchik *et al.*, 2013, but see Yang, Siriamornpun & Li, 2006).

90 We have previously argued that aquatic insect emergence plays a key role in global
91 transfer of the essential PUFA from water to land (Gladyshev, Arts & Sushchik, 2009).
92 However, fluxes of insect biomass across the aquatic–terrestrial interface vary over several
93 orders of magnitude among climate zones and landscapes (Bartels *et al.*, 2012). Furthermore,
94 insect taxonomic composition may vary among types of freshwater habitat and according to
95 climate (Polhemus & Polhemus, 2008). Recent findings have shown that phylogeny (as well as
96 diet) can substantially affect fatty-acid levels and PUFA contents in freshwater invertebrates
97 (Makhutova *et al.*, 2011; Lau *et al.*, 2012). Thus, a major part of the current study was to
98 investigate the magnitude of taxon-specific variability in the PUFA composition and contents of
99 waterbugs associated with various wetland types.

100 True waterbugs (Heteroptera: Nepomorpha) play many ecological roles in temperate
101 landscapes, including serving as the first colonizers of newly created aquatic systems (Bloechl et

102 al., 2010) and important food sources for waterfowl and bats (Papacek, 2001); some are also
103 notably successful invasive species (Coccia, Boyero & Green, 2014). Although both larval and
104 adult stages of true waterbugs are primarily aquatic, the adults periodically make short flights
105 through terrestrial landscapes (Csabai *et al.*, 2006; Boda & Csabai, 2013) and may undertake
106 frequent migrations among water bodies (Pajunen & Pajunen, 2003; Simonis, 2013) in search of
107 habitats for feeding, aestivation or overwintering (Boda & Csabai, 2013). These dispersal events
108 can include mass mixed-species flights (Stevens *et al.*, 2007; Boda & Csabai, 2013). As a result,
109 migrating waterbugs may transfer organic carbon, energy and (or) specific biochemical
110 compounds from water to land or among water bodies (e.g., Kraus, Pletcher & Vonesh, 2011).

111 Waterbugs are taxonomically diverse in Central Siberia, region characterized by steppe,
112 forest-steppe and forest landscapes with substantial expanses of wetlands (Kanyukova, 2006).
113 Unfortunately, data on the composition of the wetland macroinvertebrate fauna are scant (Batzer
114 & Ruhi, 2013). Accordingly, we studied the abundance of the dominant waterbug taxa to
115 estimate and compare their potential contribution to water-to-land subsidies among the major
116 ecoregions of Central Siberia. Because there is no quantitative approach for estimating the flight
117 intensity of waterbugs, we suggest that measuring their abundance in water bodies could serve as
118 a proxy for estimating numbers of migrating individuals and hence the , magnitude of the water-
119 to-land flux or subsidy among ecoregions.

120 Changes in diet the life cycle markedly affect the fatty acid composition of insect larvae
121 and adults (Stanley-Samuelsen *et al.*, 1988; Howard & Stanley-Samuelsen, 1996; Hood-
122 Nowotny *et al.*, 2012; Sighinolfi *et al.*, 2013; Mustonen *et al.*, 2015). There may also be taxon
123 specific differences among waterbugs: e.g., corixids are considered to be omnivorous feeders
124 that have wide spectra of potential food sources (Papacek, 2001), whereas most gerrids and
125 notonectids are viewed voracious predators that attack a variety of prey (Papacek, 2001). By
126 combining data on waterbug abundance with information on the taxon-specific and age-specific
127 differences in the contents of essential PUFAs, we hoped to estimate the relative importance of

128 different waterbugs as potential foods for terrestrial consumers in Central Siberian ecoregions
129 and thereby assess their contribution to water-land subsidies.

130

131 **Materials and methods**

132

133 *Study area and sampling sites*

134

135 The study was carried out in four adjacent areas located in Central Siberia, Russia (Fig. 1),
136 which corresponded to various ecoregions: Kazakh steppe (PA0810), Kazakh forest-steppe
137 (PA0809), Western Siberian hemiboreal forests (PA0444) and Sayan montane conifer forests
138 (PA0519); ecoregional codes (in parentheses) are given according to Olson *et al.* (2001). A
139 detailed description of the ecoregional characteristics is provided by Isachenko *et al.* (1988). In
140 each ecoregion, we established one to three rectangular areas (for more details, see below) and
141 sampled 5-7 typical water bodies (sites) during the growing seasons of 2013 and 2014, between
142 May and October.

143 The climate of all ecoregions is continental with sharply variable weather conditions. In the
144 steppe, vegetation is mostly grasses and herbs, while trees occupy only 3-5% of the area.
145 Habitats for waterbugs comprise the River Karasuk, two lakes and many temporary water bodies
146 that appear after the spring snow melt and river flooding. The forest steppe is a mosaic landscape
147 of lowlands and hills with patches of birch (*Betula pendula*) and aspen (*Populus tremula*) forests.
148 A significant part of this area, nearly 15-20 %, is occupied by lakes, ponds, river floodplains,
149 bogs and wetlands. This ecoregion is relatively large and heterogeneous; therefore, we
150 established the sampling sites that were comparatively widely spaced (Fig. 1). The hemiboreal
151 forest ecoregion is characterized by a relatively high relief, an extensive riverine and stream
152 network, and many floodplain water bodies. The montane forests are the only studied region
153 where coniferous vegetation dominates, predominately Siberian fir *Abies sibirica*. Here, aquatic

154 habitats for waterbugs were relatively large rivers (Berd and Inya), streams, and sparse
155 permanent and temporary ponds. In all ecoregions, we excluded rivers and streams with high
156 current velocities (>1 m/s) from sampling as they are typically not suitable habitats for
157 waterbugs.

158 Because the four ecoregions markedly differed in shape, size and ecological conditions, our
159 sampling effort varied among them. Five or six sites within rectangular areas of 250, 375 and
160 700 km² were sampled in steppe, montane forests and hemiboreal forests, respectively. In the
161 forest-steppe, which is an extensive ecoregion of 1500 km², 7 sampling sites were established
162 within three rectangles that were located 80-190 km from each other and had areas of 2, 8 and 90
163 km². We chose sampling sites that represented typical aquatic habitats for a given ecoregion.
164 They included rivers, streams, lakes, swamps and temporary water bodies, such as ditches and
165 potholes. For temporary water bodies, we chose those located 1-2 km from a permanent aquatic
166 habitat that was a site of waterbug reproduction. For relatively large water bodies, only shallow
167 zones (up to 1 m in depth) were sampled, and extended lake shores and wetlands were divided
168 into two or three sections that were sampled separately. Samples were taken from both riffles
169 and pools within rivers and streams.

170

171 *Insect sampling*

172

173 For each sample, a standard pond net (ca. 0.8 mm mesh, 'D' shaped opening with 0.3 m
174 diameter) was swept three times forward and backward through an approximately 1-m long
175 stretch of water; during the sweep, the net was moved across the bottom in an undulating
176 manner. This approach allowed us to include different types of vegetation, substrates and open-
177 water areas in proportion to their relative abundance, and obtain broadly representative
178 collections of waterbug taxa. We sampled monthly or bi-monthly in two or three sections for
179 relatively large water bodies, e.g., lakes, rivers and swamps, and in one or two sections for small

180 water bodies, such as ditches and ponds. The samples taking in different sections were
181 considered as replicates, i.e., on each sample occasion, 2-3 and 1-2 replicates for larger and
182 smaller water bodies, respectively.

183 All collected waterbugs were preserved and stored in 75% ethanol. All individuals that
184 were >1 mm in length were identified to the lowest taxonomic level possible using keys by
185 Kanyukova (2006), and counted. To estimate an average weight of an individual, we collected 3–
186 5 replicates (approximately 25 individuals per replicate) of the various life stages of each taxon
187 (larvae of the last stage and adults) and wet weighed them with accuracy ± 1 mg using a
188 precision balance (Ohaus, Parsippany, NJ).

189 For biochemical analyses, 3-5 samples of each dominant species from the ecoregions
190 where they occurred were taken. To form a biochemical sample, 5-30 live individuals, depending
191 on their average weight, were withdrawn from biomass samples prior to ethanol preservation or
192 obtained by additional collections made on the same date at the same sampling sites. The live
193 individuals were brought to the laboratory within 1-2 hours and held in tap water for 6-8 hours so
194 that they could empty their guts.

195

196 *Biochemical analyses*

197 The body surfaces of waterbugs were gently wiped with filter paper prior to biochemical
198 analysis. Then, nearly a half of the individuals were wet weighed with accuracy ± 1 mg, placed
199 in a chloroform:methanol mixture (2:1, v/v) and kept at -20 °C prior to fatty-acid analysis. The
200 remaining individuals were used for analysis of water content or organic carbon. To measure
201 water content, subsamples that included 2-20 individuals were dried at 75 °C to constant weight
202 and then weighed with accuracy ± 1 mg. The samples were kept in a desiccator until analysed for
203 carbon content.

204 Lipid extraction and subsequent preparation of fatty acid methyl esters (FAMES) were
205 performed as described previously (e.g., Sushchik *et al.*, 2013). A gas chromatograph equipped

206 with a mass spectrometer detector (model 6890/5975C; Agilent Technologies, Santa Clara) and
207 with a 30-m long, 0.25-mm internal diameter capillary HP-FFAP column was used for FAME
208 analysis. Detailed descriptions of the chromatographic and mass spectrometric conditions are
209 given elsewhere (Gladyshev *et al.*, 2014). The FAMEs were quantified according to the peak
210 area of the internal standard, nonadecanoic acid, which we added to samples in a chloroform
211 solution prior to lipid extraction.

212 Organic carbon measurements of waterbug samples were performed with a Flash EA 1112
213 NC Soil/MAS 200 elemental analyser (ThermoQuest, Milan). Calibration curves were generated
214 using aspartic acid and standard soil reference material (ThermoQuest, Milan).

215

216 *Data and statistical analyses*

217 We used the average abundance for during the two sampling season (2013 and 2014) and
218 average individual weight to calculate mean biomass for each taxon per square metre of each
219 study sites. In case of relatively large water bodies which sampled in two of three sections, data
220 of sections were preliminarily averaged before the subsequent calculations. Then, the biomass
221 values of the study sites within a particular ecoregion were averaged. Standard errors (SE),
222 ANOVAs and Fisher's LSD *post hoc* tests for fatty-acid contents (mg g^{-1} of wet weight), organic
223 carbon content (% of the dry weight) and water content (% of the wet weight) in waterbug taxa
224 were calculated conventionally. We expressed quantitative data for fatty acids on wet mass basis,
225 as a measure of nutritional quality of the studied insects. As far as data on insect productivity or
226 biomass are often given per unit of dry weight or organic carbon unit, we also provide water and
227 organic carbon content of the each group for comparative purposes.

228 To test whether taxonomic identify, ecoregion or life stage (i.e., adults *versus* larvae of
229 the last stage) affected the biochemical composition of waterbugs within the same family, we
230 conducted canonical correspondence analyses (CCA) using the percentage fatty-acid (FA)
231 composition (see list of FAs below). If CCA revealed significant differences among the species

232 or genera, we considered them individually in further statistical analysis. If not, i.e., life stage or
233 ecoregion predominantly affected FA composition, we combined taxa within the same family in
234 subsequent analysis. Then, we conducted multivariate discriminant analysis of FA percentage
235 composition to estimate overall differences among waterbug taxa. All tests were completed using
236 STATISTICA software, version 9.0 (StatSoft, Inc., Tulsa).

237 The following FAs were biomarkers and subject to statistical analysis: biomarkers of
238 various microalgae and organic matter of aquatic origin were 14:0, 14:1, PUFA with 16 carbon
239 atoms, 18:3n-6, 18:4n-3, EPA, DHA and n-3 LC-PUFA (sum of n-3 long-chain PUFA with >18
240 carbon atoms, apart from EPA and DHA) (Dijkman & Kromkamp, 2006; Taipale *et al.*, 2013);
241 biomarkers of green algae were 16:1n-9 and 18:n-3 (Napolitano, 1999; Fuschino *et al.*, 2011);
242 biomarkers of terrestrial plants and organic matter from terrestrial food webs were 18:2n-6 and
243 saturated FA with 20-24 carbon atoms (Napolitano, 1999; Kelly & Scheibling, 2012); biomarker
244 of terrestrial invertebrates and vertebrates was 20:4n-6 (Hixson *et al.*, 2015); biomarkers of
245 bacteria were 14-17 BFA (fatty acids with branched chains of 14-17 carbon atoms), 15:0, 17:0
246 and 18:1n-7 (Napolitano, 1999); and, biomarkers of methane oxidizing bacteria were 16:1n-6
247 (Kelly & Scheibling, 2012; Taipale *et al.*, 2015). As an indicator of carnivorous feeding by
248 waterbugs we used 18:1n-9 and the ratio between 18:1n-9 and 18:1n-7 (Stevens, Deibel &
249 Parrish, 2004; El-Sabaawi *et al.*, 2009).

250

251 **Results**

252

253 *Biomass and species composition of waterbugs*

254 Biomass of waterbug taxa averaged across all sampling dates and all sites within each
255 ecoregion (Table 1) reveal the dominance of Naucoridae, Notonectidae and Corixidae. Gerrids
256 were less abundant, but would have been underrepresented due to our sampling protocol,
257 although representatives of this family were collected separately for biochemical analyses.

258 *Gerris lacustris*, *G. odontogaster*, *Aquarius paludum*, and *Limnoporus rufoscutellatus* were
259 widespread in steppe and forest-steppe, while only *A. paludum*, was numerous in montane and
260 hemiboreal forests.

261 The sole naucoridae, *Ilyocoris cimicoides*, accounted for the majority of the total
262 heteropteran biomass, 29-55 % across the ecoregions (Table 1). Adults were especially abundant
263 in the steppe, while mean biomass of larvae and adults in forest steppe were nearly equivalent.
264 Notonectid larvae represented the greatest proportion of biomass in the steppe compared to other
265 ecoregions, 25 % versus 0 – 4 % (Table 1). *Notonecta glauca* was the dominant notonectid and
266 *Cymatia coleoptrata* the dominant corixid in all ecoregions. Other corixids in the genera *Sigara*
267 and *Paracorixa* were also abundant in steppe and forest-steppe ecoregions. The steppe generally
268 supported the highest mean biomass of waterbugs (Table 1), and some notonectids and corixids
269 that were present in steppe and forest-steppe, were not found in montane and hemiboreal forests;
270 this may be related to the lower sampling efforts in the two forested ecoregions (Table 1).

271

272 *FA composition of waterbug families*

273

274 CCA of the naucorid *I. cimicoides* showed the most prominent differences in FA
275 composition along the first dimension between larvae and adults collected from the forest-steppe
276 (Fig. 2A), while ecoregional differences among adults also contributed to the inertia associated
277 with the first dimension (Fig. 2A). These differences between larvae and adults and among
278 adults from various ecoregions were mainly due to variations in levels of PUFA with 16 carbon
279 atoms, 18:3n-3 and 16:1n-9, which are dietary markers of algae, on the one hand, and levels of
280 20:4n-6, EPA and 16:1n-6, which are markers of animal and bacterial foods, on the other hand
281 (Fig. 2B). In the second dimension, adults inhabiting the montane forests were significantly
282 separated from other ecoregions due to increased levels of 16:1n-6, and the sum of 17:1, 15:0
283 and 18:1n-7; i.e. markers of bacterial- food sources (Fig.2A, B).

284 CCA of notonectids taxa revealed a marked difference between *Notonecta* larvae
285 collected from the steppe and samples from other ecoregions, which included adults of three
286 species and larvae from forest-steppe (Fig. 3A). This difference was related to greater levels of
287 DHA and n-3 LC-PUFA in *Notonecta* larvae from the steppe and to greater 18:1n-9 and 16:0
288 levels in notonectids from other ecoregions (Fig. 3B). Increased levels of long-chain n-3 PUFA
289 in *Notonecta* larvae likely indicated their reliance on algae and protists, while high levels of
290 18:1n-9 in adults were markers for predatory feeding. The second dimension mostly revealed
291 differences between adults of notonectid species: *N. lutea* was contained FA biomarkers of
292 terrestrial food sources (18:2n-6, 22:0, 20:4n-6), while *N. glauca* had more biomarkers of aquatic
293 foods such as PUFAs with 16 carbon atoms (Fig. 3A, 3B).

294 Because of strong influence of the life stage on the overall notonectid analysis, we ran a
295 separate CCA for only adult *Notonecta* (Fig. 4). In the first dimension, separation of *N. glauca*, *N.*
296 *reuteri* and *N. lutea* was due to mainly the same FA biomarkers as seen in Fig 3B. There was no
297 obvious trend along the second dimension suggesting that taxonomic identity determined the FA
298 composition of notonectid adults, although the low total variance of CCA (61.9 %) and sample
299 scattering indicated that some intraspecific variation was present (Fig. 4A).

300 The first dimension of the Corixidae CCA separated *C. coleoptrata* adults and
301 *Micronecta* larvae (Fig. 5A). This was due to differences between levels of 16:1n-9, 14-17 BFA
302 and 18:1n-7, which were higher in *C. coleoptrata*, and PUFA with 16 carbon atoms, 14:0 and
303 16:1n-7 that were greater in *Micronecta* (Fig. 5B). The variation in the second dimension was
304 related to differences between *C. coleoptrata* and *Sigara* spp., and the latter were enriched in
305 15:0. In general, FA composition of corixids mainly reflected phylogeny because the same taxa
306 collected in different ecoregions tended to group together; for example, *C. coleoptrata* and
307 *Sigara* spp. (Fig. 5A). These differences appeared to reflect variations in the relative amounts of
308 algal and bacterial biomarkers in corixid diets (Fig 5B).

309 The most prominent difference observed along the first CCA dimension for Gerridae was
310 among populations in different ecoregions (Fig. 6A). Interestingly, the most variation in this
311 dimension was related to *A. paludum*, which was only numerous in montane and hemiboreal
312 forests and forest-steppe. The second dimension showed that *G. odontogaster*, which inhabited
313 the steppe ecoregion, was markedly richer in 14:1, 14:0 and 18:3 n-6 (markers of aquatic food
314 sources), compared to forest-associate species such as *G. lacustris* and *A. paludum*, which were
315 enriched with biomarkers indicative of terrestrial food sources (i.e., 22:0, 20:0 and 20:4n-6: Fig.
316 6B).

317 Overall, CCA analyses showed that ecoregion played a major role in variability of FA
318 composition of Gerridae, but not Corixidae (Figs. 5-6). Conversely, the biochemical composition
319 of *I. cimicoides* and Notonectidae was strongly affected by life stage (Figs. 2-3). Phylogeny
320 prevailed over ecoregional factors in separating among notonectid adults; however, its effect was
321 lower than in the other waterbug families (Figs. 4, 5). Due to these findings, for further statistical
322 analysis by MDA, we used only data derived from adults, combined at the family level for
323 notonectids and gerrids, while corixids were subdivided into three groups (*C. coleoptrata*, *Sigara*
324 spp., and a combination of *Paracorixa*, *Corixa* spp. plus *Callicorixa* spp.).

325 MDA based on 23 of the most abundant individual fatty acids or their structural groups
326 and revealed relatively clear separation of these six waterbug taxa (Fig. 7). Both discriminant
327 functions (Root 1 and 2) were statistically significant (Table 2) and a cumulative proportion of
328 the variance explained (discriminatory power) was up to 82.6 %. Root 1 best discriminated
329 corixid species, *C. coleoptrata* and *Sigara* spp., from other waterbugs (Fig. 7). Variables that
330 gave the highest contribution to the first discriminant function were 14-17BFA and DHA on the
331 one hand, and 18:1n-9 and 16:0 on the other (Table 2). Root 2 revealed differences between
332 *Sigara* and Gerridae, primarily due to the contributions of 15:0 and 18:1n-9 (Table 2). Although
333 the FA compositions of *I. cimicoides*, Notonectidae and the combined corixid species were

334 separated by the two discriminant functions, they were nonetheless placed relatively close to
335 each other (Fig. 7).

336

337 *FA contents of waterbugs*

338

339 Mean contents of the major fatty acids in waterbugs differed among taxa as did water
340 content and organic carbon (Table 3). Total FA content ($\text{mg}\cdot\text{g}^{-1}$ of wet weight) and organic
341 carbon content (% of dry weight) were significantly higher in gerrids and corixids, except for *C.*
342 *coleoptrata*, and larvae of all families had higher water content than adults. Content of essential
343 linoleic acid was almost two-fold higher in gerrids than in other waterbugs (Table 3), whereas *I.*
344 *cimicoides* and most corixid taxa were enriched in n-3 PUFA, namely linolenic acid. The only
345 exception among the corixids was *M. minutissima*, which accumulated mostly PUFAs with 16
346 and 20 carbon atoms (Table 3). Contents of arachidonic acid, 20:4n-6, were significantly higher
347 in corixid adults compared to other waterbugs. It is notable that adults of *I. cimicoides* and
348 Notonectidae had 20:4n-6 levels almost two-fold higher than those of their larvae, while the
349 highest EPA contents were recorded in larvae of *M. minutissima* and adults of *Sigara* and other
350 corixids (Table 3). DHA contents of gerrids, notonectids and *I. cimicoides* were almost
351 negligible, but high DHA contents were typical of all corixids and were highest in *C. coleoptrata*
352 adults (Table 3).

353

354 **Discussion**

355

356 Our study confirmed that the steppe and, to some degree, the forest-steppe have support the
357 highest biomass of waterbugs in the Central Siberian region. It is well known that these aquatic
358 insects prefer macrophyte-dominated, fishless lentic or slow-flowing lotic habitats (Tolonen *et*
359 *al.*, 2003; Schilling, Loftin & Huryn, 2009), and such water bodies are prevalent in the steppe

360 ecoregion. Overall, the same species of waterbugs were dominant in both the steppe and forested
361 ecoregions reflecting the fact that many of the studied species are widely distributed. For
362 instance, *N. glauca*, *Sigara limitata* and *I. cimicoides* have ranges that include Germany, Poland
363 and Spain (Kurzatowska, 2008; Florencio *et al.*, 2009; Bloechl *et al.*, 2010). Corixids, such as
364 *Paracorixa concinna* and *C. coleoptrata*, are likewise widespread in Europe although they tend
365 to favour water bodies with high levels of dissolved salts (Kurzatowska, 2008; Skern,
366 Zweimuller & Schiemer, 2010); they tended to occur most commonly in the steppe where ponds,
367 rivers and temporary water bodies with high salinity were ubiquitous (Savchenko, 2010).
368 Overall, our results confirmed previous finding that the local and regional environmental
369 variables primarily determine waterbug assemblages, while geographic location has a lesser least
370 influence (Karaouzas & Gritzalis, 2006).

371 Quantitative data on waterbug biomass are scarce, although estimates for eutyhaline
372 *Sigara selecta* from a hypersaline Mediterranean stream have been reported to reach 0.57 g DW
373 m⁻² (Barahona, Millan & Velasco, 2005). Using the mean water content of Corixidae (67 %:
374 Table 3), the biomass of this family in the steppe and forest-steppe ecoregions accounted for 0.09
375 and 0.12 g DW m⁻² respectively, considerably less than the value for *S. selecta*. Total biomass of
376 Baltic rock-pool corixids, expressed in terms of organic carbon, were as high as 150 – 400 mg C
377 m⁻² (Pajunen & Pajunen, 2003) compared to mean values of 48 and 64 mg C m⁻² in the steppe
378 and forest-steppe. While the corixids appeared to be less abundant than in some parts elsewhere
379 in the Palearctic, total biomass of Heteroptera in a shallow, fish-free, eutrophic lake in Poland
380 was estimated as 2.2 g wet weight m⁻² (Prejs, Koperski & Prejs, 1997), which is similar to the
381 mean waterbug biomass in the forest-steppe ecoregion. *Notonecta glauca* was a dominant
382 species in that lake, as was also the case in Central Siberia (Table 1).

383 We found that life stage-related differences in FA composition and contents were
384 prominent among Notonectidae, which likely shifted their diet during development, but varied
385 little between larvae and adults of *I. cimicoides*; it may be relevant that this naucorid is flightless.

386 Compared to the larvae, *Notonecta* adults had a significantly higher content of 18:1n-9, which is
387 regarded as a trophic marker for carnivorous feeding (Stevens *et al.*, 2004). They had similar
388 content of n-3 PUFAs, including EPA, as their larvae, but content of linoleic acid, 18:2n-6, was
389 almost twice as high as in larvae. Contents of this n-6 PUFA were highest in notonectid and
390 gerrid adults (both in absolute and relative terms) while n-3 PUFAs were more abundant in other
391 waterbug taxa (Table 3). Linoleic acid, is essential for most insects as it influences immunity and
392 reproduction (Stanley-Samuelson *et al.*, 1988) and, while *de novo* synthesis has been confirmed
393 only in a small number of terrestrial species (Zhou *et al.*, 2008), most insects must obtain it
394 through their diet. Trichoptera can accumulate linoleic acid only when it was present in food in
395 high quantities (Torres-Ruiz *et al.*, 2010), and we suggest that adult notonectids obtain this FA
396 through predation on chironomids or (perhaps less likely) terrestrial insects, as they are
397 comparatively rich in linoleic acid (Goedkoop, Demandt & Ahlgren, 2007; Makhutova *et al.*,
398 2011; Fontaneto *et al.*, 2011).

399 Conspecific adults of gerrids and some notonectids (*A. paludum*, *N. glauca*, and *N.*
400 *reuteri*) collected from different ecoregions, varied markedly in their FA profiles, while the
401 corixids *C. coleoptrata*, and *P. concinna* did not show such differences, and had high contents
402 of 14-17 BFA, which are biomarkers for gram-positive bacteria (Napolitano, 1999). Corixids
403 such as *Cymatia* spp. prey on mosquito larvae (Papacek, 2001) which tend to contain high
404 contents of BFA (Sushchik *et al.*, 2013). We suspect that the corixids in our study obtain FA
405 biomarkers through direct ingestion of fine detritus enriched with bacteria or via predation on
406 mosquito larvae. Corixid species or genera tended to differ in FA composition, regardless of
407 ecoregion, suggesting taxon-specific dietary specialization, in contradiction of the notion that
408 corixids have broad and overlapping diets (Papacek, 2001). However, it is worth noting that our
409 study of the diet of these insects was based on the biomarkers, and not direct observations of
410 feeding mode or ingested items.

411 Gerrids and notonectids appeared to be obligate carnivores (see also Kanyukova, 2006),
412 but their FA composition indicated that diets varied among ecoregions suggesting plasticity in
413 types of prey eaten. For instance, forest-steppe population of the gerrid *A. paludum* fed mostly
414 on aquatic preys because it had high levels of 16PUFA and 18:4n-3 that are biomarkers of
415 various microalgae and the organic matter of aquatic origin. In contrast, populations in the
416 montane forests fed mostly on terrestrial food sources, and were rich in 18:2n-6 and long-chain
417 saturated FAs that are biomarkers of organic matter from terrestrial food webs.

418 Data on the FA profiles and essential PUFA contents of aquatic Heteroptera confined to
419 only two publications (Bell, Ghioni & Sargent, 1994; Yang, Siriamornpun & Li, 2006).
420 *Notonecta* from a Scottish river analysed by Bell *et al.* (1994) had markedly higher levels of n-3
421 PUFA, 18:3n-3 and EPA, and lower levels of n-6 PUFA, 18:2n-6 and 20:4n-6, than *Notonecta*
422 adults from Central Siberia, whereas Corixidae from both the Scottish river and the Siberian
423 water bodies had similar levels of long-chain PUFA (EPA, DHA and 20:4n-6). However, Scottish
424 corixids contained higher levels of 18:3n-3 and 18:2n-6. The giant water bug, *Lethocerus indicus*
425 (Belostomatidae), from South Asia contained similar levels of EPA (Yang *et al.*, 2006) to the
426 majority of waterbugs in Central Siberia, but had unusually high content of total FAs relative to
427 our data as well as values reported in the literature (Tzompa-Sosa *et al.*, 2014).

428 Despite the general observations that long-chain PUFAs are absent in terrestrial insects
429 (Stanley-Samuelson *et al.*, 1988; Rumpold & Schluter, 2013; Sanchez-Muros, Barroso &
430 Manzano-Agugliaro, 2014), EPA has been reported to constitute significant proportions of total
431 phospholipids in some terrestrial Heteroptera such as *Monostertia unicostata* (Tingidae) and its
432 predator *Piocoris luridus* (Lygaeidae) as well as the pentatomid *Eysarcoris inconspicuus*
433 (Cakmak, Bashan & Bolu, 2007; Cakmak, 2010). We studied the total lipid fraction, which
434 contains less PUFA than the phospholipid fraction, but the proportions of EPA in waterbugs that
435 we studied were higher than or at least similar to those in the phospholipids of the terrestrial
436 Heteroptera. Only the terrestrial carnivore *P. luridus* had EPA levels similar to those of

437 waterbugs. Indeed, a study of two terrestrial heteropterans, *Agonoscelis pubescens*
438 (Pentatomidae) and *Aspongopus viduatus* (Dinidoridae), failed to detect any long-chain PUFAs
439 (Rumpold & Schluter, 2013).

440 EPA contents in Central Siberian waterbugs (1.4 to 9.9 mg g⁻¹ wet weight, generally
441 coincided well with values reported for larvae (1.4-5.2 mg g⁻¹) and adults (2.3-2.6 mg g⁻¹) of
442 other aquatic insect orders (Gladyshev *et al.*, 2011; Kalacheva *et al.*, 2013; Sushchik *et al.*,
443 2013). EPA values in adult waterbugs were likewise similar to adult chironomids,
444 Ephemeroptera and Trichoptera (3.7 - 4.2 mg g⁻¹) elsewhere in Siberia (N.N. Sushchik,
445 unpublished data).

446 Unlike other PUFAs, DHA is commonly absent or occurs only in trace levels in the
447 majority of insect taxa (Stanley-Samuelson *et al.*, 1988; Rumpold & Schluter, 2013; Sanchez-
448 Muros *et al.*, 2014; but see Lau *et al.*, 2012), and in modest level in terrestrial vertebrates
449 (Hulbert, Rana & Couture, 2002; Valencak & Ruf, 2007), but occurs in high or moderate
450 amounts in other non-insect aquatic invertebrates and fish (Makhutova *et al.*, 2013; Gladyshev *et*
451 *al.*, 2013). We found that corixids, especially *C. coleoptrata*, had the highest mass-specific DHA
452 content thus far reported for insects and, at 0.20-0.37 mg g⁻¹ wet weight, were two to four-fold
453 higher than other aquatic insects (Gladyshev *et al.*, 2011; Kalacheva *et al.*, 2013; Sushchik *et al.*,
454 2013). DHA level or DHA/EPA ratios are markers of carnivorous feeding and correlate well
455 with the trophic position, although there may also be a correlation between DHA and the
456 proportion of diatoms and flagellates eaten (El-Sabaawi *et al.*, 2009). Based on FA biomarkers,
457 the Central Siberian corixids that we studied were omnivorous but also corixids accumulated
458 DHA, while notonectids, which are known to be obligate carnivores (Kanyukova, 2006)
459 contained only traces of this PUFA. Evidently, the accumulation of DHA in aquatic insects is not
460 necessarily related to trophic level.

461 Among the studied Heteroptera taxa and other aquatic insects, corixids appeared to be the
462 best source of the essential long-chain PUFAs, such as EPA and DHA. Overland dispersal by

463 flying likely makes corixids a good potential source of the essential PUFA for waterfowl and
464 terrestrial insectivores (Papacek, 2001), constituting a major prey of the vespertilionid bat
465 *Lasiurus cinereus* (Reimer *et al.*, 2010), and a substantial dietary item of ducks such as marbled
466 teal (*Marmaronetta angustirostris*: Green & Sanchez, 2003). Based on their abundances in the
467 freshwater bodies, we conclude that where environmental factors are favourable for the
468 development of waterbug populations, such as in the steppe and forest-steppe of Central Siberia,
469 waterbugs are likely to represent an important food source for terrestrial consumers and
470 constitute a significant water-to-land flux of biomass and essential compounds that can be
471 utilized by terrestrial consumers. FA composition of adult corixids do not appear to vary
472 significantly among ecoregions (i.e., they are largely determined by species identity), and they
473 contain higher contents of EPA and especially DHA than other aquatic insects. This means that
474 even if corixids are less abundant than other aquatic insects, their dispersal through terrestrial
475 landscapes could represent a significant subsidy of essential long-chain PUFA for terrestrial
476 consumers.

477

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479

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484

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700

701 **Figure legends**

702

703 **Fig. 1** Map of the studied area. Sampling sites in ecoregions: circles, Kazakh steppe; crosses,
704 Kazakh forest-steppe; diamonds, Western Siberian hemiboreal forests; and triangles, Sayan
705 montane conifer forests; see text for details.

706

707 **Fig. 2** CCA of FA composition (% of the total) in *Ilyocoris cimicoides* collected from various
708 ecoregions in Central Siberia: S – Kazakh steppe, FS – Kazakh forest steppe, MF - Sayan
709 montane conifer forests. a) Ordination of *I. cimicoides* populations according to their FA
710 composition. b) Ordination of *I. cimicoides* FAs. Dimension 1 (D1) and Dimension 2 (D2)
711 accounted for 51.8 and 18.5 % of the total variance, respectively.

712

713 **Fig. 3** CCA of FA composition (% of the total) of notonectids collected from various ecoregions
714 in Central Siberia: S – Kazakh steppe, FS – Kazakh forest steppe, MF - Sayan montane conifer
715 forests, HF- hemiboreal forests. Adults were identified to species, and larvae to genus. a)
716 Ordination of notonectid populations according to their FA composition. b) Ordination of
717 notonectid FAs. Dimension 1 (D1) and Dimension 2 (D2) accounted for 48.8 and 22.2 % of the
718 total variance, respectively.

719 **Fig. 4** CCA of FA composition (% of the total) of adult notonectids collected from various
720 ecoregions in Central Siberia: for abbreviations see Fig. 3. a) Ordination of adult notonectid
721 populations according to their FA composition. b) Ordination of adult notonectid FAs.
722 Dimension 1 (D1) and Dimension 2 (D2) accounted for 39.4 and 21.5 % of the total variance,
723 respectively.

724 **Fig. 5** CCA of FA composition (% of the total) of corixids collected from various ecoregions in
725 Central Siberia: for abbreviations see Fig. 3. Adults were identified to species, and larvae to
726 genus. a) Ordination of corixid populations according to their FA composition. b) Ordination of

727 corixid FAs. Dimension 1 (D1) and Dimension 2 (D2) accounted for 51.7 and 20.1 % of the total
728 variance, respectively.

729 **Fig. 6** CCA of FA composition (% of the total) of gerrids collected from various ecoregions in
730 Central Siberia: for abbreviations see Fig. 3. a) Ordination of gerrid populations according to
731 their FA composition. b) Ordination of gerrid FAs. Dimension 1 (D1) and Dimension 2 (D2)
732 accounted for 51.7 and 24.7 % of the total variance, respectively.

733 **Fig. 7** Scatterplot of canonical scores for the two discriminant functions, Root 1 and Root 2 (see
734 Table 2), after multivariate discriminant analysis (MDA) of the FA % composition of adult
735 waterbugs collected in Central Siberia: Nau-II, *Ilyocoris cimicoides*; Not, Notonectidae; Ger,
736 Gerridae; Cor-Cy, *Cymatia coleoptrata*; Cor-Si, *Sigara* spp.; Cor-pcc, *Paracorixa concinna*,
737 *Corixa dentipes*, and *Callicorixa gebleri* combined.

738

739 **Table 1** Biomass of waterbugs (mean \pm SE, g wet weight \cdot m⁻²) inhabiting water bodies in four
 740 Central Siberian ecoregions (2013 and 2014).

Family	Ecoregion	Montane forests	Hemiboreal forests	Forest-steppe	Steppe
	Sampling period	June-September	August-October	May-October	May-September
	Number of samples	69	51	96	73
Naucoridae	<i>Ilyocoris cimicoides</i> *	0.11 \pm 0.05	0.14 \pm 0.04	0.47 \pm 0.06	2.06 \pm 0.39
Naucoridae	larvae <i>I. cimicoides</i>	0.06 \pm 0.03	0.02 \pm 0.01	0.44 \pm 0.09	0.16 \pm 0.06
Naucoridae	total	0.17 \pm 0.06	0.16 \pm 0.05	0.91 \pm 0.15	2.22 \pm 0.45
Notonectidae	<i>Notonecta glauca</i>	0.29 \pm 0.11	0.17 \pm 0.05	0.14 \pm 0.04	0.16 \pm 0.05
Notonectidae	<i>Notonecta reuteri</i>	0.04 \pm 0.02	n.f.	0.04 \pm 0.02	0.12 \pm 0.03
Notonectidae	<i>Notonecta lutea</i>	n.f.	n.f.	0.07 \pm 0.03	0.13 \pm 0.04
Notonectidae	larvae <i>Notonecta</i> sp.	0.02 \pm 0.01	n.f.	0.07 \pm 0.03	1.05 \pm 0.44
Notonectidae	other species#	0.04 \pm 0.02	n.f.	0.23 \pm 0.06	0.01 \pm 0.01
Notonectidae	total	0.39 \pm 0.13	0.17 \pm 0.05	0.55 \pm 0.17	1.46 \pm 0.57
Corixidae	<i>Cymatia coleoprata</i>	<0.01	0.04 \pm 0.02	0.01 \pm 0.00	0.05 \pm 0.00
Corixidae	<i>Paracorixa concinna</i>	n.f.	n.f.	0.05 \pm 0.02	0.11 \pm 0.02
Corixidae	<i>Sigara limitata</i>	n.f.	n.f.	0.01 \pm 0.00	<0.01
Corixidae	<i>Sigara fallenoidae</i>	n.f.	n.f.	0.14 \pm 0.04	0.01 \pm 0.01
Corixidae	<i>Sigara</i> spp..	n.f.	<0.01	0.02 \pm 0.01	0.08 \pm 0.02
Corixidae	larvae <i>Sigara</i> spp.	n.f.	n.f.	<0.01	0.02 \pm 0.01
Corixidae	larvae <i>C. coleoprata</i>	<0.01	0.02 \pm 0.01	<0.01	<0.01
Corixidae	larvae Corixidae	0.02 \pm 0.01	<0.01	0.02 \pm 0.00	0.03 \pm 0.03
Corixidae	other species*	<0.01	<0.01	0.02 \pm 0.01	0.07 \pm 0.05
Corixidae	total	0.03 \pm 0.01	0.07 \pm 0.03	0.27 \pm 0.08	0.37 \pm 0.13
Other Heteroptera		<0.01	<0.01	<0.01	0.01 \pm 0.00
Total		0.58 \pm 0.17	0.40 \pm 0.08	1.73 \pm 0.22	4.05 \pm 0.89

741 n.f., taxon not found; *, if not specified, data for adults are given; #, adults of species found
 742 sporadically
 743

744 **Table 2** Results of a discriminant analysis for the FA composition (% of the total) of waterbug
 745 adults in Central Siberia. FAs 16:1n-9 and 18:1n-7 were excluded from the model because of
 746 their insignificant *F* values.

	Root 1	Root 2
Canonical <i>R</i>	0.991	0.988
χ^2	952	663
d.f.	115	88
<i>P</i>	0.00000	0.00000
Means of canonical variables:		
Naucoridae	2.94	-1.89
Notonectidae	-3.30	-1.51
Gerridae	-3.63	-6.20
<i>Cymatia coleoptrata</i> (Corixidae)	19.76	3.91
<i>Sigara</i> spp. (Corixidae)	-9.41	19.94
Other Corixidae	1.28	2.72
Factor structure coefficients:		
14:0	0.033	0.037
Σ 14:1	0.046	-0.015
15:0	-0.005	0.506
14-17 BFA	0.273	0.090
16:0	-0.113	0.082
16:1n-7	0.108	0.154
16:1n-6	0.090	0.037
16 PUFA	-0.038	0.138
17:0	0.118	0.134
Σ 17:1	0.140	0.193
18:0	-0.008	-0.062
18:1n-9	-0.178	-0.216
18:2n-6	-0.031	-0.129
18:3n-6	0.028	0.040
18:3n-3	0.093	0.014
18:4n-3	0.117	0.058
20:0	0.026	-0.039
20:4n-6	0.048	0.047
20:5n-3	0.008	0.089
22:0	0.013	-0.037
20-22 PUFA n-6	0.056	0.190
20-22 PUFA n-3	0.076	0.173
22:6n-3	0.165	0.154

Table 3 Content of major FAs (mean \pm standard error, mg g⁻¹ of wet weight), as well as organic carbon (% of dry weight) and water content (% of wet weight) of waterbugs collected in Central Siberia. *N* – number of samples. Means labelled with the same letter are not significantly different at *p* < 0.05 after Fisher's LSD *post-hoc* test.

Species or group	Gerridae@	<i>Ilyocoris cimicoides</i>		Notonectidae+		<i>Cymatia coleoptrata</i>	<i>Sigara</i> spp.*	other corixids#	<i>Micronecta minutissima</i>
Stage	adults	larvae	adults	larvae	adults	adults	adults	adults	larvae
<i>N</i>	18	6	12	12	29	8	6	12	3
15:0	0.1 \pm 0.01 ^A	0.1 \pm 0.01 ^A	0.1 \pm 0.02 ^B	0.1 \pm 0.01 ^{AB}	0.1 \pm 0.02 ^B	0.3 \pm 0.03 ^C	1.3 \pm 0.07 ^F	0.4 \pm 0.04 ^D	0.6 \pm 0.00 ^E
16:0	6.9 \pm 0.70 ^{BC}	4.2 \pm 0.44 ^{AB}	3.3 \pm 0.37 ^A	2.7 \pm 0.40 ^A	5.9 \pm 0.65 ^B	3.5 \pm 0.18 ^A	9.1 \pm 1.02 ^{CD}	9.8 \pm 1.29 ^D	16.0 \pm 0.41 ^E
18:0	2.7 \pm 0.21 ^C	1.7 \pm 0.07 ^B	1.0 \pm 0.07 ^A	1.5 \pm 0.05 ^B	1.6 \pm 0.11 ^B	1.6 \pm 0.06 ^B	1.7 \pm 0.05 ^B	2.4 \pm 0.14 ^C	2.7 \pm 0.04 ^C
14-17 BFA	0.2 \pm 0.02 ^A	0.3 \pm 0.02 ^A	0.3 \pm 0.05 ^A	0.2 \pm 0.03 ^A	0.2 \pm 0.02 ^A	0.9 \pm 0.10 ^B	0.3 \pm 0.03 ^A	1.0 \pm 0.16 ^B	0.1 \pm 0.01 ^A
18:1n-9	15.2 \pm 1.59 ^C	3.6 \pm 0.42 ^A	3.9 \pm 0.34 ^A	2.9 \pm 0.42 ^A	7.7 \pm 0.73 ^B	3.4 \pm 0.10 ^A	6.6 \pm 0.53 ^{AB}	8.9 \pm 1.00 ^B	7.3 \pm 0.07 ^{AB}
18:1n-7	1.1 \pm 0.14 ^{AB}	0.7 \pm 0.05 ^A	1.1 \pm 0.13 ^{AB}	0.8 \pm 0.03 ^A	1.0 \pm 0.12 ^{AB}	2.2 \pm 0.16 ^D	0.7 \pm 0.08 ^A	1.9 \pm 0.18 ^{CD}	1.5 \pm 0.02 ^{BC}
16 PUFA	0.3 \pm 0.07 ^B	0.4 \pm 0.10 ^B	0.3 \pm 0.06 ^{AB}	0.2 \pm 0.02 ^A	0.2 \pm 0.03 ^A	0.2 \pm 0.03 ^{AB}	1.0 \pm 0.09 ^D	0.6 \pm 0.11 ^C	6.3 \pm 0.14 ^E
18:2n-6	5.0 \pm 0.44 ^E	2.3 \pm 0.13 ^{BCD}	2.1 \pm 0.16 ^{BC}	1.5 \pm 0.11 ^{AB}	2.7 \pm 0.18 ^{CD}	2.0 \pm 0.08 ^{ABC}	1.1 \pm 0.07 ^A	2.9 \pm 0.23 ^D	1.1 \pm 0.02 ^{AB}
18:3n-3	2.3 \pm 0.32 ^{AB}	3.9 \pm 0.93 ^C	2.8 \pm 0.31 ^{BC}	1.5 \pm 0.16 ^A	2.0 \pm 0.22 ^A	3.2 \pm 0.25 ^{BC}	2.5 \pm 0.12 ^{AB}	2.9 \pm 0.25 ^{BC}	1.3 \pm 0.01 ^A
20:4n-6	0.9 \pm 0.05 ^B	0.5 \pm 0.05 ^A	0.9 \pm 0.06 ^B	0.6 \pm 0.02 ^A	1.1 \pm 0.08 ^B	1.5 \pm 0.05 ^C	1.7 \pm 0.09 ^C	1.5 \pm 0.12 ^C	0.4 \pm 0.01 ^A
20:5n-3	3.3 \pm 0.23 ^C	1.4 \pm 0.22 ^A	1.4 \pm 0.08 ^A	2.5 \pm 0.10 ^B	2.4 \pm 0.18 ^B	3.2 \pm 0.08 ^C	4.8 \pm 0.22 ^D	4.2 \pm 0.22 ^D	9.9 \pm 0.24 ^E
22:6n-3	0.01 \pm 0.00 ^A	0.04 \pm 0.01 ^{AB}	0.04 \pm 0.01 ^A	0.09 \pm 0.01 ^B	0.04 \pm 0.00 ^A	0.37 \pm 0.06 ^D	0.25 \pm 0.03 ^C	0.20 \pm 0.02 ^C	0.24 \pm 0.02 ^C
FA, total	43.7 \pm 3.6 ^D	23.4 \pm 1.8 ^{AB}	21.9 \pm 1.8 ^{AB}	17.6 \pm 1.2 ^A	29.1 \pm 2.5 ^B	31.3 \pm 1.4 ^{BC}	41.1 \pm 2.8 ^{CD}	47.2 \pm 4.7 ^D	77.1 \pm 1.2 ^E
Water	56.7 \pm 1.8 ^A	74.0 \pm 1.0 ^{DE}	70.0 \pm 0.6 ^{CD}	76.6 \pm 0.6 ^E	68.3 \pm 1.2 ^C	64.3 \pm 0.23 ^B	66.3 \pm 0.9 ^{BC}	64.4 \pm 1.6 ^B	72.6 \pm 0.2 ^{CDE}
Organic C	54.8 \pm 0.4 ^E	50.1 \pm 0.4 ^B	50.5 \pm 0.6 ^{BC}	48.3 \pm 0.6 ^A	51.1 \pm 0.5 ^{BC}	49.1 \pm 0.27 ^A	55.0 \pm 0.1 ^E	53.8 \pm 0.4 ^{DE}	52.4 \pm 0.8 ^{CD}

@ *Aquarius paludum*, *Gerris lacustris*, *G. odontogaster* and *Limnopus rufoscutellatus*; + *Notonecta reuteri*, *N. glauca* and *N. lutea*; * *Sigara fallenoidea* and *S. limitata*; # *Paracorixa concinna*, *Corixa dentipes* and *Callicorixa gebleri*