Waterbugs (Heteroptera: Nepomorpha, Gerromorpha) as sources of essential n-3
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 waterbugs 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 waterbugs 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.

Introduction

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

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

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

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

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

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

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

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

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

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

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

Materials and methods

Study area and sampling sites

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

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

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

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

Insect sampling

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

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

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

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

Biochemical analyses

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

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

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

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

Data and statistical analyses

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

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

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

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

Results

Biomass and species composition of waterbugs

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

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

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

FA composition of waterbug families

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

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

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

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

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

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

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

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

FA contents of waterbugs

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

Discussion

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

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

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

We found that life stage-related differences in FA composition and contents were prominent among Notonectidae, which likely shifted their diet during development, but varied 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.

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

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

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

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

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

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

Among the studied Heteroptera taxa and other aquatic insects, corixids appeared to be the 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.

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Figure legends

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

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

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

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

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

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

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

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

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 coleoptrata</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. coleoptrata</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

Table 2 Results of a discriminant analysis for the FA composition (% of the total) of waterbug adults in Central Siberia. FAs 16:1n-9 and 18:1n-7 were excluded from the model because of 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 *Limnporus rufoscutellatus*; + *Notonecta reuteri*, *N. glauca* and *N. lutea*; * *Sigara fallenoidea* and *S. limitata*; # *Paracorixa concinna*, *Corixa dentipes* and *Callicorixa gebleri*