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Biological aspects of the associations of biting midges (Diptera: Ceratopogonidae) in two saline rivers of the Elton Lake Basin, Russia

Larisa V. ~~Golovatyuk~~[~~1~~]*Golovatyuk*[A,D], *Tatiana D. ~~Zinchenko~~*[~~1~~]*Zinchenko*[A], *Nadezhda N. Sushchik*[~~2,3~~B,C], *Galina S. ~~Kalachova~~*[~~2~~]*Kalachova*[B], *and Michail I. Gladyshev*[~~2,3~~B,C]

[~~1~~]~~Institute~~ [A]Institute of Ecology of the Volga River Basin, of Russian Academy of Sciences, Komzina str. 10, Togliatti, 445003, Russia. ~~E-mail: gollarisa@mail.ru~~

[~~2~~ B]Institute of Biophysics of Federal Research Center 'Krasnoyarsk Science Center' of Siberian Branch of Russian Academy of Sciences, Akademgorodok, Krasnoyarsk 660036, Russia.

[~~3~~]~~Siberian~~ [C]Siberian Federal University, Svobodny av. 79, Krasnoyarsk, 660041, Russia.

[D]Corresponding author. Email: gollarisa@mail.ru

Abstract. We studied species composition, density, biomass and production of larvae of the family Ceratopogonidae in two saline rivers (Volgograd region, Russia). Ceratopoponids make up an important part of macroinvertebrate community in these rivers. Average monthly production (dry weight) of ceratopogonid larvae in the rivers was 3.5–~~–~~4.8 g m^{-2} $month^{-1}$ in May~~,~~ and ~~in August it was around ~~~0.9 g m^{-2} $month^{-1}$ in August. For the first time, feeding spectra of ceratopogonid larvae, *Palpomyia schmidti* Goetghebuer, 1934, was studied using fatty acid analyses. The larvae of *P. schmidti* appeared to selectively consume diatoms and other algae and to avoid bacteria and decomposed dead organic matter (detritus) of low nutritive quality.

TOC Option 1. We studied species composition, density, biomass and production of biting midges in two saline rivers (Volgograd region, Russia). For the first time, feeding spectra of biting midges, *Palpomyia schmidti* Goetghebuer, 1934, was revealed using fatty acid analyses. The larvae of *P. schmidti* appeared to selectively consume diatoms and other algae but avoid bacteria and decomposed dead organic matter (detritus) of low nutritive quality.

TOC Option 2. We studied species composition, density, biomass and production of biting midges in two saline rivers (Volgograd region, Russia). They are a substantial seasonal food source for birds in this arid region. Average monthly production of biting midges during the study period in the saline rivers was much higher than annual production in some fresh rivers and lakes of world. For the first time, feeding spectra of one of the species of biting midges was studied using fatty acid analyses.

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Additional keywords: biomarker fatty acids, ceratopogonid larvae, ~~Saline~~ saline rivers~~, Ceratopogonid larvae~~, ~~Secondary~~ secondary production~~, . Biomarker fatty acids~~

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Introduction

Saline rivers are widespread in arid areas of the world and play a major role in maintaining biodiversity in these regions (Gallardo-Mayenco 1994; Moreno *et al*. 2001; Piscart *et al*. 2005~~; Velasco *et al*. 2006~~; Palmer and Bennett 2006; Velasco *et al*. 2006). Riparian fringes serve as corridors and refugia in arid environments and could ameliorate ecological issues related to land use and environmental quality (Naiman *et al*. 1993; Simmons and Seymour 2010).

Saline rivers also are particularly interesting because of their halotolerant or halophilic biota that often have restricted geographical ranges and occur as highly isolated populations. A long period of evolutionary adaptation to the saline environment has created endemism of the fauna of saline rivers (Velasco *et al*. 2006).

Saline rivers are highly productive ecosystems exporting matter and energy to low-productive terrestrial ecosystems of arid zones (Ballinger and Lake 2006; Zinchenko *et al*. 2014). Aquatic productivity is transferred to terrestrial ecosystems by invertebrates, amphibians, reptiles, birds and mammals (Ballinger and Lake 2006). Despite these, our knowledge on a quantitative role of aquatic macroinvertebrates in the transfer of matter and energy in arid areas ~~are~~ is limited.

Larvae of Ceratopogonidae are one of the most important components of macroinvertebrate community of saline rivers in the arid basin of the hypersaline Lake Elton (Zinchenko and Golovatyuk 2010). Average sum density and biomass of ceratopogonid larvae is more than 25% of total density and biomass of all zoobenthos taxa in the Chernavka River and the Solyanka River (Zinchenko and Golovatyuk 2010).

Larvae of the family Ceratopogonidae (Diptera) are an important component of macroinvertebrate communities in various fresh, brackish and saline waters. Biting midges include a high number of species and often have a mass development (Glukhova Przhiboro 1995; Blackwell 2001; Borkent and Spinelli 2007; Borkent 2015). Larvae of biting midges are a substantial food source for other invertebrates, fish and birds in different water bodies (Borkent and Spinelli 2007; Andrei *et al*. 2009; Sukharev 2015). Although ceratopogonid larvae are believed to play an important ecological role, this role is poorly studied yet (Borkent~~,~~ 2007). For instance, feeding spectra of Ceratopogonidae larvae are practically unknown (Glukhova 1979; Ronderos and Diaz 2002). Besides, there is a paucity of information on ceratopogonid production (Bowen 1983; Golubkov 2000).

The saline rivers in the basin of Lake Elton are used by several migratory birds as stopover sites. The birds fly over long distances to use the rich seasonal source of food in these saline rivers (Kasatkina and Shubin 2012; Sukharev 2015). Shorebirds *Charadrius hiaticula*, *Ch. alexandrinus*, *Calidris alpina*, *C. ferruginea*, *C. minuta*, *C. alba* and *Limicola falcinellus* consume larvae of Diptera, including ceratopogonid larvae in saline rivers in the basin of Lake Elton (Sukharev 2015). Therefore, it is important to evaluate the production of ceratopogonid larvae in the feeding grounds of migratory birds.

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One of the main components, which determines a nutritive value of aquatic prey for birds, especially during migrations, is the content of polyunsaturated fatty ~~acids (PUFAs) (~~; Maillet and Weber 2006~~;~~ 2007; Klaiman *et al*. 2009; Rodríguez-Turienzo *et al*. 2010; Gladyshev *et al*. 2016; Twining *et al*. 2016). Indeed, PUFAs are known to be 'pacemakers' for the metabolism of animal cells, i.e. they enhance activity of membrane-bound enzymes in high-frequency contraction muscles and activate a lipid fuel pathway from adipose tissue, which are crucial for long-distance migratory birds (Infante *et al*. 2001; Hulbert *et al*. 2002; Turner *et al*. 2003; 2005; Hulbert 2007; Weber 2011). Thus, production and PUFA ~~contents~~ concentrations of ceratopogonid larvae may be of considerable importance concerning the subsidy of matter and energy for terrestrial consumers in the arid landscape.

The aim of our work was to report the species composition, biomass and production of ceratopogonid larvae in two saline rivers in the basin of Lake Elton along with physic~~al~~ o-chemical properties of the rivers. Additionally, we evaluated the fatty acid composition and ~~content~~ concentration of the essential PUFAs~~s~~ in the biomass of ceratopogonid larvae~~,~~ of *P. ~~schmidti~~ schmidti*, so as to determine methods of larval food consumption and used the fatty acid biomarkers to trace their food sources.

Materials and methods

Study area

The Chernavka River and the Solyanka River are saline rivers in the basin of hypersaline Lake Elton, located 49°13′N 46°40′E in the Volgograd region of the Russian Federation (Fig. 1). The area belongs to the zone of desert steppes. The air temperature in summer is up to 41.~~1~~ 1°C (Zinchenko and Golovatyuk 2010) and annual rainfall less than 280 mm (http://www.weatherbase.com/weather).

The length of the Chernavka River is 2 km, with a catchment area of 18.4 ~~km^2~~ km^2, and the Solyanka River is 6.7 km long with a catchment area of 11.2 km^2. The rivers have permanent flow in the middle and lower reaches, ~~but~~ whereas the flow is intermittent at the headwater, especially in summer. Rivers are shallow. The maximum depth of the rivers is 0.8 m. Bottom sediments are black silt or silty sand. Values of main physico-chemical parameters in the Chernavka and Solyanka rivers are given in Table 1.

Sampling

Samples were collected from the Chernavka River in April 2007, in May 2011, 2014 and 2015, in August 2007–2015 and in September 2008, and the Solyanka River in May 2011, 2014 and 2015, in August 2007–2008, 2010–2015 and in September 2008. Two sites were chosen in each river, namely, in the middle section and in the mouth (Fig. 1). Quantitative samples were taken ~~by~~ with an Ekman-type grab sampler (25 cm^2), ~~in~~ with eight replicates, ~~(8 ×)~~ and/or ~~by~~ with a handle blade trawl (Zinchenko *et al*. 2014). Sampling was ~~done~~ undertaken in August and May when ceratopogonid larvae are expected to have the highest density and are actively consumed by migratory birds. Additionally, they were collected from the habitats where they were most likely to occur (on silty sand). Samples were preserved in 4%

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formaldehyde. The density and biomass of macroinvertebrates in each section ~~was~~ were estimates from two replicate samples. Total number of samples was 60.

Fatty acid analysis was conducted ~~based on~~on the basis of a previously established method for Diptera larvae (Zinchenko *et al.* 2014). Using this method, instar IV of *Palpomyia schmidti* larvae were collected in August 2014 from the mouth of Chernavka River and Solyanka River. The live animals were placed into beakers immediately after sorting and were allowed to empty their guts for several hours. To form a biochemical sample, 20–30 live individuals were pooled, their body surfaces were gently wiped with filter paper, and the animals were weighed. Immediately after weighing, the animals were placed in a chloroform–methanol mixture (2 : 1, v/v), and samples were frozen at –20°C until further analyses. Silt (sediments) samples (August, 2014) were taken simultaneously with those of the zoobenthos by using the same samplers. They were placed in the chloroform–methanol mixture and frozen, and then analysed similarly to the zoobenthos samples. For total organic carbon analyses, samples that included 20–30 larvae were air-dried for 2 days, sealed in foil and kept in a desiccator for further elemental analysis. The total organic carbon was measured with a Flash EA 1112 NC Soil/MAS 200 elemental ~~analyzer~~ analyser (ThermoQuest, Italy), as described by Gladyshev *et al.* (2007).

Calculation of production

Daily production P (g m^{-2} day^{-1}) of ceratopogonid larvae was estimated as:

$$P = GB \quad (1)$$

where G (day^{-1}) is the daily instantaneous growth rate and B (g m^{-2}) is the biomass, dry weight. Values of G were calculated according by the following formula:

$$G = 0.0041e^{0.116T} \quad (2)$$

where T (°C) is temperature (Golubkov 2000).

On each date, temperature ~~were~~ was measured at ~~15~~ 15-min intervals during 24 h (WTW, MultiLine). The average temperature was used for the calculation.

Monthly production was calculated by multiplying average daily production for all sampling dates by 30 days (April, September) and 31 days (May, July, August). Average monthly production of ceratopogonid larvae was calculated for the growing season.

~~Chlorophyll~~ Chlorophyll-~~«~~*a*~~»~~ concentration was measured to assess the relationship between primary production and the production of ceratopongonid larvae. ~~Chlorophyll~~ Chlorophyll-~~«~~*a*~~»~~ concentration was determined in the Chernavka River and the Solyanka River in May 2011, 2012, in August 2008, 2010, 2012 and in September 2008, by spectrophotometry, using extraction in acetone.

Fatty acid analysis

A detailed description of the fatty acid analysis is given elsewhere (Sushchik *et al.* 2013). Briefly, before the analysis, a fixed volume of internal standard solution (non-adecanoic acid, Sigma, USA) was

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added to a sample. Then, lipids were extracted by a modified Folch method with a mixture of chloroform–methanol (2 : 1, v/v) three times, simultaneously with mechanical ~~homogenization~~ homogenisation of the tissues with glass beads. Methyl esters of fatty acids (FAMEs) were prepared in a mixture of methanol–sulfuric acid (20 : 1, v/v) at 85°C for 2 h. FAMEs were then analysed ~~using~~ with a gas chromatograph–mass spectrometer (~~model~~ Model 6890/5975C, Agilent Technologies, USA) equipped with a 30-m-long, 0.25-mm ~~internal~~ internal-diameter HP-FFAP capillary column. Data were collected and analysed using the GC ChemStation program (Agilent Technologies~~, USA~~). Peaks of FAMEs were identified by their mass spectra, comparing them to those in the integrated NIST-2005 database and to those of available authentic standards (Sigma~~, USA~~). To determine ~~double~~ double-bond positions in monoenoic and polyenoic acids, gas chromatography–mass spectrometry ~~GC–MS~~ of dimethyloxazoline derivatives of ~~FA~~ fatty acids (FAs) was used (Makhutova *et al.*~~,~~ 2003). Each sample of ~~fatty acids~~FA was analysed in a single replicate. Ten replicate injections of a standard gave a coefficient of variation of the response values, i.e. analytical precision~~,~~ 0.6%. The FAMEs were quantified according to the peak area of the internal standard, i.e. non-adecanoic acid. For the following considerations, the biochemical data were presented in the following several ways, according to the aim of the analyses: (1) for ~~revealing~~ showing the feeding spectra~~,~~ — as a percentage of total FAs; (2) for inter-habitat comparison~~—~~, as ~~mg~~ milligrams of FA per gram of wet weight; (3) for calculation of highly unsaturated fatty acid (HUFA) production and export on the basis of ceratopogonid production and potential emergence~~—~~, as ~~mg~~ milligrams of FA per gram of dry weight.

We used a conventional notation for ~~fatty acid~~FAs of the form *A*:*B*n–*X*, where *A* gives the number of carbon atoms in the molecule, *B* represents the number of double carbon–carbon bonds and *X* gives the position of the first double bond, counting from the methyl end of the molecule.

Statistics

For comparisons of mean values of percentages and ~~contents~~ concentrations of each FA in bottom sediments and in biomass of the larvae in the rivers, ANOVA with Fisher's l.s.d. *post hoc* test was used. To ~~reveal~~ show putative differences in the overall composition of FAs of bottom sediments and larvae, multivariate canonical correspondence analysis (CCA) was conducted. Percentages of 25 FAs (see their list below in Results) were used as variables, and samples of bottom sediments (number of samples, n = 4) and larvae (n = 8) were the cases. The above statistical tests were ~~carried out~~performed conventionally (Legendre and Legendre 1998), using STATISTICA software, version 9.0 (StatSoft~~,~~ Inc. Tulsa, OK, USA).

Besides, two-way ANOVAs were conducted to assess differences in production of ceratopogonid larvae among rivers and seasons, using statistical environment R v. 3.02.

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Results

Species composition, biomass and production

The macroinvertebrate community was characterised by a low species richness. In the Chernavka River and in the Solyanka River, 25 and 23 taxa, respectively, were found during the study period. Among them, there were two species (taxa) of Ceratopogonidae. Larvae of *Palpomyia schmidti* had high frequency values (76–84%), whereas the frequency of *Culicoides* sp. was only 5% (Table 2). Larvae of *P. schmidti*, together with larvae of *Chironomus salinophilus* Zinchenko, Makarchenko et Makarchenko and *Chironomus salinarius* Kieffer 1915, have the highest density and biomass in the total benthos of Chernavka and Solyanka rivers (Zinchenko and Golovatyuk 2010; Zinchenko *et al.* 2014; Table 2, Fig. 2). In the Chernavka River, the average density of *P. schmidti* was 25% of the total density of all zoobenthos taxa, and in the Solyanka River it was 42% (Fig. 2). The density of 48 000 individuals m^{-2} recorded in the Chernavka (28 May 2015) seems to be the maximum value recorded for larvae of this species in saline rivers in the basin of Lake Elton (Fig. 3). In the Chernavka River, average biomass of *P. schmidti* was 26% of the total biomass of all zoobenthos taxa, and in the Solyanka River it was 30%

Results of calculations of daily and monthly production of ceratopogonids are given in Table 3. Daily production was lowest in the Chernavka River in August 2007 and September 2008 and highest in the same river in May 2015 (Table 3).

Average daily production in the Chernavka River in August 2007–2015 was 0.030 g m^{-2} day^{-1} and in May 2011, 2014, 2015 it was 0.156 g m^{-2} day^{-1}. Average daily production in the Solyanka River in August 2008–2015 was 0.029 g m^{-2} day^{-1} and in May 2011, 2014, 2015 it was 0.111 g m^{-2} day^{-1}. Average monthly production of ceratopogonid larvae in the Chernavka River in August (2007–2014) was 0.91 g m^{-2} $month^{-1}$ and in May (2011, 2014, 2015) it was 4.85 g m^{-2} $month^{-1}$. Average monthly production of ceratopogonid larvae in the Solyanka River in August (2007, 2008, 2010–2014) was 0.91 g m^{-2} $month^{-1}$ and in May (2011, 2014, 2015) it was 3.50 g m^{-2} $month^{-1}$.

The two-way ANOVA test showed significant ($F = 9.33$, $P < 0.01$) differences in the monthly production of ceratopogonid larvae among months (May, August), $F = 9.33$, $p < 0.01$. The influence of the River's river factor (Chernavka and Solyanka Riversrivers), as well as the interaction Monthmonth: × River river was statistically insignificant (Table 4).

Average monthly production during the study period in the Chernavka River and in the Solyanka River was 1.61 g m^{-2} $month^{-1}$ and 1.43 g m^{-2} $month^{-1}$, respectively.

Concentration of chlorophyll «*a*» in saline rivers was high (up to 341 mg L^{-1} in the Solyanka River) (Table 1).

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Fatty acids

Fatty acid composition of the larvae of ~~Ceratopogonidae, *P. schmidti*,~~ Ceratopogonidae, differed significantly from that of bottom sediments in both rivers. Larvae had in their bodies significantly higher percentages of 16:3n-4, 16:3n-3, 16:4n-1, 18:2n-6, 18:3n-3, 18:4n-3, 20:5n-3 and 22:6n-3 (Table 5). In turn, ~~levels~~ percentages of ai15:0, 15:0, i16:0, 16:0, 17:0 and 24:0 were higher in the bottom sediments than in ~~larvae~~ larval bodies (Table 5). Besides, in the Chernavka River, there were significantly higher percentage of 20:4n-6 and significantly lower percentages of 18:0 and 18:1n-9 in the biomass of ceratopogonids, than ~~those~~ there were in sediments (Table 5). Percentage of 18:3n-3 in the larvae from the Chernavka River was significantly higher, than that in the larvae from the Solyanka River (Table 5). Similarly, the percentage of 18:3n-3 in the sediments from the Chernavka River was significantly higher, than that in the sediments from the Solyanka River (Table 5).

The a~~A~~verage ~~content~~ concentration (mg g^{-1} of wet weight) of the sum of ~~fatty acid~~FAs in the biomass of larvae ~~from both rivers~~ did not differ significantly between the two rivers ~~from each other~~, ~~as well as those~~ neither did that of sediments ~~from~~ between these rivers (Table 5). The ~~contents~~ concentrations of FAs in the biomass of larvae were more than one order of magnitude higher than those in sediments (Table 5).

According to CCA (Fig. 4), the overall ~~fatty acid~~FA compositions of larvae from both rivers were very close to each other. In contrast, the overall ~~fatty acid~~FA compositions of sediments from the two rivers differed ~~re~~mark~~able~~dly in the second dimension ~~due to~~because of the percentage of FA 24:0 (Fig. 4), which was significantly higher in the sediments of the Solyanka River than ~~that~~ in those of the Chernavka River (Table 5). The differences in the overall FA composition between larvae and sediments were provided mainly by differences in the first dimension ~~between~~ among the percentages of ai15:0, 16:1n-9 and i16:0, on the one hand, and the differences of 16:3n-3, 18:4n-3, 22:6n-3, 20:4n-3, 20:4n-6, 18:3n-6, 16:4n-1 and 20:5n-3, on the other hand (Fig. 4).

Average ~~content~~ concentration of the essential long-chain ~~highly unsaturated fatty acid (~~HUFA~~)~~, 20:5n-3, in larvae was 2.36 ± 0.50 mg g^{-1} of wet weight, and that of 22:6n-3 was 0.04 ± 0.01 mg g^{-1}. Average ~~content~~ concentration of organic carbon in larvae biomass was 49.7 ± 0.5-% of dry mass, and the ~~content~~ concentration of organic nitrogen was 9.4 ± 0.2-%.

Discussion

Distribution and ecology of the dominant species ~~Palpomyia~~ P. schmidti in saline rivers of Lake Elton's basin

The biting midge~~s~~ *P. schmidti* is a widespread species in the Palearctic, with occurrence in Hungary (Goetghebuer 1934b), Spain (Delécolle *et al.* 1997), Slovakia, Russia, Azerbaijan, Tadjikistan, Kazakhstan, Iraq, Iran, Mongolia and China (Szadziewski *et al.* 2016). ~~They~~ It also occur~~s~~ in high abundance in the steppe~~s~~ and desert~~s~~ habitats (Szadziewski *et al.* 2016). The larvae usually inhabit ~~in~~ freshwater rivers (Remm 1976). However, like other species of the tribe Palpomyiini, *P. schmidti* is

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tolerant of high salinity. Szadziewski *et al.* (2016) ~~have~~ considered the species to be halobiontic. However, given that this species inhabits rivers of Lake Elton's basin with a salinity of 5.8 ~~to~~ and 31.7 mg L^{-1} (The Khara River and Chernavka River respectively~~) (~~; Zinchenko, Golovatyuk, 2013), the most likely conclusion is that the species is a euryhalin~~ic~~e with halophilic tendency (i.e. based on Gallardo-Mayenco 1994).

Larvae of *P. schmidti* inhabit ~~in~~ black and grey sandy mud in the saline rivers of Chernavka, Solyanka, Lantsug, Khara and Bolshaya Samoroda of the Lake Elton's basin. They also mass among dense filamentous algae and *Enteromorpha intestinalis*. Larvae occurred at a depth~~s~~ of up to 0.8 m where the water was flowing at 0.01–0.4 m s^{-1}. These larvae were also collected at the bottom of Lake Elton, where the salinity was 112.5 g L^{-1} (Szadziewski *et al.* 2016).

Methods of d~~D~~iet analyses ~~methods~~

A standard method of diet analysis, i.e. visual examination of gut contents under a microscope, is known to have several shortcomings. First, food items without rigid cell walls, such as infusorians and flagellates, are rapidly digested and, thus, are not detected by microscopic examination (Knisley and Geller~~,~~ 1986). Moreover, even diatoms can be broken down to unidentified debris in the alimentary tract of some benthic invertebrates (Quigley and Vanderploeg~~,~~ 1991). Thus, microscopic examination often results in a high percentage of shapeless organic material~~s~~. Second, many ingested microalgae are not digested and assimilated, but remain viable after gut passage (Porter~~,~~ 1976; Gladyshev *et al.*~~,~~ 2000; Kolmakov and Gladyshev~~,~~ 2003). These limitations of standard techniques can be overcome by using biochemical tracers, such as ~~fatty acid~~FAs, which allow the ~~studying~~ study of assimilated food (e.g. Ederington *et al.*~~,~~ 1995; Sushchik *et al.*~~,~~ 2003; Whiles *et al.*~~,~~ 2010; Makhutova *et al.*~~,~~ 2012). Fatty acid tracers (biomarkers) were used in our previous studies to evaluate food sources of chironomid, ephemeropteran and trichopteran larvae (Gladyshev *et al.*~~,~~ 1999; Sushchik *et al.*~~,~~ 2003; Zinchenko *et al.* 2014), which proved to be a superior method in analysing the diet content of ~~aquatic~~ aquatic-insect~~'s~~ larvae. Potential food sources of aquatic invertebrates, bacteria, algae, vascular plants and detritus~~,~~ have specific biomarker ~~fatty acid~~FAs (see below) and give reliable information on assimilated and preferable food.

The differences observed ~~between~~ in the ~~fatty acid~~FA composition ~~of~~ between the larvae and the bottom sediments indicated selective feeding by the species studied, i.e. *P. schmidti*. The larvae had significantly higher ~~levels (~~percentages~~)~~ of 16:3n-4, 16:4n-1, 20:5n-3 and 22:6n-3~~, compared to that of~~ than did the sediments. These FAs are known to be biomarkers of diatom algae (Sushchik *et al.*~~,~~ 2004; Graeve *et al.*~~,~~ 2005). ~~Besides~~In addition, also the ~~levels~~ percentages of 16:3n-3, 18:2n-6, 18:3n-3 and 18:4n-3 ~~also~~ were higher in the bodies of ~~the~~ larvae~~,~~ than ~~that~~ in the sediments. The above FAs are biomarkers of many ~~algae~~ algal taxa (Napolitano~~,~~ 1999; Sushchik *et al.*~~,~~ 2004). In turn, the sediments had significantly higher ~~levels~~ percentages of odd-number and branched ~~fatty acid~~FAs~~,~~ of ai15:0, 15:0 and i16:0 ~~compared to~~than were the ~~level~~ percentages of these acids in the larvae. These odd-number and

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branched FAs are biomarkers of bacteria (Desvilettes *et al.*, 1997; Napolitano, 1999). BesidesIn addition, levels concentrations of saturated acids, 16:0 and 24:0 were higher in the sediments than in the larvae. These saturated FAs are indicators of dead organic matter at a high degree of decomposition (Hama, 1999). Thus, the comparison of levels the concentrations of the above biomarkers in the sediments and in the biomass indicated, that the larvae of *P. schmidti* selectively consumed diatoms and other algae and avoided bacteria and decomposed dead organic matter (detritus) of low nutritive quality.

The dDiet of larvae Ceratopogonidae larvae have has rarely or scarcely been studied. Data on the nutrition of ceratopogonid larvae in the available literature were based on the gut analyses and observations of their food behaviour (Weerekoon, 1953; Hair and Turner 1966; Aussel and Linley 1994). Often, when calculating the production of macrozoobenthos, all the larvae of the tribe Palpomiini are regarded as predators (Smock, and Gilinsky, 1985; Gladden Smock 1990). Basing onOn the basis of a functional morphology, larvae of genus *Palpomyia* can prey on aquatic invertebrates, since because they have a special pharyngeal apparatus, unique for dipterans, which that works like a pump and sucks internal contents of a prey (Weerecoon, 1953; Glukhova 1979). In contrast, basing onon the basis of the fatty acidFA analysis, we found that larvae of *P. schmidti* selectively consume microalgae and can be assigned to a collector-gatherer group.

For freshwater zoobenthos, carbon is known to be, on average, 45% of dry biomass (Strayer and Likens, 1986). According to our data, the average percentage content concentration of carbon in the larvae of Ceratopogonidae that we studied was higher, namely ~50%.

The average value concentration of the essential PUFA, 20:5n-3, content for the *P. schmidti* larvae examined, was 2.4 mg g^{-1} wet weight, falls falling in the range of the contents concentration for larvae of another Diptera family, i.e. Chironomidae, of 0.8–4.0 mg g^{-1},; these larvae which iinhabited the Chernavka River and were are thought to be a valuable food source for the migratory birds regarding the PUFA content concentration (Zinchenko *et al.* 2014). Thus, larvae of *P. schmidti* in the studied salt rivers, due owing to the high PUFA contentsconcentrations, appeared to also have a high nutritive value for migratory birds.

Biomass and production

Average ceratopogonid biomasses in the Chernavka and Solyanka Rivers rivers was were similar (Table 3). Also, there are no statistically significant differences in the production of ceratopogonid larvae between these rivers (Table 4). Since Because these two rivers have nearly similar hydrological and hydrochemical parameters (Table 1), it is not of the least surprising, that their benthic communities have comparable larvae larval biomass and production (Zinchenko *et al.* 2014).

There is a lack of data on the production of ceratopogonid larvae in rivers and lakes (Golubkov 2000). We do not have enough data to calculate the annual production of ceratopogonids in the studied rivers, but we can use the average monthly production during the study period, namely, 1.61 g m^{-2} $month^{-1}$ in the Chernavka River and 1.43 g m^{-2} $month^{-1}$ in the Solyanka River, for comparison with annual production

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from the other rivers and lakes. Average monthly production of ceratopogonids during the study period in the saline rivers was much higher than was annual production in ~~the~~ some rivers and lakes. For example, the annual production of *Palpomyia* spp. complex in the Colliers Creek floodplain (south-eastern USA) ~~was~~ is 0 0.176 g m^{-2} $year^{-1}$ and, in the Buzzards Branch floodplain (south-eastern USA), it ~~was~~ is 0.019 g m^{-2} $year^{-1}$ (Gladden and Smock 1990). Also, annual production of ceratopogonid larvae in the Mayfield Creek River (USA, Alabama) ~~was~~ is 0.366 g m^{-2} $year^{-1}$ (Wright~~,~~ 2011). Production of predaceous midges of the tribes Sphaeromiini and Palpomyiini collected from sublittoral and littoral depths in Lake Norman, North Carolina, ~~were~~ ranged from 0.002 to 0.022 g m^{-2} $year^{-1}$ (Bowen 1983). ~~Besides~~ Furthermore, production of *Bezzia* spp. in the eutrophic Lake Batorin in western Russia ~~was~~ is 0.05 g m^{-2} $year^{-1}$ (Winberg 1971).

It is also worthwhile to compare the average monthly production of ceratopogonid larvae in August in the Chernavka River, with the production of chironomid larvae in this river. The monthly production of chironomid larvae in August, i.e. 16.7 g m^{-2} $month^{-1}$ (Zinchenko *et al*. 2014), ~~was~~ is ~~eighteen~~ 18 times ~~more than~~ the production of ceratopogonid larvae.

Biomass and production of microalgae in Chernavka and Solyanka rivers was high according to the values of chlorophyll ~~«~~*a*~~»~~. These great sources of food are one of the reasons for the high production of ceratopogonid larvae in saline rivers (Bowen 1983).

Conclusions

Ceratopogonid larvae in the Chernavka River and in the Solyanka River had a high average monthly production in the study period of 1.61 ~~g m^{-2} $month^{-1}$~~ and 1.43 g m^{-2} $month^{-1}$ respectively, which ~~was~~ were comparable to the annual production in other rivers and lakes.

The highest production of larvae in these rivers was in May (8.78 g m^{-2} $month^{-1}$ in the Chernavka River~~,~~ in 2015). The larvae of *P. schmidti* selectively consumed algae, primarily diatoms, and ignored bacteria and detritus, ~~which~~ as was ~~revealed~~ shown by ~~FA~~ FA-biomarker analysis. The selection of high-quality food provided the larvae with the high ~~content~~ concentration of essential PUFAs. In turn, the high ~~content~~ concentration of PUFAs in the larvae~~'s~~ biomass ~~meant~~ indicated their high potential nutritive value for migratory birds~~, which~~ that had a stopover at the two salt rivers.

Acknowledgements

This study was partly supported by the grants from Russian Foundation for Basic Research (RFBR), numbers 13-04-00740, ~~number~~ 15-04-03341~~,~~ and 17-04-00135. The study was also supported by Russian Federal Tasks of Fundamental Research (project ~~No~~No~~n~~number~~.~~ 51.1.1), by Federal Tasks of Ministry of Education and Science of the Russian Federation for Siberian Federal University (project number 6.1504.2017/PCh) and by the Council on grants from the President of the Russian Federation for support of Leading Scientific Schools (~~grant~~ grant numberNSh-9249.2016.5)

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Примечание [bul07454]: eXtyles does not recognize the journal "Boletín del Museo Nacional de Historia Natural de Paraguay" (in reference "Ronderos, Díaz, 2002"). If this is a valid journal title, please advise.

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Примечание [bul07458]: Reference "Szadziewski, Dominiak, Lewanczyk, 2009" is not cited in the text. Please add an in-text citation or delete the reference.

Примечание [bul07459]: eXtyles does not recognize the journal "Polish Journal of Entomology" (in reference "Szadziewski, Dominiak, Lewanczyk, 2009"). If this is a valid journal title, please advise.

Примечание [bul07460]: CrossMark reports an erratum (or similar issue) with reference "Twining, Brenna, Lawrence, Shipley, Tollefson, Winkler, 2016". The CrossMark type is "correction". Additional information can be found at http://dx.doi.org/10.1073/pnas.1616962113.

Примечание [bul07461]: Please provide a publisher and publication location, and please verify information cited is still available at the listed web address (and provide a date you have done this) or, if it is not any more, when it last was accessed in the preparation of your paper.

Publisher: CSIRO; Journal: MF:Marine and Freshwater Research
Article Type: research-article; Volume: ; Issue: ; Article ID: MF17125
DOI: 10.1071/MF17125; TOC Head:

1 <jrn>Weber, J.-M. (2011). Metabolic fuels: regulating fluxes to select mix. *The Journal of Experimental Biology*
2 **214**, 286–294. doi:10.1242/jeb.047050</jrn>

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12 Received 3 May 2017, accepted 12 November 2017

Примечание [bul07462]: Reference "Weerecon, 1953" is not cited in the text. Please add an in-text citation or delete the reference.

Примечание [bul07463]: eXtyles does not recognize the journal "Arid Ecosystems" (in reference "Zinchenko, Golovatyuk, 2010"). If this is a valid journal title, please advise.

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Table 1. Physico-chemical parameters and chlorophyll-*a* at the two sampling sites in the Chernavka and Solyanka rivers in the study period

Parameter	Chernavka	Solyanka
Depth (m)	0.05–0.80	0.05–0.80
Width in mouth (m)	7.0–8.0	4.0–5.0
Current velocity (m s^{-1})	0.05–0.40	0.02–0.40
Temperature (°C)	12.5–31.5	15.1–30.2
pH	6.5–8.4	6.9–8.4
Dissolved O_2 (mg L^{-1})	2.9–33.8	2.6–35.0
Salinity (g L^{-1})	17.2–31.7	24.0–29.0
Chlorophyll *a* (mg L^{-1})	13.0–221.0	7.0–341.0
Na^{+}+K^{+} (g L^{-1})	3.43–10.53	7.59–9.41
Ca^{2+} (g L^{-1})	0.30–1.60	0.72–1.22
Mg^{2+} (g L^{-1})	0.04–1.22	0.51–0.96
Cl^{-} (g L^{-1})	10.24–19.17	15.13–17.40
SO_4^{2-} (g L^{-1})	0.40–0.96	0.09–0.84
HCO^{-} (g L^{-1})	0.21–0.45	0.09–0.41
Total P (m g L^{-1})	0.053–0.250	0.131–0.421
NH_4^{+}-N (m g L^{-1})	30.80–45.92	13.10–45.30
NO_3^{-}-N (m g L^{-1})	0.125–2.386	0.387–6.580

Table 2. List of benthic macroinvertebrates, their frequency (*F*, percentage of samples) and maximum biomass (MB, g m^{-2}) in the Chernavka River and Solyanka River during the study period, in the basin of Lake Elton, Russian Federation

Taxon	Chernavka		Solyanka	
	F	MB	*F*	MB
Oligochaeta				
Enchytraeus issykkulensis	7	0.4		
Henlea stolli	2	0.1		
Paranais simplex	7	0.1	6	0.04
Branchiopoda				
Artemia sp.			3	0.1
Insecta				
Heteroptera				
Sigara nigrolineata	5	1.0		
Sigara assimilis	21	6.0	3	0.6
Sigara lateralis			3	0.9
Sigara sp.	14	0.2	12	1.2
Coleoptera				
Berosus bispina	18	1.8	3	1.6
Berosus fulvus	5	4.6	15	3.0
Berosus frontifoveatus	5	1.9		

Примечание [i64]: Or ‘salt concentration’?

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Примечание [i65]: AU: please adjust the values so that they have the same number of decimal points (either 1 or 2 decimal points) in both columns for MB.

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Berosus sp.	11	1.3		
Enochrus quadripunctatus	16	0.1		
Enochrus sp.	2	0.1	15	0.4
Helochares obscurus	2	0.1		
Hygrotus enneagrammus	9	0.4	9	1.3
Ochthebius sp.	2	0.1		
Paracymus aeneus			6	0.1
Diptera				
Psychodidae				
Psychoda sp.			3	0.1
Chaoboridae				
Chaoborus sp.			3	0.1
Culicidae				
Aedes sp.	2	0.1	3	0.04
Culex sp.			9	0.03
Ceratopogonidae				
Culicoides sp.	5	0.2	3	0.1
Palpomyia schmidti	84	40.6	76	16.0
Chironomidae				
Cricotopus salinophilus	98	61.2	94	12.7
Glyptotendipes salinus			3	0.6
Chironomus salinarius	41	69.5	26	8.2
Stratiomyidae				
Nemotelus sp.	7	0.1	3	0.9
Odontomyia sp.	11	0.5	6	0.02
Stratiomys sp.	2	0.9	12	13.6
Ephydridae				
Ephydra sp.	32	3.2	3	10.5
Muscidae				
Lispe sp.	2	0.01		

Table 3. Average biomass (*B*, g m^{-2}, dry weight) of ceratopogonid larvae, water temperature (*T*, °C ~~°C~~), daily instantaneous growth rate (*G*, day^{-1}), daily production (~~*P*~~$_{day}$ *p*$_{day}$, g m^{-2} day^{-1}, dry weight) and monthly production (~~*P*~~$_{month}$ *p*$_{month}$, g m^{-2}, month^{-1} dry weight) and their ~~(~~means ± s.e.~~)~~ in the Chernavka River and the Solyanka River, in the basin of Lake Elton, Russian Federation

Date	*B*	*T*	*G*	p_{day}	p_{month}
Chernavka					
24-Apr-2007	0.170	13.2	0.02	0.003	0.10
15-Aug-2007	0.008	23.4	0.11	0.001	0.03
13-Aug-2008	0.131	20.2	0.04	0.006	0.17
25-Sep-2008	0.005	12.5	0.02	0.001	0.01

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Примечание [bul07466]: Please confirm change of *P* to *p* to differentiate this variable from probability.

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Отформатировано: Шрифт: 11 пт, подстрочные, Узор: Нет

Отформатировано: Шрифт: 11 пт, курсив, Узор: Нет

Отформатировано: Шрифт: 11 пт, подстрочные, Узор: Нет

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20-Aug-2009	0.126	21.9	0.05	0.006	0.20
19-Aug-2010	0.360	23.8	0.06	0.023	0.71
26-May–2011	1.344	26.9	0.09	0.122	3.79
21-Jul-2011	0.328	23.8	0.06	0.021	0.65
18-Aug-2011	0.464	25.2	0.07	0.035	1.08
15-Aug-2012	0.130	26.8	0.09	0.012	0.36
14-Aug-2013	0.352	19.0	0.04	0.013	0.40
14-May–2014	0.720	26.7	0.09	0.064	1.98
13-Aug-2014	1.570	26.7	0.09	0.140	4.33
28-May–2015	4.060	24.6	0.07	0.283	8.78
Mean for May	2.04 ± 1.03	26.1 ± 0.74	0.083 ± 0.007	0.156 ± 0.066	4.85 ± 2.04
Mean for August	0.39 ± 0.18	23.4 ± 1.01	0.069 ± 0.009	0.030 ± 0.016	0.91 ± 0.50
Mean for all months	0.70 ± 0.29	22.5 ± 1.27	0.064 ± 0.007	0.052 ± 0.021	1.61 ± 0.66
Solyanka					
16-Aug-2007	0.060	24.8	0.07	0.004	0.13
18-Aug-2008	0.060	26.3	0.08	0.005	0.16
25-Sep-2008	0.334	15.1	0.02	0.008	0.23
21-Aug-2010	0.522	20.6	0.04	0.023	0.71
21-Jul-2011	0.124	24.1	0.07	0.008	0.25
17-Aug-2011	1.840	24.7	0.07	0.130	4.03
26-May–2011	0.048	25.3	0.08	0.005	0.12
15-Aug-2012	0.148	25.3	0.08	0.011	0.35
15-Aug-2013	0.324	25.1	0.07	0.024	0.74
14-May–2014	1.460	22.2	0.05	0.077	2.40
14-Aug-2014	0.090	25.6	0.08	0.007	0.22
27-May–2015	2.400	28.2	0.11	0.253	7.86
Mean for May	1.30 ± 0.68	25.2 ± 1.73	0.080–0.017	0.111 ± 0.074	3.50 ± 2.30
Mean for August	0.70 ± 0.29	24.6 ± 0.70	0.070 ± 0.005	0.029 ± 0.017	0.91 ± 0.53
Mean for all months	0.62 ± 0.23	23.9 ± 0.97	0.068 ± 0.007	0.046 ± 0.022	1.43 ± 0.68

Table 4. Results of a two-way ANOVA test for significant ~~both~~ main effects (~~Month~~ month and ~~River~~river) and ~~M~~month~~:~~ × ~~River~~ river - interaction on dependent variable monthly production

Parameter	d.f.	Sum square	Mean square	*F*-value	Pr (>*F*)
Month	1	45.184	45.184	9.3263	0.0072
River	1	0.843	0.843	0.1741	0.6817
Month × river	1	2.055	2.055	0.4241	0.5236
Residuals	17	0.0854	0.005	–	–

Table 5. Average values of quantitatively and qualitatively prominent fatty acids (percentage of total ~~FA~~fatty acids) ± ~~standard errors~~s.e., and sum content of ~~FA~~ fatty acids (mg g^{-1}, wet weight) in bodies of *Palpomyia* larvae from the Solyanka River (number of samples, *n* = 2) and from the Chernavka River (*n* = 4), and in bottom sediments of the Solyanka River (*n* = 2) and Chernavka River (*n* = 4), in the basin of Lake Elton, Russian Federation

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Means within a row ~~labelled with~~followed by the same letter or no letters are not significantly different from each other at ~~*p* *P* <~~ = 0.05, after Fisher ~~l.s.d.~~LSD *post-hoc* test for ANOVA~~. When ANOVA is insignificant, letter labels are absent.~~

Примечание [bul07467]: Generally difference is indicated by different letters. Should this be changed to 'Means within a row followed by different letters are significantly different from each other at $P < 0.05$, after Fisher LSD *post hoc* test for ANOVA'?

Fatty acid	*Palpomyia*, Solyanka	*Palpomyia*, Chernavka	Sediments, Solyanka	Sediments, Chernavka
14:0	1.5 ± 0.4a	2.4 ± 0.4ab	3.8 ± 1.2bc	4.5 ± 0.2c
Σ14:1	0.6 ± 0.2a	0.6 ± 0.1a	0.4 ± 0.4ab	0.0 ± 0.0b
ai15:0	0.1 ± 0.0a	0.2 ± 0.0b	0.4 ± 0.1c	0.8 ± 0.0d
15:0	0.4 ± 0.0a	0.5 ± 0.0a	1.1 ± 0.1b	1.1 ± 0.1b
i16:0	0.1 ± 0.0a	0.2 ± 0.0a	0.6 ± 0.1b	0.6 ± 0.1b
16:0	14.2 ± 1.0a	14.9 ± 0.1a	24.6 ± 0.4b	27.4 ± 1.0c
16:1n-9	0.3 ± 0.1a	0.5 ± 0.1a	1.2 ± 0.0ab	1.6 ± 0.4b
16:1n-7	14.1 ± 2.3a	15.4 ± 0.7a	14.0 ± 0.9a	4.8 ± 0.6b
16:2n-4	2.3 ± 0.4a	2.2 ± 0.1a	0.9 ± 0.4ab	0.2 ± 0.1b
17:0	1.2 ± 0.1ac	1.0 ± 0.1a	2.9 ± 0.3b	1.6 ± 0.2c
16:3n-4	1.0 ± 0.2a	0.7 ± 0.0a	0.3 ± 0.1b	0.2 ± 0.1b
16:3n-3	0.4 ± 0.2a	0.4 ± 0.1a	0.0 ± 0.0b	0.0 ± 0.0b
16:4n-1	0.4 ± 0.2a	0.6 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b
18:0	11.1 ± 0.7a	9.4 ± 0.3a	12.5 ± 3.3ab	18.8 ± 2.6b
18:1n-9	9.2 ± 1.2a	8.7 ± 0.4a	13.3 ± 3.0a	25.7 ± 3.4b
18:1n-7	6.0 ± 0.2	7.1 ± 0.6	7.6 ± 1.5	5.4 ± 0.5
18:2n-6	8.2 ± 1.3a	8.2 ± 0.8a	4.1 ± 1.1b	1.6 ± 1.0b
18:3n-3	3.6 ± 0.5a	1.5 ± 0.3b	1.5 ± 0.4b	0.0 ± 0.0c
18:4n-3	1.8 ± 0.3a	1.1 ± 0.4a	0.0 ± 0.0b	0.0 ± 0.0b
20:0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.7	0.3 ± 0.2
20:4n-6	1.2 ± 0.3ab	1.4 ± 0.5b	0.0 ± 0.0a	0.0 ± 0.0a
20:5n-3	15.9 ± 0.9a	16.6 ± 1.2a	2.4 ± 0.4b	0.5 ± 0.3B
22:0	0.6 ± 0.1	0.4 ± 0.1	1.3 ± 0.2	0.7 ± 0.5
24:0	0.2 ± 0.1ac	0.1 ± 0.0a	3.5 ± 0.2b	0.8 ± 0.3c
22:6n-3	0.2 ± 0.1a	0.3 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c
Sum (mg g^{-1})	19.9 ± 9.1a	12.8 ± 3.8a	0.3 ± 0.1b	0.9 ± 0.2b

Fig. 1. Map of the study area.

Fig. 2. Average density (ind~~.~~ividuals m^{-2}) of macrozoobenthos taxa and their percentages in the Chernavka River, in 2007–2008, and Solyanka River, in 2007–2010, in the basin of Lake Elton, Russian Federation.

Fig. 3. Average density of ceratopogonid larvae in different years in the Chernavka and Solyanka rivers, in the basin of Lake Elton, Russian Federation.

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Fig. 4. Canonical correspondence analysis of fatty acid composition (% of the total) of sediments from the Chernavka River (Sc) and the Solyanka River (Ss), and bodies of *Palpomyia* larvae from the Chernavka River (Pc) and the Solyanka River (Ps). , in the basin of Lake Elton, Russian Federation. Percentage of inertia, Dimension 1: 72.86%, %; Dimension 2: 10.01%.