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Saline rivers provide arid landscapes with a considerable amount of biochemically valuable production of chironomid (Diptera) larvae

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Abstract Saline rivers are supposed to be 'hot spots' of high biological productivity in arid landscapes. To test this, we quantified the production of chironomid larvae, because river production is known to be transferred to arid landscapes primarily by birds fed on these larvae. In addition, we studied the potential biochemical quality of the larvae for birds based on the essential highly unsaturated fatty acid (HUFA) contents in their biomass. We studied species composition and measured production of chironomid larvae in two saline rivers (Volgograd region, Russia). We also evaluated the fatty acid composition and contents of the dominant taxa and estimated the flux of HUFA from the studied saline rivers to land via chironomid potential emergence. Average monthly production of

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M. I. Gladyshev · N. N. Sushchik Siberian Federal University, Svobodny av. 79, Krasnoyarsk 660041, Russia chironomids measured for only 1 month, August, was quite comparable to annual production in some freshwater rivers. All the dominant chironomid larvae had comparatively high essential eicosapentaenoic acid contents, especially *Cricotopus salinophilus*, which showed the highest value, reported for Chironomidae. The monthly flux of HUFA from the studied rivers to land due to the chironomid potential emergence was roughly comparable to the global average estimation of annual water–land HUFA export via emerging insects.

Keywords Saline rivers · Chironomid larvae · Secondary production · Essential fatty acids

Introduction

Exchange of energy and nutrients between aquatic and terrestrial ecosystems is increasingly regarded to be necessary for providing general ecological insights and managing these systems for biodiversity and functionality (Ballinger & Lake, 2006; Gratton & Vander Zanden, 2009). In many landscapes, rivers are pivotal environmental components. Since rivers are highly productive, fluxes of energy and nutrients from the channel to the riparian area may exceed terrestrial production in the surrounding landscape, especially in low-productive regions, such as tundra or arid steppes (Ballinger & Lake, 2006; Gratton & Vander Zanden,

2009). In some arid areas, rivers are saline. In saline rivers, aridity and soil salinity diminish the presence of riparian vegetation, which allows light to easily reach the streambed, and thereby saline rivers are autotrophic ecosystems (Millán et al., 2011). However, there is comparatively poor knowledge on the export of biological production from autotrophic saline rivers to riparian ecosystems. Studies that quantify and characterize the routes and mechanisms used to supply the matter and energy from saline rivers to other ecosystems would be of global scientific interest (Millán et al., 2011).

Production of aquatic ecosystems is transferred to land by diverse 'aquatic-terrestrial interface specialists,' such as invertebrates, amphibians, reptiles, birds and mammals (Ballinger & Lake, 2006). In freshwater rivers of temperate regions, all these riparian predators and scavengers are of more or less comparable importance. In contrast to temperate regions, in arid areas namely birds are more abundant and diverse in riparian corridors (Ballinger & Lake, 2006). Many migratory birds use saline waters as stopover sites (Sanchez et al., 2006; Estrella & Masero, 2010; Kasatkina & Shubin, 2012). It is worth mentioning that long-distance migrations are energetically costly, and many migratory shorebirds selectively consume larvae of Chironomidae (Diptera) with high nutritive value in saline waters. This allows the birds to replenish their energy reserves (Sanchez et al., 2006; Estrella & Masero, 2010; Kasatkina & Shubin, 2012). Emerging insects, especially Chironomidae, are also known to be one of the main conduits of aquatic productivity to terrestrial ecosystems (Baxter et al., 2005). Thus, knowledge on production of chironomid larvae in saline rivers is of key importance for quantification of fluxes of matter and energy in both aquatic and terrestrial ecosystems of arid areas.

Besides carbon (energy) and inorganic nutrients (e.g., nitrogen), 'interface specialists' and emerging insects are known to transfer specific biochemicals to terrestrial ecosystems, such as highly unsaturated fatty acids (HUFAs) (Gladyshev et al., 2009, 2011). Recently, HUFAs have come to be recognized as biochemicals essential for animals, including humans, at all trophic levels (Arts et al., 2001). The most physiologically important HUFAs are eicosapentae-noic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). Only some taxa of microalgae, rather than higher plants, can synthesize large amounts of

EPA and DHA de novo (Cohen et al., 1995; Harwood, 1996; Tocher et al., 1998). Therefore, aquatic ecosystems play a unique role in the biosphere as the principal source of HUFA for most animals, including omnivorous inhabitants of terrestrial ecosystems (Gladyshev et al., 2009).

Thus, the goal of our work was to study the species composition of chironomid larvae in two saline rivers and to measure their production. We also aimed to evaluate the fatty acid composition of dominant taxa and to estimate the accumulation of the essential HUFAs in the biomass of chironomid larvae in saline rivers. Additionally, we aimed to use fatty acid biomarkers to trace food sources of chironomid larvae.

Materials and methods

Study area

Two saline rivers, the Lantsug and the Chernavka Rivers, flow into a closed basin of hypersaline Lake Elton, located 49°13'N 46°40'E in the Volgograd region of the Russian Federation (Fig. 1). The climate in the area is arid; air temperature in summer is up to 41.1°C (Zinchenko & Golovatyuk, 2010). Values for the main physical and chemical parameters of the rivers are given in Table 1. Bottom sediments are black silt or silty sand. Water flows of the rivers are regulated by both underground and rain water (Zinchenko & Golovatyuk, 2010). Banks and mouths of the rivers are important landing sites for abundant migratory birds, for instance, little stints Calidris minuta Leisler (Kasatkina & Shubin, 2012). These wader birds feed primarily on chironomid larvae (Kasatkina & Shubin, 2012).

Sampling

Samples were taken from the Lantsug River in 2006–2010 and the Chernavka River in 2007–2010 (Table 2). Due to the rather poor accessibility of the rivers, sampling was primarily done in August when chironomid larvae are believed to have the highest abundance and diversity and their production is the most important for migratory birds. However, one sample set was also collected in April 2007 and one set in September 2008 (Table 2). On each sampling date, each river was sampled at two reaches, the middle



Fig. 1 Map of the study area

section and mouth (Table 2; Fig. 1), and when river widths were large enough, samples were taken in both the margin and the channel (Table 2). Thus, each river was sampled several times per sampling date. Samples were taken by an Ekman-type grab sampler (25 cm^2) in replicate $(8\times)$ and/or by a handle blade trawl. The handle blade trawl (Zhadin, 1960) had a 20-cm mouth width with a blade at the lower side (Fig. 2). The mesh cone (Fig. 2) had 300-310-µm mesh size. This trawl is used for sampling the zoobenthos in shallow rivers with small width and soft (muddy) bottom sediments (Zhadin, 1960). In our study, the trawling distance was 1 m. Therefore, we used the handle blade trawl to sample locations that were too shallow for the Ekmantype sampler and used the Ekman-type sampler for locations that were too deep for the hand net.

In general, from both rivers 42 samples of zoobenthos were taken: 18 samples from each river in August 2006–2010, 2 samples in April 2007 and 1 sample in September 2008 (Table 2). In all samples, species composition of zoobenthos, densities and biomass were determined. When both samplers were used together (Table 2), average values for abundance and biomass of zoobenthos from two samples were taken for the following considerations.

Samples were immediately washed using a screen with 300-310-µm mesh size. Samples were fixed with 4% formaldehyde. In the laboratory, specimens were identified under a stereomicroscope. For measuring of biomass, animals were placed on filter paper for a short time to wipe off surface moisture, and then the animals were weighed (wet weight). To convert the measured wet weight to dry weight, which was necessary for the following calculation of HUFA production and potential export, a moisture content of 78% was used (Sushchik et al., 2007).

For fatty acid analyses, live stage IV chironomid larvae of dominant species were withdrawn from the benthos samples in August 2010 (Table 2). In the Chernavka River, *Cricotopus salinophilus* Zinchenko, Makarchenko et Makarchenko, 2009, were collected, and in the Lantsug River, *Chironomus salinarius*

	Lantsug River	Chernavka River
Water catchment area (km ²)	126	18.4
Length (km)	14.0	2.0
Depth (m)	0.1 - 1.0	0.1-0.3
Width in mouth (m)	30	5
Current velocity (m s ⁻¹)	0.2	0.4
рН	6.9-8.9	7.3-8.1
$O_2 (mg l^{-1})$	2.2-15.5	4.8-22.3
Total mineralization (g l ⁻¹)	6.8–16.9	28.7-31.6
$Na^{+} + K^{+} (g l^{-1})$	1.84-4.63	8.30-10.40
Ca^{2+} (g l ⁻¹)	0.29–0.76	1.02 - 1.44
Mg^{2+} (g l ⁻¹)	0.13-0.71	0.40-0.97
Cl^{-} (g l^{-1})	2.10-8.17	17.0–18.6
SO_4^{2-} (g l ⁻¹)	2.10-4.30	0.47-0.96
HCO_{3}^{-} (g l ⁻¹)	0.17-0.38	0.26-0.36
$PO_4^{3-}-P (mg l^{-1})$	0.48-3.30	0.33-4.5
$NH_4^+-N \ (mg \ l^{-1})$	1.9–25.1	5.5-51.5
$NO_3^{-}-N (mg l^{-1})$	0.10 - 1.00	0.10-0.14
$NO_2^{-}-N \ (mg \ l^{-1})$	0.14-2.25	0.18-1.95

Table 1 Values of the physical and chemical parameters inthe Lantsug and Chernavka Rivers in the summer low waterperiods

Zinchenko and Golovatyuk (2010). The data were kindly provided by the Center for Monitoring the Aquatic and Geological Environment, Ltd. (Samara, Russia), which has a federal license for chemical analyses of surface waters

Kieffer 1915 and Chironomus aprilinus Meigen, 1838, were taken. The live animals were placed into beakers immediately after sorting and allowed to empty their guts for several hours. Then the animals were weighed, as described above. Immediately after weighing, the animals were placed in a chloroformmethanol mixture (2:1, v/v), and samples were frozen at -20° C until further analyses. Silt (sediments) samples (August, 2010, Table 2) were taken simultaneously with those of the zoobenthos using the same samplers. They were placed in the chloroform-methanol mixture and frozen, and then analyzed similarly to the zoobenthos samples. For total organic carbon analyses, larvae were air-dried for 2 days, sealed in foil and kept in a desiccator for further elemental analysis. The samples for total organic carbon were analyzed with a Flash EA 1112 NC Soil/MAS 200 elemental analyzer (ThermoQuest, Italy) as described by Gladyshev et al. (2007).

 Table 2 Dates and locations of sampling of the two salt rivers in the basin of Lake Elton, Russian Federation

Date	Lantsug River			Chernavka River				
	Middle section L1		Mouth L2		Middle section Ch1		Mouth Ch2	
	В	М	В	М	В	М	В	М
16 August 2006	-	e	h	e	_	_	-	_
24-25 April 2007	_	h	_	h	h	-	h	-
15 August 2007	_	e	h	e	h	e	h	e
13-14 August 2008	h	e	h	e	h	e	h	e
25 September 2008	_	_	_	h	_	_	h	-
20 August 2009	h	e	h	e	h	e	h	h, e
18-21 August 2010	h	e	h, f	e, f	h	e	h, f	h, e, f

Sampling sites, designated by the letters L (the Lantsug River) and Ch (the Chernavka River) and by numbers 1–2, are depicted in Fig. 1

B river bank, M mainstream; h handle blade trawl, e Ekman-type grab sampler, f fatty acids, '-' no sample



Fig. 2 Handle blade trawl

Calculation of production

Daily production, P (g m⁻² day⁻¹), was calculated by the formula

$$P = GB,\tag{1}$$

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

$$G = -0.725 + 0.122T - 0.0028T^2, \tag{2}$$

where T (°C) is temperature (Hauer & Benke, 1991; Benke, 1998).

Monthly production for the most frequently sampled month, August, was calculated multiplying average daily production for all sampling dates by 31 days. Potential emergence of chironomids was calculated from the monthly production in August using emergence intensity of 16% of zoobenthos production in streams (Huryn & Wallace, 2000) as the proxy for the rough estimation.

Fatty acid analysis

A detailed description of the fatty acid analysis is given elsewhere (Sushchik et al., 2007). Briefly, prior to the analysis, a fixed volume of internal standard solution (nonadecanoic acid, Sigma, USA) was added to a sample. Then, lipids were extracted by a modified Folch method with chloroform:methanol (2:1, v/v) three times simultaneously with mechanical homogenization 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 analyzed using a gas chromatograph-mass spectrometer (model 6890/5975C, Agilent Technologies, USA) equipped with a 30-m-long, 0.25-mm internal diameter HP-FFAP capillary column. Data were collected and analyzed 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 bond positions in monoenoic and polyenoic acids, GC-MS of dimethyloxazoline derivatives of FA was used (Makhutova et al., 2003). Each sample of fatty acids was analyzed 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, nonadecanoic acid.

For the following considerations, the biochemical data were presented in several ways according to aim of analyses: (1) for revealing the feeding spectra—as percent of total FA; (2) for interspecies comparison—as mg of FA per g of wet or dry weight; (3) for calculation of HUFA production and export on the basis of chironomid production and potential emergence—as mg of FA per g of dry weight.

Diet analyses

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 & Geller, 1986). Moreover, even diatoms can be broken down to unidentified debris in the alimentary tract of some benthic invertebrates (Quigley & Vanderploeg, 1991). Thus, the microscopic examination often results in a high percentage of 'shapeless organic materials.' Second, many ingested microalgae are not digested and assimilated, but remain viable after gut passage (Porter, 1976; Gladyshev et al., 2000; Kolmakov & Gladyshev, 2003). These limitations of standard techniques can be overcome using biochemical tracers, such as fatty acids, which allow studying assimilated food (e.g., Ederington et al., 1995; Sushchik et al., 2003; Whiles et al., 2010; Makhutova et al., 2012). Thus, we used fatty acid tracers (biomarkers) to evaluate food sources of chironomid larvae in the studied rivers, like in our previous studies (Gladyshev et al., 1999; Sushchik et al., 2003; Whiles et al., 2010; Makhutova et al., 2012).

Statistics

Fisher's LSD post hoc test with multivariate canonical correspondence analysis (CCA) was carried out conventionally using STATISTICA software, version 9.0 (StatSoft, Inc.)

Results

Species composition, biomass and production

In the Lantsug River, 36 taxa of benthic invertebrates were found, while in the Chernavka River there were only 12 taxa (Table 3). Taxa of Chironomidae had the highest frequency values, especially *C. salinophilus* in the Chernavka River (Table 3). In the Lantsug River, average sum abundance of *Ch. salinarius*, *C. salinophilus* and *Ch. aprilinus* was 49% of total abundance of all zoobenthos taxa (Fig. 3). In the Chernavka River, average abundance of *C. salinophilus* was 66% **Table 3** List of benthic macroinvertebrates, their frequency (F, % of samples) and maximum abundance (MA, ind. m⁻²) in August in the Lantsug River (2006–2010) and Chernavka River (2007–2010), the basin of Lake Elton, Russian Federation

Taxa	Lantsu	ıg	Chernavka	
	F	MA	F	MA
Oligochaeta				
Enchytraeus issykkulensis Hrabě, 1935			17	1,760
Henlea stolli Bretscher, 1900			6	10
Homochaeta naidina Bretscher, 1896	11	570		
Limnodrilus claparedeanus Ratzel, 1868	6	10		
L. grandisetosus Nomura, 1932	17	5,100		
L. hoffmeisteri Claparède, 1862	6	190		
L. profundicola (Verril, 1871)	6	10		
Limnodrilus sp.	6	10		
Nais communis Piguet, 1906	17	500		
N.pseudobtusa Piguet, 1906	17	10		
Paranais simplex Hrabě, 1936	6	20,609		
Uncinais uncinata (Oersted, 1842)	17	2,240		
Crustacea				
Gammarus pulex (Linnaeus, 1758)	6	160		
Insecta				
Heteroptera				
Sigara sp.	17	200	11	400
Coleoptera				
Anacaena sp.	6	10		
Berosus sp.	6	10	28	500
Enochrus sp.	6	80	11	600
Ochthebius sp.	11	80		
Diptera				
Culicidae				
Aedes sp.			5	175
Ceratopogonidae				
Culicoides (Monoculicoides) sp.	56	7,800	83	8,400
Mallochohelea sp.	6	820		
Stratiomyidae				
Nemotelus sp.	11	10	11	20
Odontomyia sp.	17	10	11	370
Ephydridae				
Parydra sp.	6	10		
Ephydra sp.	17	10	28	1,200
Sphaeroceridae				
Thoracochaeta zosterae (Haliday, 1833)	6	80		
Chironomidae				
Tanypus punctipennis Meigen, 1818	6	10		
Cricotopus ornatus (Meigen, 1818)	22	760		
C. salinophilus Zinchenko, Makarchenko et Makarchenko, 2009	61	11,520	100	8,920
Chironomus aprilinus Meigen, 1838	39	2,840		



Taxa	Lants	ug	Chernavka	
	F	MA	\overline{F}	MA
Ch. gr. plumosus	6	20		
Ch. salinarius Kieffer 1915	50	19,000	33	440
Cladopelma gr. lateralis	6	10		
Glyptotendipes glaucus (Meigen, 1818)	6	10		
G. paripes (Edwards, 1929)	6	100		
G. salinus Mihailova, 1987	22	100		
Microchironomus tener (Kieffer, 1918)	33	11,800		
Paratanytarsus inopertus (Walker, 1856)	6	10		
Tanytarsus kharaensis Zorina et Zinchenko, 2009	11	400		

Number of samples in each river, n = 18



1323 20% 1713 32% 330 5% 20 0.4% 1623 58 24% 1007 1% 15% Cricotopus salinophilus Chironomus aprilinus Chironomus salinarius other Chironomidae

Lantsug

of the total abundance (Fig. 3). The total average biomass of chironomids was significantly higher than that of all other taxa in both the Lantsug and Chernavka Rivers (Fig. 4).



Fig. 4 Average biomass (g m⁻² of wet weight) of macrozoobenthos taxa in the Lantsug River, 2006–2010, and the Chernavka River, 2007–2010, the basin of Lake Elton, Russian Federation. *Bars* represent standard errors

Results of calculations of daily production of chironomids are given in Table 4. Average production in August 2006–2010 in the Lantsug River was $0.58 \text{ gm}^{-2} \text{ day}^{-1}$ dry weight, and in the Chernavka River in August 2007–2010 it was 0.49 g m⁻² day⁻¹. Average daily production in August in both rivers was $0.54 \text{ gm}^{-2} \text{ day}^{-1}$. Average monthly production in August was $16.7 \text{ gm}^{-2} \text{ month}^{-1}$, dry weight. Estimation of average monthly potential emergence of chironomids in August was $2.7 \text{ gm}^{-2} \text{ month}^{-1}$, dry weight.

Chernavka

Fatty acids

Fatty acid composition of chironomid larvae differed significantly from that of bottom sediments of both rivers. Larvae had significantly higher percents in their bodies of 15:0, 16:1n-7, 16:2n-4, 18:2n-6, 18:3n-3,

3517

66%

other taxa

Table 4 Average biomass (B, g m⁻², dry weight) of Chironomidae larvae, water temperature (T, °C), daily instantaneous growth rate (G, day^{-1}) and daily production $(P, g m^{-2} day^{-1})$, dry weight) in the Lantsug River and the Chernavka River, the basin of Lake Elton, Russian Federation

Date	В	Т	G	Р
Lantsug				
16 August 2006	0.46	27.2	0.52	0.24
25 April 2007	0.29	15.5	0.49	0.15
15 August 2007	0.55	24.4	0.58	0.32
13 August 2008	1.97	19.9	0.59	1.17
25 September 2008	0.80	13.5	0.41	0.33
20 August 2009	0.80	24.2	0.59	0.47
19 August 2010	1.14	21.5	0.60	0.69
Chernavka				
24 April 2007	0.34	13.2	0.40	0.14
15 August 2007	2.11	23.4	0.60	1.26
14 August 2008	0.03	20.2	0.60	0.02
25 September 2008	0.31	12.5	0.36	0.11
20 August 2009	0.22	21.9	0.60	0.13
20 August 2010	0.94	23.8	0.59	0.56

18:4n-3, 20:0 and especially 20:5n-3 (Table 5). However, levels of 12:0, 16:0, 18:0 and 18:1n-9 were higher in the bottom sediments than in larvae bodies (Table 5). Fatty acid 22:6n-3 was not detected in chironomid larvae and sediments of the Chernavka River, but in traces in sediments of the Lantsug River (Table 5). The content (mg g^{-1} , wet weight) of the sum of fatty acids in the biomass of larvae was 3-4 times higher than that in the mass of sediments (Table 5). Fatty acid composition of larvae of C. salinophilus differed from that of Ch. salinarius + Ch. aprilinus. Larvae of Cricotopus had higher percent levels of 16:1n-7, 16:2n-4, 16:3n-4, 18:3n-3, 18:4n-3 and 20:5n-3. In turn, Chironomus had higher levels of 14:0, i15:0, 17:0, 18:1n-7, 18:1n-9 and 18:2n-6 (Table 5). The content of the sum of fatty acids in the biomass of Cricotopus was about 1.5 times higher than that of *Chironomus* (Table 5). The average content of the physiologically important EPA (20:5n-3) in the biomass of *Cricotopus* was $4.0 \pm 0.4 \text{ mg g}^{-1}$ of wet weight, while in the biomass of Chironomus, it was only $0.8 \pm 0.2 \text{ mg g}^{-1}$ (the difference was statistically significant according to Fisher's LSD post hoc test).

Table 5 Average values of quantitatively and	Fatty acids	Cricotopus	Chironomus	Sediments Chernavka	Sediments Lantsug
qualitatively prominent	12:0	$0.3\pm0.0^{\mathrm{a}}$	$0.2\pm0.0^{\mathrm{a}}$	$1.5 \pm 0.1^{\mathrm{b}}$	$0.9 \pm 0.1^{\rm c}$
fatty acids (% of total FA) + standard errors and	14:0	$4.1 \pm 0.0^{\mathrm{a}}$	$5.0\pm0.2^{\mathrm{b}}$	$4.3 \pm 0.1^{\mathrm{a}}$	$3.8\pm0.2^{\rm a}$
sum content of FA (mg g^{-1} ,	i15:0	$0.3\pm0.1^{\rm a}$	$1.2\pm0.1^{\mathrm{b}}$	$0.3 \pm 0.1^{\mathrm{a}}$	$0.2\pm0.0^{\mathrm{a}}$
wet weight) in the bodies of two species of chironomid	15:0	$1.1 \pm 0.1^{\mathrm{a}}$	$1.1 \pm 0.0^{\mathrm{a}}$	$0.8 \pm 0.1^{\mathrm{b}}$	$0.7\pm0.0^{\mathrm{b}}$
	16:0	$19.1\pm0.6^{\rm a}$	19.8 ± 0.0^a	$28.5\pm0.0^{\rm b}$	$28.0\pm0.7^{\rm b}$
(n = 2) and <i>Ch</i> .	16:1n-7	$11.9\pm0.7^{\rm a}$	$9.6\pm0.3^{\mathrm{b}}$	$2.2 \pm 0.1^{\circ}$	$3.2 \pm 1.2^{\rm c}$
salinarus + Ch. aprilinus	16:2n-4	$3.1\pm0.0^{\mathrm{a}}$	$1.5\pm0.1^{\rm b}$	$0.0 \pm 0.0^{\rm c}$	$0.0\pm0.0^{ m c}$
(n = 3), and in bottom	17:0	$0.8\pm0.0^{\mathrm{a}}$	$1.2 \pm 0.0^{\mathrm{b}}$	1.4 ± 0.2^{b}	1.4 ± 0.0^{b}
sediments of the Chernavka River $(n - 2)$ and Lantsug	16:3n-4	$2.1\pm0.1^{\mathrm{a}}$	$0.3 \pm 0.1^{\mathrm{b}}$	$0.2 \pm 0.1^{\mathrm{bc}}$	$0.0\pm0.0^{ m c}$
River $(n = 2)$, the basin of	18:0	$9.8\pm0.8^{\rm a}$	15.2 ± 0.8^{a}	26.0 ± 3.1^{b}	$26.7\pm1.4^{\rm b}$
Lake Elton, Russian	18:1n-9	10.8 ± 0.3^{a}	14.0 ± 0.9^{b}	$24.2\pm0.8^{\rm c}$	$23.7\pm0.0^{\rm c}$
Federation	18:1n-7	3.5 ± 0.0^{a}	$9.1\pm0.6^{\mathrm{b}}$	$2.8\pm0.0^{\rm a}$	$3.5\pm0.1^{\rm a}$
	18:2n-6	$4.8\pm0.5^{\rm a}$	$7.2 \pm 0.0^{\mathrm{b}}$	$1.2 \pm 0.7^{\rm c}$	$0.7 \pm 0.2^{\rm c}$
	18:3n-3	$1.1 \pm 0.0^{\mathrm{a}}$	$0.8\pm0.0^{\mathrm{b}}$	$0.1 \pm 0.1^{\circ}$	$0.2\pm0.1^{\rm c}$
	18:4n-3	$1.0 \pm 0.1^{\mathrm{a}}$	$0.4 \pm 0.1^{\mathrm{b}}$	$0.0 \pm 0.0^{\rm c}$	$0.0 \pm 0.0^{\rm c}$
	20:0	$1.2\pm0.1^{\rm a}$	$1.1 \pm 0.1^{\mathrm{a}}$	$0.5\pm0.1^{\mathrm{b}}$	$0.4 \pm 0.2^{\mathrm{b}}$
	20:4n-6	$1.0 \pm 0.0^{\mathrm{a}}$	0.5 ± 0.0^{ab}	0.3 ± 0.3^{ab}	$0.3 \pm 0.3^{\mathrm{b}}$
Means labeled with the same letter are not significantly different from each other at $P < 0.05$ after Fisher's LSD post hoc test	20:5n-3	$17.3\pm0.5^{\rm a}$	$5.9\pm0.9^{\mathrm{b}}$	$0.1 \pm 0.1^{\circ}$	$0.0\pm0.0^{ m c}$
	22:0	$0.2\pm0.0^{\mathrm{a}}$	$0.2\pm0.0^{\mathrm{a}}$	$0.1 \pm 0.1^{\mathrm{a}}$	$0.4 \pm 0.1^{\mathrm{a}}$
	22:6n-3	$0.0\pm0.0^{\mathrm{a}}$	$0.0\pm0.0^{\mathrm{a}}$	$0.0\pm0.0^{\mathrm{a}}$	$0.1 \pm 0.1^{\mathrm{a}}$
	Sum (mg g^{-1})	23.2 ± 2.5^a	$13.2 \pm 2.2^{\mathrm{b}}$	$4.0 \pm 0.4^{\rm c}$	$5.6 \pm 0.1^{\circ}$

same lett significat each othe Fisher's LSD post hoc test



Fig. 5 Canonical correspondence analysis of fatty acid composition (% of the total) of sediments from the Chernavka River (*triangles*) and Lantsug River (*asterisks*), and bodies of larvae of *C. salinophilus (squares, the Chernavka River) and Ch. salinarus* + *Ch. aprilinus (diamonds, the Lantsug River)*. Basin of Lake Elton, Russian Federation

Percent levels of 12:0 were higher in sediments of the Chernavka River than those in sediments of the Lantsug River, while levels of the other acids as well as the content of the sum of FA in sediments of both rivers did not differ significantly (Table 5).

The overall fatty acid compositions of bottom sediments in both rivers were very close to each other according to the multivariate correspondence analysis (Fig. 5). In contrast, the fatty acid composition of *Chironomus* differed significantly from that of *Cricotopus* (Fig. 5). Both larvae compositions were also far from the compositions of sediments (Fig. 4). The differences between larvae and sediments were provided mainly by differences in the first dimension between levels of 12:0, 18:0 and 18:1n-9 on the one hand and 16:3n-4, 20:5n-3, 18:4n-3 and 16:2n-4 on the other hand (Fig. 4). The differences between *Chironomus* and *Cricotopus* were provided first of all by differences in levels of i15:0 and 16:3n-4 in the second dimension (Fig. 5).

Average content of the essential long-chain HUFA, 20:5n-3, in C. salinophilus was 18.2 mg g⁻¹ dry weight, and in Ch. salinarius + Ch. aprilinus it was 3.5 mg g^{-1} dry weight. Thus, the average content of EPA in the chironomid larvae was 10.8 mg g^{-1} dry weight. In the Lantsug River, frequencies of C. salinophilus and Ch. salinarius + Ch. aprilinus in samples were roughly similar, 61 and 39 + 50%, respectively (Table 3). Thereby, using the above average EPA contents in the three species and the data on chironomid production (Table 4), monthly production of EPA in August in Lantsug River was 193 mg m⁻² month⁻¹. In the Chernavka River, the frequency of C. salinophilus was 100% and of Ch. salinarius was 33% (Table 3). Thus, the weighted average content of EPA in the chironomid larvae in the Chernavka River was 14.6 mg g^{-1} dry weight, and monthly production of EPA in August in Chernavka River was 221 mg m⁻² month⁻¹. Average monthly production of EPA in August for both rivers was 207 mg m⁻² month⁻¹. Thus, the monthly flux of EPA from the studied rivers to land due to chironomid potential emergence was about 33 mg m⁻² month⁻¹.

Carbon and nitrogen

The carbon content in *Cricotopus* and *Chironomus* was 467 \pm 3 mg g⁻¹ dry weight and 434 \pm 1 mg g⁻¹, respectively (number of samples, n = 2). The nitrogen content in *Cricotopus* and *Chironomus* was 54.3 \pm 2.7 and 68.0 \pm 2.6 mg g⁻¹ dry weight, respectively (number of samples, n = 2). Using the above data on chironomid production in dry weight units and average content of carbon in the dominant taxa, their average production in carbon units in both rivers was 0.24 g m⁻² day⁻¹.

Discussion

Dominant species

Larvae of the dominant species, *Ch. salinarius* and *Ch. aprilinus*, are known to be halophilous organisms inhabiting saline and brackish waters (Cartier et al., 2010; Estrella & Masero, 2010; Zinchenko et al., 2011). These species are widespread and found in Europe, America and Asia (Fuentes et al., 2005; Ree & Yum, 2006; Zinchenko et al., 2011). *C. salinophilus*

was described recently as a new species (Zinchenko et al., 2009). At present, it is only known from saline rivers of the Elton Lake basin. At all stages of ontogenesis, it is similar to *Cricotopus zavreli* Szadziewski et Hirvenoja 1981, which was found in saline water bodies near Ciechocinek Spa, Poland (Szadziewski & Hirvenoja, 1981). Larvae of *C. salinophilus* at present can be included into *zavreli* group. Our data on the dominant species composition of the studied rivers are in a good agreement with the generalization of Millán et al. (2011) that communities of saline streams include physiological specialists adapted to stressful conditions (high levels of temperature and salinity) and endemic species, which often occur as highly isolated populations.

Chironomus salinarius was often found to be a dominant taxon in the zoobenthos of saline water bodies (Arias & Drake, 1994; Fuentes et al., 2005). The abundance of *Ch. salinarius* larvae reported in the available literature was up to ~44,000 ind. m⁻² (Cartier et al., 2010). Thus, the abundance of 19,000 found in the Lantsug River (Table 3) appeared to be an average population value for larvae of this species. *Ch. salinarius* is believed to be a very important environmental component. First, larvae of *Ch. salinarius* (together with *Ch. aprilinus*) are dominant taxa in brackish wetlands, which are a very important habitats for water birds (Fuentes et al., 2005).

Diet analyses

There are no data on feeding of larvae of Ch. salinarius and Ch. aprilinus, not to mention C. salinophilus, in the available literature. The found differences between fatty acid composition of the chironomid larvae and the bottom sediments indicated a selective feeding of larvae of both genera. The larvae had significantly higher levels of odd-number fatty acid 15:0 synthesized by bacteria (Desvilettes et al., 1997; Napolitano, 1999) compared to the level of this acid in the sediments. Thus, the larvae selectively consumed bacteria from sediments. Besides, the larvae had comparatively high levels of FA synthesized primarily by diatom algae, 16:1n-7, 16:2n-4, 16:3n-4 and 20:5n-3 (Sushchik et al., 2004; Graeve et al., 2005). The high levels of diatom acids in the larvae bodies indicated a selective consumption of these algae from the sediments. Levels of acids synthesized by many algae taxa, 18:2n-6, 18:3n-3 and 18:4n-3 (Napolitano, 1999; Sushchik et al., 2004), also were higher in bodies of larvae of the studied species. In contrast, levels in detritus of saturated acids, 16:0 and 18:0, which are indicators of dead organic matter at a high degree of decomposition (Hama, 1999), were higher in the sediments than in the larvae. Probably, the larvae avoided consuming organic debris of low nutritive value. Thereby, the larvae of *C. salinophilus, Ch. salinarius* and *Ch. aprilinus* from the saline rivers appeared to be capable of selective feeding, and they primarily consumed bacteria and microalgae from bottom sediments. This finding is in a good agreement with data on selective feeding of larvae of *Chironomus plumosus* in a freshwater reservoir (Gladyshev et al., 1999).

The larvae had higher levels of the long-chain saturated acid, 20:0, compared to those in the sediments. Thereby, 20:0 was probably synthesized *de novo* by the larvae rather than obtained from food. The absence of highly unsaturated 22:6n-3, essential for many animals, in larvae of the studied species of *Cricotopus* and *Chironomus* was also reported for larvae of some other species of Chironomidae (Gladyshev et al., 1999; Goedkoop et al., 2000). This acid was also practically absent in bottom sediments of both studied rivers.

The main difference in fatty acid composition of *C. salinophilus* and *Ch. salinarius* + *Ch. aprilinus* consisted of the comparatively high levels of fatty acids synthesized by diatoms, 16:3n-4, 16:1n-7, 16:2n-4 and 20:5n-3, in *C. salinophilus* on the one hand and in high levels of bacterial i15:0 and 17:0 in *Ch. salinarius* + *Ch. aprilinus* on the other. Thus, in the studied saline rivers *C. salinophilus* primarily consumed diatom algae, while *Ch. salinarius* and *Ch. aprilinus* preferred bacteria.

It is important to note that we detected only negligible levels of markers of vascular plants, longchain saturated acids, 22:0, 24:0, etc., in the studied bottom sediments and in the chironomid larvae. This is in a good agreement with the generalization of Millán et al. (2011), who noted that in saline rivers situated in arid landscapes, due to the scarcity of riparian vegetation, fine and ultrafine benthic organic matter is most commonly found, while coarse particles (i.e., leaves, stems and wood) are scarce. Our data on the feeding of the dominant taxa also seem to support the conclusion of Millán et al. (2011) that in saline rivers the most abundant functional feeding groups in biomass terms are gathering-collectors, scrapers and predators, while filtering-collectors and shredders are scarce or even absent.

It is necessary to remark that samples for FA analyses were taken in August 2010. Thus, in other seasons and years, the studied species might have different feeding spectra.

Biomass and production

Average chironomid biomass and its SE values for the Lantsug and Chernavka Rivers were quite similar, as were those of the other taxa (Fig. 4). Both studied rivers are situated very close to each other (Fig. 1) and have comparable hydrological and hydrochemical features (Table 1); this is why their benthic communities also had comparable biomass and species composition.

In the most frequently sampled month, August, the average value of daily production was 0.54 g m⁻² day⁻¹ dry weight, and the maximum value, occurring in the Chernavka River on 15 August 2007, was 1.26 g m⁻² day⁻¹ (Table 4). For instance, in the Ogeechee River (southeastern USA), the maximum daily production of chironomid larvae was only around 0.6 g m⁻² day⁻¹ (Benke, 1998), i.e., about twice lower than that in the saline rivers.

We do not have enough data to calculate the annual production of chironomids in the studied rivers, but we can use the average monthly production for August, 16.7 g m⁻² month⁻¹, for rough comparison with annual production from the other rivers. Annual production in two Polish rivers was around $25 \text{ g m}^{-2} \text{ year}^{-1} \text{ dry weight (Grzybkowska, 1989). In}$ a subtropical stream in China, annual production of chironomid larvae was about 35.6 g m⁻² year⁻¹ (Yan & Li, 2006, recalculated from Table 1 therein). Thus, the monthly production in August in the studied saline rivers was nearly comparable with annual production in some freshwater rivers. We can suppose that annual production of chironomid larvae in the studied rivers was significantly higher than that in many freshwater rivers. High production of chironomid larvae seems to be a characteristic of all saline waters. For instance, in a brackish pond in a coastal lagoon system of the northern Adriatic Sea (Italy) annual production of Ch. salinarius was up to 69.2 g of ash-free dry weight per m^2 per year (Ponti et al., 2007).

Carbon and HUFA contents

For freshwater zoobenthos, carbon is known to be on average 45% of dry biomass (Strayer & Likens, 1986). According to our data, average percent content of carbon in two dominant chironomid taxa in both rivers was exactly 45%.

The average value of EPA content for C. salino*philus*, 18.2 mg g⁻¹ dry weight, appeared to be significantly higher than all values published in the available literature for chironomid larvae, such as Chironomus spp., Procladius spp. (Goedkoop et al., 2000), Diamesa spp. (Sushchik et al., 2003), Prodiamesa spp. and Ch. plumosus (Makhutova et al., 2011). The average value of EPA content in Ch. salinarus + Ch. aprilinus from the studied saline rivers, 3.5 mg g^{-1} dry weight, was practically equal to that of Chironomus spp. from freshwater Lake Erken (Goedkoop et al., 2000), but higher that those of Ch. plumosus from some other freshwater bodies (Makhutova et al., 2011). Thereby, the studied species of the Chironomus genus, Ch. salinarus and Ch. aprilinus, appeared to have very high biochemical value for consumers of the following trophic levels.

Indeed, in various ecosystems migratory birds selectively consumed larvae of Ch. salinarius among other potential prey, which were abundant and readily available (Sanchez et al., 2006; Estrella & Masero, 2010). The larvae represented up to ~97% of the total number of water birds' captured prey (Estrella & Masero, 2010). There are no quantitative data on consumption of larvae of C. salinophilus by birds, but we can suppose that regarding the highest EPA content in its body, this species is also a very valuable food item. For instance, locally abundant migratory birds, little stints Calidris minuta, feed primarily on chironomid larvae (Kasatkina & Shubin, 2012). Moreover, there are literature data showing that migratory birds of the same genera, semipalmated sandpipers (Calidris pusilla), during their refueling stopover on the coast of Canada fed on amphipod Corophium volutator, which is particularly rich in EPA and DHA (Maillet & Weber, 2006). More than 50% of total dietary EPA and DHA is converted to oleic acid, 18:1n-9, since birds store monounsaturates over other fatty acids to achieve a compromise between high energy density and ease of mobilization/oxidation (McWilliams et al., 2002; Maillet & Weber, 2006, 2007). However, the capacity for oxidative metabolism increases, and relevant enzyme activities are positively related to changes in the abundance of n-3 HUFA in membranes that are essential during long-distance flight (Maillet & Weber, 2006, 2007). Thus, membranes of many birds' high-performance muscles contain unusually high amounts of EPA and DHA (Maillet & Weber, 2006, 2007).

It is also worth mentioning that huge emergences of *Ch. salinarius* from water bodies near settlements cause a serious nuisance problem for humans in several countries (Ree & Yum, 2006; Cartier et al., 2010). Thus, data on ecological traits of this species, including the geographical distribution, limits of tolerance and diet peculiarities, are very important for environmental management.

The higher value of EPA content in *C. salinophilus* compared to *Ch. salinarus* and *Ch. aprilinus* seemed to be due to its peculiar diet, which was found to consist primarily of diatom algae. As mentioned, diatom algae are known to have especially high levels of EPA (e.g., Sushchik et al., 2004).

The monthly flux of EPA in August from the studied rivers to land due to chironomid potential emergence, 33 mg m⁻² month⁻¹, was roughly comparable to the global average estimation of annual water-land HUFA export via emerging insects, $40 \text{ mg m}^{-2} \text{ year}^{-1}$ (Gladyshev et al., 2009). Evidently, the annual export of HUFA from these saline rivers would be significantly higher than the average global export. Thereby, potential chironomid emergence from the studied saline rivers appeared to be a very important source of the essential biochemicals for surrounding terrestrial ecosystems. It should be noted that since the HUFA contents and the production (potential emergence) were only measured and calculated for August, at present we cannot estimate probable seasonal variations in HUFA export.

It is worth noting that communities of saline streams including both physiological specialists and endemic species are highly vulnerable (Millán et al., 2011). Thus, saline rivers, as quantitatively and qualitatively important components of ecosystems of arid landscapes, should be peculiarly protected from anthropogenic impacts. For instance, anthropogenic pollution was found to significantly decrease the accumulation of HUFA in the biomass of river zoobenthos (Gladyshev et al., 2012). Thus, anthropogenic pollution is expected to diminish the peculiar function of the zoobenthos of saline rivers as the important source of essential HUFA for organisms of progressively higher trophic levels, including migratory birds.

Conclusions

Saline rivers appeared to be high-productive ecosystems of arid areas. Chironomid larvae in two studied saline rivers had high monthly production in August, 16.7 g m⁻² month⁻¹, dry weight, which was nearly comparable to annual production in some freshwater rivers. The endemic species, C. salinophilus, had the highest content of essential EPA reported for chironomid larvae. Thereby, larvae of this species are believed to be a very valuable food source for consumers of higher trophic levels, first of all for water birds. The dominant species of the studied saline rivers, C. salinophilus, Ch. salinarus and Ch. aprilinus, appeared to be quantitatively important conduits of essential EPA to terrestrial ecosystems. C. salinophilus, Ch. salinarus and Ch. aprilinus from the saline rivers were found to be selective feeders. C. salinophilus primarily consumed diatom algae, while Ch. salinarus and Ch. aprilinus preferred bacteria.

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