Chironomidae larvae in hypersaline waters of the Crimea: diversity, distribution, abundance and production

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Abstract

Chironomidae larvae may represent more than 70% of total Arthropoda numbers in hypersaline waters. Crimea, the largest peninsula of the Black Sea, has more than 50 hypersaline water bodies of marine and continental origin. Chironomidae larvae are common components of their ecosystems, but they still are poorly understood. This paper summarizes the results of a long-term study (2007–2016) of chironomids in Crimean hypersaline waters. More than 400 samples from 38 water bodies were used for analysis. The maximum salinity of water bodies containing Chironomidae larvae was between 320 and 340 g/L. At first it was shown that Baetotendipes noctivagus (Kieffer, 1911) is the most halotolerant chironomid species in the world. Frequency of larvae occurrence varied and was negatively dependent on salinity. Four chironomid species were found: B. noctivagus, Cricotopus gr. cylindraceus (Kieffer, 1908), Tanytarsus gr. mendax Kieffer, 1925 and Paratanytarsus sp. Ceratopogonidae larvae were also found twice, at salinities of 150 and 270 g/L. B. noctivagus was the most common species, which occurred in 81% of samples with chironomids. Abundance of larvae fluctuated widely and reached high numbers: in plankton – to 8 thousand/m³, in floating green algae mats – up to 3 thousand/m², and in benthos – up to 9 thousand/m². Nonlinear dependence of chironomid abundance from salinity was observed; maximum abundance was at salinity levels of between 150 and 170 g/L. The average weight of larvae of 0.05–1.50 mm in length varied little in the samples; however, larvae of greater length had a significantly different average weight. Larvae of 8 mm in the samples had the average actual weight, which ranged from 0.750 to 2.203 mg.

Keywords: Chironomidae, extreme environment, species composition, size structure, productivity

Introduction

Hypersaline water bodies are among the most extreme habitats on the planet, and therefore biodiversity in them is not very high (Vareschi 1987; Williams 1998; Wharton 2002; Belmonte et al. 2012). The majority of animal species successfully living in such harsh conditions are Arthropoda, which are represented by crustaceans and insects (Bayly 1972; Brock & Shiel 1983; Belmonte et al. 2012). The greatest variety and number of insects is represented by Diptera (Tipulidae, Culicidae, Simuliidae, Stratiomyidae, Ceratopogonidae, Ephydridae, Dolichopodidae, Chironomidae), among which in hypersaline lakes Ephydridae and/or Chironomidae usually dominate (Beadle 1969; Bayly 1972; Brock & Shiel 1983; Przhiboro & Shadrin 2012; Przhiboro 2014). Chironomidae larvae can represent more than 70% of the total number of Arthropoda in hypersaline waters (Kokkinn & Williams 1988; El-Shabrawy & El Sayed 2005).

As a component of diverse water ecosystems, the larvae of Chironomidae play an important functional role in the various inland waters and in the diet of water birds (Balushkina 1987; Armitage et al. 1995), can contribute to the spread of pathogenic organisms (Broza et al. 2005), and can cause allergic reactions in humans (Armitage et al. 1995). These factors indicate the importance of their comprehensive study. To date there are more than 15,000 described species of Chironomidae. Their larvae occupy a variety of aquatic habitats, including some very extreme, at latitudes ranging from 81°49’N to 68°00’S (Armitage et al. 1995). The maximum altitude at which there are representatives of chironomids is...
5600 m above sea level (Himalayas), where Diamesa spp. live at subzero temperatures in water drainage channels in glaciers (Kohshima 1984; Sæther & Willassen 1987). There are typical marine species, such as Clunio marinus Haliday, 1855 (Palmégn & Lindeberg 1959), or the genus of exclusively marine flightless midges Pontomyia Edwards, 1926 (Huang & Cheng 2011). Chironomidae larvae also inhabit hypersaline waters of different regions; it has been shown that larvae of chironomids can reach high abundance and dominate (in density and biomass) in water bodies at salinities of up to 70–120 g/L (Szadziewski & Hirvenoja 1981; Drake & Arias 1995; Balushkina et al. 2009; Zerguine 2014). Tanytarsus barbitarsis Freeman, 1961 dwells in Australian waters at a salinity of up to 177 g/L and can reach high abundance – up to 200,000 ind./m² (Kokkinn 1986).

Crimea, which is the largest peninsula of the Black Sea (27,000 km²), has more than 50 hypersaline water bodies of marine and continental origin (Kurnakov et al. 1936; Shadrin 2009) (Figure 1). Studies of biota, including Chironomidae, in hypersaline Crimean lakes, were previously carried out on eight lakes (Balushkina & Petrova 1989; Ivanova et al. 1994; Balushkina et al. 2009; Shadrin et al. 2010; Litvinenko & Shlyakhov 2011; Belmonte et al. 2012). It was shown that chironomid larvae are common components of the ecosystems of seven of them. The larvae were not found in Lake Koyashskoye, which has low productivity and where salinity did not fall below 160 g/L during the study period (Balushkina et al. 2009; Belmonte et al. 2012). The total number of chironomid larvae in seven lakes was very high in some cases, reaching 15,250 ind./m² (Balushkina et al. 2009) and even 69,000 ind./m² (Litvinenko & Shlyakhov 2011). Species identification of chironomid larvae was made in only two studies, and only Baeotendipes tauricus Tshernovskij – junior synonym of Baeotendipes noctivagus (Kieffer, 1911) – was found (Balushkina & Petrova 1989; Balushkina et al. 2009). There is commercial harvesting of chironomid larvae in some Crimean hypersaline water bodies to use them as food for ornamental fish. Despite the important environmental and economic role of Chironomidae they are still poorly understood in the hypersaline waters of the Crimea, and have been studied in only a few water bodies.

The authors conducted chironomid larva research in the hypersaline waters of Crimea between 2007 and 2015. The goal of this paper is to describe, analyze and discuss the results of this long-term research, comparing them with published data from other regions of the planet.

Materials and methods

Study area

Fifty relatively large and many smaller hypersaline water bodies are located in Crimea; they include Bay Sivash (the Sea of Azov) which is the largest lagoon in Europe (around 2560 km²). By origin and ionic composition, the Crimean natural water bodies are divided into marine (talassohaline) and continental (atalassohaline-sulfate). In addition to natural water bodies, on the Kerch peninsula there are artificial water bodies that are increasing in salinity and becoming hypersaline due to increasing climatic aridity (Figure 1; Kurnakov et al. 1936; Shadrin 2009; Anufriieva et al. 2014). All Crimean lakes are shallow and polymixic, and vary in size, range of abiotic factors (salinity, temperature, pH, etc.) and biotic composition. In 2000–2015, biota and ecology of saline water bodies in Crimea were studied and results of this were partially published (Zagorodnyaya et al. 2008; Balushkina et al. 2009; Belmonte et al. 2012; Shadrin & Anufriieva 2013a; Anufriieva et al. 2014; etc.).

Sampling and processing

A total of 416 samples were collected from 38 water bodies in 2007–2015; 389 samples of zooplankton, 12 benthic samples and 15 samples of floating filamentous algae mats. Of these samples, 324 were collected from hypersaline water bodies and 92 from brackish waters. It has been shown in previous studies that in hypersaline conditions (Zagorodnyaya et al. 2008; Belmonte et al. 2012), most of the benthic animals, including chironomid larvae, are present in the water column rather than in the benthos; therefore, larvae were taken primarily from plankton samples. There are two reasons why benthic animals occupy the water...
Benthic samples were collected in areas with a depth of at least 0.2 m by means of a benthic tube (area 0.018 m²). Samples of filamentous algae mats were collected from an area of 0.25 m². Samples were dried to air-dry mass, which was converted into absolute dry weight (adm) using a coefficient of 0.93 (Korelyakova 1977). The mass of sampled algae mat was determined on an electronic balance. The relative number of animals in the mats was determined by dividing the number of counted individuals by the weight of the mat piece. The samples were processed using an Olympus SZ-ST and LOMO MBS-9 stereo microscope. In 27 samples from hypersaline water bodies (Table I), larva species were identified using articles and keys for identification (Pankratova 1970, 1983; Hirvenoja 1973; Wiederholm 1983; Makarchenko & Makarchenko 1999). Developmental stage was evaluated and size was measured. The length of the chironomid larvae was measured under a STEMI DV4 (Zeiss) stereo microscope with an ocular micrometer. The larval mass was determined by the weighing of larvae (preshaved on the filter paper) on a torsion balance WT-250. Large individuals were weighed individually; small individuals of similar size were weighed together and average mass was calculated.

**Data processing**

To assess the frequency of occurrence of chironomid larvae in different ranges of salinity, all 416 samples from brackish and hypersaline water bodies were used:

\[ Y = \frac{Kc}{Kg} \times 100\% \quad (1) \]

where \( Kc \) is the number of samples in a certain range of salinity, which contained chironomids; and \( K \) is the total number of samples in the interval.

To analyze the relationship between chironomid abundance and environmental factors, only quantitative samples (Table I) were used. Taking into account the temperature correction coefficient \( Q10 = 2.25 \), production was calculated using the formula for growth of chironomid larvae (Balushkina 1987):

\[ P = 0.247W^{0.739}N \quad (2) \]

where \( P \) – production, J/m²·day or J/m³·day, \( N \) – abundance, ind./m² or ind./m³, \( W \) – individual mass, mg.

Data were subjected to standard statistical processing. The regression equations between chironomid abundance and environmental factors were calculated by the least squares method in the standard program MS Excel 2007. The significance of differences in mean values was evaluated by Student’s \( t \)-test, and the confidence level of the correlation coefficients was determined (Müller et al. 1979).

**Results**

Chironomids are common inhabitants of the Crimean hypersaline lakes, among which there are water bodies both maritime and continental in origin. Chironomids are also abundant in Bay Sivash and in saline/hypersaline artificial ponds. The maximum salinity at which Chironomidae larvae were found was between 320 and 340 g/L. Frequency of larva occurrence at different salinities varied and was negatively dependent on salinity if higher than 30–50 g/L (Figure 2). Dependency may be reliably approximated by a linear equation (\( R = 0.935; p = 0.0005 \)):

\[ Y = (0.634 - 0.0017S) \times 100\% \quad (3) \]

where \( Y \) is the frequency of occurrence (%); and \( S \) is the salinity (g/L; average of interval).

Thus, 87% of probability to find chironomid larvae in plankton at a salinity of above 50 g/L was determined by salinity. It should be noted that if salinity was below 50 g/L, Chironomidae larvae were usually 5–20 times less abundant in plankton samples than in the benthos or floating mats. There were also benthic or mat samples without chironomid larvae, despite their presence in the plankton. With a rise of salinity above 100–110 g/L, the portion of cases in which the most part of chironomid larvae lived in the plankton increased. The largest number of samples (127) was collected in Lake Chersonessus (near Sevastopol) from 2007 to 2015. The seasonal occurrence of larvae in plankton was studied; chironomid larvae were absent in samples collected from November to March. In April they were present, but not every year. Chironomid larvae were not observed during these months in other lakes.

In total, four chironomid species were found in 27 samples from hypersaline waters: *B. noctivagus*, *Cricotopus gr. cylindraceus* (Kieffer, 1908), *Tanytarsus*...
Ceratopogonidae larvae were found twice, at salinities of 150 and 270 g/L, but were not identified to the species group level. *B. noctivagus* was the most common chironomid species, which was represented in 81% of chironomid samples at salinities between 25 and 280 g/L. *Cricotopus* was represented in 22% of samples at salinities between 30 and 65 g/L. *Tanytarsus* was in 8% of chironomid samples at salinities between 30 and 180 g/L, and *Paratanytarsus* occurred once at a salinity of 58 g/L (Figure 3). As a rule, only one chironomid species was represented in a sample, and only in two samples was *B. noctivagus* found together with other species: once with *Cricotopus* at a salinity of 65 g/L, when *Cricotopus* contributed 66% of the total number of chironomids; the other with *Tanytarsus* at a salinity

| No. | Date         | Water body            | Coordinates          | S (g/L) | T (°C) | pH |
|-----|--------------|-----------------------|----------------------|---------|--------|----|
| 1   | 11 August 2015 | Sivash Bay (plankton) | 45°24'N, 35°19'E     | 65      | 30     | –   |
| 2   | 11 August 2015 | Sivash Bay (mats)     | 45°24'N, 35°19'E     | 65      | 30     | –   |
| 3   | 11 August 2015 | Sivash Bay (benthos)  | 45°24'N, 35°19'E     | 65      | 30     | –   |
| 4   | 15 August 2015 | Lake Aygulskee        | 45°56'N, 34°03'E     | 82      | 31     | –   |
| 5   | 16 August 2015 | Lake Bakalskiye       | 45°45'N, 33°11'E     | 58      | 29     | –   |
| 6   | 10 August 2015 | LakeAktashskoye, Point 4 | 45°24'N, 35°51'E | 25      | 28     | –   |
| 7   | 15 August 2015 | Lake Kiyatskoye       | 46°00'N, 33°58'E     | 185     | 28     | –   |
| 8   | 3 October 2014 | Lake Aktashskoye, Point 1 | 45°23'N, 35°48'E   | 220     | 17     | –   |
| 9   | 1 October 2014 | Lake Kiyatskoye       | 46°00'N, 33°58'E     | 180     | 15     | 7.87|
| 10  | 5 October 2014 | LakeDzharylhatc     | 45°34'N, 32°51'E     | 105     | 16     | –   |
| 11  | 1 October 2014 | Sivash Bay            | 45°24'N, 35°19'E     | 65      | 19     | 8.04|
| 12  | 5 October 2014 | LakeMoinakskoye       | 45°11'N, 33°19'E     | 45      | 16     | –   |
| 13  | 4 October 2014 | Lake Bakalskiye       | 45°45'N, 33°11'E     | 37      | 15     | 8.44|
| 14  | 4 October 2014 | LakeKuchuk-Adjigol    | 45°06'N, 35°27'E     | 30      | 11     | 8.63|
| 15  | 9 August 2013  | LakeBolshoi Kipchak   | 45°22'N, 32°31'E     | 145     | 34     | 7.72|
| 16  | 5 August 2013  | LakeAktashskoye, Point 1 | 45°23'N, 35°48'E | 130     | 29     | 8.77|
| 17  | 5 August 2013  | LakeAktashskoye, Point 2 | 45°24'N, 35°50'E | 130     | 30     | 7.96|
| 18  | 9 August 2013  | Lake Adijaichikskoye  | 45°15'N, 33°05'E     | 127     | 35     | 8.77|
| 19  | 9 August 2013  | LakeOyburskoye        | 45°18'N, 33°04'E     | 114     | 31     | 8.26|
| 20  | 8 August 2013  | Lake Achi             | 45°09'N, 35°25'E     | 65      | 29     | 8.69|
| 21  | 7 August 2013  | Pond near v. Ptashkino | 45°09'N, 36°10'E     | 14      | 28     | 8.89|
| 22  | 5 August 2013  | LakeAktashskoye, Point 3 | 45°21'N, 35°48'E | 40      | 28     | 8.55|
| 23  | 12 April 2013 | Lake Aygulskee        | 45°56'N, 34°03'E     | 150     | 17     | –   |
| 24  | 9 August 2012  | LakeBolshoi Kipchak   | 45°22'N, 32°31'E     | 280     | 27     | 6.85|
| 25  | 9 August 2012  | LakeAdijaichikskoye   | 45°15'N, 33°05'E     | 250     | 31     | 5.75|
| 26  | 8 August 2012  | Lake Adzhigol         | 45°06'N, 35°28'E     | 210     | 30     | 6.68|
| 27  | 9 August 2012  | LakeDzharylhatc      | 45°34'N, 32°51'E     | 145     | 26     | 6.67|
| 28  | 4 August 2012  | LakeTobechiikskoye    | 45°11'N, 36°18'E     | 140     | 33     | –   |

S – salinity, T – temperature.

Figure 2. Dependence of frequency of Chironomidae larvae occurrence on salinity in Crimean hypersaline waters.

Figure 3. Ranges of salinity tolerance for chironomid species in Crimean water bodies.

Gr. *mendax* Kieffer, 1925 and *Paratanytarsus* sp. Ceratopogonidae larvae were found twice, at salinities of 150 and 270 g/L, but were not identified to the species group level. *B. noctivagus* was the most common chironomid species, which was represented in 81% of chironomid samples at salinities between 25 and 280 g/L. *Cricotopus* was represented in 22% of samples at salinities between 30 and 65 g/L. *Tanytarsus* was in 8% of chironomid samples at salinities between 30 and 180 g/L, and *Paratanytarsus* occurred once at a salinity of 58 g/L (Figure 3). As a rule, only one chironomid species was represented in a sample, and only in two samples was *B. noctivagus* found together with other species: once with *Cricotopus* at a salinity of 65 g/L, when *Cricotopus* contributed 66% of the total number of chironomids; the other with *Tanytarsus* at a salinity...
of 180 g/L, when *Tanytarsus* represented about 1% of the total abundance. *Cricotopus* and *Tanytarsus* were found together in one sample at a salinity of 30 g/L.

Abundance of Chironomidae larvae fluctuated widely (Figure 4(a, b; Table II) and reached high values: in plankton, up to 8 thousand/m$^3$ or 3 thousand/m$^2$ (calculated taking into account depth), in floating mats, up to 3 thousand/m$^2$, and on the benthos, up to 9 thousand/m$^2$. As an example, in August 2015 in Bay Sivash at salinity between 61 and 65 g/L and temperature of 30°C, the abundance of Chironomidae larvae in plankton was 14 ind./m$^3$ (*B. noctivagus*), in floating mats it was 2820 ind./m$^2$ (*Cricotopus*) and in the benthos it was 1667 ind./m$^2$ (*B. noctivagus*). In this case, low abundance of Chironomidae larvae in plankton was probably due to a high concentration of juvenile fish – *Knipowitschia caucasica* (Berg, 1916) and *Atherina*

![Figure 4](image)

**Figure 4.** Dependence of log concentration of Chironomidae larvae on (a) salinity and (b) temperature in plankton of Crimean hypersaline waters.

| No. | Species                                                      | Abundance (ind./m$^3$) | Biomass (mg/m$^3$) | Production (J/m$^3$∙day) |
|-----|--------------------------------------------------------------|------------------------|--------------------|--------------------------|
| 1   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 20                     | 7.67               | 0.25                     |
| 2   | *Cricotopus* gr. *cylindraceus* (Kieffer, 1908)              | 2820*                  | 705.00***          | 24.93***                 |
| 3   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 1667*                  | 1250.00***         | 36.45***                 |
| 4   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 10                     | 7.50               | 0.22                     |
| 5   | *Paratanytarsus* sp.                                        | 10                     | 0.50               | 0.02                     |
| 6   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 100                    | 17.50              | 0.66                     |
| 7   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 2967                   | 880.00             | 30.35                    |
| 8   | *Baetendipes noctivagus* (Kieffer, 1911)                     | 1633                   | 583.33             | 6.98                     |
| 9   | *Baetendipes noctivagus* (Kieffer, 1911), *Tanytarsus* gr. *mendax* Kieffer, 1925 | 1040                   | 897.00             | 7.82                     |
| 10  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 2560                   | 1740.00            | 17.14                    |
| 11  | *Cricotopus* gr. *cylindraceus* (Kieffer, 1911)              | 25                     | 1.25               | 0.02                     |
| 12  | *Cricotopus* gr. *cylindraceus* (Kieffer, 1911)              | 25                     | 12.50              | 0.13                     |
| 13  | *Cricotopus* gr. *cylindraceus* (Kieffer, 1911)              | 20                     | 10.00              | 0.10                     |
| 14  | *Cricotopus* gr. *cylindraceus* (Kieffer, 1908), *Tanytarsus* gr. *mendax* Kieffer, 1925 | 90                     | 24.00              | 0.19                     |
| 15  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 200                    | 179.09             | 6.94                     |
| 16  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 1590                   | 467.00             | 14.91                    |
| 17  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 700                    | 1566.00            | 37.54                    |
| 18  | *Baetendipes noctivagus* (Kieffer, 1911)                     | ****                   | ****               | ****                     |
| 19  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 80                     | 31.00              | 1.10                     |
| 20  | *Baetendipes noctivagus* (Kieffer, 1911), *Cricotopus* gr. *cylindraceus* (Kieffer, 1911) | 880                    | 685.00             | 18.35                    |
| 21  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 60                     | 103.00             | 2.21                     |
| 22  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 170                    | 96.00              | 2.52                     |
| 23  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 20                     | 5.00               | 0.06                     |
| 24  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 1260                   | 700.00             | 17.02                    |
| 25  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 240                    | 150.00             | 4.89                     |
| 26  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 380                    | 296.00             | 8.58                     |
| 27  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 680                    | 234.00             | 5.73                     |
| 28  | *Baetendipes noctivagus* (Kieffer, 1911)                     | 20                     | 10.00              | 0.40                     |

Table II. Abundance, biomass and production of chironomid larvae in Crimean hypersaline waters.

No. – sample number from Table I; * – abundance, ind. /m$^2$; ** – biomass, mg/m$^2$; *** – production, J/m$^2$∙day; **** – qualitative sample.
boyeri Risso, 1810 (Shadrin et al. 2016). It should be noted that the average weight of animals in biotopes at this time also varied: 0.40 mg in plankton, 0.25 mg in mats and 0.75 mg on the bottom. Typically, the abundance of chironomid larvae was higher in the floating mats and in the benthos than in the plankton, though often there were some cases when chironomids massively presented in the plankton but had very low numbers in the mats or on the bottom.

Due to the small number of floating mat and sediment samples, subsequent analysis of abundance dependence on salinity was made only for plankton samples (Figure 4a). In general, for all lakes nonlinear dependence of chironomid larvae abundance from salinity was observed; maximum abundance was in the salinity range of 150–170 g/L. In the samples taken only from Lake Chersonessus, a significant trend of an increase of larva abundance in plankton up to 120 g/L was noted. Dependence is close to linear (R = 0.689; p = 0.005), and up to 48% of the total variability in the chironomid larva numbers in the lake plankton can be explained by the variability of salinity. The dependence of the population density on temperature was also nonlinear (Figure 4b), and the largest number of individuals was observed in the range of 16–29°C. Abundance did not correlate with pH. In the period when the larvae were represented in plankton there was a general trend of increase in the average abundance for all lakes and every year from April to July, with a subsequent decrease in October (Figure 5). In Lake Chersonessus the peak of the chironomid larva abundance occurred also in July.

| No. | S (g/L) | n (ind.) | Length range (mm) | Mass range (mg) | R     | Coefficients of the Equation (3) |
|-----|---------|----------|-------------------|-----------------|-------|---------------------------------|
| 7   | 185     | 178      | 1.5–8.0           | 0.063–0.750     | 0.997 | 0.0102 ± 0.0065 2.189 ± 0.009  |
| 8   | 220     | 49       | 2.0–9.0           | 0.063–1.750     | 0.982 | 0.0102 ± 0.0065 2.360 ± 0.019  |
| 9   | 180     | 103      | 2.0–9.0           | 0.050–2.333     | 0.985 | 0.0102 ± 0.0065 2.442 ± 0.011  |
| 10  | 105     | 128      | 2.0–10.0          | 0.056–2.250     | 0.993 | 0.0102 ± 0.0065 2.282 ± 0.011  |
| 15  | 145     | 23       | 3.0–9.0           | 0.067–2.688     | 0.973 | 0.0102 ± 0.0065 2.194 ± 0.029  |
| 16  | 130     | 169      | 2.0–7.5           | 0.063–1.938     | 0.977 | 0.0102 ± 0.0065 2.194 ± 0.022  |
| 17  | 130     | 70       | 2.0–11.0          | 0.050–4.875     | 0.992 | 0.0102 ± 0.0065 2.512 ± 0.017  |
| 20  | 65      | 32       | 5.5–11.0          | 0.635–2.667     | 0.991 | 0.0102 ± 0.0065 2.446 ± 0.024  |
| 22  | 40      | 23       | 2.0–12.0          | 0.038–4.000     | 0.981 | 0.0102 ± 0.0065 2.460 ± 0.026  |
| 24  | 280     | 63       | 2.5–9.5           | 0.050–2.000     | 0.986 | 0.0102 ± 0.0065 2.322 ± 0.011  |
| 27  | 145     | 35       | 2.0–7.0           | 0.053–1.250     | 0.991 | 0.0102 ± 0.0065 2.428 ± 0.013  |

Table III. Coefficients of power dependence (Equation 3) “mass (mg) – length (mm)” of Baetendipes noctivagus larvae in different Crimean hypersaline habitats.

No. – sample number from Table I; n – number of measured individuals; S – salinity; R – coefficient of correlation between log length and log mass. [AQ]
\[ W = a \cdot L^b \]  \hspace{1cm} (4)

where \( W \) – body weight (mg); \( L \) – length (mm); and \( a \) and \( b \) are coefficients.

Paired comparison showed that equation coefficients “\( b \)” in most cases were significantly different and the coefficients “\( a \)” were not significantly different; the average value of the coefficient “\( a \)” was calculated. The “\( b \)” coefficients were recalculated for all of the equations using this average value of “\( a \)” (Table III). The biomass and production of chironomid larvae was calculated taking into account all data (Table II). The maximum biomass of larvae in the plankton reached 2560 mg/m\(^3\), and dependence on salinity had a dome-shaped form (Figure 6a). Maximum production reached 37.5 J/m\(^3\) day, and also had a dome-shaped form of dependence on salinity (Figure 6b).

**Discussion**

As seen from new and previously published (Ivanova et al. 1994; Zagorodnyaya et al. 2008; Balushkina et al. 2009; Belmonte et al. 2012) data, Chironomidae larvae are a common component of ecosystems in Crimean hypersaline waters and play an important role in production processes. Larvae with a wide range of sizes from 2.5 to 9.5 mm were abundantly represented in water bodies of salinity up to 280 g/L, indicating the active state of larval chironomid populations. Even though larvae have been found at salinities between 320 and 340 g/L, this does not mean that they can function properly in such salinity. It has been shown previously that larvae of some Chironomidae species may spend a period of time in a dormant state, anhydrobiosis (Suemoto et al. 2004; Cornette & Kikawada 2011). It can be assumed that some \( B. \) noctivagus stages can move into anhydrobiosis due to drying or very high salinity.

The massive presence of Chironomidae larvae in the hypersaline waters of Crimea is not an exception; this phenomenon has been observed in different regions (see Table IV, where a list is given of Chironomidae species the larvae of which are able to live in hypersaline waters). Approximately 38 species belonging to different subfamilies may exist at a salinity of more than 35 g/L, and 16 species among them occur at a salinity of more than 100 g/L. Only three species were found at a salinity of 150 g/L and higher. E. K. Suworow (1908) found active stages of chironomid larvae (Chironomus sp.?) in Lake Bulak (near Caspian Sea) at a salinity of 285 g/L. Now \( B. \) noctivagus can be considered the most halotolerant Chironomidae species in the world, inhabiting in an active state hypersaline waters with a salinity of up to 280 g/L, and possibly up to 340 g/L. It is likely that Suworow found \( B. \) noctivagus larvae in Lake Bulak, which is within the area of distribution of this species.

A question arises: what adaptations allow some chironomid species to exist at high salinity? It is known that arthropods can use two strategies of osmo-adaptation to exist in hypersaline habitats (Khlebovich & Aladin 2010). Osmo-regulating animals use mechanisms of active hypo-osmotic salt regulation in the body fluids, keeping a lower concentration of salts in these fluids than in the environment. Osmo-conforming animals do not have mechanisms for salt regulation in body fluids; osmo-adaptation is carried out at the cellular level by the synthesis of compatible osmolytes and/or by obtaining them from the outside with accumulation in cells. Compatible osmolytes are low-molecular-weight organic compounds (polyols, some amino acids and methylamines, etc.); they protect proteins under conditions of osmotic stress and do not impact on the normal course of metabolic processes (Yancey 2001). Among the Diptera, including Chironomidae, there are species that use one or the other of these strategies (Sutcliffe 1960; Neumann 1961; Bradley 1987; Herbst & Bradley 1988; Patrick...
| Species | Country | References |
|---------|---------|------------|
| Baeotendipes noctivagus (Kieffer, 1911) | Crimea | Own data |
| Chironomus sp. | Lake Bulack (near Caspian Sea) | (Suworow 1908) |
| Chironomus anthracinus Zetterstedt, 1860 | Algeria | (Zerguine 2014) |
| Chironomus plumosus (Linnaeus, 1758) | Algeria | (Zerguine 2014) |
| Chironomus riparius Meigen, 1804 | Algeria | (Zerguine 2014) |
| Chironomus salinarius Kieffer, 1915 | Mediterranean region, Aral Sea | (Drake & Arias 1995; Mirabdullayev et al. 2004; Velasco et al. 2006) |
| Dicrotendipes nervosus (Staeger, 1839) | Algeria | (Zerguine 2014) |
| Glyptotendipes barbipes (Staeger, 1839) | Algeria | (Zerguine 2014) |
| Glyptotendipes gripekoveni (Kieffer, 1913) | Algeria | (Zerguine 2014) |
| Microchironomus deribae (Freeman, 1957) | Eurasia, Africa | (Laville & Tourenq 1967) |
| Microchironomus tener (Kieffer, 1918) | Algeria | (Zerguine 2014) |
| Paratanytarsus sp. | Crimea | Own data |
| Tanytarsus sp. | Algeria | (Zerguine 2014) |
| Tanytarsus affinis (Fabricius, 1794) | Algeria | (Zerguine 2014) |
| Tanytarsus barbitarsis Freeman, 1961 | Southern Australia | (Kokkinn 1986) |
| Tanytarsus gr. mendax Kieffer, 1925 | Crimea | Own data |
| Cricotopus cylindraceus (Kieffer, 1908) | Crimea | Own data |
| Cricotopus ornatus Meigen, 1818 | Algeria | (Zerguine 2014) |
| Cricotopus sylvestris Fabricius, 1794 | Algeria | (Zerguine 2014) |
| Cricotopus trifasciatus Meigen, 1810 | Algeria | (Zerguine 2014) |
| Cricotopus zavreli Szadziewski et Hirvenoja, 1981 | Europe, Mediterranean region, Kazakhstan, Turkmenistan | (Hirvenoja & Der Gaiman 1973) |
| Halocladius braunsi Goetghebuer, 1942 | North East England | (Kokkinn 1986) |
| Halocladius mediterraneus Hirvenoja, 1973 | Mediterranean region | (Por & Ben-Tuvia 1981; El-Shabrawy & El Sayed 2005) |
| Halocladius variabilis Staeger, 1839 | Spain | (Velasco et al. 2006) |
| Halocladius varians Staeger, 1839 | North East England | (Kokkinn 1986) |
| Hydrobaenus olfa Zerguine et Rossaro, 2010 | Algeria | (Zerguine 2014) |
| Limnophyes sp. | Algeria | (Zerguine 2014) |
| Orthocladius sp. | Russia (Lake Baikal) | (Zerguine 2014) |
| Psectrocladius limbatellus Holmgren, 1869 | Algeria | (Zerguine 2014) |
| Psectrocladius platypus Edwards, 1929 | Algeria | (Zerguine 2014) |
| Psectrocladius sordidellus Zetterstedt, 1838 | Algeria | (Zerguine 2014) |
| Pentaneurini | Algeria | (Zerguine 2014) |
| Pentaneurini | Algeria | (Zerguine 2014) |
| Pentaneurini | Algeria | (Zerguine 2014) |

**Note:** S = maximum salinity.
The presence of microalgal blooms may ensure the presence of chironomids not only by providing necessary energy, but also by providing osmolytes. In Crimea, blooming of the green unicellular alga *Dunalialiella salina* (Dunal) Teodoresco, 1905 in water bodies with salinity of more than 200 g/L is a common phenomenon; the concentration of glycerol (osmolyte) in their biomass can reach 80% of organic matter at that salinity (Shadrin & Anufriieva 2013b). All this leads to the conclusion that the physiological capabilities do not define the upper level of salinity at which Chironomidae naturally inhabit water bodies, and the biotic environment (algal concentration and composition) is just as important. Both elements – salinity and microalgae development – are changing in the water bodies and determine the site-to-site and temporal variability of chironomid larva composition and density in hypersaline waters, as was shown for brackish waters (Cañedo-Argüelles & Rieradevall 2009).

Hypersaline water bodies in Crimea are characterized by high variability; many of them are partially temporal or dry completely. The successful existence of animal species in these water bodies is ensured by the ability to endure, in a resting state, conditions which are incompatible with active life; the existence of dormant stages has been demonstrated for almost all crustaceans living in hypersaline lakes in Crimea (Moscatello & Belmonte 2009; Anufriieva & Shadrin 2014; Shadrin et al. 2015). Chironomid larvae *Polypedilum vanderplanki* Hinton, 1951 are able to remain in anhydrobiosis for up to 17 years, and to restore normal activity when released into the water (Cornette & Kikawada 2011). The ability of the larvae to be in an inactive state (anhydrobiosis) for quite a long time has been revealed in some other chironomid species (Suemoto et al. 2004; Jones 2009). With respect to *B. noctivagus* this issue has not been studied, but it can be assumed that the larvae of this species are also capable of long-term anhydrobiosis because they are present in significant numbers in ephemeral ponds also.

The study results clearly show that dependence of the length of the body on body weight varies depending on environmental factors, so averaging may lead to significant distortion of the actual situation. The following generalized equation was calculated for all of our samples:

\[
W = 0.0116 \cdot L^{2.328} \tag{5}
\]

Using Equation (5), the larva mass was calculated for individuals of 6 mm in length – 0.752 mg – but the average weight of larvae of such length ranged from 0.567 mg to 1.044 mg in the samples. When calculating (Equation 5) the mass of 6-mm larvae, it was overestimated or underestimated by nearly 30% compared with the real values. In the case of 8-mm larvae, the calculation gave 1.468 mg, but the average actual weight of this size larva ranged from 0.750 to 2.203 mg in the samples. Overestimation or underestimation of the average actual weight can exceed 40%. The issue requires more detailed study and discussion in future. In this study, biomass and production were calculated using actual average individual mass. According to the data above, biomass and production are dependent on salinity but other factors such as food are likely to play an equally important role. Salinity determines the maximum
possible performance. The question of interaction of factors in determining the structural and functional characteristics of larvae is interesting, but there is not enough data on the state of the environment and Chironomidae biology for an in-depth discussion. However, some things may be assumed. Calculations of chironomid production are most likely overestimating the actual values because a generalized Equation (2) of chironomid growth was used. It was shown for some Chironomidae species that the growth rate decreases and development duration increases with a salinity increase (Kokkinn 1990; Cartier et al. 2011). On this basis, it can be assumed that calculated production values are being used which may be 30–60% higher than the real values in the hypersaline lakes of Crimea. Further research is needed in this direction.

Conclusions

Chironomid larvae are a common and abundant component of the Crimean hypersaline waters, playing an important role in the functioning of the ecosystems. B. noctivagus is the most common and abundant species among them. It is likely that it is the most halotolerant Chironomidae species in the world. Despite this, the adaptation mechanisms providing for its existence in the harsh conditions of hypersaline waters are not known. Further physiological, biochemical, genetic and ecological research is needed in this direction.

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