Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 48, No. 4, December 2019
pages (305-315)

ISSN 1730-413X
eISSN 1897-3191

Does salinity affect body proportions and “size/mass” ratios of highly halotolerant *Baeotendipes noctivagus* larvae (Diptera, Chironomidae)?

by

Nickolai V. Shadrin[1],
Victor P. Belyakov[2],
Alexandra I. Bazhora[2],
Elena V. Anufriieva[1,*]

DOI: **10.2478/ohs-2019-0028**
Category: **Original research paper**
Received: **March 23, 2019**
Accepted: **June 17, 2019**

[1]*A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, 2 Nakhimov av., 299011, Sevastopol, Russia*

[2]*Institute of Limnology, Russian Academy of Sciences, Sevastyanova str. 9, St.-Petersburg, 196105, Russia*

Abstract

Measuring the linear characteristics of chironomid larvae is easier and faster than measuring their mass. The relationships between them are approximated by parabolic equations. Generalized equations are provided for all chironomid larvae. However, these relations vary in different water bodies. Measurements of 1424 chironomid larvae representing several species were taken in Crimean waters with salinity ranging from 0 to 280 PSU. There was a high correlation between three traits. In the case of *Baeotendipes noctivagus* and *Cricotopus* gr. *sylvestris*, dimorphism was found in the “head capsule length–width” relationship. Salinity affects the head capsule. The exponent “b” varied from 1.43 to 3.06 in the “body length-mass” equation for *B. noctivagus*, and from 1.943 to 2.592 for *C.* gr. *sylvestris*. It is inappropriate to use only one coefficient “b” for all chironomid larvae. In *B. noctivagus* and *Paratanytarsus confuses*, the mass of one-size larvae decreased with increasing salinity. Salinity is not the only factor affecting the size and mass of chironomid larvae.

Key words: Chironomidae, length, mass, width of head capsule, salinity

---

* Corresponding author: *lena_anufriieva@mail.ru, lena.anufriieva@gmail.com*

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at www.oandhs.pl

Introduction

Chironomid larvae (Diptera, Chironomidae) are an important ecosystem component in various inland waters and marine lagoons, as they play a key role in their production processes and nutrient dynamics as well as in matter and energy exchange between aquatic and terrestrial ecosystems (Balushkina 1987; Armitage et al. 1995; Muehlbauer et al. 2014; Shadrin et al. 2017). For example, they provide the main phosphorus flux from bottom sediments into the water column (Biswas et al. 2009). Being an important component in the diet of many fish species, they determine fish productivity in water bodies (Balushkina 1987; Armitage et al. 1995). Quantitative assessment of the functional role of chironomid larvae is an important issue in the ecology of inland waters and lagoons.

Animal body mass is an important functional indicator that determines the intensity of metabolism, growth and other processes (Menzie 1981; Balushkina 1987). Knowledge of animal mass values and patterns of variation in populations of different organisms is a necessary prerequisite for understanding the functional role of populations in ecosystems. It is often easier and faster to measure linear characteristics of chironomid larvae than the mass of their bodies (Balushkina 1987; Miyasaka et al. 2008). It has been established that the relationship between the mass of larvae and their linear characteristics (length and width of the head capsule) is well approximated by parabolic equations (Balushkina 1987; Miyasaka et al. 2008):

$$W = aL^{b} \tag{1}$$

where W – body mass, mg; L – body length/width of the head capsule, mm; a, b – coefficients.

Often the task is to calculate the body mass of chironomids retrieved from the stomachs of fish. Chitinized parts of the body are best preserved in the stomachs of fish, head capsules in particular, the width of which can be used to determine the length and mass of the consumed chironomid larvae. In the literature, generalized equations for mass relationships with linear characteristics of chironomid larvae are provided (Toderash 1984; Balushkina 1987; Miyasaka et al. 2008). However, the relationships between the body length, body mass and width of the head capsule in larvae vary in different water bodies (Balushkina 1987; Kravtsova 2007; Shadrin et al. 2017; Schütz & Füreder 2018). The exponent "b" in equation (1) describing the relationship between the body mass and the length of chironomid larvae according to values of this parameter available in the literature varies over a wide range: *P. baicalensis* – 2.282 (Kravtsova 2007), *O. nitidoscutellatus* – 2.716 (Kravtsova 2007), *Orthocladius* sp. – 2.239 (Kravtsova 2007), *C. zealandicus* – 2.571 (Graham & Burns 1983), *C. decorus* – 2.860 (Maier et al. 1990), *Diamesa cinerella* Meigen in Gistl, 1835 – 2.982 (Schütz & Füreder 2018), and from 2.340 to 3.347 for six species of New Zealand chironomids (Stoffels et al. 2003). The use of equations for specific species in real water bodies gives a more accurate result. For example, a comparison of values calculated from the generalized equation (Balushkina 1987) and the equations for specific species in real waters gives a difference of 14–21% for freshwater bodies (Kravtsova 2007) and 30–40% for hypersaline waters (Shadrin et al. 2017). Environmental factors may also affect the parameters of equation (1) for a single species as demonstrated in some cases (Fonseca & Rocha 2004; Rech et al. 2014; Shadrin et al. 2017; Schütz & Füreder 2018), but this issue has been explored to a limited extent so far. Salinity is one of the most important factors determining the structure and functioning of aquatic ecosystems (Williams 1998; Khlebovich & Aladin 2010). Salinity may also affect the parameters of equation (1) (Belyakov et al. 2018), but currently only few data support this conclusion.

In the Crimea region, which is the largest Black Sea peninsula with an area of 27 000 km², there are many water bodies with varying salinity – from freshwater to hypersaline (Anufriieva et al. 2014; Anufriieva & Shadrin 2014a; Shadrin et al. 2017). Chironomid larvae reach high abundance in these water bodies, play an important functional role and are considered a valuable biological resource (Balushkina et al. 2009; Shadrin et al. 2017). Chironomid larvae inhabit water bodies characterized by a wide range of salinity, up to 270–280 PSU, and *Baeotendipes noctivagus* (Kieffer,

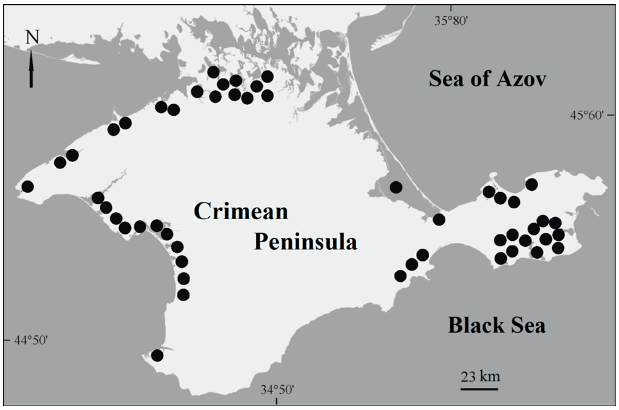

Figure 1

Distribution of Crimean water bodies surveyed in 2012–2016

1911) is the most halotolerant species among them (Shadrin et al. 2019). A wide range of salinity in lakes and lagoons in Crimea, from 0 to more than 300 PSU, makes it possible to effectively study the impact of salinity on the structural and functional characteristics of populations and communities of various organisms (Balushkina et al. 2009; Anufriieva & Shadrin 2015).

The purpose of the study was to determine the width of the head capsule, the length and body mass of larvae of *B. noctivagus* and two other chironomid species from Crimean water bodies of varying salinity, and on this basis to assess the effect of salinity on the parameters of the equations describing the relationship between the studied parameters.

Materials and methods

In Crimea, natural water bodies are predominantly saline/hypersaline. Their distribution is shown in Figure 1. There are more than 50 relatively large and many smaller hypersaline water bodies, including Sivash Bay (the Sea of Azov), which is the largest (2560 km^2) hypersaline lagoon in the world (Balushkina et al. 2009; Shadrin & Anufriieva 2013; Anufriieva et al. 2014; Shadrin et al. 2017; Shadrin et al. 2018). Based on their origin and ionic composition, Crimean saline natural water bodies are divided into marine (thalassohaline) and continental (athalassohaline-sulfate) ones. All Crimean saline lakes are shallow and polymictic with a wide range of abiotic factors (temperature, salinity, pH, etc.) and are characterized by a varied biotic composition. In 2000–2016, the biotic composition and ecology of Crimean water bodies were studied and the results were partially published (Balushkina et al. 2009; Belmonte et al. 2012; Shadrin & Anufriieva 2013; Anufriieva et al. 2014). To analyze the size and mass of chironomid larvae in 55 samples of benthos, filamentous green algae mats and plankton collected in 2012–2016 were used. Collection methods described in the literature were used (Balushkina et al. 2009; Shadrin & Anufriieva 2013; Anufriieva et al. 2014; Shadrin et al. 2017; 2018; Anufriieva et al. 2018). Chironomid larvae are not known to be planktic dwellers but in hypersaline waters many of the typically benthic animals, including chironomid larvae, live in the water column or in floating filamentous green algae *Cladophora* spp. mats (Shadrin et al. 2017; 2018). Samples were fixed with 4% formalin. Larvae were identified using available identification keys (Pankratova 1970; 1983; Hirvenoja 1973; Wiederholm 1983; Makarchenko & Makarchenko 1999). The length and width of head capsules in chironomid larvae were measured under a STEMI DV4 (Zeiss) stereo microscope with an ocular micrometer. The larval mass was determined by weighing the larvae (pre-dried on the filter paper) on the WT-250 torsion balance. A total of 1424 chironomid larvae, including 1167

Table 1

Crimean water bodies where samples were collected to measure chironomid larvae traits

Lake, date	Coordinates	Salinity, PSU	Temperature, °C	pH
Pond near the village of Chelyadinovo, 05.08.2012	45°12′28″N; 36°21′47″E	24	32	7.55
Lake Yanyshskoye, 05.08.2012	45°07′57″N; 36°25′25″E	166	36	8.01
Lake Dzharylhatch, 09.08.2012	45°33′53″N; 32°51′47″E	145	26	6.67
Lake Bolshoi Kipchak, 09.08.2012	45°22′06″N; 32°31′06″E	280	27	6.85
Lake Chersonesskoye, 23.05.2013	44°35′10″N; 33°23′33″E	43	28	–
Lake Aktashskoye, 05.08.2013	45°23′35″N; 35°48′18″E	130	29	8.77
Lake Aktashskoye, 05.08.2013	45°21′05″N; 35°48′39″E	40	28	8.55
Lake Aktashskoye, 05.08.2013	45°23′17″N; 35°48′16″E	220	17	–
Pond in the Kamysh-Burun iron ore quarry, 06.08.2013	45°16′40″N; 36°23′23″E	0	30	–
Pond near the village of Erofeevo, 08.08.2013	45°12′05″N; 35°39′01″E	9	28	9.71
Pond near Lake Kiyatskoye, 08.08.2013	45°58′53″N; 33°57′56″E	10	31	8.60
Lake Bolshoi Kipchak, 09.08.2013	45°22′06″N; 32°31′06″E	145	34	7.72
Lake Kiyatskoye, 01.10.2014	59′48.01″N; 33°57′11″E	180	15	–
Lake Dzharylhatch, 05.10.2014	45°33′53″N; 32°51′47″E	106	16	–
Lake Chersonesskoye, 12.07.2015	44°35′10″N; 33°23′33″E	122	24	–
Sivash Bay, 11.08.2015	45°31′39″N; 35°10′41″E	65	30	–
Lake Kiyatskoye, 15.08.2015	59′48.01″N; 33°57′11″E	185	27	–
Lake Kiyatskoye, 14.06.2016	59′48.01″N; 33°57′11″E	190	30	7.70
Lake Kuchuk-Adzhigol, 07.07.2016	45°06′03″N; 35°27′02″E	10	23	9.50
Lake Sakskoye, 28.07.2016	45°07′32″N; 33°35′36″E	200	29	–

B. noctivagus larvae, were measured from 55 samples collected in 2012–2016. Salinity, temperature and pH were measured during the sampling procedure, using a refractometer (Kelilong WZ212) and a pH meter (PHH-830).

For samples (Table 1) where 10 or more larvae of a size range more than 2 mm (see Tables 2–4) were measured, and parameters of the regression equations were calculated:

$$D = kL^{m} \quad (2)$$

$$W = aL^{b} \quad (3)$$

$$W = cD^{d} \quad (4)$$

where D – the width of the head capsule, mm; L – the body length, mm; W – the body mass, mg; k, m, c, d , a and b – the coefficients.

Average values, standard deviations (SD) and coefficients of variation (CV) were calculated. 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

The relationship between the body length and the width of the head capsule

***Baeotendipes noctivagus* (Kieffer, 1911).** For those samples where 10 or more larvae of a sufficient size range were measured, the parameters of equation (2) were calculated (Table 2). A high correlation between these characteristics was found in all samples; the average correlation coefficient was 0.972 (SD = 0.022, CV = 0.023). No significant differences were found in the values of the power coefficient "m"; its average value was 0.812 (SD = 0.144, CV = 0.177) and significantly differed from 1 (p = 0.001). The head capsule width of 7 mm long larvae was calculated using equation (2). Table 2 shows that the maximum differences computed for different samples did not exceed 20%. No direct effect of salinity on the calculated value of the head capsule width was found. On average, the width of the head capsule in larvae of this length was 0.254 mm (SD = 0.023, CV = 0.090). Based on the available data, no correlation was found between the width of the head capsule and the body length (or their ratio) and temperature and pH (Table 1). When the array of measurements was analyzed as a whole, all points were grouped around two lines (Fig. 2). They can be approximated by two equations, the first one (R = 0.934, p = 0.0001):

$$D = 0.109L^{0.857} \quad (5)$$

and the second one (R = 0.921, p = 0.0005):

$$D = 0.058L^{0.751} \quad (6)$$

The calculated width of the head capsule in one-size larvae (7 mm) was 0.551 mm in one group and 0.253 mm in the other; the values differed 2.2 times. The authors analyzed the effect of salinity on the width of the head capsule in two groups (Fig. 3). Salinity affected the width of the head capsule in the group with wider head capsule; it may be approximated as (R = 0.997, p = 0.005):

$$D_{7} = 0.134S^{0.280} \quad (7)$$

where S – salinity, PSU.

A weak positive correlation was found for another group (R = 0.569, p = 0.05). Salinity, temperature and pH did not determine what group would be exist in the lake. It should be noted that in one particular water body, representatives of only one group with a smaller head capsule width were usually present.

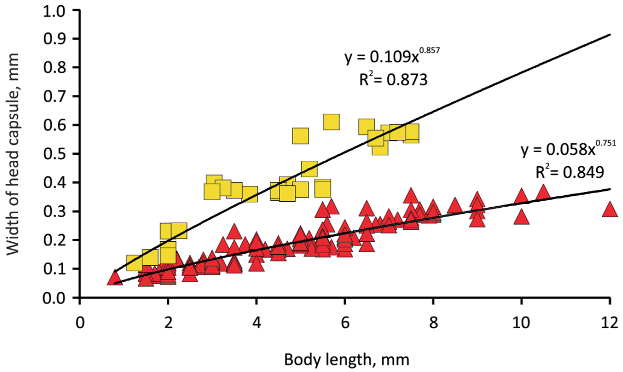

Figure 2

Relationship between the body length and width of the head capsule in *B. noctivagus* for all measured chironomid larvae in Crimean water bodies (triangles and squares represent values of two phenotypically separated groups of larvae)

Table 2

Coefficients of equation (2) describing the "body length–head capsule width" relationship for chironomid larvae from different Crimean water bodies

Lake, date	Salinity, PSU	Length range, mm	Head capsule width range, mm	n	Coefficients of equation 2		C_7, mm	R
					k	m		
Baeotendipes noctivagus								
Pond near the village of Chelyadinovo, 05.08.2012	24	2.0–5.7	0.105–0.238	28	0.056	0.862	0.300	0.913
Lake Yanyshskoye, 05.08.2012	166	3.0–8.5	0.11–0.342	39	0.037	1.010	0.264	0.935
Lake Bolshoi Kipchak, 09.08.2012	280	3.0–9.0	0.133–0.300	65	0.057	0.752	0.245	0.992
Lake Chersonesskoye, 23.05.2013	43	1.3–3.5	0.120–0.231	15	0.070	0.851	0.367	0.584
Lake Aktashskoye, 05.08.2013	130	2.0–9.0	0.074–0.311	70	0.052	0.834	0.264	0.920
Lake Aktashskoye, 05.08.2013	40	1.5–12.0	0.067–0.308	41	0.055	0.705	0.217	0.987
Lake Aktashskoye, 05.08.2013	220	2.0–8.0	0.105–0.295	159	0.062	0.702	0.243	0.973
Lake Bolshoi Kipchak, 09.08.2013	145	2.5–9.0	0.107–0.342	25	0.047	0.902	0.272	0.960
Lake Kiyatskoye, 01.10.2014	180	3.5–7.5	0.118–0.276	101	0.031	1.123	0.276	0.968
Lake Dzharylhatch, 05.10.2014	106	2.0–9.0	0.081–0.321	50	0.040	0.961	0.259	0.992
Lake Chersonesskoye, 12.07.2015	122	2.0–10.0	0.074–0.355	25	0.052	0.837	0.265	0.873
Sivash Bay, 11.08.2015	65	0.8–6.0	0.071–0.213	11	0.082	0.500	0.217	0.927
Lake Kiyatskoye, 15.08.2015	185	2.0–7.5	0.089–0.271	178	0.045	0.886	0.252	0.973
Lake Kiyatskoye, 14.06.2016	190	1.5–8.0	0.084–0.289	64	0.060	0.726	0.246	0.960
Lake Sakskoye, 28.07.2016	200	2.3–7.5	0.232–0.574	26	0.133	0.721	0.541	0.928
Cricotopus gr. *sylvestris*								
Pond near the village of Erofeevo, 08.08.2013	9	2.2–5.5	0.141–0.284	17	0.126	0.496	0.331	0.749
Pond near Lake Kiyatskoye, 08.08.2013	10	1.5–4.7	0.242–0.529	10	0.193	0.656	0.692	0.983
Lake Kuchuk-Adzhigol, 07.07.2016	10	2.3–6.5	0.105–0.269	38	0.072	0.636	0.248	0.869
Paratanytarsus confusus								
Pond in the Kamysh-Burun iron ore quarry, 06.08.2013	0	1.6–3.7	0.09–0.144	12	0.078	0.479	0.198	0.752
Lake Kuchuk-Adzhigol, 07.07.2016	10	2.3–5.5	0.103–0.194	136	0.070	0.621	0.247	0.859

n – number of measured larvae; k, m – coefficients of equation (2); C_7 – calculated width of the head capsule in 7 mm long larvae; R – coefficient of correlation.

***Cricotopus* gr. *sylvestris* (Fabricius, 1794).** This species was represented by a sufficient number of larvae to perform the calculations for only three samples (Table 2). The correlation coefficient averaged 0.867. The calculated coefficients of three equations differed significantly (p = 0.01), despite the fact that salinity was nearly the same – 9 and 10 PSU. The calculated widths of the head capsule for larvae of 7 mm in length in these samples differed 2.8 times. All points were grouped around two lines when the whole array of measurements was analyzed (Fig. 4). Salinity does not explain this separation.

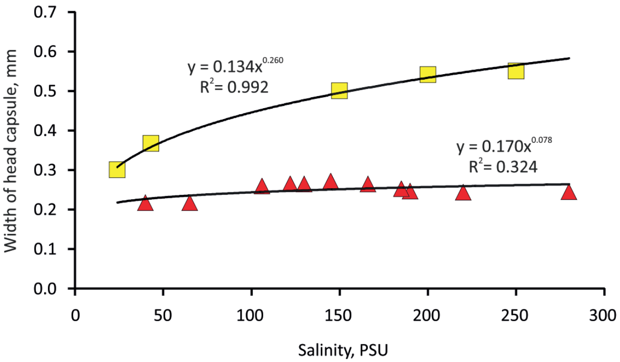

Figure 3

Relationship between salinity and the calculated width of the head capsule in 7 mm long larvae of *B. noctivagus* (triangles and squares represent values of two phenotypically separated groups of larvae in different salinity intervals)

***Paratanytarsus confuses* Palmen, 1960.** This species was represented by a sufficient number of larvae to perform calculations for only two samples collected at salinity 0 and 10 PSU (Table 2). There was no significant difference in the relationship.

The "body length/mass" ratio

***Baeotendipes noctivagus*.** Equation (3) was used for 16 samples collected in 10 lakes at different times (Table 3). On average, the correlation coefficient was 0.950 (SD = 0.037, CV = 0.039). The exponent "b" varied from 1.43 to 3.06 and was an average 2.214

Table 3

Coefficients of equation (3) "length–mass" for chironomid larvae from different Crimean water bodies

Lake, date	Salinity, PSU	Length range, mm	Mass, mg	n	Coefficients of equation 3		W_7	R
					a	b		
Baeotendipes noctivagus								
Pond near the village of Chelyadinovo, 05.08.2012	24	2.0–5.7	0.10–3.50	28	0.014	2.357	1.374	0.952
Lake Yanyshskoye, 05.08.2012	166	3.0–8.5	0.20–2.00	39	0.051	1.702	0.384	0.972
Lake Dzharylhatch, 09.08.2012	145	2.0–7.0	0.07–2.80	35	0.008	2.570	1.188	0.993
Lake Bolshoi Kipchak, 09.08.2012	280	3.0–9.0	0.12–1.83	65	0.007	2.570	1.040	0.994
Lake Chersonesskoye, 23.05.2013	43	1.3–3.5	0.04–0.40	13	0.021	2.376	2.139	0.951
Lake Aktashskoye, 05.08.2013	130	2.0–9.0	0.05–3.00	70	0.007	2.707	1.358	0.995
Lake Aktashskoye, 05.08.2013	40	1.5–12.0	0.03–4.00	41	0.009	2.518	1.208	0.976
Lake Aktashskoye, 05.08.2013	220	2.0–8.0	0.07–1.50	159	0.015	2.064	0.832	0.972
Lake Bolshoi Kipchak, 09.08.2013	145	2.5–9.0	0.07–2.60	25	0.003	3.057	1.150	0.974
Lake Kiyatskoye, 01.10.2014	180	3.5–7.5	0.20–1.30	101	0.008	2.595	1.248	0.971
Lake Dzharylhatch, 05.10.2014	106	2.0–9.0	0.06–1.80	50	0.008	2.351	0.776	0.978
Lake Chersonesskoye, 12.07.2015	122	2.0–10.0	0.20–3.50	25	0.053	1.693	1.429	0.966
Sivash Bay, 11.08.2015	65	0.8–6.0	0.07–2.80	11	0.064	1.556	1.322	0.998
Lake Kiyatskoye, 15.08.2015	185	2.0–7.5	0.04–0.95	178	0.007	2.427	0.787	0.995
Lake Kiyatskoye, 14.06.2016	190	1.5–8.0	0.08–1.00	64	0.035	1.425	0.560	0.936
Lake Sakskoye, 28.07.2016	200	2.3–7.5	0.10–1.20	26	0.036	1.425	0.576	0.923
Cricotopus gr. *sylvestris*								
Pond near the village of Erofeevo, 08.08.2013	9	2.2–5.5	0.25–1.00	17	0.039	1.943	1.710	0.890
Pond near Lake Kiyatskoye, 08.08.2013	10	1.5–4.7	0.05–0.80	10	0.018	2.438	2.068	0.997
Lake Kuchuk-Adzhigol, 07.07.2016	10	2.3–6.5	0.125–2.00	38	0.012	2.592	1.861	0.969
Paratanytarsus confusus								
Pond in the Kamysh-Burun iron ore quarry, 06.08.2013	0	1.6–3.7	0.10–0.50	12	0.026	2.067	1.451	0.879
Lake Kuchuk-Adzhigol, 07.07.2016	10	2.3–5.5	0.13–0.84	136	0.023	1.859	0.857	0.918

n – number of measured larvae; a, b – coefficients of equation (3); W7 – calculated mass of 7 mm long larvae; R – coefficient of correlation

(SD = 0.502, CV = 0.227). There was no significant correlation between this parameter and salinity. Based on the obtained parameters of the equations for each sample (Table 3), the mass of 7 mm long larvae (Table 3, W_7) was calculated. The mass ranged from 0.384 mg to 2.139 mg; the maximum mass was 5.6 times higher than the minimum one. The range of differences in the weighed mass of one-size larvae in all samples was almost the same. A general trend of mass reduction with increasing salinity was observed in one-size larvae. Based on the determination coefficient (R^2), no more than 40% of the total variability in the body mass of one-size larvae can be explained by changing salinity. The analysis of the salinity effect on the calculated mass of 7 mm long larvae shows that the data are grouped around two curves (Fig. 3). The first curve can be approximated by the equation (R = 0.946, $p = 0.001$):

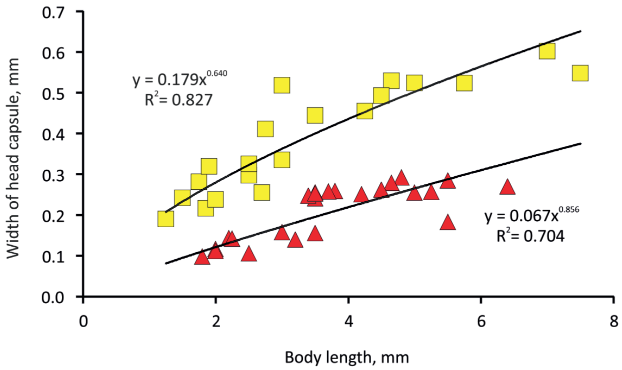

Figure 4

Relationship of the body length and width of the head capsule in *C.* gr. *sylvestris* for all measured chironomid larvae in Crimean water bodies (triangles and squares represent values of two phenotypically separated groups of larvae)

$$W_7 = 4.556 - 0.658Ln(S) \tag{8}$$

where W_7 – the mass of 7 mm long larvae, mg; S – salinity, PSU, and the equation (R = 0.902, $p = 0.001$):

$$W_7 = 2.798 - 0.421Ln(S) \tag{9}$$

No correlation was found between the mass and its ratio to size traits and temperature and pH.

The authors also calculated the equation using all measurements (1167) for this species (R = 0.898, *p* = 0.0005):

$$W = 0.027L^{1.879} \tag{10}$$

The mass of 7 mm larvae, calculated using this equation, was 1.039 mg. This value is 2 times smaller than the largest mass in the analyzed samples and 2.7 times larger than the smallest mass in those samples (Table 3).

***Cricotopus* gr. *sylvestris*.** Calculations were performed for three samples only, collected at salinity 9 and 10 PSU (Table 3). Significant differences were found in the equation parameters (*p* = 0.005) and in the calculated mass; larvae with a length of 7 mm differed 1.6 times.

***Paratanytarsus confuses*.** Calculations were performed for two samples only, collected at salinity 0 and 10 PSU (Table 3). Calculated mass of the 7 mm long larvae was significantly higher in freshwater than in saline water.

The "head capsule width/mass" ratio

Table 4 presents the parameters calculated by equation (3). Parameters for different samples differed greatly in the case of *B. noctivagus*. Correlation coefficients between the body mass and the width of the head capsule were on average lower than between the length and mass; the average value was 0.903 (SD = 0.113, CV = 0.125). The body mass of larvae with a head capsule width of 0.250 mm was calculated using the obtained equations (Table 3). The calculated mass ranged from 0.052 mg to 2.025 mg, averaging 0.897 mg (SD = 0.477, CV = 0.532). The calculated maximum mass was 8.7 times higher than the calculated minimum mass. The calculated mass was not directly correlated with salinity. This procedure was also applied to three samples of *C.* gr. *sylvestris* (Table 3). The parameters of these equations also differed significantly.

Table 4

Coefficients of equation (4) describing the "head capsule width–mass" relationship for chironomid larvae from different Crimean water bodies

Lake, date	Salinity, PSU	Head capsule width range, mm	Mass, mg	n	Coefficients of equation 4		$W_{0.250,}$ mg	R
					c	d		
Baeotendipes noctivagus								
Pond near the village of Chelyadinovo, 05.08.2012	24	0.105–0.238	0.10–3.50	28	6.597	1.833	0.521	0.682
Lake Yanyshskoye, 05.08.2012	166	0.11–0.342	0.20–2.00	39	12.032	1.788	1.022	0.724
Lake Dzharylhatch, 09.08.2012	145	0.07–0.282	0.07–2.80	35	21.923	2.32	0.887	0.988
Lake Bolshoi Kipchak, 09.08.2012	280	0.133–0.300	0.12–1.83	65	113.630	3.378	1.023	0.994
Lake Chersonesskoye, 23.05.2013	43	0.076–0.381	0.04–0.40	15	1.203	1.182	0.233	0.738
Lake Aktashskoye, 05.08.2013	130	0.074–0.311	0.05–3.00	70	45.275	2.769	0.973	0.909
Lake Aktashskoye, 05.08.2013	40	0.067–0.306	0.03–4.00	41	281.190	3.557	2.025	0.984
Lake Aktashskoye, 05.08.2013	220	0.105–0.295	0.07–1.50	159	55.700	2.941	0.947	0.999
Lake Bolshoi Kipchak, 09.08.2013	145	0.107–0.342	0.07–2.60	25	89.186	3.311	0.901	0.960
Lake Dzharylhatch, 05.10.2014	106	0.08–0.320	0.06–1.80	50	21.001	2.443	0.714	0.983
Lake Chersonesskoye, 12.07.2015	122	0.074–0.355	0.20–3.50	25	7.079	1.425	0.984	0.807
Sivash Bay, 11.08.2015	65	0.07–0.215	0.05–1.00	11	77.266	2.752	1.700	0.926
Lake Kiyatskoye, 15.08.2015	185	0.09–0.271	0.04–0.95	178	30.982	2.648	0.790	0.989
Lake Kiyatskoye, 14.06.2016	190	0.08–0.289	0.08–1.00	64	4.178	1.571	0.472	0.845
Lake Sakskoye, 28.07.2016	200	0.232–0.61	0.10–1.20	28	3.344	1.853	0.257	0.799
Cricotopus gr. *sylvestris*								
Pond near the village of Erofeevo, 08.08.2013	9	0.139–0.291	0.14–0.28	17	3.955	1.389	0.577	0.559
Pond near Lake Kiyatskoye, 08.08.2013	10	0.242–0.529	0.05–0.80	10	7.173	3.578	0.052	0.978
Lake Kuchuk-Adzhigol, 07.07.2016	10	0.105–0.269	0.11–0.27	38	124.62	3.277	1.371	0.896
Paratanytarsus confusus								
Pond in the Kamysh-Burun iron ore quarry, 06.08.2013	0	0.090–0.144	0.10–0.50	12	10.193	1.914	0.718	0.519
Lake Kuchuk- Adzhigol, 07.07.2016	10	0.103–0.194	0.13–0.84	136	1.588	1.130	0.332	0.835

n – number of measured larvae; c, d – coefficients of equation (4); $W_{0.250}$ – calculated mass of larvae with 0.250 mm width of head capsule; R – coefficient of correlation

Discussion

The relationship between the body length and width of the head capsule

Based on the available published data, the power coefficients "m" in parabolic equation (2) describing the relationship between the width of the head capsule and the body length of chironomid larvae representing seven species were calculated: *Chironomus decorus* Johannsen, 1905 – 0.679 (Maier et al. 1990), *C. ornatus* (Meigen, 1818) – 0.856 (Swanson & Hammer 1983), *C. xanthus* Rempel, 1939 – 0.664 and 0.639 (Fonseca & Rocha 2004), *C. zealandicus* Hudson, 1892 – 0.856 (Graham & Burns 1983), *Diamesa incallida* (Walker, 1856) – 1.23 (Nolte & Hoffmann 1992), *Orthocladius nitidoscutellatus* Lundström, 1915 – 1.028 (Kravtsova 2007), *Orthocladius* sp. – 0.976 (Kravtsova 2007), and *P. baicalensis* (Tshernovskij, 1949) – 0.835 (Kravtsova 2007). For 10 species, including those listed in Table 2, the average value of the exponent "m" is 0.807 (SD = 0.171, CV = 0.214). The exponent "m" is in most cases significantly below 1. This suggests that the rate of increase in the body length of chironomid larvae during their growth is generally significantly higher than the growth rate of the head capsule width. One-size larvae of different species have a varying head capsule width, which means that, in general, the equations of relationships between the body length and the head capsule width are species-specific, as previously reported (Stoffels et al. 2003). Our data on *B. noctivagus* and *C.* gr. *sylvestris* suggest that intraspecific dimorphism in the head width may exist among chironomid larvae. The factor/factors determining this dimorphism are unknown. The second coefficient "k" in equation (2) varies; it cannot be considered as a definite constant. Data on *B. noctivagus* and *C.* gr. *sylvestris* (Table 2) show that environmental conditions affect the coefficient "m" and the width of the head capsule in one-size larvae. It is possible that salinity affects the ratio of the body length and the head capsule width but without one general trend. No effect of salinity on the body proportions was previously found in other arthropods (Anufriieva & Shadrin 2015). It is likely that the trophic factor has a stronger effect on the "body length/head capsule width" ratio. This conclusion is supported, in particular, by experiments on larvae cultured on different types of nutrient medium (Fonseca & Rocha 2004; Rech et al. 2014).

Relationship between the mass and linear dimensions of the body

The exponent "b" in equation (3) describing the relationship between the body mass and the length of chironomid larvae significantly varied in our data (Table 3) – from 1.566 to 3.057. Values of this parameter provided in the available literature and calculated by the authors of this paper using published data also vary over a wide range: *P. baicalensis* – 2.282 (Kravtsova 2007), *O. nitidoscutellatus* – 2.716 (Kravtsova 2007), *Orthocladius* sp. – 2.239 (Kravtsova 2007), *C. zealandicus* – 2.571 (Graham & Burns 1983), *C. decorus* – 2.860 (Maier et al. 1990), *Diamesa cinerella* Meigen in Gistl, 1835 – 2.982 (Schütz & Füreder 2018), and from 2.340 to 3.347 in six species of New Zealand chironomids (Stoffels et al. 2003). Based on available data, i.e. our data and those derived from the literature (Graham & Burns 1983; Maier et al. 1990; Stoffels et al. 2003; Kravtsova 2007; Schütz & Füreder 2018), the mean value of "b" calculated for 15 chironomid species from different regions was 2.494 (SD = 0.833, CV = 0.334). In the past, two values of coefficients "b" were also calculated for larvae of chironomids as a whole – 2.04 (Toderash 1984) and 2.781 (Balushkina 1987). However, our data and the analysis of the published results allow us to conclude that it is inappropriate to always use one coefficient "b" for all chironomid larvae, because this may produce high under- or overestimation. This parameter is species-specific and depends on the conditions in which the larvae develop. It was suggested earlier, based on a more limited data set, that the coefficient "b" decreases with increasing salinity (Belyakov et al. 2018). However, our new data on *B. noctivagus* show that there is no overall trend in the salinity effect on this coefficient. It follows from our data (Fig. 3) as well as from equations (3) and (4) that the mass of one-size larvae of *B. noctivagus* and *P. confuses* decreases with increasing salinity. Equations (8) and (9) show that some other factors may affect this relationship. It is likely that the trophic factor is more important in determining the "body length/mass" ratio and this assumption was supported by experiments (Fonseca & Rocha 2004; Rech et al. 2014) and field study (Schütz & Füreder 2018). This effect is more pronounced in larger larvae (Shadrin et al. 2017; Belyakov et al. 2018). The decrease is probably associated with an increase in energy expenditure to maintain metabolism and a decrease in the growth rate at high salinity (Kokkinn 1990; Cartier et al. 2011).

The exponent "d" in equation (4) of the relationship between the body mass and the width of the head capsule in chironomid larvae (Table 4) varied significantly in our data – from 1.130 to 3.378, i.e. in a wider range than in the equation of the relationship between the mass and the body length. In the literature, the following values of this coefficient are available: *P. baicalensis* – 3.960 (Kravtsova 2007), *O. nitidoscutellatus* – 2.720 (Kravtsova 2007), *Orthocladius* sp. – 2.440 (Kravtsova 2007), and from 1.641 to 4.020 in six species of New Zealand chironomids (Stoffels et al. 2003). The average value of "d" for 12 species of chironomids from different regions is 2.477 (SD = 0.844, CV = 0.341). In the past, the value of the coefficient "d" was calculated for the chironomid larvae as a whole – 3.280 (Balushkina 1987), which, as demonstrated above, is impractical for chironomid larvae under real conditions. This parameter is species-specific and depends on the conditions in which the larvae develop. At the same time, our data on *B. noctivagus* show that there is no overall trend in the effect of salinity on this coefficient. Probably, the trophic factor has a strong influence on the coefficient of the equation as well as some other factors (temperature, oxygen concentration, etc.). When comparing the calculation of the body mass with the body length and the width of the head capsule, it can be observed that – as in the case of *B. noctivagus*, according to our data, and in the case of other species, according to published data (Stoffels et al. 2003; Kravtsova 2007) – the variability in values is larger and the determination coefficient (R^2) is smaller in the case of the mass/head capsule width ratio. Therefore, it is better to calculate the body mass using the body length rather than the width of the head capsule. Given the close relationship between the width of the head capsule and the body length, one can confidently use one of the parameters to calculate the other.

Two phenotypically distinct morphs differing in body size and body proportion were found among chironomid larvae of the studied species. A similar population polymorphism was observed in various animal taxa and it is assumed that it increases the adaptability of populations (Huston & DeAngelis 1987; Anufriieva & Shadrin 2014b). Such polymorphism may result from the interaction of different environmental characteristics with a population gene pool and the critical factors, which include: (1) growth rate variations among individuals resulting from intra-population metabolic communications; (2) polymorphism in a population gene pool; (3) epigenetic diversity resulting from alternative gene expression and epigenetic mechanisms (Huston & DeAngelis 1987; Goldberg et al. 2007; Anufriieva & Shadrin, 2014b). "The phenotype can be represented as a branching system of trajectories in a phase space spreading along the time axis" (Waddington 1940). *B. noctivagus* lives under a wide range of salinity, and probably it is possible only realizing different adaptive norms. It is easier to conclude that polymorphism results from genetic diversity or alternative discrete realizations of the same genotype. The available data do not provide an explanation as to whether this polymorphism is caused by genetic diversity or phenotypic plasticity. It is an intriguing issue and further research is needed.

When comparing the results obtained with fixed and non-fixed larvae of chironomids, it should be considered that with prolonged storage in formalin or alcohol, larvae can lose on average 20–30% of their original mass (Donald & Paterson 1977). Taking this into account, it should be assumed that in our study there were cases of larvae mass underestimation. It is likely that the "a" and "c" indicators in equations (3) and (4) should be increased by 20–30%. We assume that when the larvae of different sizes were fixed, their mass loss was at the same rate, and, consequently, the coefficients "b" and "d" in equations (3) and (4) for the living body mass remained unchanged.

General conclusions: when analyzing populations and taxocenes of chironomid larvae in different water bodies, it is inappropriate to use general equations for the mass–body relationship with linear traits. Salinity affects the larvae mass and "length–mass" ratio but cannot be considered as a single factor affecting the size and mass of chironomid larvae in water bodies. All factors act together. More detailed studies of the effects of various factors (salinity, temperature, oxygen concentration, quality and quantity of food, etc.) on the parameters of the equations are needed.

Acknowledgements

The authors thank Dr. Bindy Datson (Australia) for her selfless work on improving the English language of the manuscript and an anonymous reviewer for helpful comments and suggestions. Data analysis and manuscript writing were supported by the Russian Science Foundation (grant 18-16-00001 for N.V. Shadrin and E.V. Anufriieva), sampling in 2012–2016 was conducted in the framework of the state assignment of the A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (No AAAA-A19-119100790153-3), and chironomid species identification and larvae measurements were conducted by V.P. Belyakov and A.I. Bazhora in the framework of the state assignment of the Institute of Limnology of RAS (No 0154-2018-0004).

References

Anufriieva, E., Hołyńska, M. & Shadrin, N. (2014). Current invasions of Asian Cyclopid species (Copepoda: Cyclopidae) in Crimea, with taxonomical and zoogeographical remarks on the hypersaline and freshwater fauna. *Ann. Zool.* 64(1):

109–130.

Anufriieva, E. & Shadrin, N. (2014a). Resting stages of crustaceans in the Crimean hypersaline lakes (Ukraine) and their ecological role. *Acta Geol. Sin*. 88: 46–49.

Anufriieva, E.V., & Shadrin, N.V. (2014b). Factors determining the average body size of geographically separated *Arctodiaptomus salinus* (Daday, 1885) populations. *Zoological Research* 35(2): 132–141.

Anufriieva, E.V. & Shadrin, N.V. (2015). Morphometric variability of *Arctodiaptomus salinus* (Copepoda) in the Mediterranean-Black Sea region. *Zoological Research* 36(6): 328–336.

Anufriieva, E.V., Vdodovich, I.V. & Shadrin, N.V. (2018). First data on predation of *Eucypris mareotica* (Crustacea, Ostracoda) in hypersaline waters. *Food Webs* 16: e00090.

Armitage, P.D., Pinder, L.C. & Cranston, P. (1995). *The Chironomidae: Biology and ecology of non-biting midges*. London: Chapman & Hall.

Balushkina, E.V. (1987). The functional significance of chironomid larvae in inland water bodies. Leningrad: Nauka. (In Russian).

Balushkina, E.V., Golubkov, S.M., Golubkov, M.S., Litvinchuk, L.F. & Shadrin, N.V. (2009). Effect of abiotic and biotic factors on the structural and functional organization of the saline lake ecosystems in Crimea. *Zh. Obshchei Biol.* 70(6): 504–514. (In Russian).

Belmonte, G., Moscatello, S., Batogova, E.A., Pavlovskaya, T., Shadrin, N.V. et al. (2012). Fauna of hypersaline lakes of the Crimea (Ukraine). *Thalassia Salentina* 34: 11–24.

Belyakov, V.P., Anufriieva, E.V., Bazhora, A.I. & Shadrin, N.V. (2018). Effect of salinity on chironomid larvae (Diptera, Chironomidae) in hypersaline lakes of Crimea. *Biology Bulletin* 45(10): 1211–1218.

Biswas, J.K., Rana, S., Bhakta, J.N. & Jana, B.B. (2009). Bioturbation potential of chironomid larvae for the sediment-water phosphorus exchange in simulated pond systems of varied nutrient enrichment. *Ecol. Eng.* 35: 1444–1453.

Cartier, V., Claret, C., Garnier, R. & Franquet, E. (2011). How salinity affects life cycle of a brackish water species, *Chironomus salinarius* Kieffer (Diptera: Chironomidae). *J. Exp. Mar. Biol. Ecol.* 405(1): 93–98.

Donald, G.L. & Paterson, C.G. (1977). Effect of preservation on wet weight biomass of chironomid larvae. *Hydrobiologia* 53(1): 75–80.

Fonseca, A.L. & Rocha, O. (2004). Laboratory cultures of the native species *Chironomus xanthus* Rempel, 1939 (Diptera-Chironomidae). *Acta Limnologica Brasiliensia* 16(2): 153–161.

Goldberg, A.D., Allis, C.D. & Bernstein, E. (2007). Epigenetics: a landscape takes shape. *Cell* 128: 635–638.

Graham, A.A. & Burns, C.W. (1983). Production and ecology of benthic chironomid larvae (Diptera) in Lake Hayes, New Zealand, a warm-monomictic eutrophic lake. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 68(3): 351–377.

Hirvenoja, M. (1973). Revision der Gattung Cricotopus van der Wulp und ihrer Verwandten (Diptera, Chironomidae). *Ann. Zool. Fenn.* 10: 1–363.

Huston, M.A. & DeAngelis, D.L. (1987). Size bimodality in monospecific populations: a critical review of potential mechanisms. *Am. Nat.* 129: 678–707.

Khlebovich, V.V. & Aladin, N.V. (2010). The salinity factor in animal life. *Her. Russ. Acad. Sci.* 80: 299–304.

Kokkinn, M.J. (1990). Is the rate of embryonic development a predictor of overall development rate in *Tanytarsus barbitarsis* Freeman (Diptera: Chironomidae)? *Australian Journal of Marine and Freshwater Research* 41(5): 575–579.

Kravtsova, L.S. (2007). The relationships between the body mass and its linear sizes in larvae of three Chironomidae species (Diptera) from the lake Baikal littoral. *Zoologichesky Zhurnal* 86(11): 1398–1401. (In Russian).

Maier, K.J., Kosalwat, P. & Knight, A.W. (1990). Culture of *Chironomus decorus* (Diptera: Chironomidae) and the effect of temperature on its life history. *Environ. Entomol.* 19(6): 1681–1688.

Makarchenko, E.A. & Makarchenko, M.A. (1999). Chironomidae. Non-biting midges. In S.J. Tsalolikhin (Ed.), *Key to Freshwater Invertebrates of Russia and Adjacent Lands. V. 4. Higher Insects. Diptera* (pp. 210–295). St. Petersburg: Zoological Institute RAS. (In Russian).

Menzie, C.A. (1981). Production ecology of *Cricotopus sylvestris* (Fabricius) (Diptera: Chironomidae) in a shallow estuarine cove. *Limnol. Oceanogr.* 26(3): 467–481.

Miyasaka, H., Genkai-Kato, M., Miyake, Y., Kishi, D., Katano, I. et al. (2008). Relationships between length and weight of freshwater macroinvertebrates in Japan. *Limnology* 9(1): 75–80.

Muehlbauer, J.D., Collins, S.F., Doyle, M.W. & Tockner, K. (2014). How wide is a stream? Spatial extent of the potential "stream signature" in terrestrial food webs using meta-analysis. *Ecology* 95(1): 44–55.

Müller, P.H., Neuman, P. & Storm, R. (1979). *Tafeln der Mathematischen Statistik*. Leipzig: VEB Fachbuchverlag.

Nolte, U. & Hoffmann, T. (1992). Fast life in cold water: *Diamesa incallida* (Chironomidae). *Ecography* 15(1): 25–30.

Pankratova, V. (1970). Larvae and pupae of Mosquitoes of the subfamily Orthocladiinae in the fauna of the USSR (Diptera, Chironomidae = Tendipedidae). Leningrad: Nauka. (In Russian).

Pankratova, V. (1983). Larvae and pupae of Mosquitoes of the subfamily Chironominae in the fauna of the USSR (Diptera, Chironomidae = Tendipedidae) Leningrad: Nauka. (In Russian).

Rech, K.C., Guereschi, R.M., Torres, K.H. & Nuñer, A.P.D.O. (2014). Subsidies for production of *Chironomus calligraphus* larvae (Chironomidae, Diptera) in the laboratory. *Invertebr. Reprod. Dev.* 58(3): 199–206.

Schütz, S.A. & Füreder, L. (2018). Unexpected patterns of chironomid larval size in an extreme environment: a highly glaciated, alpine stream. *Hydrobiologia* 820(1): 49–63.

Shadrin, N.V. & Anufriieva, E.V. (2013). Climate change impact on the marine lakes and their Crustaceans: The case of marine hypersaline Lake Bakalskoye (Ukraine). *Turk. J. Fish. Aquat. Sc.* 13: 603–611.

Shadrin, N.V., Anufriieva, E.V., Belyakov, V.P. & Bazhora, A.I. (2017). Chironomidae larvae in hypersaline waters of the Crimea: diversity, distribution, abundance and production. *The European Zoological Journal* 84(1): 61–72.

Shadrin, N.V., Anufriieva, E.V., Kipriyanova, L.M., Kolesnikova, E.A., Latushkin, A.A. et al. (2018). The political decision caused the drastic ecosystem shift of the Sivash Bay (the Sea of Azov). *Quatern. Int.* 475: 4–10.

Shadrin, N.V., Belyakov, V.P., Bazhora, A.I. & Anufriieva, E.V. (2019). The role of salinity as an environmental filtering factor in the determination of the Diptera taxonomic composition in the Crimean waters. *Knowl. Manag. Aquat. Ec.* 420: 3.

Stoffels, R.J., Karbe, S. & Paterson, R.A. (2003). Length-mass models for some common New Zealand littoral-benthic macroinvertebrates, with a note on within-taxon variability in parameter values among published models. *New Zeal. J. Mar. Fresh. Res.* 37(2): 449–460.

Swanson, S.M. & Hammer, U.T. (1983). Production of *Cricotopus ornatus* (Meigen) (Diptera: Chironomidae) in Waldsea Lake, Saskatchewan. *Hydrobiologia* 105(1): 155–163.

Toderash, I.K. (1984). *Functional importance of chironomids in reservoir ecosystems of Moldova*. Kishinev (Chisinau): Shtiintsa Publisher. (In Russian).

Waddington, CH. (1940). *Organisers & Genes*. Cambridge: Cambridge University Press.

Wiederholm, T. (1983). Chironomidae of the Holarctic Region: Keys and diagnoses. Part 1. Larvae. *Entomologica Scandinavica* 19: 457.

Williams, W.D. (1998). Salinity as a determinant of the structure of biological communities in the salt lakes. *Hydrobiologia* 381: 191–201.