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ON THE DISTRIBUTION OF BLACK FLY LARVAE IN SMALL LOWLAND RIVERS IN LITHUANIA

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ABSTRACT – It is known that the species composition of blackflies is different in different rivers. The aim of our study was to investigate peculiarities in the distribution of blackflies in small lowland rivers in Lithuania. The investigation was carried out at 15 study sites. In addition to blackfly larvae, physical and chemical indices of the rivers were also investigated. Our investigation revealed the presence of 15 species of blackflies, the dominant species differing from site to site. The results of multiple regression analysis showed that according to our data the larval density of five blackflies [*Simulium trifasciatum* Curtis, 1839; *S. ornatum* Meigen, 1818; *S. lineatum* (Meigen, 1804); *S. morsitans* Edwards, 1915; and *S. rostratum* Lundström, 1911] was statistically correlated with some physical parameters (substrate, discharge, water temperature) and chemical properties (water hardness, pH, permanganate oxidation of organic matter, quantity of nitrates and nitrites in the water) of the rivers.

KEY WORDS: Simuliidae, lowland rivers, distribution, water parameters, Lithuania

INTRODUCTION

It is known that the species composition of blackflies is different in different rivers. Even closely situated rivers can be dominated by different blackfly species. For example, in the largest river of Lithuania, the Nemunas, *Simulium maculatum* (Meigen, 1804) and *S. reptans* (Linnaeus, 1758) larvae were dominant. A smaller tributary of the Nemunas River, the Merkys River, was characterized by dominance of *S. equinum* (Linnaeus, 1758), *S. lineatum* (Meigen, 1804), *S. morsitans* Edwards, 1915 and *S. ornatum* Meigen, 1818 (BERNOTIENE, 2006). The species composition of blackflies is often different even in rivers of similar size. What factors are important for the distribution of preimaginal stages of one or another species of blackflies along rivers?

Blackfly larvae are filter-feeding insects that are known to be indicators of physical and chemical changes in water bodies (RUBTSOV, 1978). Each species of blackfly is associated with a particular habitat and geographic area (ADLER ET AL., 2004). The chemical and physical factors most often associated with larval distribution are dissolved oxygen, pH, conductivity, ionic composition, alkalinity and hardness (KIM AND MERRITT, 1988). Some of the factors that influence the distribution of blackflies also affect their population dynamics (ROSS AND MERRITT, 1978).

The aim of our study was to investigate peculiarities in the distribution of blackflies in small lowland rivers in Lithuania and to elucidate some of the factors that condition the distribution and density of different blackfly species in these rivers.

MATERIAL AND METHODS

The investigation was carried out at 15 study sites in 13 different rivers between May and October of 2004. The annual water discharges varied from 0.3 to 33 m^3/s at the study sites (Table 1, Fig. 1). Two study sites each were selected on the Riese and Virinta Rivers. One study site on the Riese River was located close to the outlet from a lake, while another study site was located about 4 km downstream. The substrate at one study site on the Virinta River consisted of small stones, while the substrate at the other study site on the same river was rubble.

Physical and chemical indices of the water and the presence of blackfly larvae were investigated three times per year.

Table 1. Annual discharges (m^3/s) and localities of the rivers investigated.

River	*Discharge*	*Locality*
Dubysa	13	N55^{0}18`E23$^0$26`
Elme	0.5	N55^{0}32`E24$^0$ 47`
Gruda	1.9	N54^{0}06`E24^{0}16
Merkys	33.4	N54^{0}06`E24$^0$16`
Musia	0.6	N55^{0}17`E24$^0$50`
Riese*	0.5	N54^{0}46`E25$^0$19`
Skroblus	0.7	N54^{0}06`E24$^0$16`
Susiena	0.4	N55^{0}28`E24$^0$59`
Sventoji	36	N55^{0}28`E25$^0$01`
Ula	5	N54^{0}07`E24$^0$27`
Varius	0.3	N55^{0}33`E25$^0$06`
Vilnia	6	N54^{0}41`E25$^0$21`
Virinta*	3.8	N55^{0}26`E25$^0$02`

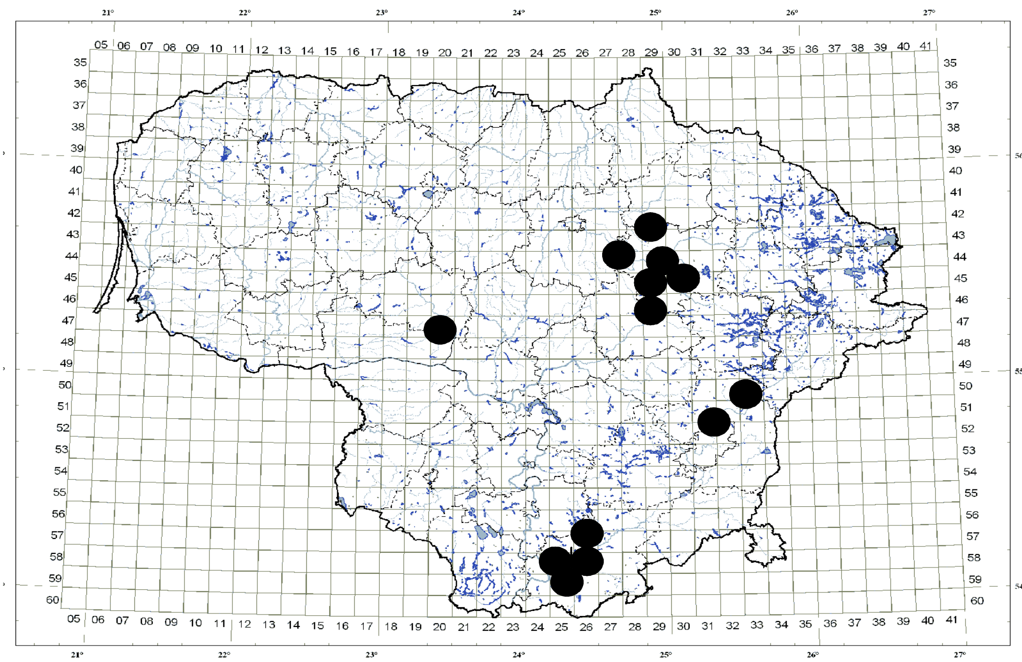

Fig. 1. Map of Lithuania and the study sites.

The abundance of blackfly larvae was estimated from samples collected on water plants: we collected three separate samples each time, and the abundance of larvae of each blackfly species was calculated per 1 dm^2 of water plant surface. More than 62,000 blackfly larvae were investigated.

In the course of this research, some physical properties (water temperature, stream velocity) and chemical indices (pH; total hardness; carbonate hardness; quantity of nitrates, nitrites, phosphates; oxygen dissolved in the water; permanganate oxidation of organic matter) were investigated at the study sites. A Merck compact laboratory for water testing was used. Permanganate oxidation of organic matter was measured in the laboratory according to the generally accepted method (international standard - LST EN ISO 8467). Correlation analysis and multiple regression were used to identify some of the factors that determine the distribution of different blackfly species along water bodies and answer the question as to which of the factors investigated may be the most important for the distribution of preimaginal blackflies.

RESULTS

Physical parameters differed at the different study sites. Water temperature varied from 6.5 (the Skroblus) to 20°C (the Dubysa), while stream velocity varied from 0.2 m/s (the Varius River) to 1.3 m/s (the Virinta and Gruda Rivers). Three types of substrate were determined: sand, small stones, and rubble (Table 2). For the calculations, sand was scored as 1 (it consisted of the small-

Table 2. Physical parameters of the rivers investigated (average ± SD).

	Temperature	*Velocity*	*Substratum*
Dubysa	14.3 ± 4	0.7 ± 0.1	rubble
Elme	11.8 ± 0.9	0.8 ± 0.1	Small stones
Gruda	10.9 ± 2.3	0.8 ± 0.1	rubble
Merkys	10.9 ± 2.7	0.7 ± 0.1	sand
Musia	16.5 ± 2	0.5 ± 0.1	Small stones
Riese	15.1 ± 3.5	0.8 ± 0.1	Small stones
Skroblus	9.1 ± 2.1	0.5 ± 0.1	sand
Susiena	13.7 ± 0.9	0.3 ± 0.1	sand
Sventoji	14.9 ± 1.5	0.7 ± 0.1	sand
Ula	11.4 ± 1.9	0.6 ± 0.2	rubble
Varius	12.5 ± 1.5	0.3 ± 0.1	sand
Vilnia	13.5 ± 3.2	0.7 ± 0.1	rubble
Virinta	13.6 ± 1.7	1.2 ± 0.1 / 1 ± 0	Rubble / Small stones

Table 3. Chemical parameters of the rivers investigated (average ± SD).

	pH	Total hardness (mmol/l)	Organic matter (mgO^2/l)	Amount of oxygen (mg/l)	-NO^2 (mg/l)	-NO^3 (mg/l)	-PO^4 (mg/l)
Dubysa	8	3.3 ± 0.1	3.3 ± 0.2	8 ± 1.6	0.02 ± 0.01	5 ± 4.1	0.08 ± 0.06
Elme	8	3.7 ± 0.3	2.5 ± 0.2	8.7 ± 0.5	0.05 ± 0	5.3 ± 3.7	0.25 ± 0
Gruda	8	2.4 ± 0.1	3.6 ± 0.9	9.8 ± 0	0.01 ± 0	0.7 ± 0.5	0.08 ± 0.06
Merkys	8	2.7 ± 0.1	3.1 ± 0.6	9.3 ± 0.8	0	2 ± 1.6	0.08 ± 0.06
Musia	8	3.5 ± 0.1	4 ± 0.9	9 ± 0.7	0.04 ± 0.02	5 ± 4.1	0.08 ± 0.06
Riese	8.1 ± 0.1	3.2 ± 0.2	3 ± 0.5	7.1 ± 1.6	0.03 ± 0.02	5± 4.1/ 0.3 ± 0.3	0.1 ± 0.1
Skroblus	8	2.2 ± 0.1	2.2 ± 0.4	9.5 ± 0.5	0	1 ± 0.8	0.08 ± 0.06
Susiena	7.8 ± 0.2	3.3 ± 0.1	4 ± 2.2	8.2 ± 0.4	0.02 ± 0.01	6.7 ± 4.7	0.08 ± 0.06
Sventoji	8	3 ± 0.2	3.2 ± 0.3	8.9 ± 1.2	0	0	0
Ula	8	2.8 ± 0.1	2.2 ± 0.2	9 ± 0.6	0.1 ± 0.1	7 ± 4.2	0.08 ± 0.06
Varius	8	2.9 ± 0.1	5 ± 0.1	9.1 ± 1	0	0	0
Vilnia	8.1 ± 0.3	3.0 ± 0.3	2.6 ± 0	8.4 ± 1.2	0.03 ± 0.01	5 ± 4.1	0.17 ± 0.12
Virinta	8.2 ± 0.1	3.3 ± 0.2	3.9 ± 0.9	9.4 ± 0.8	0.1 ± 0.01	3.3 ± 2.9	0.08 ± 0.06

est particles), small stones were scored as 2, and rubble was scored as 3. The two study sites on the Virinta River differed in their substrate and velocity (Table 2), and the two study sites on the Riese River differed statistically in some chemical parameters (quantity of $-NO^3$) (Table 3) .

Chemical parameters also differed at the different study sites. The pH varied from 7.5 (the Susiena River) to 8.5; total hardness varied from 2.3 to 4.1 mmol/l; the amount of oxygen dissolved in the water varied from 5.8 to 10 mg/l; permanganate oxidation of organic matter varied from 1 to 5.1 mg O^2/l; the quantity of nitrites varied from 0 to 0.8, that of nitrates from 0 to 10, and that of phosphates from 0 to 0.25 mg/l (Table 3).

We did not find any blackfly larvae in the Varius. A low abundance of blackfly larvae was typical of the Susiena river (from five to 547 larvae per 1 dm^2 of water plant surface). The highest densities of blackfly larvae were recorded for the Merkys (1206 ± 249 larvae/1 dm^2), Ula (1197.5 ± 472.5 larvae/1 dm^2), and Skroblus (959 ± 289 larvae/1 dm^2) Rivers. The total density of blackfly larvae was not correlated with any of the physical or chemical parameters investigated.

Our investigation revealed the presence of 15 blackfly species at the 15 study sites (Table 4).

Table 4. Blackfly species found in the rivers investigated. D – Dubysa, E – Elme, G – Gruda, Mr – Merkys, M – Musia, R – Riese, Sk – Skroblus, Sv – Sventoji, U – Ula, Va – Varius, Vl – Vilnia, Vr – Virinta.

	D	E	G	Mr	M	R	Sk	S	Sv	U	Va	Vl	Vr
Cnephia pallipes (Fries, 1824).						+							
Simulium lineatum (Meigen, 1804)	+		+		+	+			+	+		+	+
S. equinum (L., 1758)	+		+	+			+		+	+		+	+
S. erythrocephalum (De Geer, 1776)	+											+	
S. intermedium Roubaud, 1906							+					+	
S. ornatum Meigen, 1818		+	+	+	+	+	+	+	+	+		+	
S. trifasicatum Curtis, 1839		+	+				+	+		+			
S. frigidum (Rubzov, 1940)							+						
S. curvistylus Rubzov, 1957					+							+	
S. posticatum Meigen, 1838	+	+	+			+	+						
S. rostratum Lundstrom, 1911					+	+		+					+
S. morsitans Edwards, 1915	+		+	+		+				+		+	+
S. noelleri Friederichs, 1920				+	+	+						+	
S. paramorsitans Rubzov, 1956		+	+	+		+			+			+	+
S. reptans (L., 1758)				+					+			+	

Some species, such as *C. pallipes, S. intermedium,* and *S. curvistylus,* were recorded at only one or two study sites, whereas others, such as *S. lineatum, S. equinum*, and *S. ornatum,* were found frequently. The dominant blackfly species differed at the various study sites in different rivers (Table 5).

The species *Simulium ornatum* and *S. equinum* were dominant in three of the rivers investigated, *S. equinum* and *S. morsitans* were dominant in three rivers (marked * in Table 5), while one river (marked **) was characterized by dominance of *Simulium ornatum, S. equinum* and *S. morsitans. Simulium ornatum* and *S. rostratum* were dominant in three rivers (marked *** in Table 5);

and *S. ornatum* was dominant in one river (marked [x]). One river was characterized by dominance of *S. lineatum* and *S. equinum* ([xx]).

Table 5. Dominant blackfly species in different rivers (%).

	S. ornatum	*S. equinum*	*S. lineatum*	*S. morsitans*	*S. rostratum*
Sventoji	48,86	20,38			
Merkys	12,23	81,09			
Dubysa*		20,42		49,72	
Vilnia[xx]		57,89	24,17		
Ula*		44,87		37,86	
Skroblus	73,58	16,06			
Virinta*		31,71		37,5	
Gruda**	65,99	20,53		21	
Musia ***	29,81				27,57
Riese***	23,9				57,34
Susiena***	67,86				19,5
Elme[x]	93,25				

The results of multiple regression analysis showed that according to our data the larval density of five blackfly species was statistically correlated with certain physical and chemical parameters of the rivers (Table 6). The relationship between water hardness and carbonate hardness was very close ($R=0.89$, $p < 0.05$). Carbonate hardness was not used in the multiple regression analysis in order to avoid multicolinearity. The most important factor for density of *S. trifasciatum* was permanganate oxidation of organic matter. Substrate, river discharge, and water hardness were parameters of lesser importance (Table 6). Larvae of *S. trifasciatum* were found more often and more abundantly in sandy, small, clean rivers with soft water. The most important factor for density of *S. ornatum* larvae was pH. Discharge, water hardness, permanganate oxidation of organic matter and the quantity of nitrates in the water were factors of lesser importance. Larvae of *S. ornatum* were found more often and more abundantly in small rivers with hard, clean water and low pH. The most important factor for density of *S. lineatum* larvae was water discharge. Another important parameter for distribution of *S. lineatum* larvae was the quantity of nitrates in the water. Larvae of *S. lineatum* were found more abundantly in large and rich in nitrates rivers. The most important factor for density of *S. morsitans* larvae was the substrate. Permanganate oxidation of organic matter and the quantity of nitrates were factors of lesser importance. Larvae of *S. morsitans* were found more often and more abundantly in rich in organic matter rivers with a rubble substrate. The most important factor for density of *S. rostratum* larvae was the quantity of nitrates. Water temperature and pH were other important parameters for distribution of *S. rostratum* larvae (Table 6). Larvae of *S. rostratum* were found abundantly in warm, rich in nitrates rivers with a mean pH of at least 8 and higher.

Table 6. Some statistically reliable coefficients of multiple regression for five blackfly species: F, ß (beta), B, Partial correlations, p.

	ß (Beta)	B	Partial Correlations	p <
S. trifasicatum, F= 3,719, p<0,05				
Organic matter	-0.509	-5.731	-0.39	0.001
substratum	-0.476	-6.954	-0.58	0.01
discharge	-0.392	- 0.393	-0.45	0.01
hardness	-0.335	-9.139	-0.48	0.05
S. ornatum, F= 3,227, p< 0,005				
pH	-0.478	-105.947	-0.38	0.01
discharge	-0.396	-1.171	-0.45	0.05
hardness	0.379	30.63	0.42	0.05
$-NO^3$	-0.370	-3.064	-0.381	0.05
Organic matter	-0.288	-9.582	-0.36	0.05
S. lineatum, F= 2,824, p< 0,05				
discharge	0.470	1.476	0.49	0.01
$-NO^3$	0.386	3.394	0.38	0.05
S. morsitans, F= 4,99, p< 0,0005				
substratum	0.353	10.289	0.41	0.05
$-NO^2$	0.291	88.288	0.38	0.05
Organic matter	0.247	5.56	0.35	0.05
S. rostratum, F= 2,438, p< 0,05				
$-NO^3$	0.454	2.262	0.43	0.05
pH	0.439	58.52	0.40	0.05
temperature	0.377	2.551	0.38	0.05

DISCUSSION

We did not find any blackfly larvae in the Varius River, and low abundance of blackfly larvae was typical of the Susiena River too, but the main reason for this could be the low stream velocity. It is known that some species, such as *Simulium costatum*, are very resistant to low stream velocity (JENSEN, 1997), but blackfly larvae commonly do not develop in streams with a velocity of less than 0.1 – 0.2 m/s (RUBTSOV, 1956). Velocity of the Varius River in summer was 0.2 m/s, while that of the Susiena River in summer, when the greatest abundance of blackflies was detected, was slightly higher at 0.4 m/s. Larvae of *S. ornatum* and *S. rostratum*, the dominant species in the Susiena River, can live at such low velocities. The highest densities of blackfly larvae were typical of the Merkys, Ula and Skroblus Rivers. The highest densities of blackfly larvae were not typical of rivers with the highest stream velocity: the velocity of rivers characterized by the highest density of blackflies varied from 0.5 to 0.7 m/s.

The Susiena River was the only one of all those investigated in which the water was characterized by a low pH, with a mean pH that was lower than 8. Larvae of *S. ornatum* can be abundant in such conditions.

Discharge was important for three of the species investigated. *Simulium trifasciatum* and *S. ornatum* preferred small rivers, while *S. lineatum* preferred large rivers (discharge varied from 0.3 to 33 m^3/s). In a previous study the density of *S. ornatum* was shown to be negatively correlated with discharge of the river (BERNOTIENE, 2006) (the discharge of the rivers investigated varied from 1 to 500 m^3/s).

Our studies have shown that *S. lineatum* prefers larger rivers (with a discharge of from 0.3 to 33 m^3/s), and it was one of the dominant species in an even larger river, the Neris (discharge about 200 m^3/s) (BERNOTIENE, 2006).

It is difficult to say how the substrate can influence the density of one or another blackfly species. Blackfly larvae can usually be found attached to water plants and only rarely to stones. In spite of that, the densities of *S. trifasciatum* and *S. morsitans* were related to river substrate: the larvae of *S. trifasciatum* preferred sand and the larvae of *S. morsitans* preferred courser substrates, such as rubble. The substrate in our data correlated with stream velocity ($R= 0.44$, $p < 0.05$). Perhaps the distribution of these blackfly species can be attributed to the influence of two parameters.

Many of the parameters investigated are closely related with eutrophication and with each other. Input of nitrates and phosphates causes gradual eutrophication of many rivers, especially lowland rivers. Such eutrophication can also result from cumulative point sources of organic matter (GILLER AND MALMQVIST, 1998). Organic pollution reduces oxygen (GILLER AND MALMQVIST, 1998). Many organisms also show a clear preference for cold waters, which may have much to do with the effects of temperature on oxygen availability. Species of water insects do differ in their respiratory ability and oxygen requirements, and these differences may contribute to differences in species distribution (GILLER AND MALMQVIST, 1998).

Larvae of *S. trifasciatum* were found at only five study sites. Density of *S. trifasciatum* larvae is know to be negatively correlated with the total amount of nitrogen (BERNOTIENE, 2006). Our data from small rivers show similar results. A negative correlation between density of *S. trifasciatum* and the permanganate oxidation of organic matter was detected. As total nitrogen is linked with the amount of organic matter, we can conclude that larvae of *S. trifasciatum* prefer rivers with low organic pollution and ones with soft water (Table 6).

Larvae of *S. ornatum* can be found in very different rivers in Lithuania. The larvae of this species were found at 11 of the 15 study sites (Table 6). The species was dominant in eight of the 13 rivers (Table 5). We previously found that density of *S. ornatum* larvae is negatively correlated with some factors connected with water pollution, viz. permanganate oxidation of organic matter, biochemical oxygen demand (BOD), total amount of phosphorus, and total amount of nitrogen (BERNOTIENE, 2006). Our data from small rivers show a similar dependence. We found a negative correlation between density of *S. ornatum* and permanganate oxidation of organic matter as well as the quantity of nitrates in the water. In spite of the fact that *S. ornatum* is known as an “anthropophilic" species resistant to water pollution (RUBTSOV, 1956; KAPLICH ET AL., 1992), larvae of *S. ornatum* prefer rivers with low organic pollution. The difference between *S. ornatum* and *S. trifasciatum* was in the relationship between larval density and water hardness. Larvae of *S. trifasciatum* preferred soft water, whereas those of *S. ornatum* preferred hard water (Table 6).

Positive correlations were found between parameters related to organic pollution and density of the larvae of three blackfly species (*S. lineatum, S. morsitans*, and *S. rostratum*). In investigation conducted in different rivers in Lithuania density of *S. lineatum* was found to be correlated negatively with permanganate oxidation of organic matter, BOD, and the total quantity of phosphorus (BERNOTIENE, 2006). In studies showing a negative correlation between density of *S. lineatum* and parameters related to organic pollution, permanganate oxidation of organic matter varied from 5.4 to 14 (mgO^2/l), whereas the same parameter varied from 2.2 to 5 (mgO^2/l) in our studies in small rivers. Thus, the greatest abundance of *S. lineatum* can be found in rivers with permanganate oxidation of organic matter around 5 mgO^2/l.

Simulium rostratum is a Holarctic species. The larvae are prevalent in enriched flows, especially around the outlets of lakes and in beaver ponds (ADLER ET AL., 2004). These reaches of rivers can be characterized by higher water temperature and water richer in organic matter as compared with sections of rivers without ponds and far from lakes. The positive correlation between density of *S. rostratum* and water temperature as well as the quantity of nitrates in the water is therefore not surprising. *Simulium rostratum* was dominant at the study sites on the Riese and Musia Rivers about 50 - 70 m from the outlet and at study sites on the Riese and Susiena Rivers more than 4 km from the outlet.

The density of larvae of other blackfly species was not correlated with any of the physical or chemical parameters investigated. This study in small rivers showed that the species composition and density of some blackfly species depend upon the prevailing environmental factors in their habitats.

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