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Larvae of Chironomids (Insecta, Diptera) Encountered in the Mantle Cavity of Zebra Mussels, *Dreissena polymorpha* (Bivalvia, Dreissenidae)

key words: Dreissena, Chironomidae, endosymbiosis, prevalence of infection, intensity of infection, seasonal dynamics

Abstract

The paper includes data on species composition of chironomid larvae which were encountered in the mantle cavity of zebra mussels (*Dreissena polymorpha*) within 7 waterbodies in the Republic of Belarus. All were found to be free-living species commonly present in periphyton and/or benthos. A long-term study of the seasonal dynamics of these larvae in *Dreissena* did not reveal any typical pattern. Our data suppose that chironomids do not have an obligate association with zebra mussels and possibly enter their mantle cavity inadvertently.

1. Introduction

The zebra mussel, *Dreissena polymorpha* (PALLAS), is a macrofouling species whose proliferation has caused significant ecological and economic consequences within European and North American waterbodies (KARATAYEV *et al.*, 1997, 2002b; O'NEILL, 1997). Although numerous works on the biology and ecology of this bivalve have been conducted (for instance, see KARATAYEV *et al.*, 1997, 2002b), relatively little is known about their endosymbionts (i.e., commensals and parasites) and the role that they play in the population dynamics of *Dreissena* (MOLLOY *et al.*, 1997). This information gap is currently being addressed as a project of International Research Consortium on Molluscan Symbionts (IRCOMS), a network of over a dozen of scientists from the former Soviet Union, Europe, and North America (MOLLOY, 2003). In this IRCOMS project, efforts have initially focused on the development of a fundamental database characterizing the systematics, biology, ecology, and distribution of *Dreissena*'s endosymbionts (BURLAKOVA *et al.*, 1998; KARATAYEV *et al.*, 1999, 2000a, b, 2002a, 2003a, b; LARUELLE *et al.*, 1999, 2002; MOLLOY *et al.*, 1996, 1997, 2001). The present paper is a further IRCOMS contribution toward understanding the endosymbionts of *D. polymorpha.*

Among all 34 known endosymbionts of zebra mussels (MOLLOY *et al.*, 1997), chironomids are one of the less studied groups. Chironomidae larvae have been frequently encountered in *D. polymorpha*, including waterbodies of Russia (KUPERMAN *et al.*, 1994), Belarus (KARATAYEV *et al.*, 1999, 2000a; MASTITSKY, 2001) and North America (CONN *et al.*, 1994; RICCIARDI, 1994). Identification of these chironomids, however, was seldom reported to the

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species level, and the nature of their relationship to zebra mussels still remains unclear. To address these issues, we herein present a list of 14 chironomid taxa which were observed in the mantle cavity of zebra mussels within 7 waterbodies in the Republic of Belarus. Data on prevalence and intensity of infection with chironomids as well as results of the first long-term, month-to-month monitoring of chironomid larvae in *D. polymorpha* are also presented. Our data have confirmed previous observations (CONN *et al.*, 1994; KUPERMAN *et al.*, 1994; RICCIARDI, 1994) that only free-living chironomids are present in zebra mussels.

2. Methods

During 2001–2002, we sampled *D. polymorpha* populations in 7 waterbodies in the Republic of Belarus (Table 1). All samples were collected at 0.5–1.5 m depth from rocky substrates, on which mussels formed aggregated populations; samples were stored at 10–15 °C, and dissected within 72 h. Before dissections, molluscs were sorted by length into several size classes using 5.0 mm intervals.

Table 1. Morphometry of waterbodies studied, date of sampling and number of mussels collected.

Waterbody	Location	Surface (km^2)	Volume (millions m^3)	Maximal depth (m)	Date of sampling (day.month. year)	Number of mussels collected
Svisloch River	53°55′N, 27°30′E, city of Minsk	n.r.*	n.r.	2.0	08.11.2001	99
Drozdy Reservoir	along Svisloch River, city of Minsk	2.1	5.7	6.0	April 2001–November 2002	3036
Komsomolskoe Reservoir	along Svisloch River, city of Minsk	0.4	0.8	4.5	05.11.2001	129
					April–October 2002	813
Chizhovskoe Reservoir	along Svisloch River, city of Minsk	1.63	1.87	4.7	12.11.2001	63
Berezina River	54°14′N, 28°31′E, city of Borisov	n.r.	n.r.	3.0	12.06.2002	60
					21.11.2002	11
Lake Lukomskoe	54°40′N, 29 °05′E, Novolukoml town	36.7	243.0	11.5	22.09.2001	500
Lake Naroch	53°50′N, 26 °45′E, Nikoltsy village	79.6	710.0	24.8	18.07.2001	100

* n.r. = not recorded

Wherever possible, we examined the following classes: 0.1–4.9 mm, 5.0–9.9 mm, 10.0–14.9 mm, 15.0–19.9 mm, 20.0–24.9 mm, 25.0–29.9 mm and 30.0–34.9 mm. To determine the prevalence (i.e., percent of infected molluscs) and intensity (i.e., number of symbionts per single host organism) of chironomid infection, 15 to 50 mussels from each size class were dissected. Before dissections, we cleaned and dried mussel shell surfaces and measured their length with calipers to the nearest millimeter. Mussels were cut open with a scalpel and their soft tissues were transferred to a plankton counting chamber containing unchlorinated tap water. Observations for chironomids were then made using a stereomicroscope (20–70x). In total, 4,803 mussels were dissected. Larvae found were preserved in 4% formalin for subsequent identification to the lowest taxonomic level possible (CHERNOVSKY, 1949; PANKRATOVA, 1970, 1977). Sometimes some larvae were damaged during dissections. In such cases, they were not preserved and prevalence of infection was not calculated for such samples because species could not be identified.

To study the seasonal dynamics of *D. polymorpha* infection with chironomids, we conducted month-to-month dissections from the reservoirs Drozdy (April 2001 to November 2002; during July–August 2001, samples were taken twice a month) and Komsomolskoe (April to October 2002). In the Drozdy Reservoir, mussels were collected at 0.5–0.7 m depth at 3 replicate sites. Fifty mussels with shell lengths of 15.0–20.0 mm were randomly selected from each replicate and dissected. Consequently, at each sampling time we examined ca. 150 mussels. In the Komsomolskoe Reservoir, mussels were collected at 3 different locations, and dissections were made after sorting the mussels into the above-mentioned size classes. To determine infection parameters, 15 molluscs from each size class were dissected. Thus, usually ca. 45 molluscs were examined at each sampling time from each sampling site. For both Drozdy and Komsomolskoe reservoirs, we evaluated the total prevalence and intensity of infection at the family level, not at the level of individual species.

Statistical analysis was performed using STATISTICA 6.0 software (StatSoft Inc., 2001). To compare mean prevalence values, we applied Kruskal-Wallis *H*-test by ranks. The difference between infection prevalence of mussels from different size classes was evaluated with *G*-test of independence (SOKAL and ROHLF, 2001).

3. Results

3.1. Species Composition of Chironomids in D. polymorpha and Degree of Mussel Infection

We succeeded in collecting of 62 undamaged chironomid larvae representing 14 taxa and 2 subfamilies (Tables 2 and 3). Of these, 62.9% (39 larvae) were in the Chironominae and 37.1% (23 larvae) in the Orthocladiinae. The most common species was *Limnochironomus* gr. *nervosus* (24 larvae – 38.7%). A few larvae could only be identified to genus (e.g., *Cryptochironomus* sp., *Cricotopus* sp., *Prodiamesa* sp.) or to subfamily (e.g., Orthocladiinae) because they were early instars and thus did not possess the pronounced morphological characteristics required for species identification (e.g., well developed submentum, mandibles, antennae).

The prevalence of infection with larvae of individual species varied from 0.3 to 7.7% (Tables 1 and 2). There was only one case when prevalence was as high as 18.2% (*L. nervosus,* Berezina River, 21.11.2002). This high value of prevalence was not typical and, undoubtedly, should be explained by the very small number of dissected mussels (n = 11). As a rule, we observed just a single larva per mussel. The highest intensity of infection was observed in Lake Lukomskoe, i.e., 3 larvae in a 27 mm long mussel (22.09.2001).

Although in the current study larvae of chironomids were found most frequently in mussels with a shell >15 mm, there was no statistically significant correlation noted between prevalence and mussel size class in any waterbody ($0.50 < P \leq 1.0$, *G*-test). As mentioned above, the most common value of infection intensity was 1 larva/mussel and, correspondingly, this parameter of infection also did not demonstrate any relationship with mussel size.

Table 2. Chironomids from subfamily Chironominae found in *Dreissena polymorpha* in waterbodies of Belarus.

Species	Waterbody	Sampling date (day.month.year)	Infection prevalence (%)
Cryptochironomus sp.	Drozdy Reservoir	28.08.2001	0.8
Glyptotendipes gr. *gripekoveni* KIEFF.	Chizhovskoe Reservoir	12.11.2001	1.6
	Drozdy Reservoir	27.11.2002	n.r.*
Limnochironomus gr. *nervosus* STAEG.	Berezina River	21.11.2002	18.2
	Drozdy Reservoir	19.11.2001	n.r.
		16.01.2002	0.7
		25.03.2002	n.r.
		30.09.2002	n.r.
	Komsomolskoe Reservoir	05.11.2001	2.3
		03.04.2002	0.8
		21.05.2002	0.8
		25.07.2002	7.7
		27.11.2002	n.r.
	Svisloch River	08.11.2001	1.0
		09.10.2002	2.2
Limnochironomus tritomus KIEFF.	Berezina River	12.06.2002	1.9
	Drozdy Reservoir	27.05.2002	0.3
		26.08.2002	0.7
Paratanytarsus gr. *lauterborni* KIEFF.	Drozdy Reservoir	15.08.2001	0.8
		30.09.2002	n.r.
Polypedilum gr. *convictum* WALK.	Chizhovskoe Reservoir	12.11.2001	4.8
	Drozdy Reservoir	19.06.2002	0.7
		30.09.2002	n.r.
Tanytarsus gr. *gregarius* KIEFF.	Lake Naroch	18.07.2001	0.8

* n.r. = not recorded

3.2. Seasonal Dynamics of D. polymorpha Infection with Chironomids

Two years of observations in the Drozdy Reservoir and a 7-month study in the Komsomolskoe Reservoir did not reveal any notable seasonal pattern of *D. polymorpha* infection with chironomids (Figure 1). In the Drozdy Reservoir, mean monthly prevalence of infection did not differ significantly in 2001 ($P = 0.48$, *H*-test), but did varied in 2002 ($P < 0.01$, *H*-test). The highest infection prevalence in this waterbody occurred in November 2001 ($2.0 \pm 1.2\%$; mean ± SE here and elsewhere in the paper) and in October 2002 ($8.7 \pm 0.7\%$). In contrast, maximum prevalence ($7.7 \pm 7.7\%$) in the Komsomolskoe Reservoir in 2002 was observed in July.

Table 3. Chironomids from subfamily Orthocladiinae found in *Dreissena polymorpha* in waterbodies of Belarus.

Species	Waterbody	Sampling date (day.month.year)	Infection prevalence (%)
Corynoneura celeripes WINN.	Komsomolskoe Reservoir	05.11.2001	0.8
Cricotopus gr. *algarum* KIEFF.	Drozdy Reservoir	August 2001	n.r.*
		30.09.2002	n.r.
		27.11.2002	n.r.
Cricotopus gr. *silvestris* FABR.	Drozdy Reservoir	19.06.2002	0.7
		30.09.2002	n.r.
		27.10.2002	n.r.
Cricotopus sp.	Drozdy Reservoir	27.10.2002	n.r.
	Lake Lukomskoe	22.09.2001	0.2
Eukieferiella bicolor ZETT.	Svisloch River	08.11.2001	1.0
Prodiamesa sp.	Komsomolskoe Reservoir	21.05.2002	1.2
Orthocladiinae spp.	Drozdy Reservoir	30.09.2002	n.r.
		27.10.2002	n.r.
	Komsomolskoe Reservoir	03.04.2002	2.1

* n. r. = not recorded

Mean prevalence of mussel infection in the Drozdy Reservoir in 2001 was $0.65 \pm 0.26\%$. In 2002 it was $2.47 \pm 0.60\%$, significantly higher than in 2001 ($P = 0.01$, *H*-test). Mean 7-month prevalence in the Komsomolskoe Reservoir in 2001 was $1.94 \pm 0.98\%$. In both waterbodies, the intensity of infection did not exceed 2 larvae per mussel during the entire period of observations and commonly was 1 larva/mussel.

4. Discussion

Although chironomids have been frequently recorded from the mantle cavity of *D. polymorpha* (KUPERMAN *et al.*, 1994; CONN *et al.*, 1994; RICCIARDI, 1994; KARATAYEV *et al.*, 1999, 2000a; MASTITSKY, 2001), their identification to lower taxonomic levels have generally been lacking. Exceptions include: *Chironomus bathophilus* from the Volga Basin (Russia) (KUPERMAN *et al.*, 1994) and *Paratanytarsus* spp. from the St. Lawrence River (New York State and Quebec Province) (CONN *et al.*, 1994; RICCIARDI, 1994). The present work represents the first study to focus on chironomid species composition within *D. polymorpha.* All species found were common representatives of periphyton and/or benthos of freshwater waterbodies (CHERNOVSKY, 1949; PANKRATOVA, 1970, 1977). Since chironomid species vary significantly between waterbodies, the list of species that might be encountered in the mantle cavity of *Dreissena* is no doubt far more extensive than noted in the present paper.

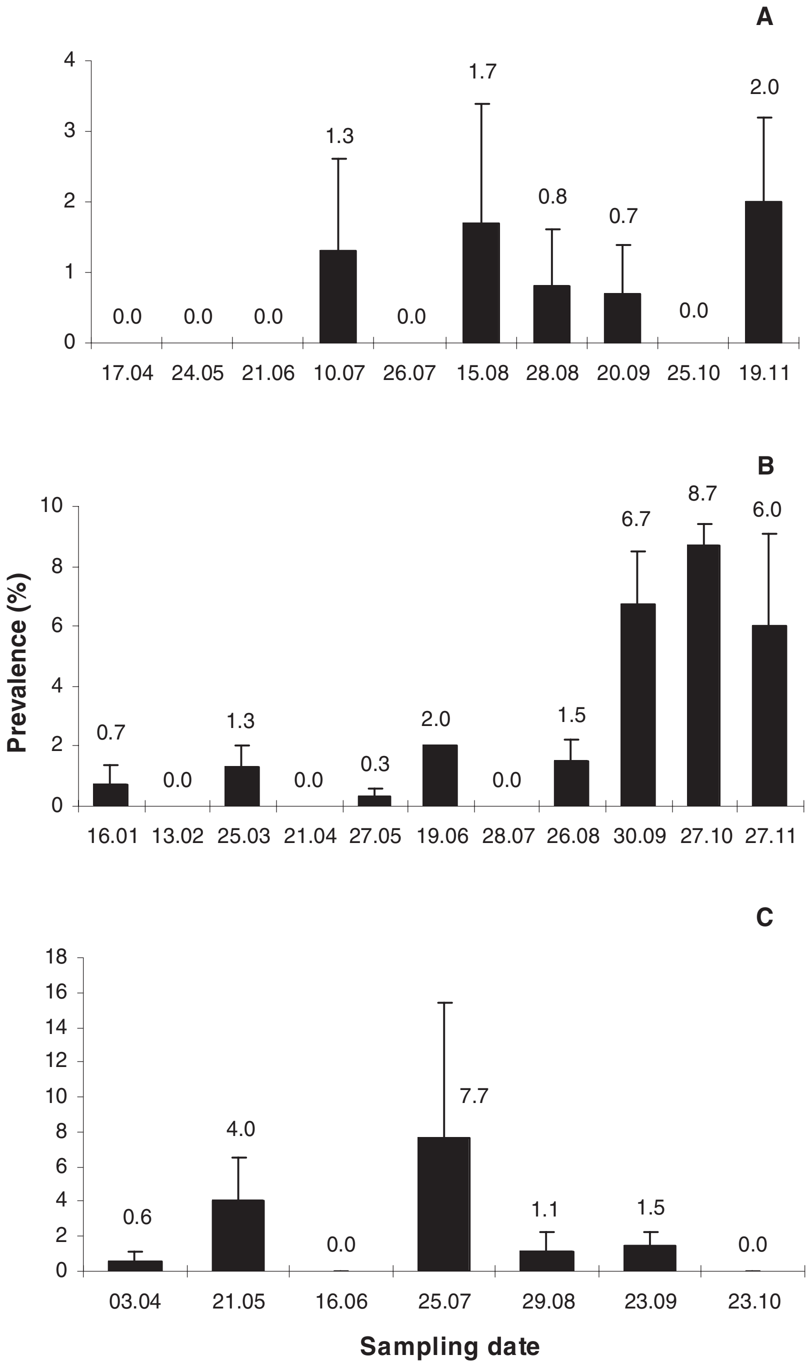

Figure 1. Seasonal dynamics of *D. polymorpha* prevalence of infection with Chironomidae larvae. A: Drozdy Reservoir, 2001; B: Drozdy Reservoir, 2002; C: Komsomolskoe Reservoir, 2001. Bar height corresponds to mean prevalence values (expressed by numbers). Vertical lines are standard errors of means.

We found that the mean prevalence of infection of mussels with chironomids of individual species varied from 0.3 to 8.7 ± 0.7% (Tables 1 and 2). This range of infection prevalence values is in accordance with previous reports (KUPERMAN *et al.*, 1994; CONN *et al.,* 1994; RICCIARDI, 1994; KARATAYEV *et al.*, 1999, 2000a; MASTITSKY, 2001). We usually observed a single larva in a mussel. Other researchers (RICCIARDI, 1994; KARATAYEV *et al.*, 1999, 2000a) reported the same low intensity of infection. The maximum chironomid intensity ever documented in *Dreissena* was 6 larvae/mussel (RICCIARDI, 1994).

There are reports of close positive correlations between size of zebra mussels and their prevalence and intensity of infection with some endosymbionts (BURLAKOVA *et al.*, 1998; KARATAYEV *et al.*, 1999, 2000b, 2002a). Although we usually found chironomid larvae in mussels longer than 15 mm, statistically significant correlations of such kind for chironomids were observed in none of waterbodies studies. Perhaps, it can be explained by the absence of any biologically obligate relationships between *Dreissena* and chironomids.

This is the first long-term study of the seasonal dynamics of chironomids in zebra mussels. Our data suggest that chironomids are not necessarily present in *Dreissena* constantly, but just tend to occur in the mantle cavity during definite periods of the year (Figure 1). All larvae found were free-living species, and we hypothesize that they inadvertently entered zebra mussels since they are species that typically abundant in periphyton and benthos.

One of the best examples to illustrate how chironomids can appear in the mantle cavity of *Dreissena* is the *L. nervosus* – the most common species in our study. Colonies of *D. polymorpha* are well known to create special environments characterized by an abundance of substrate, shelter, and food for many types of organisms (KARATAYEV *et al.*, 1994, 1997, 2002b; STEWART *et al.*, 1999), and *L. nervosus* has been observed in *D. polymorpha* aggregations by many authors (WOLNOMIEJSKI, 1970; IZVEKOVA, 1980; SOKOLOVA, 1980; SOKOLOVA *et al.*, 1980; PROTASOV and AFANASIEV, 1984). According to SOKOLOVA (1980) and IZVEKOVA (1980), *L. nervosus* is often found within druses of zebra mussels living directly on their shells. Inhabiting a druse allows a larva to be present under a real "rain" of suspended food particles which were agglutinated by mussels (i.e., feces and pseudofeces). Taking into consideration that larvae of *L. nervosus* were also often observed to crawl among *Dreissena*'s byssus threads (IZVEKOVA, 1980), it is easy to understand how these larvae might get into the mantle cavity through the pedal gape in the shell. Larvae might also be carried by water flow into the mussel's inhalant siphon during filtering activity. Most probably, both of these routes of infection occur. Irrespective of their route of entry, larvae must be small enough to get into the mussel. Indeed, our data demonstrate this since it was usually very small larvae, i.e. early developmental stages, which were found inside the mussel.

Having got into the mantle cavity inadvertently, chironomids might then gain definite benefits, e.g., protection from predators and a rather constant supply of food particles and oxygen. Assuming no detrimental effect to the host mussel, chironomid larvae could thus be regarded as commensals of *Dreissena* (RICCIARDI, 1994). Nevertheless, further field and experimental studies need to be undertaken to verify any possible benefits. In this regard, it would be valuable to compare the relative occurrence of chironomid species inside of zebra mussels and in the periphyton samples taken from the shells of mussels. If any species would appear to infect mussels more frequently than others do, it might be expected to invade its host intentionally.

SOKOLOVA *et al.* (1980) reported that most chironomids they observed in druses of zebra mussels in the Uchinskoe Reservoir (Russia) were gatherers. Besides *L. nervosus*, these included *L. pulsus* var. *objectans*, *Tanytarsus* spp., *Microtendipes pedellus*, *Lauterborniella brachylabis*, *Polypedilum nebeculosum*, and *Procladius ferrugineus*. The typical food for these species was detritus and algae. IZVEKOVA (1980) indicated that the same food was usual in the guts of the *Glyptotendipes gripekoveni*, *Tanytarsus gregarius* and *Polypedilum* spp. – species also found in our study (Tables 1 and 2). These data on feeding habits of chironomids from *D. polymorpha* druses suggest that larvae encountered in the mantle cavity of mussels do not cause any serious damage to their hosts. Yet larvae may cause some pressure on adjacent organs and tissues within the mantle cavity, thus disturbing their normal function. Experimental studies could be performed to measure such possible negative effects on host mussels.

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