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Seasonal dynamics of endosymbiotic ciliates and nematodes in *Dreissena polymorpha*

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Abstract

We report the results of a two-year study in the Svisloch River (Minsk, Belarus) on the dynamics of infection in *Dreissena polymorpha* by nematodes and three ciliate species *Conchophthirus acuminatus*, *Ophryoglena* sp., and *Ancistrumina limnica*. Although these endosymbionts were present in most of the samples, their prevalence and infection intensity differed significantly. *C. acuminatus* and *A. limnica* infection intensities in both years of the study had a maximum in summer and were positively correlated with water temperature. In contrast, *Ophryoglena* sp. and nematode infection intensities were considerably lower in summer versus winter and were negatively correlated with temperature. In the first long-term study to monitor the size and reproductive rate of *C. acuminatus*, we found that mean length was negatively correlated with temperature and that temperature was positively correlated with asexual reproduction, with a peak of cell division in April as water temperatures increased.

Keywords: Endosymbiont; Zebra mussel; Belarus; *Conchophthirus acuminatus*; Nematode; *Ancistrumina limnica*; Commensal; *Ophryoglena* sp.

1. Introduction

The zebra mussel, *Dreissena polymorpha*, is one of the most aggressive and important freshwater aquatic invaders worldwide. Once introduced, their populations can grow rapidly, allowing them to become enormously abundant. They are frequently competitively dominate over native fauna and can impact all components of the freshwater ecosystem (Karatayev et al., 1997; Karatayev et al., 2002a). The introduction of *D. polymorpha* and *D. bugensis* into the Great Lakes in the mid-1980s, and their subsequent rapid spread across much of North America has caused hundreds of millions of dollars in damage and increased operating expenses at raw water infrastructures (O'Neill, 1997). The extent of the ecological and economical impact of zebra mussels is directly related to their abundance in an ecosystem. Populations of introduced zebra mussels, however, do not stabilize and can vary widely over time (Ramcharan et al., 1992; Stanczykowska and Lewandowski, 1993). Determining the role that endosymbionts may play in these density fluctuations is a critical step toward a comprehensive understanding of the population dynamics of these pest mussels.

Although 34 symbiotic species are known from *D. polymorpha*, relatively little effort has been made to study their interactions with their host (Molloy et al., 1997). This information gap is currently being addressed by a network of over a dozen scientists from Europe, North America, and the former Soviet Union as a research project of the International Research Consortium on Molluscan Symbionts (IRCOMS) (Molloy, 2003). IRCOMS efforts are initially focusing on the development of a fundamental database characterizing the systematics, biology, ecology, and distribution of *Dreissena*'s endosymbionts.

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This paper continues our research on the dynamics of the host-specific commensal *C. acuminatus* and a parasitic *Ophryoglena* sp. (Burlakova et al., 1998; Karatayev et al., 2000a,b, 2002b) and represents the first study of the seasonal dynamics of nematodes and the ciliate *Ancistrumina limnica* within *D. polymorpha*. This is also the first long-term study to monitor the size and reproductive rate of *C. acuminatus*.

2. Materials and methods

To determine the seasonal dynamics of *D. polymorpha* symbionts, studies were conducted in the Drozdy Reservoir (surface area 2.1 km^2, volume 5.7×10^6 m^3, maximum depth 6 m) located along the Svisloch River (53°55′N, 27°32′E) within the city of Minsk, Belarus (Fig. 1). *D. polymorpha* had colonized the reservoir and the Svisloch River in the mid-1980s. In 2001–2002, samples were usually taken at monthly intervals; however, during summer 2001 we sampled at shorter intervals (ca. 15–20 days).

To compare the current infection prevalence and intensity of *D. polymorpha* with *Ophryoglena* sp. with previously published data (Karatayev et al., 2000a,b, 2002b), we collected samples in November 2001 at two other locations along the Svisloch River within Minsk, i.e., Komsomolskoye Reservoir and Chizhovskoye Reservoir (Fig. 1). Another location along the Svisloch River near Denisovskaya Street was additionally sampled in September 2002 (Fig. 1). These locations were the exact sites sampled during previous studies in 1994–1997 (Burlakova et al., 1998; Karatayev et al., 2000b).

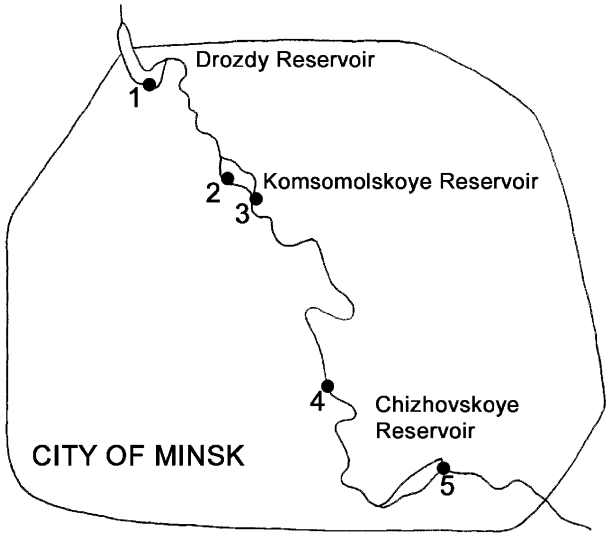

Fig. 1. Map of Minsk with sampling sites along the Svisloch River: 1, Drozdy Reservoir; 2, Komsomolskoye Reservoir; 3, Svisloch River near Ierusalimskaya Street (sampled in 1994–1996, Burlakova et al., 1998); 4, Svisloch River near Denisovskaya Street; 5, Chizhovskoye Reservoir.

At each sampling time and at each location, we recorded water temperature and made three replicate collections at a ca. 0.5–0.7 m depth from rocky substrates. At each sampling time, mussels were randomly collected from three locations 50 m apart. Each collection of 150–250 mussels from each of these three locations represented a replicate sample. Replicate samples were held separately at 10–15 °C and processed within 72 h. From each replicate, 50 mussels with shell lengths of 15.0–20.0 mm were randomly selected and dissected to determine prevalence, and 20 mussels (a subset from the latter 50) were dissected for intensity of infection. This particular size class was chosen since it was readily available at all sampling times throughout the two-year study and was known from previous studies in the Svisloch River to harbor endosymbionts (Burlakova et al., 1998; Karatayev et al., 2000a,b, 2002b). In 2001, from each replicate collection we dissected 50 mussels for *Ophryoglena* sp. and another 50 mussels for *C. acuminatus* and nematodes. In 2002, we studied all symbionts from the same 50 mussels. Thus, wherever possible, at each sampling time we examined ca. 150 mussels for prevalence and ca. 60 mussels for intensity.

Before dissections, we cleaned and dried shell surfaces and measured mussel length to the nearest millimeter with calipers. Mussels were then cut open with a scalpel and dissected in unchlorinated tap water within a plankton counting chamber using a stereomicroscope (20–70×). The whole soft body of each mussel was dissected with special attention to gills and digestive gland, each piece of which was carefully checked for the presence of symbionts. In addition, to monitor the occurrence of asexual reproduction in *C. acuminatus* we recorded the frequency of "fission pairs", i.e., individuals which were undergoing division (Beers, 1959).

To determine the seasonal dynamics of *C. acuminatus* body size at each sampling time in each replicate, 10 ciliates were isolated from each of 3 randomly selected mussels from among the 50 that were dissected for infection (i.e., 30 ciliates were measured per replicate and 90 in total per sampling date). We measured the maximum length and width of *C. acuminatus* under a microscope (400×) to the nearest 0.1 μm. Before measurements, ciliates were immobilized in 10% formalin vapor for less than 1 min. As is typical of the Conchophthiridae, *C. acuminatus* are extremely laterally compressed, and individual ciliates typically lie on their flattened left side (i.e., the portion of their cell which makes contact with a substrate is not their ventral surface). Thus, our "width" measurements might more accurately be considered "height" measurements, but "width" is the traditional term used (Raabe, 1971).

Following Saffo (1992), we have used the term "infection" in referring to all symbiotic organisms, irrespective

of whether a particular organism has a mutualistic, parasitic, or commensal association with its host.

To compare prevalence and intensity data we used Kruskal–Wallis ANOVA by ranks and median test and consequently multiple comparisons of mean ranks for all groups. Spearman rank or Pearson correlation was applied to find the relationship between water temperature and other parameters. Effects were considered statistically significant at $P < 0.05$; when multiple statistical tests were conducted on the same data simultaneously, we considered critical α for significance with Bonferroni correction. Data on *C. acuminatus* length and width were analyzed with two-factor ANOVA, I model (fixed effects), and Tukey HSD multiple comparison test was used for post-hoc analysis of means. In all cases, Statistica software was utilized (STATISTICA version 6, StatSoft, 2001).

3. Results

Nematodes and all three species of ciliates were present in almost all *D. polymorpha* samples in the Drozdy Reservoir, but differed considerably in prevalence and infection intensity (Table 1).

3.1. Conchophthirus acuminatus

Mean (±SE here and elsewhere in paper) infection prevalence and mean infection intensity were always high, ranging from 98.7 ± 0.7 to 100% and from 348 ± 32 to 1672 ± 232 ciliates/mussel, respectively (Fig. 2A, B). Mean infection intensity had a maximum in summer, i.e., July–August in 2001 and August in 2002, was considerably lower during other seasons, and positively correlated with temperature (Spearman $r_s = 0.34$, $n = 63$, $P = 0.007$).

Mean infection prevalence during 2001 and 2002 did not differ significantly among sampling dates within each year or between years (Kruskal–Wallis test: $P = 0.094$; 2001 versus 2002: $P = 0.032$, critical α with the Bonferroni correction = 0.025). In contrast, mean infection intensity did differ significantly among sample dates within each year (Kruskal–Wallis and median tests: $P < 0.001$) and was significantly higher in 2001 than in 2002 (Kruskal–Wallis and median tests: $P < 0.01$). Mean intensity in July–August was significantly higher in both years than in other months (multiple comparisons of mean ranks, $P \leqslant 0.02$). The consistently high prevalence throughout the two-year study made it impossible to find a significant correlation between infection prevalence and intensity (Spearman $r_s = -0.16$, $n = 63$, $P = 0.22$).

During the study, mean *C. acuminatus* length varied from 87.2 ± 0.9 to 100.8 ± 1.1 μm (range: 52.9–132.3 μm; total mean length 92.09 ± 0.25 μm; 95% con-

Table 1
Mean ± SE (median; sample size) endosymbionts prevalence and intensity of infection, size, and percent of fission pairs of *Conchophthirus acuminatus* in *Dreissena polymorpha* in the Drozdy Reservoir in 2001 and 2002

Endosymbiont	Prevalence (%)	Intensity (ciliates/mussel)	Length (μm)	Width (μm)	Fission pairs (%)
Conchophthirus acuminatus, 2001	99.7 ± 0.1 (100; 30)	849.3 ± 38.5 (599; 507)	91.2 ± 0.3 (91.1; 900)	53.8 ± 0.2 (52.9; 900)	1.53 ± 0.07 (0.99; 507)
Conchophthirus acuminatus, 2002	100 ± 0 (100; 33)	657.1 ± 28.9 (456; 639)	93.0 ± 0.4 (91.1; 988)	53.7 ± 0.2 (52.9; 987)	0.73 ± 0.06 (0; 639)
Ophryoglena sp., 2001	80.8 ± 4.1 (96; 30)	18.7 ± 1.8 (6; 502)	n.r.[a]	n.r.	n.r.
Ophryoglena sp., 2002	97.3 ± 0.7 (100; 33)	30.3 ± 1.5 (17; 620)	n.r.	n.r.	n.r.
Ancistrumina limnica, 2001	52.6 ± 6.0 (48.5; 30)	7.7 ± 1.0 (3; 247)	n.r.	n.r.	n.r.
Ancistrumina limnica, 2002	25.8 ± 4.1 (18; 33)	5.7 ± 0.7 (2; 241)	n.r.	n.r.	n.r.
Nematodes, 2001	33.0 ± 4.6 (28.6; 30)	2.4 ± 0.2 (1; 183)	n.r.	n.r.	n.r.
Nematodes, 2002	34.7 ± 3.7 (36; 33)	1.8 ± 0.1 (1; 237)	n.r.	n.r.	n.r.

[a] n.r., not recorded.

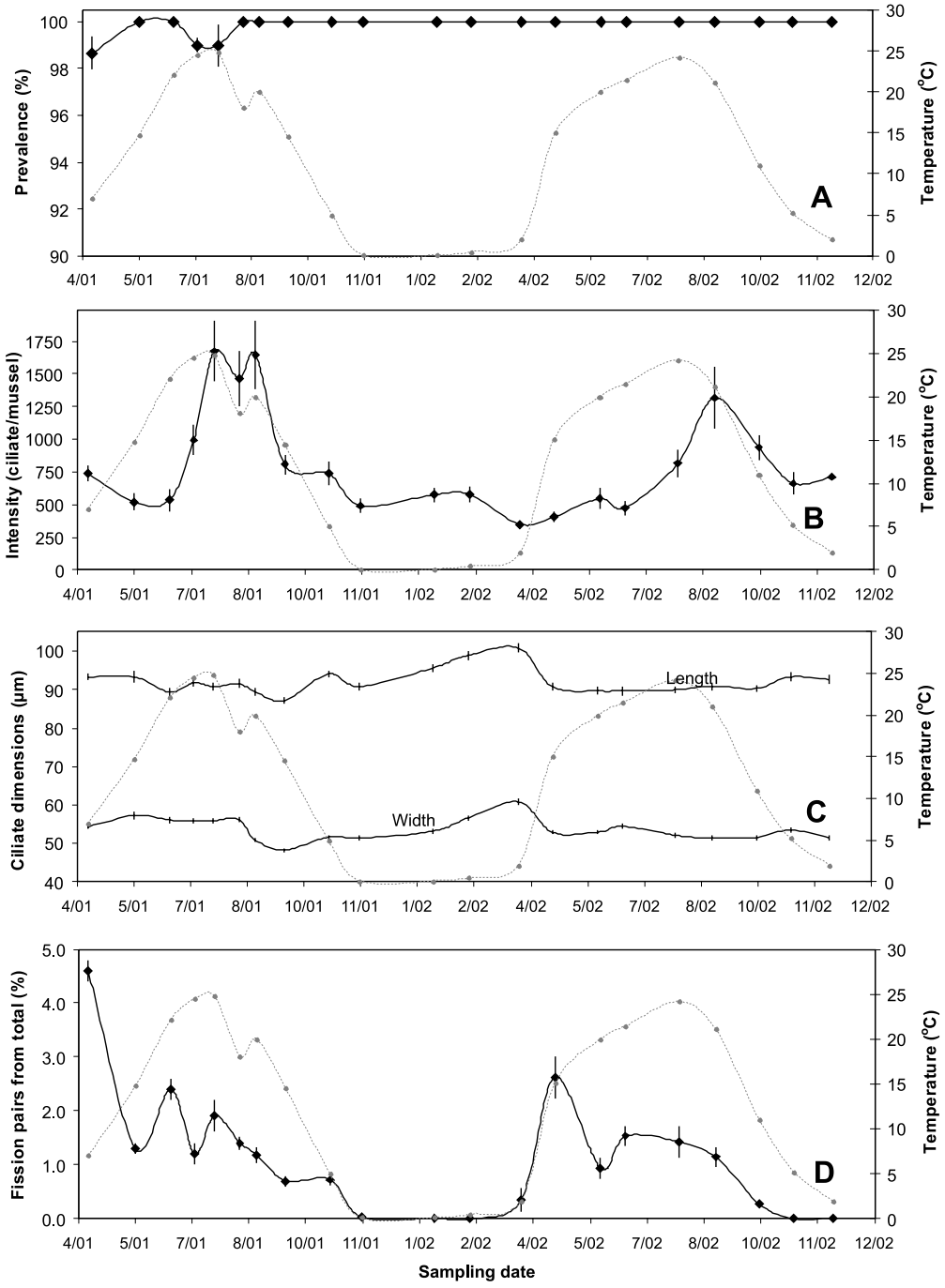

Fig. 2. Seasonal dynamics of prevalence (A), infection intensity (B), body dimensions (C, length, upper line and width, lower line) and percent of fission pairs of *Conchophthirus acuminatus* (D) in *Dreissena polymorpha* from Drozdy Reservoir (Minsk, Belarus). Data points on solid line are means ± SE. Dashed line is water temperature.

fidence interval: 91.61–92.58 μm; $n = 1888$). Mean width changed from 48.2 ± 0.5 to 60.8 ± 0.9 μm (range: 35.3–88.2 μm; total mean width 53.72 ± 0.16 μm; 95% confidence interval: 53.41–54.04 μm; $n = 1887$) (Fig. 2C). The mean body length in 2001 was slightly smaller (Table 1) and significantly different from mean average length in 2002 (Welch t test: $P < 0.001$); however, mean body width was not significantly different in both years of study (t test: $P = 0.82$). *C. acuminatus* body length varied significantly in different seasons (two-factor ANOVA: $P < 0.001$). However, both effects of sampling site and interactions were significant also ($P \leqslant 0.02$). The same was found for width seasonal dynamics (two-factor ANOVA: sampling date: $P < 0.001$; site: $P = 0.005$; interactions: $P = 0.002$). Ciliate length and width were significantly higher in March 2002 and lower in

September 2001 (Tukey HSD: $P < 0.04$, Fig. 2). Mean *C. acuminatus* body length was negatively correlated with water temperature (Pearson $r = -0.53$, $n = 63$, $P < 0.001$). However, there was no significant correlation between temperature and mean width (Pearson $r = -0.02$, $n = 63$, $P = 0.90$). Mean length of *C. acuminatus* was positively correlated with mean width (Pearson $r = 0.73$, $n = 1887$, $P < 0.001$), and the regression parameters were significant (Table 2).

The mean monthly ratio of length to width for *C. acuminatus* ranged from 1.61 ± 0.02 to 1.82 ± 0.01; total mean for both years of study was 1.724 ± 0.004 (range 1.091–2.375; 95% confidence interval: 1.716–1.730, $n = 1887$).

The mean percent of the fission pairs of *C. acuminatus* in *D. polymorpha* varied from 0 to 5% (range: 0–20%, mean ± SE: 1.08 ± 0.05, $n = 1146$), and in both years of study was the highest in April, right after the winter minimum (Fig. 2D). There was a significant correlation between the mean percent of the fission pairs of *C. acuminatus* and temperature (Spearman $r_s = 0.70$, $n = 63$, $P < 0.001$). Accordingly, there was a significant correlation between the mean percent of mussels in which fission pairs were found and temperature (Spearman $r_s = 0.58$, $n = 63$, $P < 0.001$). In addition, the percent of the fission pairs of *C. acuminatus* was negatively correlated with mean body length of the ciliates (Spearman $r_s = -0.34$, $n = 63$, $P = 0.007$).

3.2. Ophryoglena sp.

We found *Ophryoglena* sp. in *Dreissena* for the first time in July 2000 in Zaslavskoye Reservoir upstream of Minsk, and by 2001 they had spread downstream to all sampling locations on the Svisloch River (Table 3). Whenever *Ophryoglena* sp. infection was present at any site, prevalence tended to be moderate to high.

During the two-year study, mean monthly infection prevalence of *Ophryoglena* sp. in *Dreissena* ranged from 43.3 ± 6.8 to 100% (Fig. 3A). Prevalence was the lowest in August 2001 and considerably higher in autumn, winter, spring, and the beginning of summer. Mean infection intensity was low to moderate and ranged from 1.4 ± 0.1 to 65.8 ± 5.8 ciliates/mussel. During July–October 2001 and May–August 2002 infection intensity was minimal and significantly lower than in winter and spring ($P \leqslant 0.01$, multiple comparisons of mean ranks; Fig. 3B). We found a significant positive correlation between *Ophryoglena* sp. prevalence and infection intensity (Spearman $r_s = 0.78$, $n = 63$, $P < 0.001$). There was a slight negative correlation between *Ophryoglena* sp. prevalence and temperature (Spearman $r_s = -0.28$, $n = 63$, $P = 0.02$) and both intensity and temperature (Spearman $r_s = -0.26$, $n = 63$, $P = 0.04$).

The intensity of infection of zebra mussels with *Ophryoglena* sp. was significantly different depending on sampling date (Kruskal–Wallis and median tests:

Table 2
Significance and parameters for simple linear regression between length and width (in μm) of *Conchophthirus acuminatus* found in *Dreissena polymorpha*

Independent variable	Dependent variable	Regression coefficient ± SE (P)	Intercept ± SE (P)	R (P)	Significance of regression $F(1, 1885)$, P
Width	Length	1.12 ± 0.02 (< 0.001)	31.93 ± 1.32 (< 0.001)	0.73 (< 0.001)	2108.4, $P < 0.001$
Length	Width	0.47 ± 0.01 (< 0.001)	10.31 ± 0.95 (< 0.001)		

Table 3
Prevalence (%, means ± SE) of *Dreissena polymorpha* infection with *Ophryoglena* sp. in 1994–2002 at different sites along the Svisloch River

Sites studied	1994	1995	1996	1997	2000	2001	2002
Zaslavskoye Reservoir (Mastitsky, 2001)	n.r.[a]	n.r.	n.r.	n.r.	49.5	n.r.	n.r.
Drozdy Reservoir (this study)	n.r.	n.r.	n.r.	n.r.	n.r.	80.8 ± 4.1	97.3 ± 0.7
Komsomolskoye Reservoir (S. Mastitsky, unpublished data)	n.r.	n.r.	n.r.	n.r.	n.r.	60.8	40.8 ± 7.1
Svisloch River, Ierusalimskaya Street (Burlakova et al., 1998)	0	0	0	n.r.	n.r.	n.r.	n.r.
Svisloch River, Denisovskaya Street (S. Mastitsky, unpublished data)	n.r.	n.r.	0	0	n.r.	71.1	61.2
Chizhovskoye Reservoir (S. Mastitsky, unpublished data)	0	n.r.	n.r.	n.r.	n.r.	79.3	n.r.

[a] n.r., not recorded.

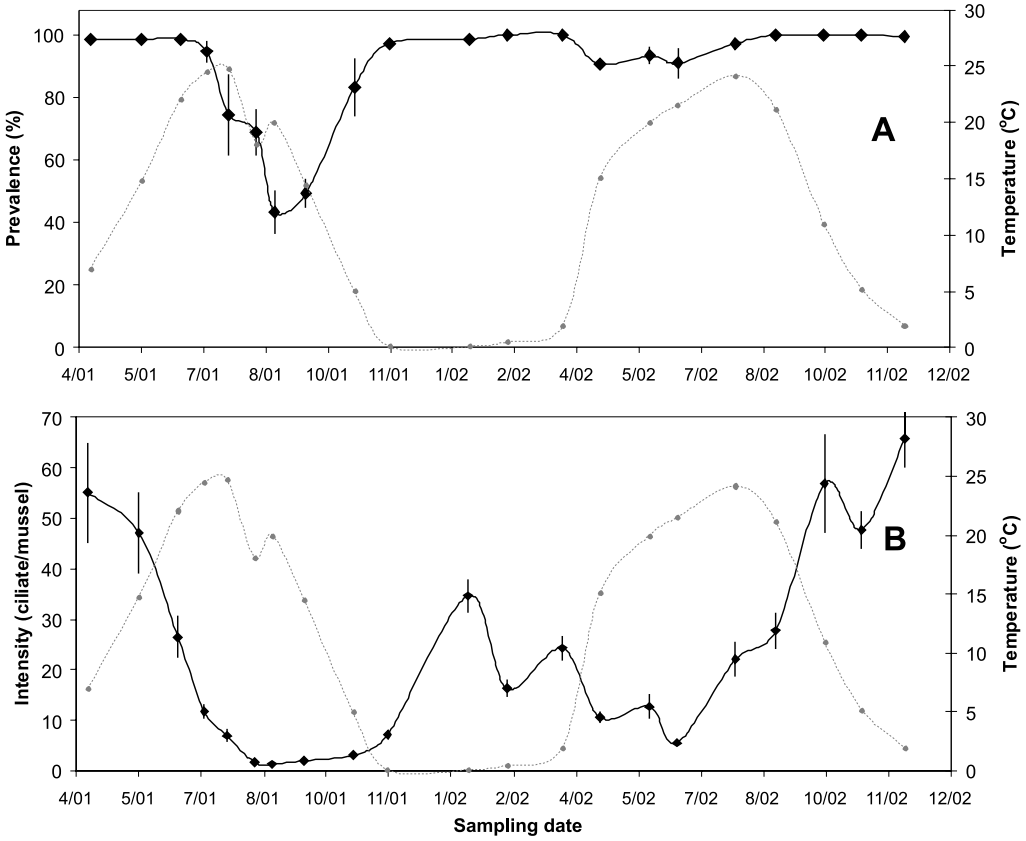

Fig. 3. Seasonal dynamics of prevalence (A) and infection intensity (B) of *Ophryoglena* sp. in *Dreissena polymorpha* from Drozdy Reservoir (Minsk, Belarus). Data points on solid line are means ± SE. Dashed line is water temperature.

$P < 0.001$) and between the 2 years sampled (Kruskal–Wallis and median tests: $P < 0.001$), with higher infection intensity in 2002 than in 2001 (Table 1). The same differences were found for prevalence among sampling dates (Kruskal–Wallis and median tests: $P < 0.01$), and between the 2 years sampled (Kruskal–Wallis and median tests: $P < 0.001$; critical α with the Bonferroni correction = 0.025) with higher prevalence in 2002 (Table 1).

3.3. Ancistrumina limnica

Ancistrumina limnica mean infection prevalence varied from 0 to 93.3 ± 6.7% (Fig. 4). Mean infection intensity was always low, from 0 to 17.3 ± 4.9 ciliates/mussel (maximum 111 ciliates/mussel). Prevalence and intensity of infection in both years were considerably higher in summer and fall than in winter and spring. We found a low, although significant, similar positive correlation between temperature and infection intensity and temperature and prevalence (Spearman $r_s = 0.36$, $n = 62$, $P < 0.005$). Intensity also positively correlated with prevalence of infection (Spearman $r_s = 0.74$, $n = 62$, $P < 0.001$).

Intensity of *A. limnica* infection varied between the two years of study (Kruskal–Wallis test: $P = 0.018$, median test: $P = 0.029$, critical α with the Bonferroni correction = 0.025) as well as among sampling dates (median test: $P < 0.001$). Prevalence of *A. limnica* infection was significantly different between years as well as among sampling dates (Kruskal–Wallis and median tests: $P < 0.001$).

3.4. Nematodes

Nematodes were identified as free-living, benthic species (V.G. Gagarin, personal communication, Institute for Biology of Inland Waters Russian Academy of Science). In samples from September and October 2002, *Chromadorina bioculata* was the most common species (total of 22 adults and 6 juveniles collected); adults of *Eumonhystera vulgaris* were found three times, and other species (*Tridentulus floreanae*, *Tobrilus tenuicaudatus*, and *Monhystrella* sp.) were observed only once.

Mean monthly infection prevalence was low to high, ranging from 6.7 ± 1.7 to 76.7 ± 8.8% (Fig. 5). The mean infection intensity was always low and varied from 1 to 3.7 ± 0.8 worms/mussel (maximum 22 worms/mussel). Prevalence and intensity of infection in both years were considerably lower in summer and much higher during winter and spring. We found a significant negative correlation between temperature and infection

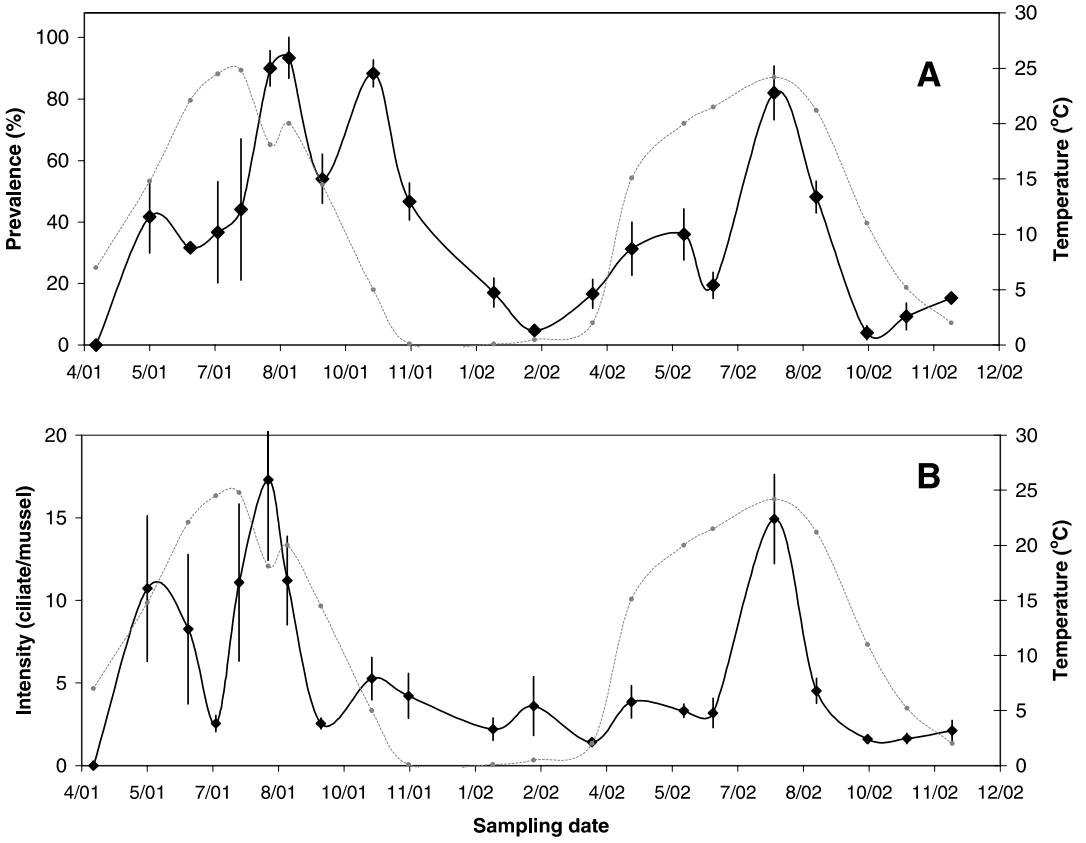

Fig. 4. Seasonal dynamics of prevalence (A) and infection intensity (B) of *Ancistrumina limnica* in *Dreissena polymorpha* from Drozdy Reservoir (Minsk, Belarus). Data points on solid line are means ± SE. Dashed line is water temperature.

prevalence (Spearman $r_s = -0.73$, $n = 63$, $P < 0.001$), and temperature and infection intensity (Spearman $r_s = -0.56$, $n = 63$, $P < 0.001$). There also was a positive correlation between nematode infection prevalence and infection intensity (Spearman $r_s = 0.72$, $n = 63$, $P < 0.001$). The number of nematodes per host mussel was significantly different among sampling dates (Kruskal–Wallis and median tests: $P < 0.001$; critical α with the Bonferroni correction = 0.025) but not between years ($P = 0.17$ and $P = 0.71$, respectively). Similarly, prevalence varied significantly among sampling dates (Kruskal–Wallis and median tests: $P < 0.01$), but was similar between years ($P > 0.36$).

3.5. Comparison of symbionts

During both years of the study, the infection intensity of the four types of symbionts was significantly different (Kruskal–Wallis and median tests: $P < 0.001$), with the highest found for *C. acuminatus* and the lowest for nematodes ($P < 0.01$, multiple comparisons of mean ranks).

Conchophthirus acuminatus and *A. limnica* infection intensity in both years of the study had a maximum in summer and was positively correlated with temperature (Figs. 2, 4). In contrast, *Ophryoglena* sp. and nematode infection intensities were considerably lower in summer versus winter and were negatively correlated with temperature (Figs. 3, 5).

4. Discussion

4.1. Conchophthirus acuminatus

Conchophthirus acuminatus has the highest prevalence of infection among all 34 symbionts known from *Dreissena* spp. (Molloy et al., 1997). Thus, high infection prevalences, some near 100%, which we recorded in *D. polymorpha* in the Svisloch River (Table 1, Fig. 2), were typical of European populations, including recent studies in Belarus (Burlakova et al., 1998; Karatayev et al., 2000a,b). The mean yearly prevalences of *C. acuminatus* infection in *D. polymorpha* size group 15.0–19.9 mm recorded in the Svisloch River in 2001 (99.7%) and 2002 (100%) were very similar to the prevalences reported in the same river in 1996 (91.4 ± 3.0%) and 1997 (98.4 ± 0.7%) (Karatayev et al., 2000b).

Conchophthirus acuminatus mean monthly infection intensities in *D. polymorpha* varied from 348 ± 32 to

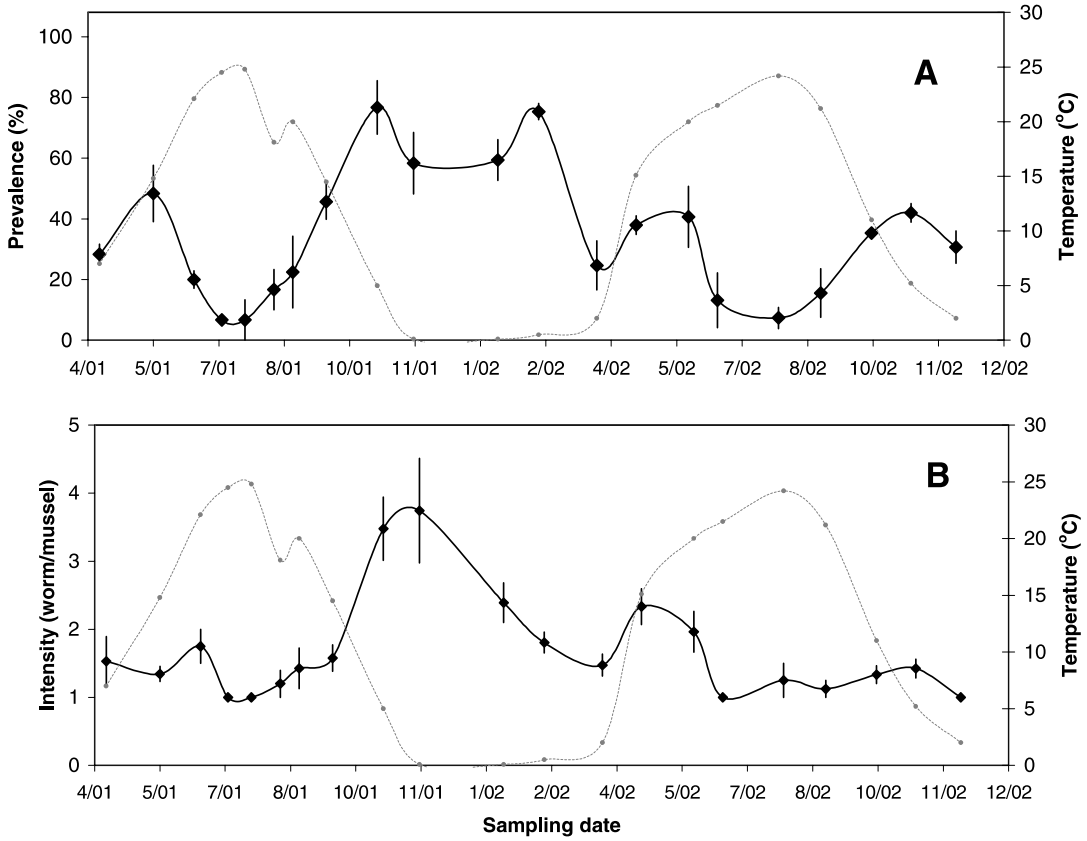

Fig. 5. Seasonal dynamics of prevalence (A) and infection intensity (B) of nematodes in *Dreissena polymorpha* from Drozdy Reservoir (Minsk, Belarus). Data points on solid line are means ± SE. Dashed line is water temperature.

1672 ± 232 ciliates/mussel and were similar to those reported from previous studies (Burlakova et al., 1998; Karatayev et al., 2000a,b). The highest *C. acuminatus* intensity ever recorded was 14,035 ciliates in a 27-mm *D. polymorpha* from Lake Lotviny, Belarus (Karatayev et al., 2000a). In a previous study (Karatayev et al., 2000b), we reported that in 1997 *C. acuminatus* intensities in the Svisloch River in Minsk and Dnieper River in Kiev (Ukraine) peaked in August, and we suggested that this was potentially in preparation for a period of mass ciliate emergence synchronized to occur when new potential hosts, i.e., the next generation of juvenile *Dreissena*, became abundant shortly thereafter. We also suggested that further studies were necessary to check if the one summer peak of infection intensity of *C. acuminatus* observed in these two rivers was truly an annual event. In both years of the current study, the *C. acuminatus* infection intensity did have a pronounced maximum peak in mid-summer (Fig. 2B), thus, providing strong evidence that this is a yearly event at least in the Svisloch River.

In a previous laboratory study we demonstrated that *C. acuminatus* infection intensity after 24 days of exposure at two different temperatures became significantly higher ($P < 0.001$) at 21 ± 1 °C (176.8 ± 10.2 ciliates/mussel) than at 14 ± 1 °C (48.5 ± 2.9 ciliates/mussel) (Karatayev et al., 2003). These data suggested that the reproduction rate of *C. acuminatus* was correlated with temperature. In our current study we found a positive correlation between infection intensity and temperature. The percent of the fission pairs in the *D. polymorpha* and in both 2001 and 2002 was maximal in April, right after the winter minimum. This increase in reproduction rate was very similar to what we observed in the above-mentioned laboratory study. We also found significant correlations between the percent of fission pairs and temperature and between the percentage of mussels in which fission pairs were found and temperature.

According to Raabe (1971), *C. acuminatus* body length and width varied from 50 to 120 μm (most often ca. 100 μm) and from 30 to 60 μm (most often 50 μm), respectively. In our study *C. acuminatus* length ranged from 52.9 to 132.3 μm and had a mean of 92.1 ± 0.3 μm. Mean width of *C. acuminatus* during the study varied from 35.3 to 88.2 μm and had a mean of 53.7 ± 0.2 μm. Raabe (1956) observed a slight decrease in the size of *C. acuminatus* with lake depth. In our study, mean *C. acuminatus* body length differed significantly among seasons and was negatively correlated with water temperature (Fig. 2C). As might be expected, the largest

C. acuminatus were found right before the rapid increase in the percent of fission pairs (respectively, in March and April 2002; Figs. 2C, D). Correspondingly, there was a slight negative correlation between mean body length of *C. acuminatus* and the percent of the fission pairs.

The average length and width of *C. acuminatus* were similar in both years of study (Table 1), and as might be expected, length correlated positively with width. The mean ratio of length to width was 1.72 ± 0.01—very similar to that recorded in a separate study of the same *C. acuminatus* population in the Svisloch River in January 2000 (1.59 ± 0.01; S. Mastitsky, unpublished data).

4.2. Ophryoglena sp.

Ophryoglena sp., although widespread in European *D. polymorpha* population (Karatayev et al., 2000a; Molloy et al., 1996, 1997), is much less common than *C. acuminatus*. From 15 lakes, a river, and a canal studied in Belarus during 1996–1997, this ciliate was found in Dnieper-Bug Canal only (Karatayev et al., 2000a). During this current study it was surprising to find an abundant population of *Ophryoglena* sp. in the Svisloch River because in our previous extensive study of zebra mussel endosymbionts in 1994–1997 (Burlakova et al., 1998; Karatayev et al., 2000b) this ciliate had not been observed. We found the ciliate for the first time in the Svisloch River in 2000, and now *Ophryoglena* sp. is widely distributed and has a moderate to high prevalence of infection. Since zebra mussels colonized the Svisloch River in the mid-1980s, we may estimate that *Ophryoglena* sp. infected their population ca. 15 years after *D. polymorpha* initial colonization. These data support our previous hypothesis that the spread of endosymbionts may depend on the duration of time after the initial zebra mussel colonization of a waterbody, and therefore, the maximum numbers of alien species associated with *D. polymorpha* are probably found in the oldest zebra mussel populations (Karatayev et al., 2000a).

In our previous monthly study (June–November 1997) conducted in Dnieper-Bug Canal (Karatayev et al., 2002b), *Ophryoglena* sp. mean population prevalence significantly ($P < 0.01$, χ^2 test) decreased from 62% in June to 11% in August, but then rose again up to 26% by November. In this current study, in 2001 we also found minimal prevalence in August ($43.3 \pm 6.8\%$); however, in 2002 the *Ophryoglena* sp. prevalence was always high (from 90.7 ± 0.7 to 100%), making it impossible to find an annual pattern of infection prevalence seasonal dynamics (Fig. 3A). Mean infection intensity was the lowest in July–October, significantly lower than in winter and spring. *Ophryoglena* sp. prevalence of infection positively correlated (Spearman $r_s = 0.78$, $P < 0.001$) with infection intensity. However, the infection intensity was not strictly predictable from the prevalence. For example, *Ophryoglena* sp. prevalence was the minimal in August and September 2001, but then it reached 97% in November 2001, when infection intensity was still very low (Fig. 3).

4.3. Ancistrumina limnica

There are few reports in the literature of *A. limnica* associated with zebra mussels (Karatayev et al., 2000a; Raabe, 1956). This non-host-specific invader of freshwater lamellibranches and gastropods was observed in the mantle cavity of *D. polymorpha* in a Polish river (Raabe, 1956) and in zebra mussels in 11 of 13 waterbodies in Belarus (Karatayev et al., 2000a). However, this is the first report on the dynamics of *A. limnica* prevalence and intensity infection in *D. polymorpha*. The prevalence and intensity of infection of *A. limnica* in these Belarussian waterbodies were always low, varying from 0.3 to 21.5% and from 3.7 ± 0.5 to 8.6 ± 1.1 ciliates/mussel, respectively (Karatayev et al., 2000a). In our current study, we observed much higher mean prevalence and infection intensity, i.e., respectively, up to 94% and up to 17.3 ± 4.9 ciliates/mussel (Figs. 4A, B). Prevalence and intensity of infection were considerably higher in summer and fall than in winter and spring. The seasonal dynamics of infection intensity and prevalence were positively correlated with temperature, and intensity strongly positively correlated with infection prevalence.

4.4. Nematodes

The presence of only free-living nematode species within the mantle cavity provided further evidence that they do not have an obligate symbiotic association with zebra mussels (Molloy et al., 1997). We hypothesize that benthic free-living nematodes probably gain entrance inadvertently to the mantle cavity by crawling along byssal threads—attachment fibers which extend through the pedal gape into mantle cavity.

Nematodes have been commonly observed in the mantle cavity of *Dreissena* populations both in Europe (Karatayev et al., 2002a; Kuperman et al., 1994) and North America (Conn et al., 1994; Toews et al., 1993). Conn et al. (1994) reported the maximum prevalence and maximum mean intensity of zebra mussel infection by nematodes in the St. Lawrence River (New York), respectively, 40% and 2.8 worms per mussel. According to Karatayev et al. (2000a), nematodes were found in *D. polymorpha* in all 17 waterbodies studied in Belarus and the prevalence varied from 2 to 52%. The mean infection intensity was always low and varied from 1 to 2.3 worms/mussel. To our knowledge, the long-term dynamics of the prevalence and intensity of nematode infection in *D. polymorpha* had never previously been

investigated. In our two-year study, mean infection prevalence ranged from 6.7 ± 1.7 to $76.7 \pm 8.8\%$ and mean infection intensity from 1 to 3.7 ± 0.8 worms/mussel (Fig. 5). There was a significant negative correlation between temperature and both infection prevalence and intensity. This suggests that during winter months higher numbers of nematodes may actively use zebra mussels for shelter or that nematode population densities are simply higher in the benthos during this season with a correspondingly higher number inadvertently entering the mantle cavity.

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