Field and laboratory studies of *Ophryoglena* sp. (Ciliata: Ophryoglenidae) infection in zebra mussels, *Dreissena polymorpha* (Bivalvia: Dreissenidae)

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Abstract

This study, conducted in the Dnieper-Bug Canal in Belarus, is the first to monitor the seasonal (June–November) dynamics of infection with the parasitic ciliate *Ophryoglena* sp. in a zebra mussel (*Dreissena polymorpha*) population. Mean population prevalence and intensity of infection varied, respectively, from 11 to 62% and from 0.9 to 24.1 ciliates/mussel. Mean prevalence was highly correlated with mussel length in mussels <20 mm (\(R^2 = 0.97\)) and was lower in larger mussels. Mean infection intensity in mussels 1–25 mm long was similarly correlated with their size (\(R = 0.98\)), reached a maximum in the 20–25 mm size-class, and then sharply decreased, thus providing evidence, albeit limited, that high intensity of infection might be lethal. Transinfection of zebra mussels by *Ophryoglena* sp. was achieved in the laboratory—a first for a protozoan parasite of *D. polymorpha*; from an initial complete lack of infection, mean prevalence and intensity rose, respectively, to 86.7% and 8.3 ciliates/mussel. © 2002 Elsevier Science (USA). All rights reserved.

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1. Introduction

The proliferation of the macrofouling zebra mussel, *Dreissena polymorpha*, within European and North American waterbodies, has resulted in significant ecological and economic impacts (Karatayev et al., 1997; MacIsaac, 1996; O’Neill, 1996, 1997). The extent of these impacts is directly related to mussel density within a habitat, and it is well documented that their population densities are not stable and can fluctuate widely (Ramcharan et al., 1992). Defining the role parasites play in these density fluctuations is important for understanding these population shifts. The present study of an *Ophryoglena* sp. infecting a *D. polymorpha* population in its European range was undertaken to address this question by studying fundamental aspects of this disease.

Although 34 species are known as parasites and/or commensals of *D. polymorpha* (including ciliates, haplosporidians, trematodes, nematodes, oligochaetes, mites, chironomids, and leaches), few parasites have been documented as being highly pathogenic (Molloy et al., 1997). Compared to the wide diversity of lethal parasites known from other bivalves, particularly commercially valuable marine species (Lauckner, 1983; Sindermann, 1990; Sparks, 1985), zebra mussels might appear to have relatively few serious diseases. Prior to the arrival of these mussels in North America, however, relatively little attention was given to parasitology-related studies. This information gap is currently being addressed as a project of the International Research Consortium on Molluscan Symbionts (IRCOMS), a
network of over a dozen scientists from the former Soviet Union, Europe, and North America (Molloy, 2001). 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 parasites, as well as other symbiotic organisms encountered within these mussels (Burlakova et al., 1998; Karatayev et al., 2000a, b; Laruelle et al., 1999; Molloy et al., 2001; Molloy et al., 1997; Molloy et al., 1996). Ciliates are the most common endosymbionts in zebra mussels (Molloy et al., 1997), and previous IRCOMS investigations have focused on species in the orders Scuticociliatida and Rhynchodida (Burlakova et al., 1998; Karatayev et al., 2000a, b; Laruelle et al., 1999). Ciliates in the order Hymenostomatida were reported from the digestive gland of *D. polymorpha* in 8 of 13 Russian waterbodies (Molloy et al., 1996) and have also been observed to be widespread in European zebra mussel populations (authors, unpublished data). These hymenostomatid ciliates have now been determined to be an undescribed *Ophryoglena* sp. (D.H. Lynn, personal communication, University of Guelph), which specifically inhabits duct and tubule lumina within the mussel’s digestive gland (authors, unpublished data) (Fig. 1). Ophryoglenid ciliates had also been reported by Stanczykowska (1977) and Zdun et al. (1994) to be potentially destructive to the digestive gland of European zebra mussels. In North America, Toews et al. (1993) observed *Ophryoglena* ciliates inside the mantle cavity of living and dead *D. polymorpha* from Lake Erie, but because they did not report these *Ophryoglena* from the digestive gland or within any other organ, it is highly probable that they were not parasitic, but rather common free-living histophagous species.

In our previous study of zebra mussel endosymbionts in Belarus, we reported an annual prevalence and intensity of *Ophryoglena* infection of 43% and 8.4 ciliates/mussel in the Dnieper-Bug Canal in 1997 (Karatayev et al., 2000a). The present paper contains a comprehensive examination of the month-to-month (June–November) dynamics of *Ophryoglena* infection during the latter 1997 study, as well as infection data from an additional sample taken in 1999. Monitoring of *Ophryoglena* infection could give us better understanding how prevalence and infection vary over time and what potential impact the parasite may have on *Dreissena*. We also report the successful laboratory transinfection of this *Ophryoglena* sp.—the first for a protozoan parasite of *D. polymorpha*.

2. Materials and methods

2.1. Fieldwork

We sampled the *D. polymorpha* population in the Dnieper-Bug Canal in the southwestern corner of Belarus. This canal (width ca. 30 m, mean depth ca. 1.6 m, and maximum depth ca. 4 m) was built in 1775 to connect the Dnieper River (Black Sea Basin) to the Zapadnyi Bug River (Baltic Sea Basin), and *D. polymorpha* likely colonized it shortly thereafter. In 1997, mussels were sampled monthly from June to November at a single site located 15 km west of the city of Pinsk. In August 1999, this same site was sampled again.

All samples were collected at ca. 1.5 m depth, held at 5–10 °C, and dissected within 72 h. At each sampling time, mussels were collected from three locations.

![Fig. 1. Cross-section of digestive gland of *D. polymorpha* infected with *Ophryoglena* sp., H&E stain. (A) Ciliates (arrows) with prominent, dark-staining macronucleus visible in three of the gland lumina (scale bar = 50 μm). (B) Ciliate in lumen of a digestive gland tubule (ciliate macronucleus (M), mussel digestive tubule epithelium (DTE), scale bar = 25 μm).](image-url)
10–15 m apart. Each collection of 400–700 mussels from each of these three locations represented a replicate sample. To determine the size–frequency distribution of the zebra mussel population, 200–600 mussels were randomly chosen from each replicate sample and measured to the nearest millimeter with a caliper. Based on available mussel sizes, each replicate was divided into a maximum of seven 5-mm size classes relative to shell length (1 \( \leq L < 5 \) mm; 5 \( \leq L < 10 \) mm; 10 \( \leq L < 15 \) mm; 15 \( \leq L < 20 \) mm; 20 \( \leq L < 25 \) mm; 25 \( \leq L < 30 \) mm; 30 \( \leq L < 35 \) mm). In each of the three replicates, mussels were then randomly selected from each size class and dissected in unchlorinated tap water within a plankton counting chamber using a stereomicroscope (20–70×). The whole soft body of each mussel was then dissected with a special attention to digestive gland, each piece of which was carefully checked for the presence of ciliates. At each sampling time and for each available size class, we dissected 17 randomly chosen mussels per replicate to determine infection prevalence and 7 mussels (a subset from the latter 17) per replicate for infection intensity. Prevalence and intensity of infection within each replicate sample were then calculated from these latter data based on the proportion of each 5-mm size class in the sample. Overall mean prevalence and intensity of infection in the population for each sampling date were then determined from these three replicate sample means. Before doing this latter calculation, we verified that there was no significant difference between the three replicate means using Kruskal–Wallis ANOVA by ranks (intensity data \( P = 0.75 \); prevalence data \( P = 0.84 \)). For all above 1997 data, intensities among different samples were compared by median test (Różsa et al., 2000) and by non-parametric Kruskal–Wallis one-way ANOVA on ranked data; prevalences were compared by \( \chi^2 \) test, Kruskal–Wallis ANOVA, and Mann–Whitney test using Statistica software (Windows Release 5.0 B, StatSoft). In 1999, this canal site was revisited, and a single sample of 221 randomly collected mussels measured for size distribution and 110 mussels (a subset from the latter 221) dissected for both prevalence and infection intensity.

2.2. Laboratory experiment

To attempt transinfection, an experiment was set up in which 50 zebra mussels from the Dnieper-Bug Canal (i.e., above-mentioned infected population) and 50 mussels from the Svisloch River (i.e., a zebra mussel population within the city of Minsk known to be uninfected by *Ophryoglena* sp.; Karatayev et al., 2000a) were placed in a tray \(( L \times W \times H = 37 \times 27 \times 4 \text{cm})\) containing 2.0 L of unchlorinated tap water. Mean mussel length of the Dnieper-Bug and Svisloch mussels was, respectively, 15.0 and 14.6 mm. The Dnieper-Bug and Svisloch mussels were separately color-coded with a 2-mm diameter tag glued to the valve of each mussel and then randomly mixed within the tray. Three replicate trays were set up and held at 13 \( \pm 0.5 \) °C for 30 days. We fed mussels every 2–3 days with the alga *Scenedesmus* sp. and at selected intervals (ca. 3 days) randomly removed and dissected five Dnieper-Bug and five Svisloch mussels from each tray to determine infection prevalence and intensity. Infection intensity and prevalence data were analyzed using the same procedures as in the above-mentioned field studies.

3. Results and discussion

3.1. Fieldwork

3.1.1. Mussel length in relation to infection prevalence and intensity

Throughout the six-month 1997 sampling period, mean infection prevalence in mussels between 1 and 20 mm long was very highly correlated with their size \((R^2 = 0.97; P < 0.014)\), reaching 41.5% in the 15.0–19.9 mm size class (Fig. 2A). Prevalence appeared to level off in larger size classes (mussels \( \geq 20 \) mm), but the number of mussels examined in these larger size classes was relatively low throughout the sampling period, and large error terms (e.g., \( \pm SE \)) did not permit clear conclusions (Fig. 2A). Thus, although the relationship between mean prevalence and size was almost linear in mussels <20 mm in length, the overall coefficient of regression was lower \((R^2 = 0.81, P < 0.01)\) when all mussel lengths were included in the analysis.

Throughout the six-month 1997 sampling period, mean infection intensity in mussels between 1 and 25 mm long was very highly correlated with their size \((R = 0.98; 96.3\% \text{ variance explained})\), reached 15.6 ± 4.0 (mean \( \pm SE, n = 98 \) ) ciliates/mussel in the 20.0–24.9 mm size class, and then sharply decreased \((R = 0.39; 14.9\% \text{ variance explained})\) (Fig. 2B). The smallest infected mussel was 2.5 mm long and contained seven ciliates, whereas maximum intensity, 291 ciliates/mussel, was observed in a 22-mm mussel in July.

The sharp decrease in infection intensity in mussels \( \geq 25 \) mm suggested that high intensity *Ophryoglena* infection may be lethal. A similar observation (i.e., death suspected as a result of high infection intensity) was made by Lyakhnovich et al. (1983) for infection of *D. polymorpha* by the parasitic trematode *Phylodistomum folium* in Lukomskoe Lake (Belarus). In this latter study, intensity of trematode infection likewise increased with mussel size, reached a maximum in mussels 24–26 mm long, and then sharply decreased. This pattern is in contrast to that which we observed in three populations of the commensal ciliate *Conchophthirus acuminatus* in which high correlations \((R^2 = 0.83–0.92)\) existed between in-
tensity and zebra mussel size, with no drop off in infection intensity in the largest size classes (Burlakova et al., 1998). Thus, the data in our present study, in combination with these other above-mentioned two studies, suggest that the inverse correlations noted between infection intensity and zebra mussel length may be the result of the death of highly infected individuals. Further experimentation is required to test this hypothesis.

3.1.2. Monthly prevalence and intensity of infection

The population prevalence and intensity of infection during the six-month 1997 sampling period (Fig. 3) varied significantly between months (both $P < 0.001$, Kruskal–Wallis ANOVA; $P < 0.001$, median test). Mean population infection prevalence declined significantly from 62% in June to 11% in August ($P < 0.01$, $\chi^2$ test), but then rose again up to 26% by November (Fig. 3A). Two years later, when this canal site was again sampled in August 1999, we observed an even lower mean population prevalence of infection, i.e., 1.8%. This very low prevalence in 1999 was also accompanied by a very low intensity, i.e., 1 ciliate/mussel versus 3.4 ciliates/mussel in August 1997. This was likely due in part to an increase in the relative abundance compared to August 1997 (Fig. 4) of 1–10 mm mussels—a size group with characteristically low intensity and prevalence (Fig. 2).

Mean population infection intensity was low (0.9–7.6 ciliates/mussel) throughout the 1997 study period, except for a statistically significant ($P = 0.01$, Kruskal–Wallis ANOVA) peak of 24.1 ciliates/mussel in July (Fig. 3B). Median intensity in July was significantly different from that in June–October ($P < 0.05$, median test). In a previous study of infection of zebra mussels by the commensal ciliate C. acuminatus, we also observed a single peak of infection intensity during the summer both in the Svisloch River (Minsk, Belarus) and Dnieper River (Kiev, Ukraine) (Karatayev et al., 2000b).

This study is the first to monitor the month-to-month dynamics of prevalence and intensity of Ophryoglena sp. infection in a zebra mussel population. Although according to our data both prevalence and infection intensity seem to have a prominent peak over a six-month period (Fig. 3), we do not suggest, however, that these patterns are typical annual events (i.e., the highest prevalence in June and intensity in July).
The overall mean infection intensity of *Ophryoglena* sp. during 1997 (8 ciliates/mussel) was very low compared to the typical infection intensity of the commensal ciliate *C. acuminatus*. A mean intensity of 553 *C. acuminatus*/mussel, for example, was recorded in this same Dnieper-Bug population during the same 1997 period (Karatayev et al., 2000b). This suggests that high intensity infections by *Ophryoglena* sp. may be pathogenic, and there is some support in the literature for this hypothesis. Kazubski (personal communication in Stanczykowska, 1977) reported observing “dangerous” infections by small and large ophryoglenids in zebra mussels. Likewise, Zdun et al. (1994) indicated that ophryoglenid ciliates might have an adverse effect on *Dreissena*.

### 3.2. Laboratory transinfection experiments

Infection of Svisloch mussels by *Ophryoglena* sp. from Dnieper-Bug mussels was achieved in the laboratory. This represented the first successful trial ever conducted to initiate parasitic protozoan infection in *Dreissena*. From an initial complete lack of infection among the Svisloch mussels, prevalence and intensity rose, respectively, to 13.3 ± 6.7% and 0.7 ± 0.3 ciliates/mussel by day 2 (Fig. 5A and B). Both prevalence and intensity in these Svisloch mussels constantly continued to increase within the first 8 days and then began to level off. During the first 5 days of the experiment, the prevalence of infection in Dnieper-Bug and Svisloch mussels was significantly different (100 ± 0% versus 13.3 ± 6.7%, day 2; 60.0 ± 11.5% versus 13.3 ± 6.7% (mean ± SE), day 5; *P* < 0.001, *χ²* test; *P* < 0.05; Mann–Whitney test). The same was true for intensity of infection (2.5 ± 0.5 versus 0.7 ± 0.3 ciliates/mussel, day 2; 2.6 ± 0.6 versus 0.7 ± 0.3 ciliates/mussel, day 5; *P* < 0.01, median test and Kruskal–Wallis ANOVA). After 8 days, however, the prevalence (46.7 ± 17.6%) and intensity (2.6 ± 1.2 ciliates/mussel) in the Svisloch mussels was not significantly different (prevalence—*P* = 0.37, Mann–Whitney test; *P* > 0.05, *χ²* test; intensity—*P* = 0.34, Kruskal–Wallis ANOVA; *P* = 0.69, median test) from that in the Dnieper-Bug mussels (66.7 ± 6.7% and 1.9 ± 0.4 ciliates/mussel). The intensity of infection during 0–5 days was statistically different for Dnieper-Bug versus Svisloch mussels (*P* < 0.001, median test and Kruskal–Wallis ANOVA), but during 8–36 days, the number of ciliates per host was similar (*P* > 0.12, median test and Kruskal–Wallis ANOVA). At the same time, throughout the experiment the mean prevalence and intensity of infec-

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**Fig. 4.** Size–frequency distribution of *D. polymorpha* from the Dnieper-Bug Canal in August 1997 and 1999.

**Fig. 5.** Dynamics of infection prevalence (A) and intensity (B) of *D. polymorpha* with *Ophryoglena* sp. and the length of *Ophryoglena* sp. (C) in experimental trays (average for three trays ±SE indicated).
tion in Dnieper-Bug mussels did not change significantly ($P = 0.21$, $P = 0.89$, Kruskal–Wallis ANOVA). The highest mean prevalence and intensity achieved in the Svisloch mussels during the 30-day experiment was, respectively, 86.7% and 8.3 ciliates/mussel.

As is typical of species in the family Ophryoglenidae, this *Ophryoglena* sp. produces small infective cells, i.e., theronts, by mitosis outside of the host in surrounding waters (authors, unpublished data). This may have occurred in these trials since the length of *Ophryoglena* observed during dissection of Svisloch mussels during the first 5 days of the experiment was significantly ($P = 0.002$, t test) smaller than the length of ciliates observed in Dnieper-Bug mussels (Fig. 5C). Once these *Ophryoglena* had infected Svisloch mussels, these young ciliates grew, and after 8 days their mean length ($56.4 \pm 5.4 \mu m$) was not significantly different ($P = 0.11$, t test) from that in the Dnieper-Bug mussels ($70.9 \pm 4.5 \mu m$).

Further studies are required to understand the life cycle of this *Ophryoglena* sp. and to formally describe the species. In addition, it is of great importance to determine their effect on zebra mussels. If the effect will be found to be highly pathogenic and if this species is host specific to zebra mussels, then potential for *Dreissena* biocontrol should be investigated.

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