Research Article

Infection of *Dreissena polymorpha* (Bivalvia: Dreissenidae) with *Conchophthirus acuminatus* (Ciliophora: Conchophthiridae) in lakes of different trophy

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Abstract

The freshwater invasive mollusc *Dreissena polymorpha* and its host-specific commensal ciliate *Conchophthirus acuminatus* represent a convenient model to study various aspects of symbiotic relationships. This study was conducted to test whether the nutrient enrichment of waterbodies inhabited by *D. polymorpha* is associated with *C. acuminatus* infection. Three lakes differing in their trophic conditions were simultaneously sampled to estimate the levels of *C. acuminatus* infection in *D. polymorpha* (eutrophic Lake Batorino, moderately eutrophic Lake Myastro, and mesotrophic Lake Naroch, Republic of Belarus). Although the percentage of infected molluscs did not differ among these waterbodies, the average number of ciliates per host showed a significant increase with respect to the lake trophic status. This pattern could be explained by favorable feeding conditions experienced by *D. polymorpha* and its commensal *C. acuminatus* in waters of higher trophy. In particular, the average size of *D. polymorpha* (i.e., carrying capacity for the infection) significantly positively correlated with the lake trophic status. Higher trophy could also directly affect reproduction potential of the ciliate *C. acuminatus*, which supposedly feeds on bacteria, algae, and other organic particles filtered out from the water by its host. The results of this study may apply to a wider spectrum of similar commensal associations in fresh waters.

Key words: Belarus, commensalism, *Conchophthirus acuminatus*, *Dreissena polymorpha*, Lake Batorino, Lake Myastro, Lake Naroch, trophic state

Introduction

The ciliate *Conchophthirus acuminatus* is known as the most common endosymbiont of the invasive bivalve mollusc *Dreissena polymorpha* (zebra mussel) in European waterbodies (Molloy et al. 1997; Karatayev et al. 2007). This protozoan can be found on epithelial surfaces of the mantle, gills and visceral mass, in the gill water tubes and suprabranchial cavities, and on the labial palps of the mollusc (Laruelle et al. 1999). High levels of infection coupled with the absence of pathological effects on the host tissues suggest a commensal relationship between *C. acuminatus* and *D. polymorpha* (Karatayev et al. 2007). Moreover, the ciliate is considered to be highly host-specific to *D. polymorpha* (Molloy et al. 1997).

The ubiquitous distribution and typically high abundance of *C. acuminatus* in European populations of zebra mussels make this ciliate and its host a very convenient model system for studies on commensalism, a type of symbiosis that, due likely to its neutrality to the host, has attracted considerably less attention and is thus less understood than other forms of close long-term relationships between species (Saffo 1992; Karatayev et al. 2007). At the same time, research on host-commensal relationships can potentially shed a great deal of light on the evolution of ecological interactions in general, and on the origins and nature of parasitism and mutualism in particular (e.g., Tokeshi 1993; Houk 1994; Yamamura 1996; Paracer and Ahmadjian 2000; Miller et al. 2006). Thus, Karatayev et al. (2007) suggested to use the system "*D. polymorpha* – *C. acuminatus*" for addressing such questions as mechanisms of symbiont dispersal, symbiont's life cycle features increasing the probability of infection, strains of symbiont and host, and factors responsible for host specificity.

Although extensive research efforts have been made to address some of these questions (Molloy et al. 1997; Burlakova et al. 1998; Karatayev et al. 2000a, 2000b, 2003a, 2003b, Mastitsky 2004), many aspects of the relationship between *C. acuminatus* and *D. polymorpha* remain unravelled (Karatayev et al. 2007). The specific focus of this study was the level of *C. acuminatus* infection in *D. polymorpha* populations inhabiting waterbodies with different nutrient enrichment. Trophic status of a waterbody is deemed an important factor shaping community composition and abundance of parasites (McKenzie and Townsend 2007; Johnson and Carpenter 2008; Johnson et al. 2010). Moreover, anthropogenic eutrophication of natural waters has been implicated with emergence of a number of infectious diseases of humans and wildlife (Johnson et al. 2006; Johnson et al. 2010; Johnson and Paull 2011). In contrast to parasites, our current understanding of the effects of environmental nutrient enrichment on commensal organisms is very limited. The present study highlights a positive link between the waterbody's trophic status and the abundance of commensal ciliate *C. acuminatus* in *D. polymorpha*, and suggests likely mechanisms underlying this association.

Material and methods

Study area

The study was conducted at the Narochanskaya Lake System (Figure 1; Table 1), a chain of three interconnected lakes located in the north-western part of the Republic of Belarus. Although located closely to each other and thus subjected to the same climatic conditions, these lakes differ considerably in their trophic states (Table 2). According to Zhukova et al. (2005), Lake Batorino, Lake Myastro and Lake Naroch may currently be defined as eutrophic, moderately eutrophic, and mesotrophic waterbodies, respectively. These lakes can, therefore, be used as a useful model for testing trophy-related ecological research hypotheses. In addition, all three lakes were approximately simultaneously invaded by zebra mussels in the 1980s (Ostapenya et al. 1993), suggesting that most of the observable differences among these three populations of *D. polymorpha* are likely to be determined by lake-specific conditions rather than time since colonization.
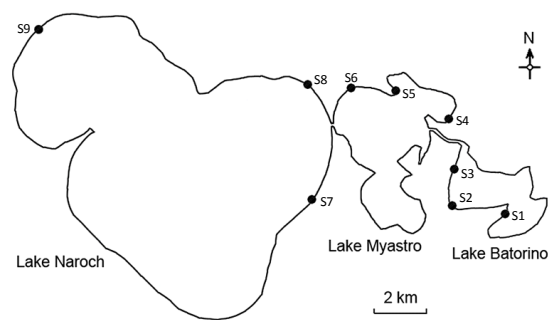

Figure 1. Sampling sites at the examined lakes (shown as numbered filled circles). See Table 1 for additional information.

Protocols of field sampling and dissections

The lakes were synchronously sampled in May, July and September 2003. In each lake, a few hundred of molluscs were collected by hand from three similar littoral sites at 0.6-0.8 m depth (Figure 1; Table 1). Samples were stored in lake water at 10-15°C and dissected within 48 hours. As the level of *C. acuminatus* infection was expected to depend on size of *D. polymorpha* (Karatayev et al. 2007), the size frequency distributions in the collected samples of zebra mussels were taken into account during dissections. To determine the size structure, shell length of 100-200 molluscs from each sampling site was measured with calipers to the nearest millimeter. Molluscs were then sorted into size classes using 5.0 mm intervals. The number of molluscs to dissect was determined based on the proportion of each size class in a sample, with 7 molluscs taken from the most abundant class. Thus, depending on the size structure of *D. polymorpha* in a sample, 10 to 25 molluscs were dissected on each sampling date from each sampling site.

Mussels were cut open with a scalpel and their soft tissues were repeatedly flushed with unchlorinated tap water from a pipette to remove ciliates from epithelial surfaces into a plankton counting chamber. Soft tissues were then also transferred into the chamber and homogenized to small pieces with forceps. The intensity (i.e., number of ciliates per infected mollusc) and prevalence of *C. acuminatus* infection (i.e., percentage of infected individuals) were determined using a stereomicroscope (×20). Following Saffo (1992), the term "infection" is used in this paper irrespective of commensal relationship between *D. polymorpha* and *C. acuminatus*.

Table 1. Records of *Dreissena polymorpha* and *Conchophthirus acuminatus* in the Narochanskaya Lake System*.

Lake	Sampling site (codes refer to those in Figure 1)	Coordinates of the sampling site		Sampling date	Water temperature, °C	Number of *D. polymorpha* dissected	Median number of *C. acuminatus* found / host
		Latitude, N	Longitude, E				
Batorino	S1	54°52′20	26°57′33	21.05.2003	14.8	14	216
Batorino	S1	54°52′20	26°57′33	21.07.2003	26.3	12	869
Batorino	S1	54°52′20	26°57′33	06.09.2003	13.2	13	1090
Batorino	S2	54°50′33	26°55′42	21.05.2003	14.8	14	75
Batorino	S2	54°50′33	26°55′42	21.07.2003	26.4	14	743
Batorino	S2	54°50′33	26°55′42	06.09.2003	13.2	14	716
Batorino	S3	54°51′03	26°55′43	21.05.2003	14.8	17	183
Batorino	S3	54°51′03	26°55′43	21.07.2003	26.8	14	1047
Batorino	S3	54°51′03	26°55′43	06.09.2003	13.8	15	1103
Myastro	S4	54°52′15	26°55′27	21.05.2003	13.6	15	164
Myastro	S4	54°52′15	26°55′27	18.07.2003	21.0	24	297
Myastro	S4	54°52′15	26°55′27	05.09.2003	14.8	24	1055
Myastro	S5	54°52′55	26°53′46	21.05.2003	13.4	18	68
Myastro	S5	54°52′55	26°53′46	18.07.2003	23.0	20	407
Myastro	S5	54°52′55	26°53′46	05.09.2003	15.0	16	488
Myastro	S6	54°52′53	26°52′19	21.05.2003	13.0	25	492
Myastro	S6	54°52′53	26°52′19	18.07.2003	24.0	18	679
Myastro	S6	54°52′53	26°52′19	05.09.2003	15.6	20	908
Naroch	S7	54°51′15	26°51′24	20.05.2003	13.4	19	174
Naroch	S7	54°51′15	26°51′24	17.07.2003	21.2	25	297
Naroch	S7	54°51′15	26°51′24	04.09.2003	12.5	16	430
Naroch	S8	54°52′59	26°50′37	20.05.2003	13.4	18	86
Naroch	S8	54°52′59	26°50′37	17.07.2003	21.9	18	15
Naroch	S8	54°52′59	26°50′37	04.09.2003	13.5	14	242
Naroch	S9	54°54′21	26°42′18	20.05.2003	13.4	18	45
Naroch	S9	54°54′21	26°42′18	16.07.2003	24.7	25	174
Naroch	S9	54°54′21	26°42′18	03.09.2003	15.2	16	110

* Raw data generated in this study are available at http://dx.doi.org/10.6084/m9.figshare.95448 and http://dx.doi.org/10.6084/m9.figshare.95449

Table 2. Selected morphometric and hydrobiological parameters* of the examined lakes (Zhukova et al. 2005).

Parameter	Lake		
	Batorino	Myastro	Naroch
Surface area (km^2)	6.3	13.1	79.6
Mean depth (m)	3.0	5.4	8.9
Maximal depth (m)	5.5	11.3	24.8
Volume (million m^3)	18.7	70.0	710.4
Total phosphorus (mg P/L)	0.030 ± 0.008	0.029 ± 0.006	0.014 ± 0.002
Total nitrogen (mg P/L)	1.20 ± 0.13	0.94 ± 0.17	0.85 ± 0.09
Secchi depth (m)	1.5 ± 0.4	4.0 ± 0.9	5.5 ± 0.6
Seston (mg/L)	5.5 ± 1.9	2.2 ± 0.8	1.1 ± 0.2
Chlorophyll *a* in seston (μg/L)	8.5 ± 4.2	3.4 ± 2.1	1.8 ± 0.7
BOD$_5$ (mg O$_2$/L)	2.6 ± 0.8	1.5 ± 0.4	1.1 ± 0.6
Bacterioplankton density (million cells/ml)	3.7 ± 3.7	2.7 ± 0.5	1.8 ± 0.4
Phytoplankton biomass (mg/L)	3.4 ± 2.2	2.1 ± 1.3	0.7 ± 0.3

* For the period of May-October 2003 (mean ± SD)

Statistical analysis

The counts of *C. acuminatus* in zebra mussels had considerably right-skewed non-normal distributions in all of the examined samples (overall mean = 640 ciliates/mussel, median = 332 ciliates/mussel, skewness = 4.8, kurtosis = 39.9, n = 476). This suggested using median and quartiles to describe the most typical values and the variation of infection in a sample (Rózsa et al. 2000).

As the same three sites were repeatedly sampled within each lake, the resultant data on *C. acuminatus* abundance in zebra mussels collected from a site on a giving sampling date were likely to be correlated. Hence a linear mixed-effects modelling approach was employed to test the effect of trophy level on the *C. acuminatus* counts (including zero values). For this purpose, a Gaussian linear mixed-effects model (referred to as Model 1 below) was fitted

to the power-transformed *C. acuminatus* counts (i.e., transformation of the form $X' = X^\lambda$). The power parameter $\lambda = 0.2$ was found to be optimal for achieving a normal-like distribution of the counts. The two fixed effects included into Model 1 were trophy level (a nominal variable with three levels corresponding to the lakes under study, with the eutrophic Lake Batorino treated as reference) and *D. polymorpha* shell length. Length of the mussels was centered by subtracting the mean from each observation. Due to such a centering, the model intercept would be interpreted as the average expected value of (power-transformed) *C. acuminatus* number in a mollusc of average length, rather than the expected number of ciliates in a mollusc with zero length (West et al. 2007). An exploratory analysis showed that correlation between the *C. acuminatus* numbers and *D. polymorpha* shell length varied among samples (Figure 2). The random part of the model thus included a random intercept (the effect of sampling site, nested within sampling month) and a random slope that accounted for varying correlation between the zebra mussel size and *C. acuminatus* numbers.

An additional mixed-effects model (Model 2) was fitted to test whether the shell length of zebra mussels differed among lakes, with the sampling site nested within sampling date being treated as random effects. This analysis was based on the raw untransformed shell length measurements obtained when establishing the size frequency distribution in each sample of zebra mussels (see above). The residual variance in Model 2 was allowed to vary among lakes in order to account for the corresponding pattern revealed during the data exploration (see Zuur et al. (2009) for details on this type of parameterization).

Both of these models were fitted using the restricted maximum likelihood method as implemented in the *lme* function from the *nlme* v3.1-103 package for R software (R Development Core Team 2012). Post-hoc comparisons of the groups of interest were conducted with the Tukey tests using the function *glht* from the package *multcomp* v1.2-12 for R (Hothorn et al. 2008). Adequacy of the models was checked using standard diagnostic plots of residuals (i.e., residuals plotted against fitted values, as well as quantile-quantile plots) (Zuur et al. 2009). Fisher's exact test was applied to compare the prevalence of *C. acuminatus* infection among lakes.
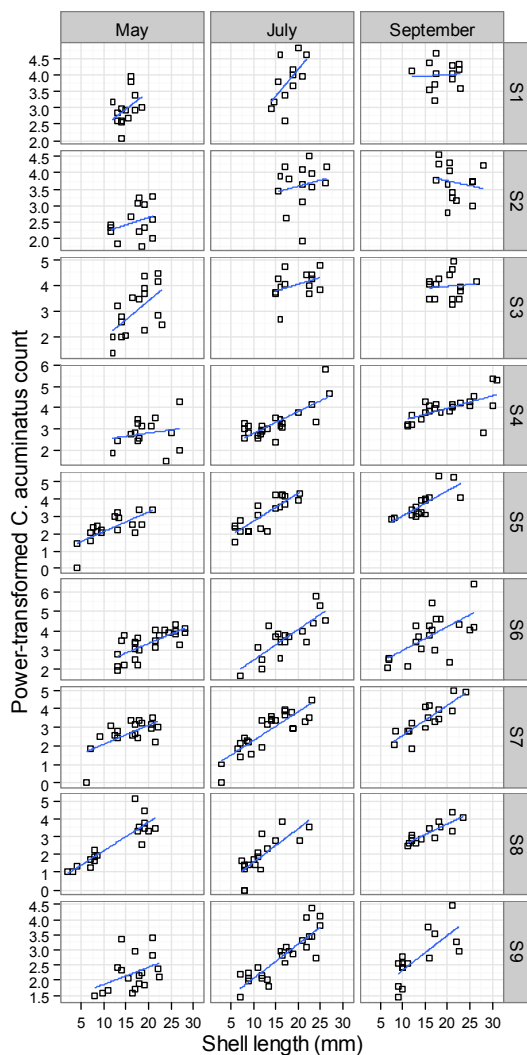

Figure 2. Relationships between the counts of *C. acuminatus* and the shell length of *D. polymorpha* in the examined samples. Linear regression lines were added to highlight the patterns. Station codes correspond to those in Figure 1.

Results

All the examined molluscs from Lake Batorino were infected with *C. acuminatus* (n = 127), and only a few zebra mussels did not harbor ciliates in Lake Myastro (99.4% infected, n = 180) and Lake Naroch (97.6% infected, n = 169). Overall, there was no statistically significant difference in the prevalence of *D. polymorpha* infection with *C. acuminatus* among these three waterbodies (P = 0.16, Fisher's exact test).
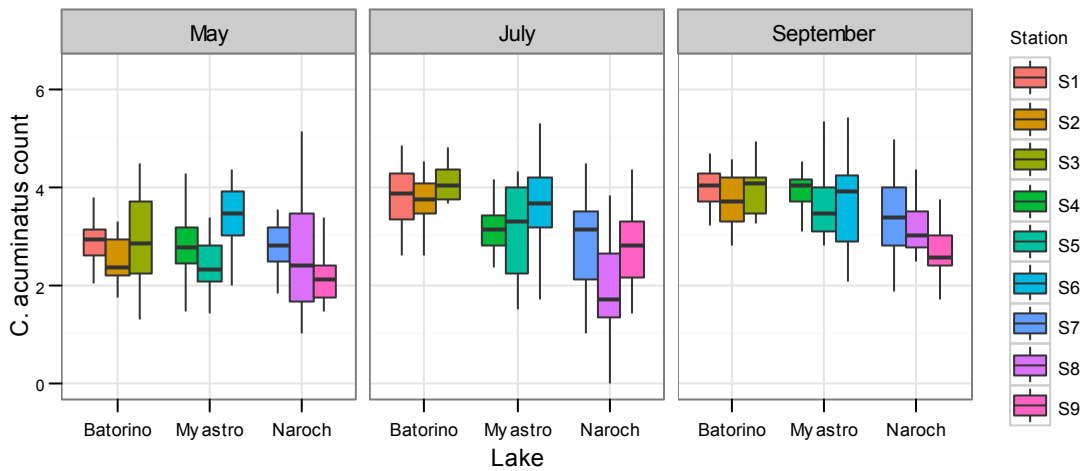
Figure 3. Seasonal dynamics of the power-transformed counts of *C. acuminatus* in *D. polymorpha* at each sampling station within a lake. Station codes correspond to those in Figure 1. Horizontal lines within the boxes represent median values. The heights of the boxes correspond to the interquartile ranges (IQR). Whiskers are 1.5×IQR.

The number of *C. acuminatus* in individual infected molluscs (i.e., intensity of infection) varied from 1 to 10782 ciliates/mussel, with the highest value observed in Lake Myastro. By pooling all data for a lake, the median counts of *C. acuminatus* per host (including zero-infected molluscs) were estimated to be 659 (224; 1177) in the eutrophic Lake Batorino, 437 (145; 1009) in moderately eutrophic Lake Myastro, and 163 (36; 430) in mesotrophic Lake Naroch (values in parentheses are the first and the third quartiles, respectively). As suggested by the results of mixed-effects modelling, the expected average number of ciliates adjusted for the host's size significantly differed among lakes ($F_{2,\ 22} = 14.2$, $P < 0.001$). Pairwise comparisons revealed no difference between Lake Batorino and Lake Myastro (P = 0.995, Tukey test), while there was a considerably lower number of *C. acuminatus* in zebra mussels from Lake Naroch than from the other two lakes (P < 0.001 in both cases, Tukey tests; Figure 3). The counts of *C. acuminatus* were also strongly associated with *D. polymorpha* shell length ($F_{1,448} = 59.6$, $P < 0.001$). However, the strength of this relationship remarkably varied among sampling stations and dates within lake (Figure 2), a pattern that was accounted for by the random slope term of Model 1 (see "Material and methods" section). The residuals of Model 1 were approximately normally distributed and showed no hetero-geneity of variance, indicating that the model adequately fitted the data (Figure 4).

The size of *D. polymorpha* significantly differed among lakes ($F_{2,22} = 7.6$, P = 0.003), as well as among sampling stations and dates within lake (Figure 5). The mean (± standard error) shell length of the molluscs was estimated to be 18.8 ± 0.11 mm in Lake Batorino (n = 1219), 15.7 ± 0.15 mm in Lake Myastro (n = 1973) and 14.3 ± 0.14 mm in Lake Naroch (n = 1876). The differences between Lake Batorino and Lake Myastro (P = 0.049, Tukey test), as well as between Lake Batorino and Lake Naroch (P < 0.001, Tukey test) were statistically significant, whereas no difference was detected between Lake Myastro and Lake Naroch (P = 0.279, Tukey test). Diagnostic plots for the residuals of Model 2 revealed no particular patterns, suggesting that the model was adequate (Figure 6).

Discussion

Despite the wide spread of symbiotic associations, only few of them have proven to be effective model systems for studying the nature and driving forces of symbiosis (Nishiguchi 2004). The widely spread invasive mollusc *D. polymorpha* and its highly host-specific symbiotic ciliate *C. acuminatus* appear to be one of such effective models (Karatayev et al. 2007).

Figure 4. Diagnostic plots for Model 1. Points on the left plot do not form any notable pattern, suggesting homogeneity of variance of the residuals. Points on the quantile-quantile plot at the right are aligned approximately into a straight diagonal line, confirming the expected normal distribution of the residuals.
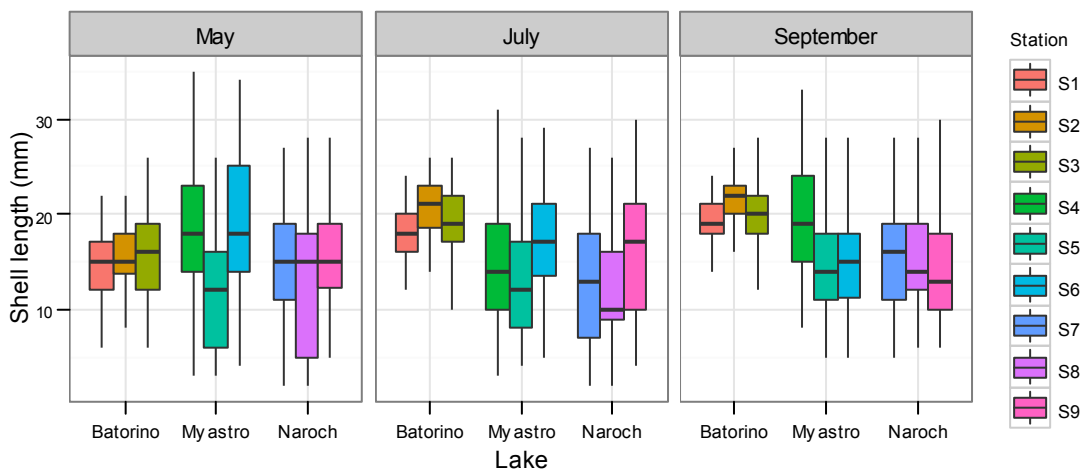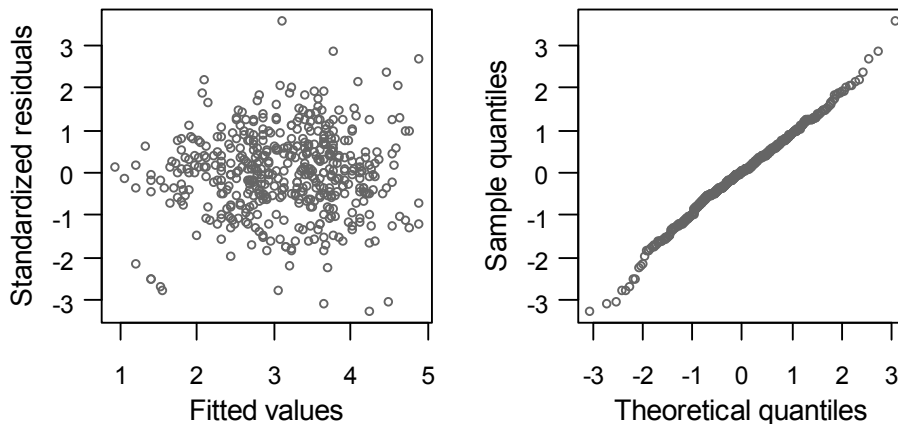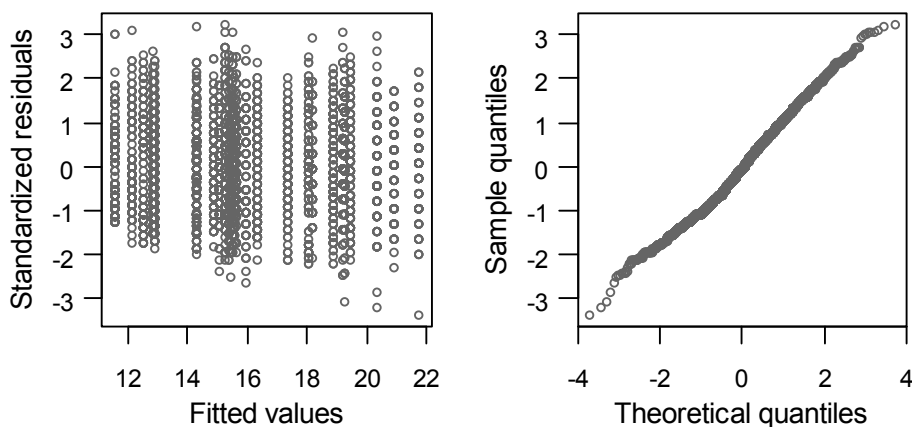

Figure 5. Seasonal dynamics of the shell length of *D. polymorpha* at each sampling station within a lake. Horizontal lines within the boxes represent median values. The heights of the boxes correspond to the interquartile ranges (IQR). Whiskers are 1.5×IQR.

Figure 6. Diagnostic plots for Model 2. Interpretation of these plots is the same as in Figure 4.

The present study, in particular, is the first to determine whether the level of *C. acuminatus* infection in *D. polymorpha* is related to the nutrient enrichment of the environment.

Conchophthirus acuminatus were found in zebra mussels from all three surveyed lakes, with the prevalence of infection being near or equal to 100%. The intensity of infection was also high, sometimes reaching more than 10,000 ciliates per mussel. Such high levels of *C. acuminatus* infection are not uncommon in European populations of *D. polymorpha* (Karatayev et al. 2007), and may provide evidence in favor of commensal character of the relationship between this ciliate and zebra mussels (Molloy et al. 1997).

The abundance of *C. acuminatus* is known to be positively associated with size of *D. polymorpha* (Karatayev et al. 2007). In the present study, the strength and even direction of this relationship was found to vary both spatially and temporally (Figure 2). When adjusted for this random variation, the average expected number of *C. acuminatus* in a zebra mussel of average length was found to significantly differ among the examined lakes, progressively increasing from the mesotrophic Lake Naroch to eutrophic Lake Batorino. This finding is likely to be explained by interplay between the following two mechanisms.

On the one hand, the revealed link between the lake's trophic status and abundance of *C. acuminatus* could be indirectly determined by size of *D. polymorpha* (i.e., carrying capacity for infection), which on average was found to increase with trophy (Figure 5). Greater size of *D. polymorpha* in eutrophic waters was documented by several authors (Stańczykowska 1977, 1984; Dorgelo 1993; Sprung 1993; MacIsaac 1994; Nalepa et al. 1995), who generally explained this by more favorable feeding and growth conditions in such waters due to higher content of seston. Stańczykowska (1997, 1984) and Nalepa et al. (1995) found that even within the same lake, zebra mussels from more eutrophic parts could be larger and heavier than those from less eutrophic sites. Similar positive associations have been reported for other molluscan suspension feeders (e.g., Kirbi and Miller 2005; Ravera et al. 2007).

On the other hand, the significant lake effect on *C. acuminatus* abundance revealed in this study, after adjusting for the effect of *D. polymorpha* size, suggests that the varying feeding conditions in the examined lakes might directly affect reproduction potential of the ciliate. Increased abundance in response to eutrophication is known for free-living ciliates (e.g., Revelante et al. 1985; Beaver and Crisman 1989; Zingel et al. 2002), and may also take place in endosymbiotic ciliates such as *C. acuminatus*. Although detailed information on the composition of food items of *C. acuminatus* is lacking, by analogy with other *Conchophthirus* spp., one can expect this protozoan to feed on bacteria, algae, and other small-sized organic particles filtered out by zebra mussels from the water column (Molloy et al. 1997; Karatayev et al. 2007). The amounts of seston, bacterioplankton, and algae in the examined waterbodies demonstrate clear patterns, with the highest values recorded in the eutrophic Lake Batorino and the lowest ones in mesotrophic Lake Naroch (Table 2).

Arguably, the results of this study may be applicable to a broader spectrum of aquatic symbiotic associations similar to the one existing between *D. polymorpha* and *C. acuminatus*. However, accumulation of additional evidence is required to verify the mechanisms suggested in this paper to explain the positive feedback between the abundance of *C. acuminatus* and the trophic conditions its host lives in. For instance, a number of factors other than trophic level can influence between-waterbody differences in size of *D. polymorpha*, such as size-selective consumption of molluscs by predators (Molloy et al. 1997), density-dependent effects (Hebert et al. 1991), variations in beginning of spawning (Haag and Garton 1992), and current velocities (Bij de Vaate 1991).

Acknowledgements

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