First report of endosymbionts in Dreissena polymorpha from the brackish Curonian Lagoon, SE Baltic Sea*

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KEYWORDS

Dreissena polymorpha Conchophthirus acuminatus Ophryoglena sp. Seasonal dynamics Brackish water

Romualda Chuševė^{1,2,*} Sergey E. Mastitsky³ Anastazja Zaiko¹

Coastal Research and Planning Institute,
Klaipeda University,
H. Manto 84, LT 92294 Klaipeda, Lithuania

Lithuania Environment Protection Agency,
Marine Research Department,
Taikos av. 26, LT-91149, Klaipeda, Lithuania;
e-mail: romualda.chuseve@corpi.ku.lt

³ RNT Consulting Inc.,
823 County Road 35, Picton, Ontario K0K 2T0, Canada

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Abstract

We report the first results of a parasitological study of *Dreissena polymorpha* (zebra mussels) from the brackish Curonian Lagoon, SE Baltic Sea. Zebra mussels were collected monthly from May to October 2011 from a site near the mouth of the River Nemunas. Three types of endosymbionts were found in the mantle

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^{*}corresponding author

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cavity and visceral mass of the molluscs during dissections, i.e. the commensal ciliate Conchophthirus acuminatus and parasitic ciliate Ophryoglena sp., and rarely encountered, unidentified nematodes. The abundances of C. acuminatus and Ophryoglena sp. were positively associated with host shell length and water temperature, but no effect of water salinity was detected. As the endosymbionts are either highly host-specific to zebra mussels (C. acuminatus and Ophryoglena sp.) or are probably free-living organisms that inadvertently infect the molluscs (nematodes), we conclude that the presence of D. polymorpha in the Curonian Lagoon does not pose any serious parasitological risk to native biota. We emphasize, however, that this conclusion should be treated with caution as it is based on a study conducted only at a single location. Our work extends the currently scarce records of D. polymorpha parasites and commensals from brackish waters, and adds to a better understanding of the ecological impact this highly invasive mollusc causes in the areas it has invaded.

1. Introduction

The Ponto-Caspian zebra mussel, *Dreissena polymorpha* (Pallas 1771), is one of the most successful and best-studied suspension-feeding invaders, capable of colonizing both fresh and brackish water bodies. Its life history and biological traits (e.g. living in clumps, and non-selective suspension-feeding) determine its dominance over other invertebrates, enabling it to become highly abundant and impacting when introduced into a new aquatic ecosystem (Karatayev et al. 2002).

However, being a powerful filter-feeder, the zebra mussel can greatly reduce algal biomass and negate or mask the ever increasing effects of nutrient pulses (Karatayev et al. 2002, Dzialowski & Jessie 2009). Several studies have, therefore, addressed the potential use of zebra mussels in water quality remediation (e.g. Reeders & Bij de Vaate 1990, Orlova et al. 2004, Elliott et al. 2008, Stybel et al. 2009, Goedkoop et al. 2011) or sewage sludge treatment (Mackie & Wright 1994). These issues are particularly relevant to large transitional ecosystems, such as the Baltic Seas brackish lagoons, with well-pronounced, anthropogenic eutrophication.

When considering the pros and cons of zebra mussel cultivation for water quality improvement, it is important to identify and assess all possible ecological risks the species may pose. One of the negative ecological effects of the zebra mussel is associated with its ability to host a diverse range of endosymbionts, including potentially pathogenic parasites of fish and waterfowl (Molloy et al. 1997, Karatayev et al. 2000a, Mastitsky 2004, 2005, Mastitsky & Gagarin 2004, Mastitsky & Samoilenko 2005, Mastitsky & Veres 2010). Increased abundances of such parasites hosted by D. polymorpha in invaded water bodies have repeatedly been documented in Europe (Molloy et al. 1997, Mastitsky 2005, Mastitsky & Veres 2010).

Although *D. polymorpha* tolerates salinities of up to about 6 PSU and is thus not uncommon in brackish waters (Karatayev et al. 1998), it is essentially unknown whether the diversity and abundance of *D. polymorpha* endosymbionts in the invaded brackish waters differ from fresh waters. The only exception we are aware of is the work by Raabe (1956), who observed a considerable negative correlation between salinity and the prevalence of *D. polymorpha* infection with its commensal ciliate *Conchophthirus acuminatus* in the Vistula Lagoon, Baltic Sea.

Studying the parasites and other endosymbionts of *D. polymorpha* (e.g. their species composition, prevalence and intensity of infection under varying conditions) is deemed an essential part of the integrated assessment of the environmental impact this mollusc can potentially have. Accordingly, we conducted a half-year-long study of the seasonal dynamics of endosymbionts in *D. polymorpha* from the Lithuanian part of the Curonian Lagoon, SE Baltic Sea. This work adds to a better understanding of the parasitological risks posed by the mollusc in brackish water bodies, and also highlights relevant implications for potential *D. polymorpha* cultivation (e.g. utilization of zebra mussel biomass in husbandry).

2. Material and methods

2.1. Study area

The Curonian Lagoon is a large (1.584 km²), shallow (average depth ~ 3.8 m) coastal water body connected to the south-eastern Baltic Sea by the narrow (0.4–1.1 km) Klaipeda Strait (Figure 1). The River Nemunas brings 98% of the total freshwater runoff and enters the lagoon in its central area, dividing the water body into two different parts (Gasiūnaitė et al. 2008). The northern part is a transitory riverine-like system transporting freshwater into the sea, where the salinity ranges from 0.5 to 5–6 PSU during short-term wind-driven inflow events. Seawater inflows of 1–6 days duration are the most common, but the seawater intrusions are usually restricted to the northern part of the lagoon, only rarely propagating ≥ 40 km into the lagoon. The lacustrine southern part is characterized by a relatively closed water circulation and lower current velocities. It therefore serves as the main depositional area of the lagoon (Gasiūnaitė et al. 2008).

Dreissena polymorpha was probably introduced into the Curonian Lagoon in the early 1800s. The molluscs were presumably attached to timber rafts and reached the lagoon via the central European invasion corridor (Olenin et al. 1999, Karatayev et al. 2008). Currently, zebra mussels are highly abundant in the lagoon, occupying the littoral zone down to 3–4 m depth and occurring on both hard substrates and soft bottoms

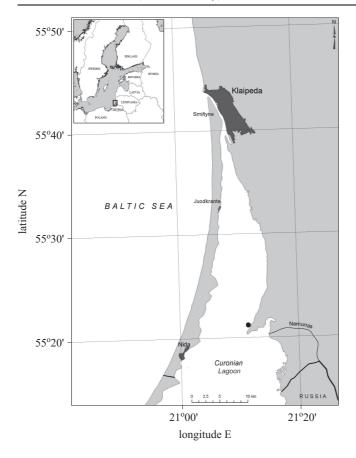


Figure 1. Location of the sampling site in the Curonian Lagoon (filled circle)

(Zemlys et al. 2001). The largest area occupied by the mussels is located in the central part of the lagoon (Zaiko et al. 2010).

Dreissena polymorpha sampling and dissection protocol

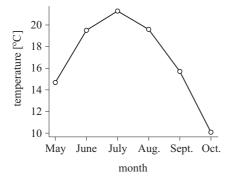
From May to October 2011, zebra mussels were collected monthly with a hand net from a depth of 0.5–1.0 m at a site in the central part of the Curonian Lagoon near the mouth of the River Nemunas (21°11′27, 55°21′15; Figure 1). Live mussels were immediately transported to the laboratory in plastic buckets filled with 5 L of lagoon water.

In the laboratory, the molluscs were divided into two size classes according to their shell length, i.e. <10 mm and >15 mm long, and 20 individuals were randomly selected from each of these groups and dissected within 72 h. Before dissection, shells were rinsed with tap water and wiped with a paper towel. Mussels were cut open with a scalpel,

and the fluid trapped between the valves was collected into a plankton counting chamber and examined for the presence of large-bodied organisms (e.g. oligochaetes, chironomid larvae). The visceral mass was rinsed with a portion of tap water to collect any additional symbionts. The entire soft body was then detached from the shell with a scalpel and dissected under a stereomicroscope ($\times 20$ –70) (Karatayev et al. 2002). The symbionts found were identified to the lowest possible taxonomic level (Molloy et al. 1997, Mastitsky 2004) and counted. All the parasitological terms used in this paper, such as intensity of infection (i.e. number of symbionts per infected host) and prevalence of infection (i.e. percentage of the host individuals infected), are in accordance with Bush et al. (1997).

Statistical analysis

An exploratory data analysis showed that the counts of endosymbionts in D. polymorpha (including zero observations) had considerably right-skewed distributions, representing the family of negative binomial distributions (tested using the Cullen & Frey (1999) technique). Mathematically, a negative binomial distribution is equivalent to an overdispersed Poisson distribution (Hilbe 2011). Thus, we fitted Poisson log-linear models accounting for overdispersion (Breslow 1984) to identify environmental predictors of the abundance of endosymbionts. Each model initially included water temperature and salinity as predictors of the abundance (the average monthly records of temperature and salinity for the sampling site were kindly provided by the Environment Protection Agency, Marine Research Department, Lithuania). Both of these parameters varied considerably over the duration of study (Figure 2), so to avoid redundancy time was not incorporated into the models. The numbers of endosymbionts were strongly correlated with shell length of the zebra mussels (see below).



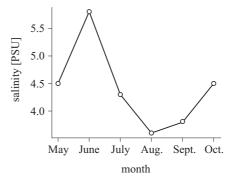


Figure 2. Average monthly values of water temperature (°C; left) and salinity (PSU; right) at the sampling site

To adjust for this effect, shell length was included in the models as an offset term (Hilbe 2011). The analysis was conducted using the functionality of the package *dispmod* v1.1 (Scrucca 2012) in the R v2.14.0 statistical computing environment (R Development Core Team 2011). Here we report the models that contain only significant terms. Insignificant terms were stepwise backward eliminated from the initial models.

3. Results

Dreissena polymorpha was found to be infected with its two host-specific endosymbionts: the commensal ciliate Conchophthirus acuminatus Claparéde et Lachmann, 1858 and the parasitic ciliate Ophryoglena sp. Both of these species were present in all samples of the zebra mussels, but differed in abundance and seasonal dynamics.

The ciliate C. acuminatus was encountered in almost all of the dissected zebra mussels (Figure 3). Uninfected molluscs were only come across in May, resulting in a 90% prevalence of infection in that month. The highest intensity of infection (i.e. number of ciliates in infected hosts) in Dreissena with shell length <10 mm was recorded in July, while in larger molluscs it was observed in August (Figure 3).

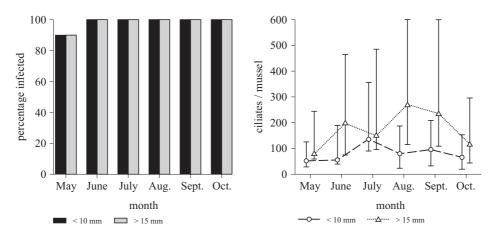


Figure 3. Seasonal dynamics of the prevalence (left) and intensity (right) of *Conchophthirus acuminatus* infection in zebra mussels from the two size classes examined. Intensity of infection is presented as median monthly values, with vertical lines corresponding to the first and the third quartiles

Overall, the intensity of infection was rather moderate, ranging from a monthly median of 56.5 [20.3, 90.8] to 143.0 [49.5, 238.3] ciliates/mussel, with the maximum recorded in July (the values in square brackets after

the medians are the first and third quartiles respectively). The maximum and the minimum numbers of *C. acuminatus* recorded in individual infected zebra mussels were 1203 and 2 ciliates respectively.

The parasitic ciliate *Ophryoglena* sp. was considerably less abundant than *C. acuminatus* (Figure 4). Monthly prevalence of infection with this parasite varied from 17.5% in October to 82.5% in July. The intensity of infection was consistently low over the entire period of observations (Figure 4), not exceeding a median monthly value of 4.0 [2.0, 6.0] ciliates/mussel (July). The minimum and maximum numbers of *Ophryoglena* sp. found in individual infected zebra mussels were 1 and 18 ciliates, respectively.

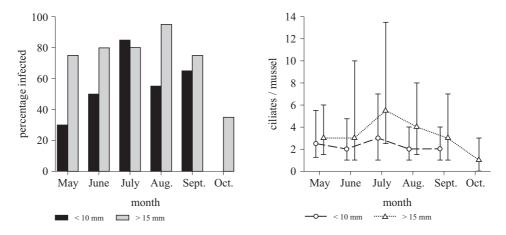


Figure 4. Seasonal dynamics of the prevalence (left) and intensity (right) of *Ophryoglena* sp. infection in zebra mussels from the two size classes examined. Intensity of infection is presented as median monthly values, with vertical lines corresponding to the first and the third quartiles

The abundance of both ciliates (i.e. their counts, including zero values; Bush et al. (1997)) was moderately but statistically significantly correlated with shell length of D. polymorpha (Spearman $r=0.421,\ n=240,\ p<0.001$ for C. acuminatus and Spearman $r=0.318,\ n=240,\ p<0.001$ for Ophryoglena sp.). As concluded by the corresponding Poisson log-linear models, the numbers of ciliates were also positively associated with water temperature, but not salinity (Tables 1, 2).

In addition to the host-specific C. acuminatus and Ophryoglena sp., we occasionally encountered zebra mussels whose mantle cavities contained live nematodes. These unidentified worms were observed in D. polymorpha collected from August to October, and were consistently found only in molluscs with shell length > 15 mm. The number of nematodes in infected

Table 1. Parameters of the log-normal Poisson model describing the effect of water temperature on *Conchophthirus acuminatus* counts in zebra mussels. The residual deviance unexplained by this model is 201.8 on 238 degrees of freedom

Coefficients	Estimate	Standard error	z-value	p-value
intercept	-5.511	0.418	-13.186	<< 0.001
temperature	0.200	0.023	8.813	<< 0.001

Table 2. Parameters of the log-normal Poisson model describing the effect of water temperature on *Ophryoglena* sp. counts in zebra mussels. The residual deviance unexplained by this model is 201.8 on 238 degrees of freedom

Coefficients	Estimate	Standard error	z-value	p-value
intercept	1.313	0.251	5.238	<< 0.001
temperature	0.061	0.015	4.220	<< 0.001

zebra mussels never exceeded 1, with the prevalence of infection being 10% in August and September, and 15% in October.

4. Discussion

Although *Dreissena polymorpha* has been present in the Curonian Lagoon for about 200 years (Leppäkoski & Olenin 2000), our study is the first report of endosymbionts in the mollusc from this part of the Baltic Sea, and also the first record of the ciliates *Conchophthirus acuminatus* and *Ophryoglena* sp. in Lithuanian populations of zebra mussels. There have been occasional studies of the parasites of *D. polymorpha* in Lithuania related to the cytogenetics of the trematodes *Phyllodistomum folium* Olfers, 1817 and *Bucephalus polymorphus* Baer, 1826, hosted by the mollusc in freshwater lakes; however, no data on the levels of infection have been reported for these parasites (Petkevičiūtė et al. 2003, Stunžėnas et al. 2004).

In the 1950s, a study similar to ours was conducted by Raabe (1956) in the brackish Vistula Lagoon (0.5–6.5 PSU (Chubarenko & Margonski 2008)) of the Baltic Sea, Poland. The author found two species of ciliates infecting zebra mussels, i.e. *C. acuminatus* and *Hypocomagalma dreissenae* Jarocki & Raabe, 1932. The presence of *C. acuminatus* in both the Curonian and Vistula Lagoons is not surprising and in line with the ubiquitous distribution of this protozoan in European populations of *D. polymorpha* (Molloy et al. 1997, Karatayev et al. 2007, Mastitsky et al. 2008). There could be two major reasons for the absence of *H. dreissenae* in our samples, the first one being the ecology of this ciliate. *H. dreissenae* prefers saline waters

(Raabe 1956, Jankowski 2001), so that the rather low salinity levels in the central part of the Curonian Lagoon (Figure 2) as compared to the truly brackish Vistula Lagoon (Raabe 1956, Rolbiecki & Rokicki 2008) could have prevented H. dreissenae from developing a detectable population. The second reason could be associated with the dissection technique used in our study: H. dreissenae are of rather small size (length 32–50 μ m; Molloy et al. 1997), which makes it difficult to detect this ciliate without histological analysis. In contrast, Ophryoglena sp. is easily detectable during parasitological dissections. The absence of this ciliate in the zebra mussels examined by Raabe (1956) is more difficult to interpret as very little is currently known about its ecology and biology.

The levels of infection of *D. polymorpha* with *C. acuminatus* and *Ophryoglena* sp. recorded in our study are comparable to those in other European water bodies (Molloy et al. 1997, Mastitsky 2004, Karatayev et al. 2007). The quantitative dominance of *C. acuminatus* over *Ophryoglena* sp. observed in our samples is also consistent with previous studies. Such a dominant position of *C. acuminatus* is generally explained by its commensal relationship with *D. polymorpha*, allowing the ciliate to reach high numbers without causing any significant harm to its host (Molloy et al. 1997, Karatayev et al. 2007). In contrast, *Ophryoglena* sp. is a true parasite (Molloy et al. 1997, Karatayev et al. 2002), whose levels of infection are likely to be inversely related to the fitness of its host.

The numbers of *C. acuminatus* and *Ophryoglena* sp. in zebra mussels were significantly positively associated with temperature (Tables 1, 2). Whereas such a positive relationship has been well documented in previous works for *C. acuminatus* (Karatayev et al. 2000a, 2003), the existing data for *Ophryoglena* sp. are controversial. As in our results (Figure 4), the highest levels of the prevalence and intensity of *Ophryoglena* sp. infection in *D. polymorpha* from the Dnieper-Bug Canal, Belarus, were observed in summer months (Karatayev et al. 2002). However, considerably lower levels of the *Ophryoglena* sp. infection in zebra mussels were recorded in summer than in winter months in the Drozdy Reservoir, Belarus (Karatayev et al. 2003) and in the River Meuse, NE France (Minguez & Giambirini 2012). Additional investigations would help to better understand the seasonal dynamics of this parasitic ciliate in *D. polymorpha* and the role of temperature and other environmental factors in this process.

In his study in the Vistula Lagoon, Raabe (1956) found *C. acuminatus* to be less tolerant to salinity than its host *D. polymorpha*, so that the prevalence of infection declined to 0% with increasing salinity. Neither *C. acuminatus* nor *Ophryoglena* sp. demonstrated such a pattern in the Curonian Lagoon. This, however, could be explained by the relatively

low average monthly salinities we observed (≤ 4.5 PSU most of the time), preventing confident statistical inference.

In addition to the ciliates, we found D. polymorpha to be infected with unidentified nematodes. Several studies conducted in freshwater lakes in Europe (Karatayev et al. 2003, Mastitsky & Gagarin 2004, Mastitsky et al. 2008) suggest that these worms were probably free-living species typically inhabiting periphyton. The most common species of nematodes documented thus far in zebra mussels are oxyphilic representatives of the family Chromadoridae (Mastitsky & Gagarin 2004, Mastitsky et al. 2008). A similar observation was made by Reid et al. (2012), who performed parasitological dissections of *Dreissena rostriformis bugensis* from the Colorado River basin in California, USA. As suggested by Mastitsky & Gagarin (2004), oxygenation of the water due to the filtration activity of zebra mussels may attract such oxyphilic nematodes to *Dreissena* clumps, and then the worms may be accidentally sucked into the mantle cavity through the mollusc's inhalant siphon. The levels of nematode infection observed in our samples of zebra mussel are consistent with a number of studies performed in freshwater water bodies (e.g. Molloy et al. 1997, Karatayev et al. 2000b, 2003, Mastitsky & Gagarin 2004, Mastitsky et al. 2008).

In summary, our work extends the currently scarce records of D. polymorpha parasites and commensals from brackish waters, thus adding to a better understanding of the ecological impacts this highly invasive mollusc has in the areas it has invaded. We found three types of endosymbionts in D. polymorpha from the Curonian Lagoon. The commensal ciliate C. acuminatus and the parasitic ciliate Ophryoglena sp. are considered to be highly host-specific endosymbionts of D. polymorpha (Karatayev et al. 2007, Dr. Daniel P. Molloy, personal communication). It is thus unlikely that these ciliates will switch to any new hosts in the Curonian Lagoon. The nematodes we found in a few zebra mussels were presumably native species that penetrated the mantle cavity of the molluscs inadvertently. Therefore, our data suggest that the introduction of D. polymorpha has not caused any adverse parasitological effects in the Curonian Lagoon, and that the mollusc does not pose any additional risks if cultured for remediation purposes with subsequent biomass utilization in husbandry. We would emphasize, however, that this conclusion should be treated with great caution as it is based on a study conducted at one single location only. The additional sampling of D. polymorpha population on a larger spatial scale in this water body would help verify our results.

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