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DREISSENA POLYMORPHA AND *CONCHOPHTHIRUS ACUMINATUS*: WHAT CAN WE LEARN FROM HOST-COMMENSAL RELATIONSHIPS

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ABSTRACT Host specificity, extremely high prevalence and infection intensity, and easy sampling make the mantle-cavity ciliate *Conchophthirus acuminatus* a very convenient model to address numerous fundamental questions relating to symbiosis and commensalism. The acquisition of food by the ciliate as a result of *Dreissena* filtration activity is probably the basis of the symbiosis, with additional benefits to *C. acuminatus* being shelter, provision of oxygen, and dispersal. The number of *C. acuminatus* in a *Dreissena* population depends on the mussel's size-frequency distribution as there is a direct correlation between *Dreissena* size and infection intensity. Lack of a correlation between host density and commensal infection intensity may indicate that *D. polymorpha* and *C. acuminatus* have a different environmental optimum. Zebra mussels of a given length in each population may have their own carrying capacity of *C. acuminatus* infection intensity. Upon reaching this carrying capacity, a symbiont population may have density-dependent feedback mechanisms, which slow its reproduction rate within a host and/or increase its emigration from the mussel to maintain an optimal density within its host. Massive emergence of ciliates into open water may be synchronized with a mass occurrence of recently settled *Dreissena* juveniles to maximize the infection.

KEY WORDS: zebra mussel, *Conchophthirus acuminatus*, commensal, host specificity, dispersal mechanisms

INTRODUCTION

Symbiosis is an extremely widespread association between two or more different species, common in all biological kingdoms, in which one of the partners, or symbiont, benefits at the other's expense (parasitism), whereas the other remains unaffected (commensalism) or both may benefit (mutualism) (Ahmadjian & Paracer 1986, Odum & Barret 2005).

The term *commensal* was first used by P. J. van Beneden in 1876 for an association in which one animal shares food caught by another animal, and currently is used in a broader sense, including other benefits such as protection (in Ahmadjian & Paracer 1986). This type of symbiotic relationship, probably because of its neutrality to the host, attracts much less attention than parasitism or mutualism (e.g., Henry 1966, Cheng 1970, Gotto 1970, Read 1970, Street 1975, Ahmadjian & Paracer 1986).

According to Starr's classification of the symbiosis continuum (Ahmadjian & Paracer 1986), the ciliate *Conchophthirus acuminatus* (Claparède & Lachmann, 1858) (Scuticociliatida: Conchophthiridae) is endosymbiotic (residing most of its lifespan inside the mantle cavity of *Dreissena*), persistent (remains within its host for a long time); obligate (so highly adapted to its symbiotic existence that it cannot survive long outside its host), and highly specific (found virtually only in *Dreissena* spp.).

Among over 45 endosymbionts known from *Dreissena polymorpha* (Pallas, 1771), *C. acuminatus* is definitely the most common endosymbiont of European *Dreissena* (reviewed in Molloy et al. 1997, Karatayev et al. 2000a, Mastitsky 2004, Mastitsky & Gagarin 2004, Mastitsky & Samoilenko 2005). *Conchophthirus acuminatus* (Fig. 1) is localized on epithelial surfaces of the mantle, gills and visceral mass, in the gill water tubes and suprabranchial cavities, and on the labial palps of zebra mussels (Laruelle et al. 1999). This species was found in all European populations of *Dreissena* examined, including Eastern and Western Europe, former Soviet Union and recently colonized Ireland (reviewed in Molloy et al. 1997, Burlakova et al. 2006). In contrast, *C. acuminatus* has never been found in North America (Toews et al. 1993, Camp et al. 1999, Karatayev et al. 2000b; authors, unpublished data).

Conchophthirus acuminatus has the highest prevalence of infection (percent of infected individuals in a population) among all *Dreissena* endosymbionts, with often 100% infection in a population (Molloy et al. 1997, Karatayev et al. 2000a, Mastitsky 2004). This ciliate is also the most numerous endosymbiont found in *Dreissena*, with the mean infection intensity (number of ciliates per infected mussel) in a population often exceeding thousands of ciliates per mussel (Burlakova et al. 1998, Karatayev et al. 2000a, Karatayev et al. 2000b, Karatayev et al. 2003a). In 1934, Kidder reported that all *Conchophthirus* spp. have an obligate association with bivalves and likely can tolerate only short periods outside their hosts, as during their transfer to new hosts. Karatayev et al. (2003a) demonstrated in laboratory trials that *C. acuminatus* can survive independently up to 6 days.

High host specificity, extremely high prevalence and infection intensity in European dreissenids, and relative ease of sampling make this ciliate a very convenient model to address numerous fundamental questions relating to symbiosis and commensalism. As suggested by Saffo (2001), such questions include, but are not limited to: (1) host and symbiont factors responsible for specificity, (2) trophic interactions, (3) symbiont dispersal mechanisms, (4) synchronization of host and symbiont life cycles, (5) life cycle features increasing infection probability, and (6) environmental factors affecting infection prevalence and intensity. In this current paper we address some

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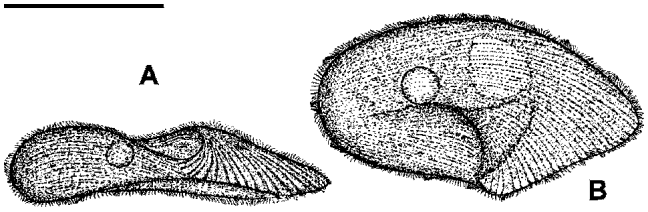

Figure 1. ***Conchophthirus acuminatus*****. Views from ventral side (A) and right side (B). Scale bar = 50 μm. (adapted from Fenchel 1965)**

of these questions as well as discuss directions for future research. This paper is a part of an extensive investigation that we, as members of the International Research Consortium on Molluscan Symbionts (http://www.nysm.nysed.gov/ircoms), are conducting to characterize the systematics, biology, ecology, and distribution of *Dreissena's* endosymbionts (Molloy et al. 1997, Burlakova et al. 1998, Laruelle et al. 1999, Karatayev et al. 2000a, Karatayev et al. 2000b, Karatayev et al. 2003a, Karatayev et al. 2003b, Fokin et al. 2003, Mastitsky 2004, Mastitsky & Gagarin 2004, Molloy et al. 2005, Mastitsky & Samoilenko 2005, Burlakova et al. 2006, Mastitsky & Vezhnovets 2006).

NATURE OF THE RELATIONSHIP

Although the contents of the food vacuoles of *C. acuminatus* have never been comprehensively characterized, evidence suggests that this species is essentially a commensal rather than a parasite (Molloy et al. 1997, Burlakova et al. 1998). This is based in part on studies done on *Conchophthirus* spp. infecting other bivalve species (Kirby 1941, Antipa & Small 1971). As a further indication of commensalism, there was no evidence of host tissue damage (Laruelle et al. 1999) even when intensity of *Dreissena* infection exceeded thousands of ciliates per mussel (Karatayev et al. 2000a). There is also a generally accepted concept that the prevalence of infection is inversely related to pathogenicity (Anderson & May 1981). Therefore, the ubiquitous high prevalence of infection in European *Dreissena* spp., which often reaches 100% (Table 1), is indirect evidence suggesting that *C. acuminatus* is commensal rather than parasitic.

HOST SPECIFICITY

Field sampling suggests that *C. acuminatus* is host specific to *Dreissena* spp. According to Raabe (1971), this ciliate is found exclusively in *D. polymorpha*. This author wrote: "It should be emphasized that I have never observed the immigration of this species (*C. acuminatus*) into Unionidae, even though their shells were sometimes completely covered by infested *Dreissena*" (Raabe 1950). In contrast to Raabe (1950), Yurishinets (1999) reported *C. acuminatus* from *Unio pictorum*, but he found this ciliate only in unionids overgrown with *Dreissena* suggesting that *U. pictorum* was an accidental host, inadvertently ingesting the *C. acuminatus* associated with *Dreissena*. *Conchophthirus acuminatus* has also been reported from *Dreissena rostriformis bugensis* Andrusov (cited as *D. bugensis* in Karatayev et al. 2000b). Although *C. acuminatus* was consistently observed in *D. r. bugensis* in this latter two-year study, both prevalence and infection intensity of *C. acuminatus* in *D. polymorpha* were always significantly higher than in *D. r. bugensis*. Because both *Dreissena* spp. were sampled from the same exact locations, Karatayev et al. (2000b) suggested that *D. r. bugensis* may not be as suitable a host as *D. polymorpha* or possibly *D. r. bugensis* may just be an accidental host in which *C. acuminatus* cannot survive and/or reproduce. This suggestion is also supported by the fact that both the prevalence and infection intensity of *D. r. bugensis* were much lower than any reported from *D. polymorpha* except in shallow areas of the River Svisloch, Belarus (Table 1). However, because the data on *D. r. bugensis* are very limited, further study is required to test these hypotheses.

Raabe (1971) also reported another *Conchophthirus* species, *C. klimentinus*, from the mantle cavity of *D. polymorpha* sampled from Lake Ohrid (Republic of Macedonia). Later the *Dreissena* from this lake were described as a new species, *Dreissena stankovici*, endemic for this lake (Lvova & Starobogatov 1982). Recently, Molloy et al. (2007) again recorded both *C. klimentinus* and *C. acuminatus* from *D. stankovici* in Lake Ohrid. The presence of the endemic ciliate *C. klimentinus* in *D. stankovici* suggests coevolution of these two endemic species.

The degree of specificity of a symbiont may relate to the evolutionary stage of the symbiosis (Ahmadjian & Paracer 1986). Presumably, the more highly evolved a symbiotic association, the longer the symbionts have had to adapt to each other and the more specific would be the association.

DEPENDENCE OF *C. ACUMINATUS* ON THE SYMBIOSIS

For a ciliate residing in the mantle cavity of a mussel, water currents in the cavity are important for the nutritional and respiratory needs. Because the water flow from the mussel's exhalant siphons can be expelled over quite a distance, in addition to rheotactic response, filtering may also act as a sort of chemical beacon to guide the commensals in their search for the appropriate partner (Gotto 1970).

Although the acquisition of food caused by *Dreissena* filtration activity is probably the basis of the symbiosis, this partnership is more complex, with additional benefits to *C. acuminatus* including shelter provided by the host bivalve and also the provision of oxygen for respiratory needs. In addition, *Dreissena* also provides a dispersal means to *C. acuminatus* (Burlakova et al. 2006).

Food

One of the most essential primary benefits that *C. acuminatus* receives from *D. polymorpha* are nutrients. The diet of *Conchophthirus* spp. isolated from other bivalves is known to consist of algae, bacteria, and even sloughed-off host epithelial cells (Kirby 1941, Antipa & Small 1971). In addition to the direct nutritional piracy, this ciliate may also ingest some of the host's cells. Laruelle et al. (1999) reported zebra mussel sperm cells within food vacuoles of *C. acuminatus*. Therefore, trophic relationships between *C. acuminatus* and *D. polymorpha* could be described as a biotrophic symbiosis, where at least one of two involved organisms obtains nutrients from the other (Ahmadjian & Paracer 1986).

Protection

Protective associations in varying degrees of intimacy are widespread in aquatic environments (Gotto 1970). The partnership *Dreissena–Conchophthirus* is not strictly on a food-sharing basis, as the host also provides *Conchophthirus* with a physical

TABLE 1.

Mean (±SE) prevalence and intensity of infection of *Dreissena* spp. with *Conchophthirus acuminatus*.

Waterbody	Prevalence, %	Intensity, Ciliates/mussel	References
Dreissena polymorpha			
Two Danish lakes	100	Very high	Fenchel 1965
Lake Naroch (Belarus): 1994	100	2–5100	Burlakova et al. 1998
2001	96.2 ± 3.9	482.8 ± 258.2	Mastitsky 2003
2002	95.2 ± 2.9	n.r.	Mastitsky 2003
Lake Myastro (Belarus): 1994	100	Very high	Burlakova et al. 1998
2003	99.4	807.6 ± 95.8	Mastitsky unpublished
Lake Lukomskoe (Belarus): 1994	100	Very high	Burlakova et al. 1998
1997	91.6 ± 7.0	357.9 ± 128.3	Karatayev et al. 2000b
Stream Skema (Belarus) 1994	100	Very high	Burlakova et al. 1998
River Svisloch (Belarus):			
1994, 0.3-m depth	0	0	Burlakova et al. 1998
1995, 0.5-m depth	58	2.8	Burlakova et al. 1998
1995, 1.5-m depth	100	High	Burlakova et al. 1998
1996, 1.5–2.5-m depth	86.1 ± 4.3	n.r.	Karatayev et al. 2000b
1997, 1.5–2.5-m depth	79.5 ± 3.8	118.1 ± 19.9	Karatayev et al. 2000b
River Berezina (Belarus): June 2002	100	442.9 ± 61.6	Mastitsky & Vezhnovets 2006
November 2002	100	296.0 ± 52.0	Mastitsky & Vezhnovets 2006
River Mukhavets (Belarus) 1999	10.9	12.5 ± 1.5	Karatayev et al. 1999
River Pina (Belarus) 1999	91.8	112.0 ± 12.0	Karatayev et al. 1999
Reservoir Drozdy (Belarus): 2001	99.7 ± 0.1	849.3 ± 38.5	Karatayev et al. 2003b
2002	100	657.1 ± 28.9	Karatayev et al. 2003b
Reservoir Zaslavskoe (Belarus) 2000	100	n.r.	Mastitsky 2001
Reservoir Osipovichskoe (Belarus) 2000	90	n.r.	Mastitsky 2001
Reservoir Komsomolskoe (Belarus) 2002	100	n.r.	Mastitsky et al. 2003
Reservoir Chizhovskoe (Belarus) 2001	100	n.r.	Mastitsky unpublished
Dneiper-Bug Canal (Belarus) 1997	96.7 ± 1.1	525.2 ± 68.5	Karatayev et al. 2000b
Lake Batorino (Belarus) 2003	100	772.8 ± 58.3	Mastitsky unpublished
Lake Nedrovo (Belarus) 1999	27.4	5.2 ± 3.0	Karatayev et al. 1999
Lake Snudy (Belarus) 1999	97.8	110.0 ± 29.0	Karatayev et al. 1999
Lake Lepelskoe (Belarus) 1997	99.6 ± 0.2	154.0 ± 39.5	Karatayev et al. 2000b
Lake Drivyaty (Belarus) 1996	81.9	n.r.	Karatayev et al. 2000a
Lake Strusto (Belarus) 1996	99.1	n.r.	Karatayev et al. 2000a
Lake Severnyi Voloso (Belarus) 1996	98.0	n.r.	Karatayev et al. 2000a
Lake Voiso (Belarus) 1996	95.7	n.r.	Karatayev et al. 2000a
Lake Bolduk (Belarus) 1998	99	632 ± 86	Karatayev et al. 2000a
Lake Dolzha (Belarus) 1998	100	2979 ± 258	Karatayev et al. 2000a
Lake Lotviny (Belarus) 1998	100	3224 ± 556	Karatayev et al. 2000a
Lake Myadel (Belarus) 1998	99	1003 ± 85	Karatayev et al. 2000a
Lake Malye Shvakshty (Belarus) 1998	35	266 ± 168	Karatayev et al. 2000a
Lake Bolshie Shvakshty (Belarus) 1998	93	432 ± 102	Karatayev et al. 2000a
Lake Spory (Belarus) 1998	86	482 ± 76	Karatayev et al. 2000a
Lake Svir (Belarus) 1998	98	705 ± 79	Karatayev et al. 2000a
Lake Volchin (Belarus) 1998	81	67 ±7	Karatayev et al. 2000a
River Dnieper (Ukraine): 1996	78.9 ± 5.0	n.r.	Karatayev et al. 2000b
1997	74.9 ± 4.9	26.2 ± 3.5	Karatayev et al. 2000b
River Shannon (Ireland) 2004: Clonmacnoise	100	High	Burlakova et al. 2006
Terryglass	100	High	Burlakova et al. 2006
Rossmore	100	High	Burlakova et al. 2006
Tuamgraney	100	High	Burlakova et al. 2006
Dreissena rostriformis bugensis			
River Dnieper (Ukraine) 1996	39.2 ± 3.7	n.r.	Karatayev et al. 2000b
River Dnieper (Ukraine) 1997	16.0 ± 3.1	4.6 ± 0.8	Karatayev et al. 2000b
Dreissena stankovici			
Lake Ohrid, (Macedonia) 2003, 2004*	83	Low	Molloy et al. 2007

* In Lake Ohrid two *Conchophthirus* species were present (*C. acuminatus* and *C. klimentinus*).
n.r. = not recorded.

protection. Although the maximum survival period of *C. acuminatus* outside *Dreissena* has been estimated as 144 h, most ciliates died within 48 h (Karatayev et al. 2003a); this is very similar to results obtained on ciliates from other bivalves (see later). The inquilinism of this association strongly suggests continuous adaptation over a long period of time.

Dispersal

Dispersal is one of the most important tasks for a species. Under the risk of extinction, organisms develop adaptations, which facilitate dispersal to regions further than those colonized by their parents. In our following discussion of *C. acuminatus* dispersal, we will consider two different processes: microdispersal (or diffusion, across hospitable terrain), that is, finding new hosts within the waterbody or connected waterbodies; and macro-dispersal (or "jump dispersal" across unsuitable terrain), when *via* symbiotic relationships with *Dreissena* the species can "jump" over terrestrial barriers and increase their ultimate area of distribution.

Conchophthirus acuminatus has been found in all freshwater *Dreissena* populations in Europe including Switzerland (Claparède & Lachmann, 1858), Bulgaria (Raabe 1934), Poland (Raabe 1934, Dobrzanska 1958), Hungary (Raabe 1950), Denmark (Fenchel 1965), Macedonia (Raabe 1966), Belarus (Burlakova et al. 1998, Karatayev et al. 2000a, Mastitsky 2004), Russia (Laruelle et al. 1999), Netherlands, France, Greece (Laruelle et al. 1999), Ukraine (Yurishinets 1999, Karatayev et al. 2000b), and recently in Ireland (Burlakova et al. 2006). This extremely wide distribution suggests that this ciliate has a very effective system for dispersal and infection of new mussels. How does *C. acuminatus* maintain its infection in expanding zebra mussel populations? To infect new mussels, *C. acuminatus* must leave their host and spend a certain period of time in open water. Zebra mussels may spread to other waterbodies as adults attached to various objects transported by humans and by the downstream dispersal of their planktonic larvae, sometimes being carried hundreds of kilometers from their origin (Stoeckel et al. 1997). *Dreissena* larvae lack a mantle cavity and are too small to contain *C. acuminatus* whose average size is 100 μm × 50 μm (Raabe 1971). The smallest zebra mussel infected with *C. acuminatus* ever reported was 1.1 mm long, which had only one ciliate (Karatayev et al. 2000a). Nevertheless, freshwater European zebra mussel populations are commonly infected with *C. acuminatus*. It was shown that *Dreissena* larvae can stay in plankton in downstream currents for more than a week during summer temperatures (Hillbricht-Ilkowska & Stanczykowska 1969, Skalskaya 1976) and in the fall, when water temperature decreases; this period may last for more than a month (reviewed in Lvova et al. 1994). Therefore we suggest that to be able to follow downstream *Dreissena* larvae spread, *C. acuminatus* should be able to stay in open water for a similar amount of time.

Kidder (1934) reported that *Conchophthirus* spp. have an obligate association with bivalves and can tolerate only brief periods (not longer than 24 h) in open waters, as occurs during their transfer to new hosts. Later Beers (1959) found that for *Conchophthirus mytili* DeMorgan (syn. *Peniculistoma mytili* (DeMorgan)), an inhabitant of the mantle cavity of marine bivalve *Mytilus edulis* Linneaus, a period of 84 h in open water was fatal at 14°C, and that ciliates died even faster at 22°C (48 h) and 30°C (10 h). According to Fenchel (1965), who also studied the survival of different bivalve-inhabiting ciliates in open water, 50% of the following species survived different time intervals: *P. mytili*, 100 h; *Ancistrum mytili* (Quennerstedt), 100 h; *Ancistrocoma myae* (Kofoid and Busch), ca. 50–100 h; *Ancistrum caudatum* Fenchel, ca. 50–100 h; and *Thigmophrya saxicavae* Fenchel, ca. 50–100 h. With the *Dreissena* experiments, it was shown that the maximum survival period of *C. acuminatus* outside their host was 144 h (6 days), but that most ciliates died within 48 h (Karatayev et al. 2003a). These data are very similar to results obtained on ciliates from other bivalves and may suggest that *C. acuminatus* would not be able to survive for as long as the zebra mussel larvae, which are at the leading edge of the dispersing population. Over time, however, it is likely that *C. acuminatus* would be able to eventually establish itself throughout the entire expanded population by relatively smaller incremental steps of dispersion (Karatayev et al. 2003a). In addition, survival experiments on *C. acuminatus* in Karatayev et al. (2000a) were obtained by dissection of zebra mussels. Ciliates, which naturally emerge from their hosts, may contain greater food reserves than these laboratory specimens giving greater longevity in open water; this could be very similar to the duration of the *Dreissena* planktonic stage.

Because *C. acuminatus* is found commonly in European freshwater zebra mussel populations and has not been found in North America yet, Karatayev et al. (2000b) hypothesized that: (1) larval rather than adult *Dreissena* invaded North America and (2) European waterbodies invaded by *Dreissena*, where *C. acuminatus*-infestations are found, were either colonized by adult mussels or alternatively, if a waterbody was colonized by planktonic larvae, the connecting waterbody had an upstream source of veligers and *C. acuminatus*.

Ireland, being an island, is not directly connected by freshwater to any previously existing source population of zebra mussels. Colonization by zebra mussels is believed to have taken place in Ireland in 1993/1994 (Minchin et al. 2005). The recent report of *C. acuminatus* and other species specific endosymbionts of *D. polymorpha* from the River Shannon system (Burlakova et al. 2006) supports the hypothesis proposed by Pollux et al. (2003) that Ireland was colonized by adult zebra mussels and that these were infected with *C. acuminatus*. Therefore, analysis of *Dreissena* endosymbionts may help us to reconstruct the mechanisms of invasion of their hosts.

ADAPTATION TO THE HOST LIFE CYCLE

To effectively infect a new host, a symbiont should synchronize its life cycle with the life cycle of its host. Therefore, we suggest that instead of leaving *Dreissena* randomly, *C. acuminatus* will synchronize their massive emergence from the hosts either with certain conditions and/or periods in the season, to maximize the effectiveness of infection. Fenchel (1965) suggested that ciliates in nondreissenid bivalves are likely to occasionally leave their hosts, but certainly if the hosts are damaged or dying. Burlakova et al. (1998) experimentally proved that *C. acuminatus* rapidly leave their dying zebra mussel hosts, and they suggested that these mussels are likely a major source for the spread of *C. acuminatus* infection. In these experiments fatally injured mussels were kept in trays with uninfected *Dreissena*. The number of ciliates in the dead and dying mussels decreased from initial 291 ± 65 to 0 within three

days, whereas within the first 24 h all uninfected mussels became infected and intensity of infection increased from 0 to 258 ± 101. The transinfection was much slower in control trays with live infected and uninfected mussels, and after the first 24 h the infection intensity in initially uninfected mussels reached only 3.9 ± 1.2 ciliate.

Later in their laboratory experiments, Karatayev et al. (2003a) demonstrated that the rate of *C. acuminatus* emergence from live zebra mussels varied considerably through time at 14°C and 21°C. Very often a sampling period (2–3 days) in which no emergence was observed was followed by a period of high emergence (e.g., at 14°C from 0–25 ciliates/mussels and at 21°C from 0–720 ciliates/mussel per day). The intensity of emergence was temperature dependent and the total mean number of ciliates emerging from each mussel during the 24-day experiment was significantly higher at 21°C (207 ciliates/mussel) than at 14°C (29 ciliates/mussel). Therefore, massive emergence of ciliates may occur not only from injured or dying hosts, but also often occurs from live *Dreissena*. This may be associated with the reaching of the carrying capacity of ciliates inside their hosts and also with transinfection mechanisms.

Two separate studies have shown that infection intensity in *D. polymorpha* is considerably lower in winter, spring, early summer, and late autumn and has a pronounced peak in late summer, which is coincident with a mass occurrence of recently settled *Dreissena* juveniles (Karatayev et al. 2000b, Karatayev et al. 2003b, Lucy 2006). Because *C. acuminatus* can tolerate only brief periods in open waters to minimize the time required for transfer to a new host, Karatayev et al. (2000b) suggested that a period of mass ciliate dispersal may be synchronized to occur when new potential hosts (i.e., juvenile *Dreissena*) become abundant. Many of the older *D. polymorpha* in a population die within a brief period of time after their last spawning (Lvova 1980). Because *C. acuminatus* rapidly leave dying *D. polymorpha* (Burlakova et al. 1998), dying *Dreissena* could be a major source of *C. acuminatus* to initiate infection in other members of the population, especially young-of-the-year mussels. In infected populations, this would theoretically result in mass emigration of *C. acuminatus* from dead mussels in search of new hosts (Karatayev et al. 2000b). Therefore, we suggest that the life cycle of commensal *C. acuminatus* is highly synchronized with that of *Dreissena* to optimize their infection of new hosts and minimize symbiont's mortality associated with host death and transition to new mussels.

FACTORS CONTROLLING INFECTION

Although we defined the relationships between *D. polymorpha* and *C. acuminatus* as commensalism, for convenience and clarity in this article we use terminology developed for parasitic relationships (i.e., prevalence of infection; percent of mussels with ciliates) and intensity of infection (i.e., number of ciliates per infected mussel) (Saffo 1992).

Conchophthirus acuminatus has the highest prevalence and intensity of infection among all commensals and parasites found in zebra mussels (Molloy et al. 1997), with often 100% infection occurring in a population (Table 1). In contrast to prevalence, which is almost always high, the infection intensity varies greatly from a few to several thousands ciliates per infected mussel and depends on various factors (Fenchel 1965, Molloy et al. 1997, Burlakova et al. 1998, Karatayev et al. 2000a, Karatayev et al. 2003a, Karatayev et al. 2003b), many of which are still not well understood (Karatayev et al. 2000b).

Abiotic Factors

It was shown (Raabe 1956) that *C. acuminatus* is less tolerant to salinity than *D. polymorpha* and thus infection prevalence declined from 100% to 0% with increasing salinity in a Polish bay. Although *D. polymorpha* flourished in freshwater, it has evolved from brackish water (Karpevich 1955) and can tolerate salinity up to 6 ppt (Karatayev et al. 1998). If *C. acuminatus* is an obligate commensal of *Dreissena* spp. and has a long history of coevolution with zebra mussels, why then do they have different salinity tolerances? If they had a long-term evolutionary history together, it would be logical to suggest that *C. acuminatus* would have a salinity tolerance similar to zebra mussels and therefore have a similar distribution range, rather than being restricted to fresh waters only. This is a clear call for future studies, including the examination of *Dreissena* populations in their native range in areas of varying salinity in the Caspian Sea and Dnieper-Bug Liman for the presence of ciliate species other than *C. acuminatus*.

Infection could also be limited by depth. Burlakova et al. (1998) reported that in shallow sites of the River Svisloch (Belarus) the majority of zebra mussels were eliminated over winter by a combination of factors, including ice scour, mallard duck predation, and fluctuating water levels. Therefore, mussels at depths $\leq$0.5 m were much smaller (i.e., younger) and zebra mussel densities were lower than those present at >1.5 m sampling site. As a result, the prevalence of infection was significantly lower at the 0.5-m deep site (58%) than at the 1.5-m deep site (100%). Raabe (1966) also reported that the prevalence of infection of *Dreissena* with *C. acuminatus* in Lake Ohrid (Republic of Macedonia) was highest at depths of ~0.5–20 m compared with the shallow (<0.5 m) rocky shoreline, but he did not report whether mussels on the "rocky shoreline" were smaller in size (i.e., younger) and thus less likely to be infected. In their survey of 17 Belarusian waterbodies Karatayev et al. (2000a) found that infection intensity of *C. acuminatus* varied from 67 ± 7 to 3224 ± 556 ciliates/mussel (Table 1) without a significant correlation between infection intensity and water oxidizability, transparency, water color, hydrocarbonates, or trophic status of the waterbody.

A two-year study of the seasonal dynamics of *C. acuminatus* in *D. polymorpha* conducted by Karatayev et al. (2003b) in the Reservoir Drozdy (Belarus) revealed a positive significant correlation between intensity of infection and water temperature. This relationship also showed a significant positive correlation between temperature and rate of *C. acuminatus* reproduction expressed as percentage of dividing cells from total number of ciliates per mussel.

Biotic Factors

It was found that *C. acuminatus* infection intensity in three Belarusian lakes correlated directly with *D. polymorpha* length ($R^2 = 0.83$–0.92) (Burlakova et al. 1998). In this particular study the smallest infected mussel was 2-mm long and contained only a single *C. acuminatus*, whereas the maximum number (5100 ciliates) was recorded in a 28-mm *D. polymorpha*. Later

Karatayev et al. (2000b) also found a significant correlation between size and infection intensity in *Dreissena* spp., with correlation coefficient varying from 0.50–0.91. The lowest correlation coefficient in this study was found in the Dnieper River ($R^2 = 0.50$) and occurred simultaneously with the lowest recorded mean infection intensity (i.e., 26 ciliates/mussel). In contrast to *D. polymorpha*, little evidence of correlation was found between host size and infection intensity for *D. r. bugensis* ($R^2 = 0.14$).

Burlakova et al. (1998) conducted a field experiment where highly infected ($\sim 1.8 \times 10^3$ ciliates/mussel) zebra mussels were held in a cage together with low infected (2.8 ciliates/mussel) *D. polymorpha* of a similar size (20–23 mm). During the first 60 days of this experiment, infection intensity in low infected mussels reached the level of the highly infected individuals and then leveled off (i.e., infection intensities rose to and remained at $1.5–2.0 \times 10^3$ ciliates/mussel). These data suggest that zebra mussels of a given length in each population may have its own carrying capacity of infection intensity. Upon reaching this carrying capacity, a symbiont population may have density-dependent feedback mechanisms, which slow its rate of reproduction within a host and/or increase its emigration from the mussel (see "dispersal" section) to maintain an optimal density within its host (Karatayev et al. 2000b). The hypothesis that *D. polymorpha* of a given length in each population may have its own carrying capacity of infection intensity is also supported by the fact that infection intensity of zebra mussel over a 8-mo period of study was always higher in the River Svisloch compared with the River Dnieper (Karatayev et al. 2000b).

In addition, no correlation between infection intensity and host density was found in 17 Belarusian waterbodies (Karatayev et al. 2000a, Table 1). For example, the highest mean intensity of infection (3224 ± 556 ciliates/mussel) was found in Lake Lotviny, which had the lowest *D. polymorpha* density (<1 m^2). Similar low *Dreissena* density (<1 m^2) was found in Lake Malye Shvakshty, but infection intensity was also low (266 ± 168 ciliates/mussel). In Lake Dolzha, both zebra mussel density (183 m^{-2}) and infection intensity (2979 ± 258 ciliates/mussel) were high. Therefore, the carrying capacity of infection intensity does not seem to depend on the host density, but is controlled by some other factors that should be a subject of future investigations.

CONCLUSIONS AND DIRECTIONS FOR FUTURE STUDY

Nature of the Relationship

We have only indirect evidence indicating that *C. acuminatus* is a commensal (e.g., no evidence of host pathology and high prevalence of infection). It is very important to study comprehensively the contents of the food vacuoles of the ciliate, to find out whether there is competition for food between *C. acuminatus* and their host, and also to determine whether mussel hosts ever feed on this ciliate.

Host Specificity

Conchophthirus acuminatus was found exclusively in *Dreissena* spp. In addition, there is evidence suggesting that *D. r. bugensis* may not be as suitable host for *C. acuminatus* as *D. polymorpha*, or possibly *D. r. bugensis* may just be an accidental host in which *C. acuminatus* cannot survive and/or reproduce. Because so far only mixed populations of *D. polymorpha* and *D. r. bugensis* were studied for *C. acuminatus* infection, it is important to check if pure *D. r. bugensis* populations also have prevalence and infection intensities of *C. acuminatus* similar to those found in *D. polymorpha* populations.

Co-evolution

Dreissena can live in fresh and brackish (<6‰) waters, whereas *C. acuminatus* was reported to be absent in brackish *Dreissena* populations. However, *Dreissena* populations from brackish waters in their native range have never been checked for the presence of *C. acuminatus*. In addition, it will be important to check for the presence of *C. acuminatus* or related ciliate species in other species and subspecies of *Dreissena* present in the Caspian Sea (e.g., *D. polymorpha andrusovi*). Therefore, a study of *Dreissena* populations from the Azov and Caspian seas may help to explain whether *D. polymorpha* and *C. acuminatus* have different origins, and shed some light on their coevolution history.

Dispersal

Dreissena may spread in their attached and planktonic stages, but *C. acuminatus* colonizes only the attached stage of *Dreissena*. Therefore, presence/absence of *C. acuminatus* in *Dreissena* population may help to reconstruct the vectors of zebra mussel spread.

Free-living Stage of C. acuminatus

There is evidence indicating that massive emergence of ciliates into open water is coincident with a mass occurrence of recently settled *Dreissena* juveniles to maximize successful infection. Another important question concerning the free-living stage of *C. acuminatus* is whether they feed and reproduce at that stage. If they are able to feed, their mortality may be lower than estimated in laboratory experiments. This may increase the chance of infection of new hosts, especially if they are able to reproduce outside the host. The intensive emergence of *C. acuminatus* from zebra mussels, in addition to the high level of *C. acuminatus* infection in often abundant population of *D. polymorpha*, may result in a high density of *C. acuminatus* in the open water. Knowledge of the density of free-living ciliates in combination with data on their mortality may yield a better understanding of the dynamics of *C. acuminatus* in zebra mussels.

Mechanism of Infection

Investigating if there are specific metabolic cues—chemotaxis between host and symbiont—for finding appropriate *Dreissena* hosts will be an interesting task in future studies of the *C. acuminatus—D. polymorpha* relationship.

Carrying Capacity of the Host

There is a direct correlation between *Dreissena* size and infection intensity. Therefore, numbers of *C. acuminatus* in *Dreissena* population will depend on the size-frequency distribution of

their host and small populations represented by large mussels may have more ciliates than large populations of small mussels. However, data also suggest that zebra mussels of a given length in each population may have their own carrying capacity of *C. acuminatus* infection intensity. Upon reaching this carrying capacity, a symbiont population may have density-dependent feedback mechanisms, which slow its rate of reproduction within a host and/or increase its emigration from the mussel to maintain an optimal density within its host. The carrying capacity of infection intensity does not seem to depend on host density, but it may be controlled by other factors that should be a subject of future investigations.

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