ENDOSYMBIONTS OF *DREISSENA POLYMORPHA* IN IRELAND: EVIDENCE FOR THE INTRODUCTION OF ADULT MUSSELS

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ABSTRACT

Although zebra mussels (*Dreissena polymorpha*) have invaded waters across Europe for over 200 years, they colonized Ireland only within the past decade. To test the hypothesis that Ireland was colonized by adult *D. polymorpha*, we examined mussels from different sites along the Lower Shannon River system in Ireland for the presence of host specific and generalist endosymbionts.Within the mantle cavity and/or associated with zebra mussel tissues we found species specific-ciliates (*Conchophthirus acuminatus* and *Ophryoglena hemophaga*) and generalist symbionts (the ciliate *Ancistrumina limnica*, nematodes, oligochaetes and chironomids). We found a significant difference in the prevalence of symbionts among sites, but all mussels at all sites harboured one specialist species *C. acuminatus*, and all of the mussels at three of the four sites also had the second specialist, *O. hemophaga*. Thus, with the introduction of *D. polymorpha* into Ireland, at least two additional species, their host-specific symbionts *C. acuminatus* and *O. hemophaga*, have also been introduced. The presence of these symbionts in Ireland supports the hypothesis that adult zebra mussels were introduced into Ireland, rather than larval stages. This contrasts with the introduction of zebra mussels to North America, where adult zebra mussels are devoid of host-specific symbionts.

INTRODUCTION

Although zebra mussels (*Dreissena polymorpha* (Pallas)) have been spreading throughout Europe since the beginning of the 19th century, they colonized Ireland only within the past decade (Minchin, 2000). This aggressive invader can become very abundant within freshwater ecosystems and has caused significant ecological and economic impacts in Europe and North America (Karatayev, Burlakova & Padilla, 1997, 2002; O'Neill, 1997). In addition to being an invader of freshwaters, the zebra mussel has also been a vector for the spread of other species, most notably its endosymbionts (Karatayev *et al.*, 2000). In their review of the natural enemies of zebra mussels, Molloy *et al.* (1997) found 34 taxa of endosymbionts, including ciliates, trematodes, nematodes, chironomids, oligochaetes, mites and leeches, associated with *Dreissena*. Among the parasites and commensals associated with zebra mussels, there are at least five species of ciliates, *Conchophthirus acuminatus* (Claparède & Lachmann), *C. klimentinus* Raabe, *Hypocomagalma dreissenae* Jarocki & Raabe, *Sphenophrya dreissenae* Dobrzanska and *S. naumiana* Raabe, known to be specific to dreissenid mussels (Molloy *et al.*, 1997). In addition, a newly described species, *Ophryoglena hemophaga* Molloy, Lynn & Giamberini, is believed to be a specific symbiont of dreissenid mussels (Molloy, Lynn & Giamberini, 2005). All reported *Dreissena* endosymbionts are found only in benthic juveniles and adults, and have never been found associated with their planktonic larvae. Thus far, all populations of zebra mussels in Europe that have been examined have one to four host-specific endosymbionts; however, none of these host-specific endosymbionts has been found in North American *Dreissena* populations (Molloy *et al.*, 1997).

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In order to control the spread of invaders, we need to identify the principal vectors for their spread as well as the life stages most likely transported by each vector. In addition, knowledge of the life stage that is transported when invasions are successful can provide important information regarding the most important vectors for the dispersal of invading species. The presence of commensals or parasites, known to be found only in adults, can confirm that adult life stages, not larvae, were introduced when new invasions are found.

We examined *D. polymorpha* from different sites along the Lower Shannon River system in Ireland for the presence of host-specific and generalist endosymbionts, to test the hypothesis proposed by Pollux *et al.* (2003) that Ireland was colonized by adult *D. polymorpha*.

MATERIAL AND METHODS

In order to determine endosymbiont species composition, prevalence (percent of mussels with endosymbionts) and intensity (number of symbionts per infected individual) of infection, zebra mussels were collected in September 2004 from 4 sites along the Lower Shannon River system (Fig. 1): Clonmacnoise (Site 1), a jetty on a meandering section of the Shannon River between two large lakes, Lough Ree and Lough Derg; Terryglass (Site 2), a sheltered quay on the northeast shore of Lough Derg; Rossmore (Site 3), a sheltered jetty, near the entrance to a river, where there is little boat or human activity, on the west side of Lough Derg; Tuamgraney (Site 4), a quay on a small river 2 km upstream of Lough Derg, where there is no zebra mussel recruitment (Minchin & Lucy, 2004), but loose adult mussels could be obtained.

Live zebra mussels were collected from 0.5–2.5 m depth below water level from the vertical surfaces of jetties and quays using a scraper with a pocket net mounted on a pole.

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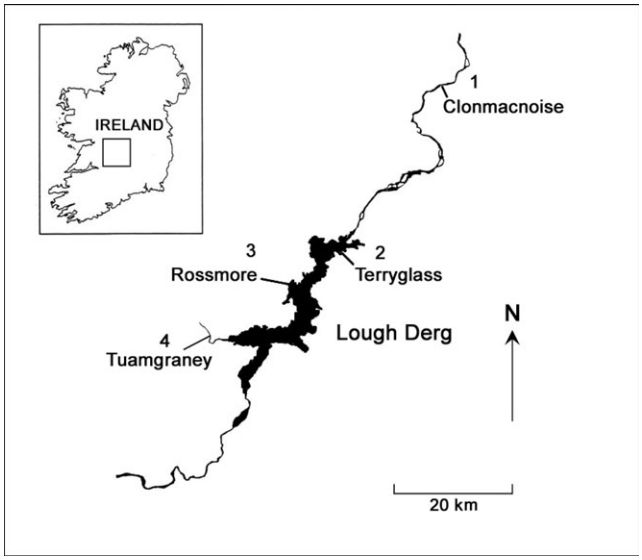

Figure 1. Map of sampling sites along the Lower Shannon River, Ireland. The inset shows the location of the Lower Shannon River.

All mussels were dissected within 12 h of collection. Ten mussels 19–32 ± 1 mm maximum dimension were examined from each of the four sites. Before dissection, shells were cleaned with tap water and then wiped with paper towel. Mussels were opened with a scalpel and all of the water trapped between the valves was collected and examined for symbionts. The mantle cavity was flushed with fresh water to rinse out any additional symbionts. The visceral mass was removed from the shell and the whole body was dissected and examined for the presence of trematodes, ciliates and other symbionts using a stereomicroscope (20–70X). Only those species that could be found by dissection and did not require histology for detection were included.

The number of endosymbionts per infected mussel (intensity) was recorded for *Ophryoglena hemophaga* and *Ancistrumina limnica*, as well as for species of nematodes, chironomids, and oligochaetes. Because of the lack of special chambers required to count *C. acuminatus* in *D. polymorpha* (Burlakova, Karatayev & Molloy, 1998), we did not determine the intensity for this ciliate. For *O. hemophaga* and *A. limnica* intensity was classified as follows: low intensity (<5 ciliates/mussel), moderate intensity (5–10 ciliates/mussel), and high intensity (>10 ciliates/mussel).

RESULTS

The mean size of mussels examined was similar among sites (Table 1). Although mussels at Clonmacnoise were significantly smaller than those examined at the other sites (ANOVA $P = 0.006$, Tukey HSD $P < 0.004$), the size of mussels examined did not differ among the other three sites ($P > 0.09$, Tukey HSD).

We found six types of endosymbionts within the mantle cavity and/or associated with zebra mussel tissues, including ciliates (*C. acuminatus*, *O. hemophaga* and *A. limnica*), nematodes, oligochaetes and chironomids (Table 2). *Conchophthirus acuminatus* is host specific to zebra mussels, and *O. hemophaga* is likely to be specific to dreissenids as well.

We found a significant difference among sites in the prevalence of generalist symbionts ($G = 14.7$, 3 df, $P < 0.001$) and a significant difference in prevalence among the symbionts ($G = 11.9$, 3 df, $P < 0.01$), including nematodes ($G = 4.46$, 1 df, $P < 0.05$). For the specialist symbionts there was a significant difference among sites and among species (sites $G = 33$, 3 df, $P < 0.001$, species $G = 15.3$, 3 df, $P < 0.001$). However, differences were driven by one site, where none of the mussels harboured *O. hemophaga*. There was no significant difference among sites for *C. acuminatus* ($P = 1$), as all mussels at all sites had this symbiont, but there was a highly significant difference among sites for *O. hemophaga* ($P < 0.001$).

Table 1. Size of mussels examined for endosymbionts at four sites along the Shannon River in Ireland.

Site	*n*	Mean size (range) (mm)	Median size (range) (mm)	Density (mussels m^{-2})
Clonmacnoise	10	22.9 (22–34)	14 (4–24)	1,030
Terryglass	10	26.1 (22–32)	14 (1–30)	2,300
Rossmore	10	27.8 (25–30)	10 (1–30)	2,750
Tuamgraney	10	24.9 (19–32)	25 (18–30)	<1

n, sample size; median size and range of size of mussels and mussel density at each site from Minchin & Lucy (2004).

The intensity of *Dreissena* infection with *O. hemophaga* was moderate (~5–10 ciliates per mussel) at Lake Terryglass and Clonmacnoise, and low (1–5 ciliates per mussel) at Rossmore; no *O. hemophaga* were found in mussels at Tuamgraney. *Ancistrumina limnica* was found only at Tuamgraney and the infection intensity was usually low to moderate (<10 ciliates per mussel); only one mussel had a high intensity (>10).

The median number of nematodes, chironomides, and oligochaetes found among sites was one per infected mussel; however at Clonmacnoise we found nine nematodes in one individual and at Tuamgraney three chironomids were found in one zebra mussel (Table 3).

DISCUSSION

Conchophthirus acuminatus is the most common endosymbiont found in *D. polymorpha* in Europe (Molloy *et al.*, 1997; Burlakova *et al.*, 1998; Karatayev *et al.*, 2000). It is usually found on the surface of the visceral mass and gills of zebra mussels, where it moves about using cilia. Their relationship with zebra mussels, although obligate, is far more likely to be commensal than parasitic (Molloy *et al.*, 1997). *Conchophthirus acuminatus* is known to be extremely host-specific and has been reported only from *D. polymorpha* and *D. bugensis* (Molloy *et al.*, 1997; Karatayev *et al.*, 2003a). Raabe (1950) reported that this species is never found in unionid bivalves, even when they are completely covered by *C. acuminatus*-infested zebra mussels. This ciliate has also been found to have the highest prevalence of infection among all zebra mussel symbionts. Often, all zebra mussels in a population are infected (Molloy *et al.*, 1997; Karatayev *et al.*, 2000, 2003a). In this study we also found that all mussels at all sites were infected with *C. acuminatus*. Although we were unable to assess infection intensity, we saw several dozens to hundreds of *C. acuminatus* per mussel. High levels of intensity are not unusual for *C. acuminatus* and this ciliate is the most numerous endosymbiont found in *Dreissena* (Burlakova *et al.*, 1998; Karatayev *et al.*, 2000). The highest infection intensity for *C. acuminatus* ever reported was 14,035 ciliates/mussel and was observed in a 26.4 mm mussel from Lake Lotviny (Belarus) (Karatayev *et al.*, 2000).

Conchophthirus acuminatus is a relatively large ciliate (length 50–120 μm; Molloy *et al.*, 1997) and has never been found in *Dreissena* veligers, which are probably too small (50–250 μm; Karatayev, 1983) to harbour this ciliate. The smallest mussel ever reported to be infected with this ciliate was 1.1 mm long and had a single *C. acuminatus* in its mantle cavity (Karatayev

Table 2. Prevalence of symbionts in zebra mussels in the Shannon River system, Ireland.

Site	Species-specific symbionts		Generalist symbionts			
	Conchophthirus acuminatus	*Ophryoglena hemophaga*	*Ancistrumina limnica*	Nematode	Chironomid	Oligochaete
1	100	100	0	50	0	0
2	100	100	0	10	0	0
3	100	100	0	20	0	10
4	100	0	50	30	40	0

Cell values are the percent of individuals examined ($n = 10$ for each site) that were infected with each symbiont. The sites were: 1, Clonmacnoise; 2, Terryglass; 3, Rossmore; 4, Tuamgraney. *Conchophthirus acuminatus* and *Ophryoglena hemophaga* are specific to zebra mussels; the other four symbionts are generalists, found associated with multiple species.

Table 3. Intensity of symbionts (numbers of symbionts per infected mussel).

Site	Species-specific symbionts	Generalists symbionts			
	Ophryoglena hemophaga	*Ancistrumina limnica*	Nematode	Chironomid	Oligochaete
1	Moderate	0	2.6 (1; 1–9)	0	0
2	Moderate	0	1	0	0
3	Low	0	1	0	1
4	0	Moderate	1	1.5 (1; 1–3)	0

Cell values are mean number of symbionts per infected mussel with median and range in parenthesis for *Ancistrumina limnica*, nematodes, chironomids and oligochaetes. For *Ophryoglena hemophaga* a semi-quantitative ranking was used: Low = <5 per mussel, Moderate = 5–10 per mussel, High = >10 per mussel. The sites were: 1, Clonmacnoise; 2, Terryglass; 3, Rossmore; 4, Tuamgraney.

et al., 2000). *Conchophthirus acuminatus* has been found in zebra mussels in virtually all European waterbodies examined (reviewed in Molloy *et al*., 1997; Burlakova, 1998; Karatayev *et al*., 2000). Zebra mussels can spread downstream though connected waterways by the dispersal of their planktonic larvae, and are sometimes carried hundreds of kilometres from their origin (Stoeckel *et al*., 1997). The veligers can stay suspended in downstream currents for more than a week (Hillbricht-Ilkowska & Stanczykowska, 1969; Skalskaya, 1976). In contrast, *C. acuminatus* can survive six days at most outside of its host (Karatayev *et al*., 2003b). Therefore, the transport of *C. acuminatus* to new sites must be within adults, not larval mussels.

Because *C. acuminatus* is found in all *Dreissena* populations in Europe and has not been found in North America, Karatayev *et al*. (2000) hypothesized that (1) larval rather than adult zebra mussels invaded North America, and (2) that recent European waterbodies invaded by *Dreissena* where *C. acuminatus*-infestations are found were more likely to have been colonized by adult mussels than by veligers. If a water body was colonized by veligers, the water connection that was the source of veligers would have to be the source of *C. acuminatus* as well. Ireland, being an island, is not directly connected by freshwater to any existing source population of zebra mussels. Therefore, the high prevalence of *C. acuminatus* throughout the Shannon River system suggests that infected adults were introduced into Ireland.

Although the ciliate *Ophryoglena* sp., which has recently been described as a new species *Ophryoglena hemophaga* (Molloy *et al*., 2005), is widespread in Europe, it is much less common in zebra mussels than *C. acuminatus* (Molloy *et al*., 1996; Karatayev *et al*., 2000). Several authors have suggested that ophryoglenine ciliates may have a pathogenic effect on *Dreissena* (e.g. Kazubski, personal communication in Stanczykowska, 1977; Zdun *et al*., 1994). However, no other ciliate in the suborder Ophryoglenina has ever been found to be a parasite of bivalves. It is likely that this species is host-specific to *Dreissena* as it has only been found in this genus. *Ophryoglena hemophaga* was found in all mussels at three of the Irish sites sampled, Terryglass, Clonmacnoise, and Rossmore. At Tuamgraney, where it was absent, there were very low densities of *Dreissena*. Very low host population density can lead to the loss of symbionts in a population. When populations have low densities, as can happen during the very early stages of colonization or when few individuals are introduced, individual hosts can lose their symbionts, especially species like *O. hemophaga*, which must leave its host to reproduce and then must recolonize the host. At very low host population densities, the probability that all individuals will be rediscovered by the symbiont is low.

Ancistrumina limnica is common in European *D. polymorpha* populations. This ciliate is a nonspecific commensal of freshwater lamellibranchs and gastropods (Molloy *et al*., 1997). We found this species only at Tuamgraney, where they likely invaded *Dreissena* by moving from native molluscs at the site. Nematodes, chironomids, and oligochaetes found in the mantle cavity of *Dreissena* are also nonspecific symbionts, and are occasionally observed in zebra mussels populations both in Europe and North America (Molloy *et al*., 1997; Karatayev *et al*., 2000, 2003a).

Seven genera of trematodes have been reported in the literature as parasites of species of *Dreissena*. Zebra mussels can serve as first intermediate hosts (e.g. *Bucephalus polymorphus* (Baer) and *Phyllodistomum* spp.), second intermediate hosts (e.g. *Echinoparyphium recurvatum* (Linstow)), or the only host (e.g. *Aspidogaster* spp.) for trematode parasites (Molloy *et al*., 1997). Because low rates of trematode prevalence are typical in *D. polymorpha* (Molloy *et al*., 1997; Karatayev *et al*., 2000), it was not surprising that we did not find trematodes in zebra mussels in Irish waters. To confirm their absence or presence in Irish waters, larger sample sizes of *Dreissena* would be required.

Thus, with the introduction of *D. polymorpha* into Ireland, at least two additional species, their host-specific symbionts *C. acuminatus* and *O. hemophaga*, have also been introduced. The presence of these symbionts in Ireland supports the hypothesis that adult zebra mussels, attached to boat hulls, were introduced into Ireland, rather than larval stages (Pollux *et al*., 2003). Their presence in this newly established invasion contrasts with the introduction of zebra mussels to North America, where adult zebra mussels are devoid of host specific symbionts.

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