Correlating incidences of *Dreissena polymorpha* attachment with Trematode parasitism in *Stagnicola emarginata* snails

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Abstract – The invasive zebra mussel (*Dreissena polymorpha*) has negatively impacted many northern Michigan aquatic species in the past twenty years (Strayer, 2009). One of the species affected has been *Stagnicola emerginata,* a freshwater snail. This species of snail also acts as the intermediate host for a number of parasitic schistosome species which increase the size of the snail, possibly making them more vulnerable to zebra mussel colonization (Horak et al., 2002). The purpose of this study was to determine if there is a relationship between Schistosomatidae parasitism in the freshwater snail *Stagnicola emarginata* and attachment of *Dreissena polymorpha*. Snails were collected from Douglas Lake in northern Michigan lake, shed under artificial lighting to induce cercarial emergence, and observed to determine parasite infection. *D. polymorpha* attachment was noted before snail release. Findings indicate no significant relationship between schistosome parasitism and the presence of *D. polymorpha*. We suggest the absence of a relationship may be due to the schistosomes acting as prudent parasites.

Key Words – *Stagnicola emarginata*, *Dreissena polymorpha*, schistosomes, swimmer's itch, cercarial dermatitis, prudent parasitism.

INTRODUCTION

Since its introduction to the Great Lakes in 1988, *Dreissena polymorpha*, commonly known as the zebra mussel, has wreaked havoc upon populations of indigenous freshwater species, including the *Stagnicola emarginata* snail (M.D.N.R., 1995). Using byssel threads, juvenile *D. polymorpha* attach to a variety of aquatic objects, ranging from boat hulls to biotic life, such as snail shells (Stoeckel et al., 2004). The encumbrance caused by the attached *D. polymorpha* has presumably applied a strong selective pressure upon many snail species. Resultant morphological responses have occurred, including selection for decreased shell size as well as impeded growth rate and burrowing ability in related snail species (Van Appledorn et al., 2007). It is possible that *D. polymorpha* attachment may limit *S. emarginata*'s burrowing to such an extent that it spends less time semi-submerged in the sand. As a result, *S. emarginata* may be exposed to increased parasitism.

S. emarginata is afflicted by at least eight known trematode parasites in the Schistosomatidae family (Blankespoor and Keas, 1997). The schistosome begins its complex life cycle in waterfowl feces which contain fertilized schistosome eggs. Once in water, the eggs hatch to form miracidia, a ciliated transmission stage with an approximate twenty hour life span (Neuhaus, 1952). During this stage, the miracidia infects an intermediate mollusk host, such as *S. emarginata*. The miracidia develops into a sporocyst within the mollusk host and can survive *in vitro* up to ten weeks before individual cercariae emerge daily from the snail (Horák et al., 2002). The cercarial stage then penetrates a waterfowl definitive host and completes its life cycle.

While appearing to cause no physical injury to the snail host, the sporocyst does modify the snail's internal physiology (Sluiters et al., 1980; Sluiters, 1981). Parasites can cause heightened respiration and heart rates as well as varied protein, carbohydrate, nitrogen, and lipid levels (Meyer et al., 1986; Thompson, 1997). A related snail species, *Lymnaea stagnalis,* has been shown to have increased shell and body size when parasitized, resulting in giant growth (McClelland and Bourns, 1969). Also, schistosomes have the ability to manipulate host neurological gene expression to their own benefit, as the parasite terminates host gamete reproduction and subsequently sequesters reproductive energy for itself (Hoek et al., 1997). A combination of these factors may reduce the snail's ability to evade *D. polymorpha* attachment, since resultant shell gigantism may inhibit burrowing ability. If zebra mussels have a higher probability of attaching to larger objects than small ones, larger *S. emarginata* will be more prone to zebra mussels. The energy consumed by the parasite could also weaken *S. emarginata* and reduce its burrowing rate.

The purpose of our study was to determine if there was a relationship between schistosome parasites in *S. emarginata* and *D. polymorpha* attachment. As both *D. polymorpha* attachment and schistosomal parasitism cause negative effects upon the snail host, we expected to observe a direct relationship between parasitism and *D. polymorpha* attachment.

METHODS AND MATERIALS

S. emarginata were collected on five separate occasions at five locations in White Goose Bay in Burt Lake, near Cheboygan, Michigan. Collection site locations were chosen based on zebra mussel abundance, sandy shores, depth, and presence of waterfowl, specifically the Common Merganser. Burt Lake was chosen over surrounding lakes since its *D. polymorpha* abundance is higher than most lakes, which made collection of *S. emarginata* with *D.*

polymorpha more probable (Cain et al., 2008). Sandy beaches were chosen as *S. emarginata* prefer sandy substrate as they are bottom feeders (Cain et al., 2008). As *S. emarginata* abundance does not increase with depth, our collections were limited to water approximately 1 m deep to simplify collection. The presence of waterfowl, specifically the Common Merganser, which is a definitive host of schistosome parasites, increased the possibility of collecting infected snails; Common Merganser was sighted in the Burt Lake area by a knowledgeable source the week prior to collection. Specimens were collected by hand between the hours of 6:00 AM and 7:00 AM between July 19th and July 24 th. The snails were placed in buckets with lake water and transported to the lab.

Shedding, the term given to when a parasite leaves the snail in an intermediate host, was completed using methods described by Blankespoor and Reimink (1998) (see Figure 1). Using forceps, individual snails were placed in plastic communion cups, which were half-filled with oxygenated, filtered water from Douglas Lake. Plexiglas disks with 55 circular cut-outs, each holding a communion cup, were used to hold the snails. Each disk was then placed into a wooden holder under a fluorescent light to induce cercarial shedding. Light was used to stimulate shedding as cercariae typically emerge within six hours after dawn, most likely due to sunlight (Anderson et al., 1976; Cort and Talbot, 1936). Shedding was permitted for 2 hrs, approximately from 8:00 AM to 10:00 AM.

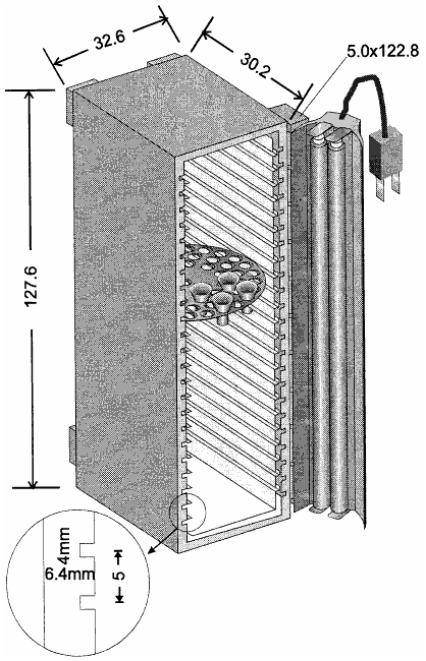

Figure 1. Apparatus used to shed *S. emarginata*. Measurements are in cm unless stated otherwise (from Blankespoor and Reimink, 1998).

Each communion cup was inspected using a Bausch and Lomb Stereo Zoom 4 dissecting microscope to determine cercarial presence or absence. The number of *S. emarginata* with *D. polymorpha* attached was also counted by hand and recorded. Snails were promptly returned to the collection buckets and subsequently released to their respective collection sites by approximately 1:00 PM. A Chi-square test was used to determine if there was a significant difference between *D polymorpha* attachment and schistosome parasitism in *S. emarginata*.

RESULTS

Table 1 shows the relationship between *S. emarginata*, *D. polymorpha*, and schistosome parasites. As Table 1 conveys, approximately 2% of *S. emarginata* collected were parasitized, while approximately 22% had *D. polymorpha* attached. There appears to be no significant relationship between *D. polymorpha* attachment and schistosome parasitism on *S. emarginata (Chi-square=0.663, N=2587, df=3, p=0.882)*.

Table 1. Total number of *S. emarginata* collected with and without *D. polymorpha* as well as with and without schistosome parasitism. Percentages are out of the total 2,587 snails collected.

	D. polymorpha Present	*D. polymorpha* Absent	Total
Schistosomes Absent	575 (22.2%)	1965 (76.0%)	2540 (98.2%)
Schistosomes Present	13 (0.5%)	34 (1.3%)	47 (1.8%)
Total	588 (22.7%)	1999 (77.3%)	

DISCUSSION

As apparent by the data, *D. polymorpha* attachment is not pervasive enough to negatively effect *S. emarginata* by causing increased schistosome infection; additionally, schistosome parasitism does not physically affect *S. emarginata* to a high enough degree to cause increased *D. polymorpha* attachment.

Throughout the course of this study, several possible improvements have been noted which may prove useful in future investigations. As shown in Table 1, the rate of infection is limited, comprising only 47 of the 2,587 *S. emarginata* sampled. It is possible that due to the low rate of infection, an increased sample size would have been able to yield more conclusive data. The laboratory methods used to determine schistosome infection were possibly inaccurate, since it was difficult to confirm the presence of translucent cercariae using white light and clear cups. A more costly technique which has proved effective in detecting as few as one, and as many as three hundred cercariae, is a polymerase chain reaction assay (Driscoll et al., 2005). However, this level of sophistication was outside of the means of our study. An additional source of error may have involved the dislodgment of mussels from snail shells during transport. During collection, snails with *D. polymorpha* attached should have been placed in a container separate from snails without *D. polymorpha*. One-tailed schistosome cercariae, misidentified as annelids

or possibly a *D. polymorpha* larval stage, were also not counted. The one-tailed schistosome cercariae were noticeably abundant during sheddings and we hypothesize that data analysis would have been significantly affected if they had been included in data collection.

After considering these errors, there is probably still cause for the lack of a relationship between schistosome parasitism and *D. polymorpha* attachment. Specifically, it is possible that the schistosome parasites may be acting as prudent parasites (Buckling and Brockhurst, 2008). The term "prudent parasitism" refers to parasitic organisms limiting their negative impact upon their host in order to avoid reducing their own fitness (Holmes, 1983). Thus, the parasite is 'prudent' and does not fully exploit the host. In the schistosome and *S. emarginata* symbiosis, it would not be beneficial to the schistosomes to negatively affect the host's ability to function to a degree where the host is unable to survive.

Prudent parasitism has also been observed in relationships between parasitic schistosomes and their host snails (Minchella, 1985). Schistosomes are known to alter the physiology of *S. emarginata* in many ways, including its immune system (Coustau, 2008). Thus, schistosomes manipulate the immune system to the extent that their own safety is ensured, without compromising the ability to fight off other pathogens (Bayne et al., 2001). There are also many unknown effects of schistosomes on snail hosts, such as changes in metabolism, heart rate, and respiration (Meyer et al., 1986; Thompson, 1997). Nonetheless, the snail is seemingly as healthy as when not parasitized, possibly due to prudent parasitism (Sluiters et al., 1980; Sluiters, 1981).

It would not be difficult to imagine that the schistosomes are minimizing their effect on the behaviors and mechanisms *S. emarginata* uses to evade *D. polymorpha* attachment to propagate their own survival. *D. polymorpha* attachment would seemingly not be beneficial to

the schistosome, as *D. polymorpha* inhibits *S. emarginata* foraging and burrowing ability (Van Appledorn et al., 2007). Thus, less energy is yielded for the parasite to exploit and probability of predation increases, killing both the host and parasite. Therefore, as a parasite's fate is tied closely to that of its host, it should minimize its effect on the host, as any detrimental effect would be analogous to committing a suicidal action upon itself.

We made the initial assumption that gigantism, caused by the schistosome, may decrease burrowing ability; the incapability to burrow would then possibly result in increased *D. polymorpha* attachment. For that reason, it would be logical that the schistosomes increase shell size to a certain threshold, where the ability of *S. emarginata* to effectively evade *D. polymorpha* attachment is not inhibited.

Similarly, the schistosome's energy exploitation should be limited so as to allow *S. emarginata* to effectively avoid *D. polymorpha* attachment. It would not benefit *S. emarginata* to have the ability to burrow, but not the energy to do so. Therefore, the schistosome must utilize a portion of the host's energy, but to a limit. This gives further cause to why the schistosome terminates the host gamete production (Hoek et al., 1997) and seizes the energy for itself: it is able to receive energy but does not interfere with the host's ability to survive.

It is probable to assume that highly virulent schistosomes that caused detrimental affects to *S. emarginata* were evolutionarily selected against. Specifically, they caused *S. emarginata* to die prematurely before they were able to fully exploit the host. Consequently, the schistosome parasite should be under selective pressure to become less virulent over time.

A new paradigm of parasitic relationships theorizes that parasitic relationships will evolve over time to become progressively less virulent until the symbiosis becomes a mutualism. Two parameters to the theory exist: that the host population is not extensive and that dispersal

between hosts is difficult (Cain et al., 2008). In this case of the schistosomes, switching snail hosts is impossible; once the miracidia develop into sporocysts they are unable to switch intermediate hosts (Horak et al., 2002).

Having no natural predators, *D. polymorpha* increases in population size every year, and consequently applies a stronger selective pressure on much abiotic and biotic freshwater life (Nalepa et al., 1996; Strayer, 1999; Van Appledorn, 2007). One may assume *S. emarginata* populations are unable to feed due to lack of detritus owed to increased consumption by *D. polymorpha* (Strayer 1999). In addition to the burden caused by attached *D. polymorpha*, then *S. emarginata* populations may decrease in size (Van Appledorn, 2007). The reduction in number of *S. emarginata* may cause a stronger selective pressure on schistosome parasites, causing them to possibly exploit their host less and stay on the host for longer periods of time. However, the fact that schistosomes have complex life cycles conflicts with this hypothesis as the parasite would be unable to remain on one host and still produce offspring. Nonetheless, it may be possible that *D. polymorpha* could continue to apply selective pressure and cause the schistosome to become less virulent. It would be an interesting concept if one of North America's most notorious invasive species may actually be indirectly benefiting an indigenous species.

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