

**The Effects of Colonization by Zebra Mussels, *Dreissena polymorpha*
on Fitness in two Anisopteran species: *Hagenius brevistylus* and *Didymops
transversa* from**

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Abstract

The colonization of two Anisopteran larvae (*Hagenius brevistylus* and *Didymops transversa*) by *Dreissena polymorpha* was studied in Douglas Lake after the recent mussel invasion from the Lake Huron watershed. Our objectives included measuring the frequency of the zebra mussels on larvae and exuviae, and measuring fitness indirectly by means of “righting” tests, or how long it took the *D. transversa* larvae to “right” itself after being placed upside down. We hypothesized that larvae with zebra mussels attached would show a decrease in fitness because of the increased energy costs. Because we found a low frequency of larvae with mussels, colonization was induced. Our results indicated that as the number of zebra mussels per larva increased, so did “righting” time. “Righting” time also increased as the ratio of mussel weight to larva weight increases. None of the *H. brevistylus* were able to right themselves when observed. There was a significant difference between the average “righting” time for *D. transversa* larvae before after colonization. The larvae with mussels had a higher “righting” time than larvae without mussel attachment, supporting our hypothesis. Because the majority of our larvae were induced with zebra mussels, they had a higher ratio of mussel weight to larval weight than is found in naturally occurring populations in Douglas Lake from previous studies. This indicates that although a large load of zebra mussels may decrease fitness, the occurrence is unusual and may not have an effect on large populations in Lakes.

Introduction:

The zebra mussel, *Dreissena polymorpha* originates from Aralocaspian Basin in Eastern Europe, and was spread unintentionally in the ballast water of ships throughout Europe in the early 1800s (USGS 2006). They were similarly spread to the Great lakes in the mid-1980s when they reached North America. In the absence of natural predators, and the availability of unexplored niches in the Great Lakes, they have spread quickly, at the cost of native community structures (Nalepa 1994; USGS 2006). As an invasive species, they have adversely affected many of the native species directly by attachment to organisms, as is exhibited in snails as well as indirectly through competition (Nalepa 1994). *D. polymorpha* use their byssal threads to attach to substrates when they are larvae, juvenile mussels, or even as adults if they are removed from their original substrate (Lewandowski, K. 1982). During the pediveliger, the last larval stage, *D. polymorpha* will crawl with its foot or swim with its velum to a substrate where they become juvenile mussels. They may disperse again as juveniles either passively floating with the current, typically on a plant, or by crawling at night to find a more permanent substrate which they leave as adults only if removed by force as a result of stress. Chance and the limits of the substrate available primarily dictates final settlement of the mussels.

Colonization of Anisopteran Larvae by zebra mussels has been observed in Germany, near the native habitat of *D. polymorpha* (Weihauch and Borcharding 2002) as well as in areas of recent invasion such as Douglas Lake in the Great Lakes region (McCauley, Wehrly 2007). In the study done at Douglas Lake by McCauley and Wehrly (2007), colonization occurred as high as 63% of all Anisopteran larvae collected: *Didymops transversa*, and *Hagenius brevistylus*. Of the 30 *H. brevistylus* larvae collected, 77% carried at least one mussel. Fewer *D. transversa* were found with one mussel or more, 69% of the 13 larvae. These two species were most frequently colonized by the mussels in comparison the three other species which occur in Douglas Lake (Santiago and Fincke unpublished).

Hagenius brevistylus is from the family Gomphidae. It exhibits the sprawler lifestyle, resting on top of the substrate using its long extended legs for support (Corbet 1999) . The larval stage can take up to 4 years, and they will emerge from their exuviae in late June and early July in Michigan (UMMZ 1999). It has a larger surface area exposed because of this lifestyle, and this may allow it to be more vulnerable to colonization by *D. polymorpha*. *Didymops transversa* comes from the family Micromiidae and it adopts the burrowing lifestyle, digging under the

sediment with its legs to hide from predators and potential prey (Corbet 1999). This lifestyle may make it more difficult for *D. polymorpha* to attach to it. They emerge from their larval stage in late June in Michigan (UMMZ 1999).

The objective of this study was to record the frequency of zebra mussel colonization in late July at Douglas Lake, and to measure a fitness correlate of individuals with zebra mussels relative to the larvae without mussels. We hypothesize that the zebra mussel colonization will decrease larval fitness. We measured fitness indirectly, as a measurement of “righting” time of Anisoptera larvae with and without zebra mussels.

Materials and Methods:

The study site, Douglas Lake in Pellston Michigan, is a mesotrophic kettle lake formed in the Wisconsin Glaciation period that also formed the Great Lakes, and derives its water from Lake Huron. In 2002 *D. polymorpha* were first recorded and have spread since then (McCauley and Wehrly 2007). Fifty-seven Anisoptera exuviae were collected July 9, 2007 from the shore of Pine Point. Twenty-six *H. brevistylus* were collected and 21 *D. transversa*. The number of zebra mussels on the exuviae, their distance from shore, and whether or not the Anisoptera emerged was recorded.

Anisoptera larvae were collected July 24, 25, and August 1, 2007 using D-shaped nets. Six *D. transversa* were collected July 24 by the boat-well at UMBS. Ten *H. brevistylus* and 42 *D. transversa* were collected July 25 at East Point. One *H. brevistylus* was collected August 1 at the boat-well at UMBS. Larvae were not visible from the surface, and the net was used to scoop sandy substrate near vegetation and debris, preventing any bias due to visibility of larvae. The length and width of the beach was taken where larvae were sampled, to estimate density. The larvae were taken back to the boat-well at UMBS and placed individually in 10.2 cm diameter wax covered paper cups filled with sandy substratum and water from Douglas Lake. The water was changed every few days when it got low. The number and placement of any zebra mussels colonizing the larvae were recorded. To estimate larval instars, measurements were taken of length between eyes, overall body length, and overall abdomen width.

To observe feeding behavior, Chironomids larvae were collected from the main branch of the Maple River. Chironomids were placed in a Petri dish with uncolonized *D. transversa* for 30 minutes. Then two cups were filled with water and 6 Chironomids. Sand was placed in one cup to determine if it had any effect on feeding time. A *D. transversa* was placed in each cup

overnight from 22 hours to 8 hours the next day. The number of Chironomids were counted and the larvae were set aside.

To test fitness, “righting” tests were conducted on August 3, 5, 6, 7 and 8, 2007. Because *H. brevistylus* was not observed to be able to “right” itself with mussels, they were excluded from the tests. The *D. transversa* larvae were placed in a pan of water upside down with their backs to the bottom of the pan, and then let go. The time it took for them to flip back to their natural position was recorded in seconds and rounded to the nearest integer. If the larvae did not flip by 20 seconds, the number for flip time was recorded as 20 s. Three trials were run right after another per individual per day and then averaged, to take fatigue into account.

To control for individual variation in energy, each individual found without zebra mussels acted as its own control. On July 3, after recording “righting” time for larvae without mussels, all larvae were covered in zebra mussels overnight, to induce colonization. On July 5, the trials were repeated, and any larvae without zebra mussels were again covered in mussels. This procedure was repeated once a day for the next three days to see the long-term effect on larvae “righting” ability. Some larvae were uncolonized during all tests.

The zebra mussels were removed from each larva at the end of the 3 consecutive “righting” trials. The larvae and their mussels were weighed, before conducting 3 more consecutive “righting” trials, and then releasing the larvae back at East point.

SPSS version 14.0 for Windows was used to run statistical tests on the data. Three linear regressions were run. One compared the larval area to the number of zebra mussels, and another compared the average “righting” time and the ratio of zebra mussels weight to larval weight. The last regression was run on the number of zebra mussels per larvae and the average flip time. Two unpaired t-tests were run on the data. In the first test, larvae, which started out without zebra mussels but then became induced with zebra mussels were tested. Their “righting” time before induction was compared to after induction. In the second test, all larvae were used. In addition to the average “righting” time of larvae before induction, the averages of larvae that never were induced with zebra mussels were included in the first category. In addition to the average “righting” time of larvae after induction, the averages of larvae that had mussels throughout all trials were included in the second category.

Results

The area of the beach at Pine Point sampled July 25 was 222 m². One *H. Brevistylus* and one *D. transversa* were found with zebra mussels. The frequency of *D. transversa* on the beach was 0.19 larvae per m² and the frequency of *H. brevistylus* was 0.05 larvae per m². The frequency of *H. brevistylus* with mussels was 18% (N=11) and the frequency of *D. transversa* with mussels was found to be 2% (N=48). We found 11 *H. brevistylus* (mean length= 21.98) and 48 *D. transversa* (mean length= 21.72), (Figure 1).

During our experiments, we found that 9 larvae molted (Figure 2), entering the next instar. Larvae that molted were all in the smallest 60% of the population. We found the average eye-to-eye (head width) growth ratio to be about 1.18, slightly less than the 1.2-1.3 that Corbet (1999) reports for various Odonate species. Corbet also reports that this growth ratio changes throughout the lifetime of the larvae. To investigate this, we plotted % growth of width and length as a function of larval length, which we assume to be an effective estimation of age. We found that for our small sample size, % growth, or growth ratio, for width decreased as larvae aged. At the same time, we found that length growth ratio decreases much slower (Figure 3), meaning that length increases at a higher rate than width as they become older. This means that larvae should become longer and narrower as time goes on. As shown by Figure 3**, this is exactly what seems to be occurring in *D. transversa*, of which all but one of the moltings occurred. Molting data does not seem to ever have been recorded previously for *D. transversa*.

Of the exuviae found on the beach, only 28% of all larvae had zebra mussels. Of the *H. brevistylus*, 38% had one zebra mussel or more (N=26) and of the *D. transversa* 14% had one or more zebra mussel attached (Figure 4). Of all the exuviae, only one larva did not successfully emerge: a *H. brevistylus* with 5 zebra mussels attached.

From the feeding trials, 5 out of the 6 Chironomids were gone by 8 h the next morning in the cup without sand, and 3 out of the 6 Chironomids were gone in the cup with sand. The one Chironomid in the cup without sand was not moving when found in the morning, while the Chironimidae in the cup with sand were moving.

The regression comparing the larval area to the number of zebra mussels attached was not significant. As the ratio of zebra mussel weight to larval weight increased, so did “righting” time ($R^2=0.221$, $p=0.23$)(Figure 5). As the number of zebra mussels per larva increases, the average “righting” time increases ($R^2=0.972$, $p<<0.05$)(Figure 6). The average “righting” time per larvae is higher after zebra mussel induction ($F=52.87$, $N=26$, $p<<0.05$) (Figure 7). Overall,

larvae with zebra mussels have a lower average “right” time compared to larvae without mussel attachment ($F=57.55$, $N=34;28$, $p<<0.05$) (Figure 7). The average righting time due to starvation during the period of trials was not significant, as the average “righting” time for larvae without any mussels did not increase over a period of days.

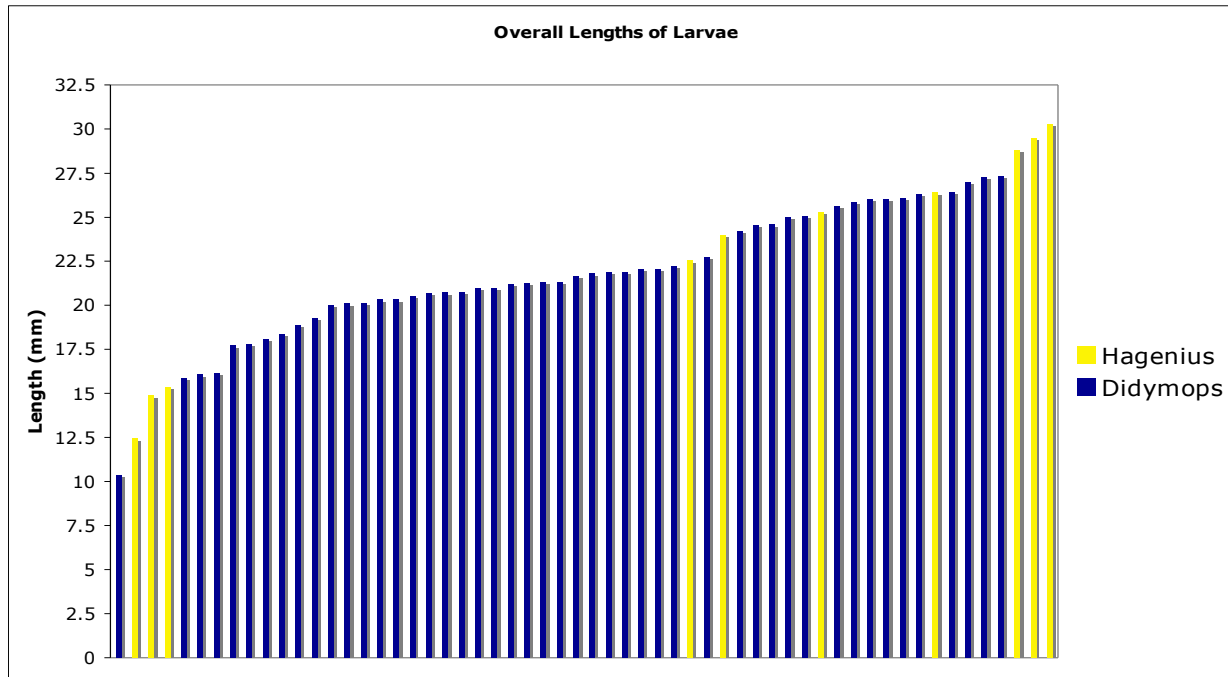


Figure 1. Hagenius were found mostly on the extremes of the size distribution.

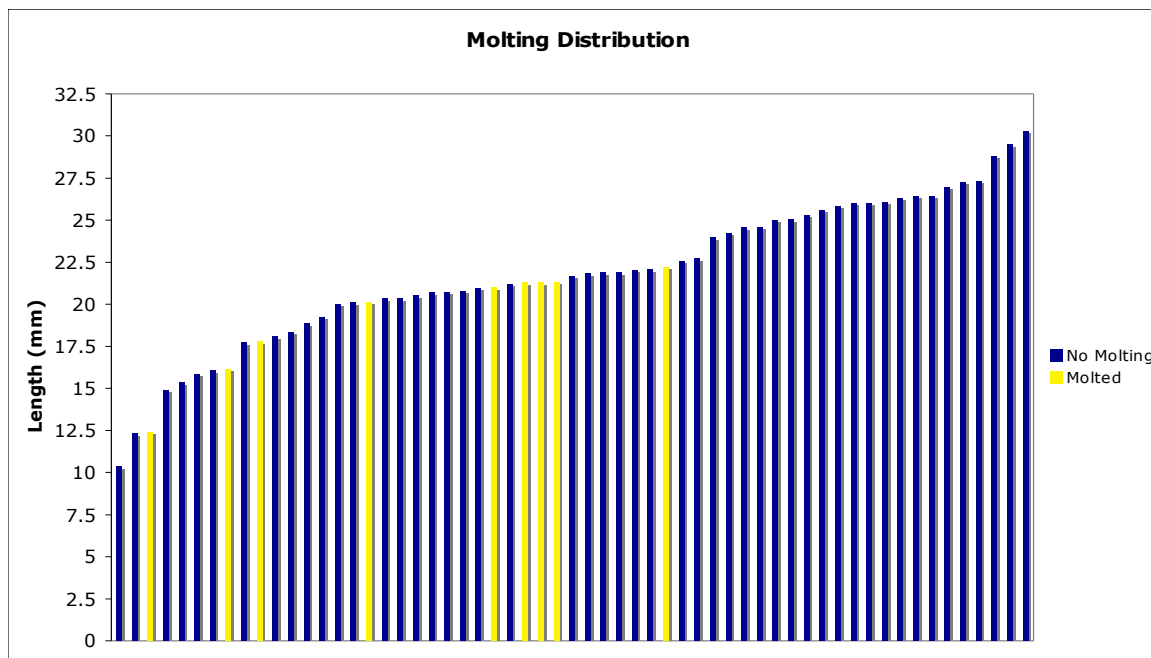


Figure 2. Molting distribution of *D. transversa*.

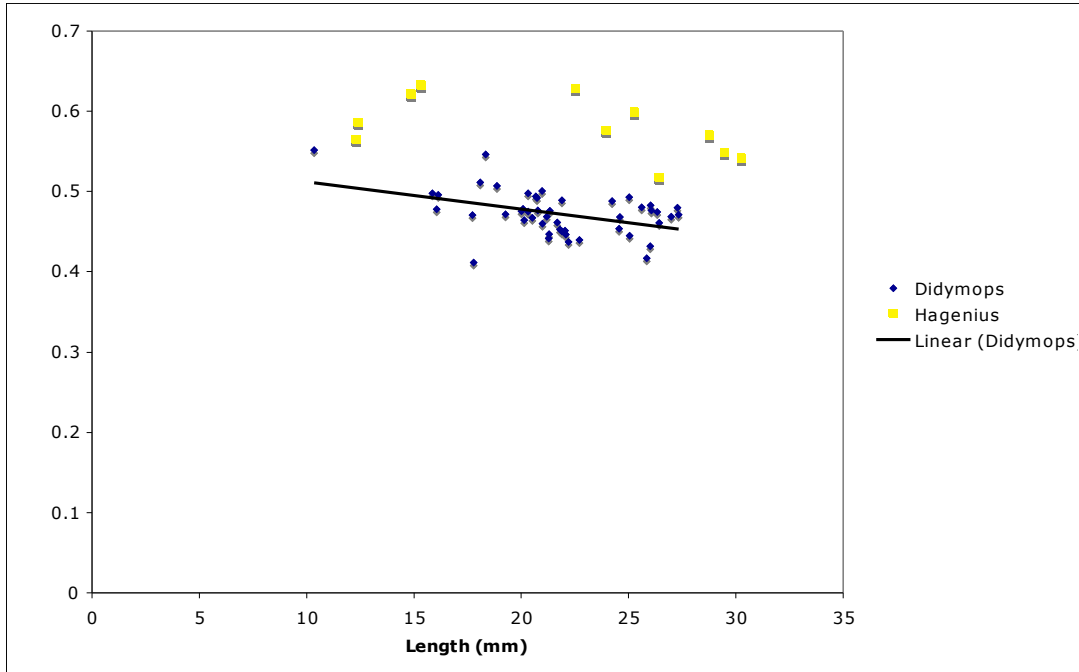


Figure 3. The ratio of width and length as a function of larval length, which we assume to be an effective estimation of age.

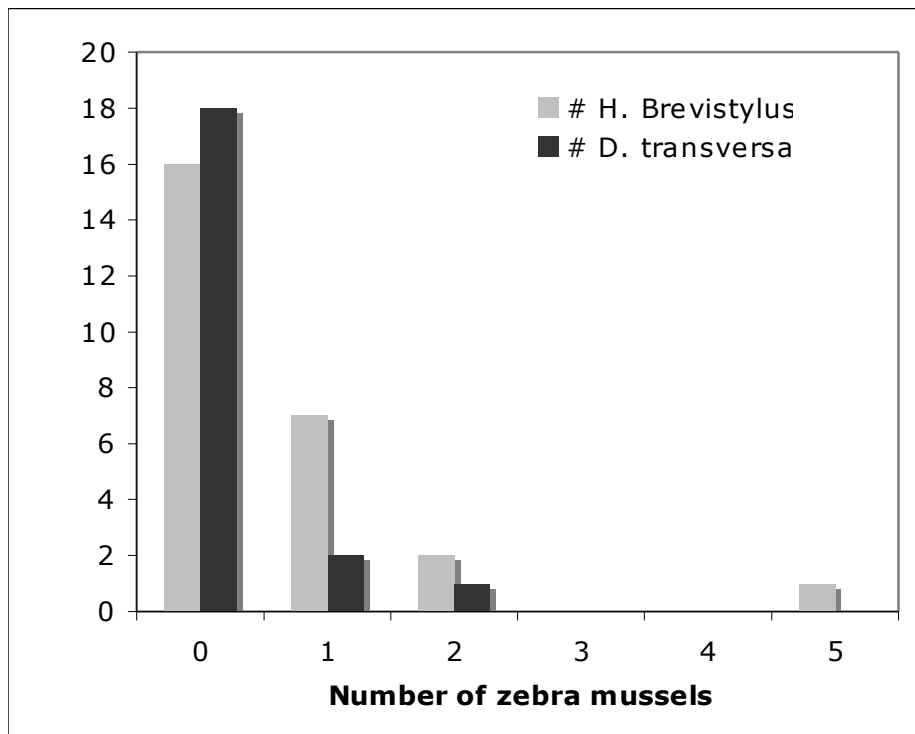


Figure 4. Exuviae were collected on the beach at Pine Point on July 9, 2007.

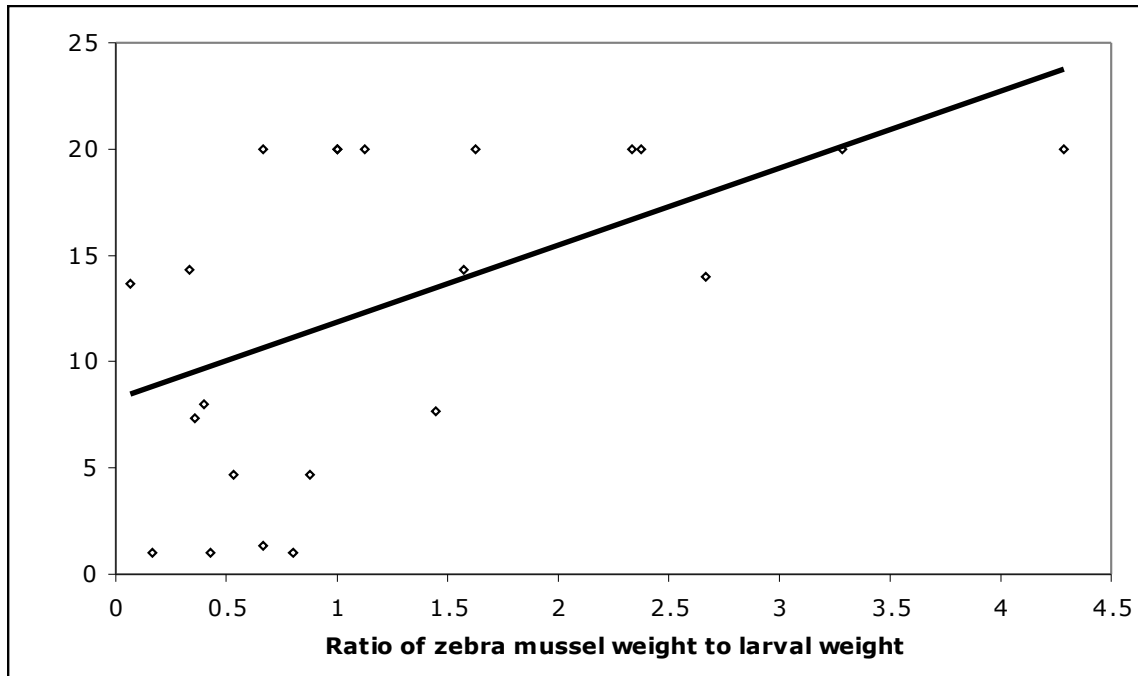


Figure 5. A regression with $r^2=0.221$ and the line $y=3.63x+8.22$. The average ratio of zebra mussel weight to larval weight was 1.25 s.

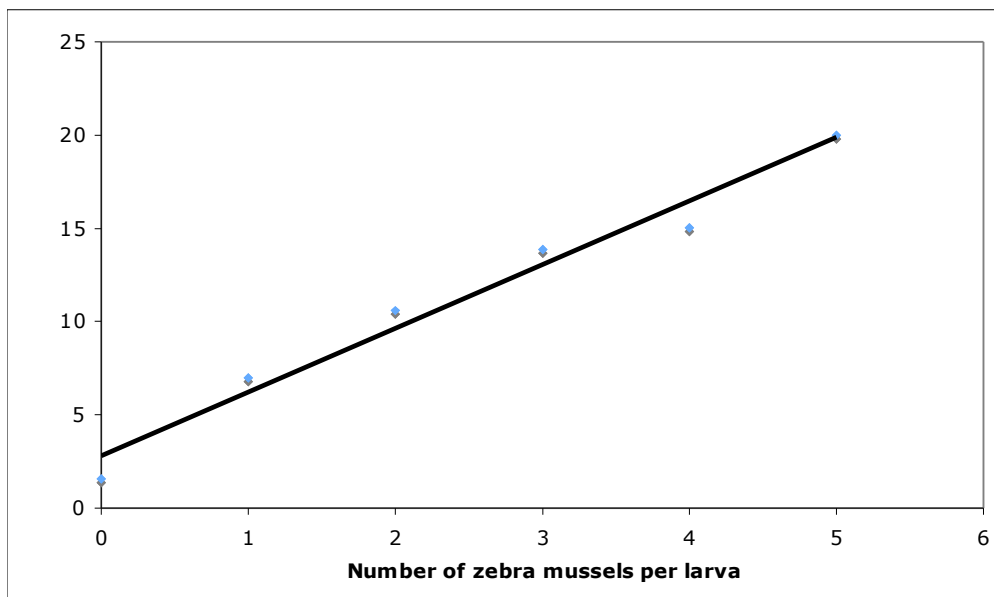


Figure 6. Mean time to "right" as a function of the number of zebra mussels. This includes data for up to only 5 mussels. As the number of zebra mussels increased, the time it took to "right" it self increased. The $r^2=0.973$ and the equation for the line is $y=3.42x+2.80$.

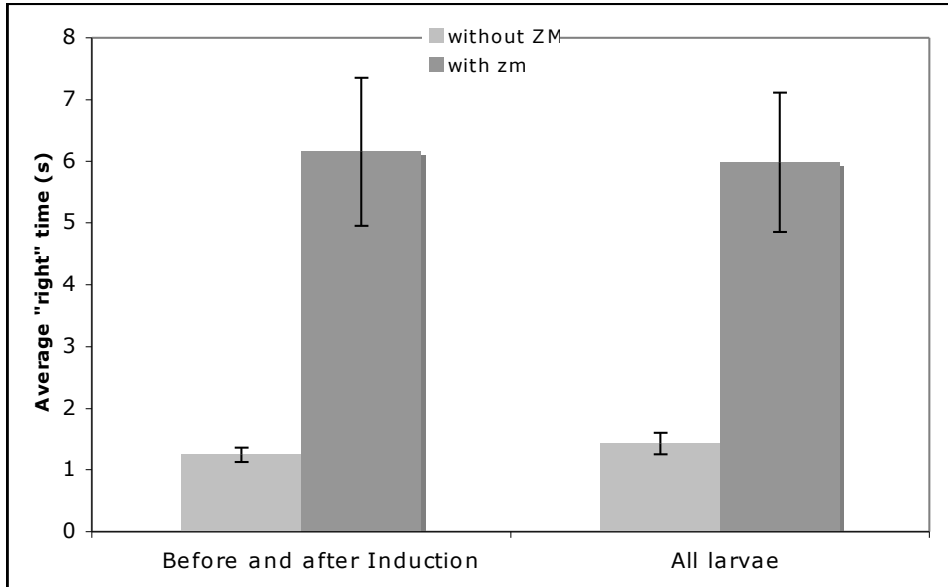


Figure 7. Independent *t*-tests show significant differences between larvae with and without mussel attachment. The group on the left includes the average time for larvae found without mussels attached before and after they were induced with zebra mussels. The group on the right compares the average righting time of larvae without mussels (both averages for larvae which never were induced with mussels, and averages for larvae before they were induced) to larvae induced with mussels (both averages for larvae found with mussels and averages for larvae after induction with mussels).

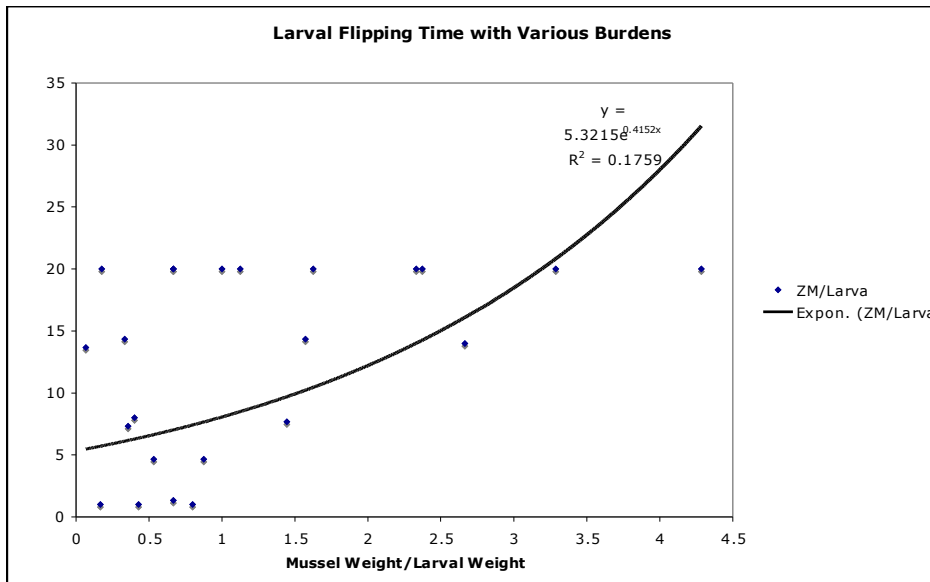


Figure 8. Exponential model for average righting times.

Discussion

Our results indicate that a large load of *D. polymorpha* may decrease fitness of *D. transversa* instars prior to emergence indirectly by increasing amount of energy required to move (Figure 7). This energy cost increases as the number of mussels per larva increases, and the burden relative to the size of the larvae increases (Figure 5 and 6). *H. brevistylus* was not observed to be able to “right” itself, even if given several hours. It was often found in the morning upside down, even though it was observed to be right side up the night before, showing that this poses a realistic problem for the species. The increased time to move as result of mussel attachment may make the larvae more vulnerable to predators, which is the prominent cause of death in the larval stages (Corbet 1999). They may become more susceptible to internal parasites such as Trematoda as their decreased energy may weaken their immune system. Decreased foraging efficiency as a result of increased energy costs and time spent may contribute to a decrease of fitness, but not as greatly as the decrease of fitness due to predation because the larvae are sit and wait predators, and use a prehensile labium to capture prey. Further tests could explore the effects of colonization on Anisoptera larvae feeding time and escape from predation.

Another vulnerable stage in the life cycle of Anisoptera is emergence from larvae to adult forms. Our study only focused on how the mussels may have prevented emergence, and our results may have not shown high mortality (1 larvae dead out of 13 with mussel attachment) because predation was not taken into account. During emergence, avian predators can consume up to 50% of the group emerging in a day (Corbet 1999). Low energy may increase the time the larvae spend crawling from shore to a refuge, and thus increasing exposure to potential predators. This mortality may not be visible from the collection of exuviae on the beach and may require other means of collecting data, such as calculating predation rates of larvae with and without zebra mussel attachment based on visual observations.

In terms of what we found in the field, our findings were in sharp contrast to what McCauley and Wehrly (2007) reported finding in May 2005. With relatively equal sample sizes (Figure 1), we found few larvae with mussel attachment, compared with this previous data. Not only did we find only small number with zebra mussels attached, the ones we did find had only 1 and 2 mussels attached, which is mildly surprising considering that McCauley and Wehrly (2007) discovered larvae with up to 8 mussels. The fact that we sampled roughly two months

later than the previous study should offer a reasonable explanation for the discrepancy; mature larvae emerge to become dragonflies somewhere during this two-month period of June and July. If our eye-to-eye measurement is the same as McCauley's head width measurement, which I believe to be true considering the lateral placement of the eyes on the larvae, then our larvae were, on average, much younger. We measured the average eye-to-eye length to be 6.02 mm, compared to McCauley and Werhly's 7.83 mm (2007); we assume smaller means younger. If our larvae were younger, because the oldest larvae had just finished emerging, then perhaps our samples showed a lower frequency of attachment because they had been exposed to zebra mussels for a shorter length of time.

In our study, there were larvae with 5, 6, 7 or even 8 *D. polymorpha* attached at a time, which resulted in a high ratio of zebra mussel weight to larval weight (Figure 5). This was a result from single larva being exposed to a large number of mussels per volume, and such high attachment numbers do not usually occur in nature. In the study done by McCauley and Werhly (2007), the mean percent body mass carried by infested individuals was $66 \pm 12\%$ for *D. transversa* while our mean percent body mass per infested individual was 125% for *D. transversa*. In our study there was a decrease in "righting" time, which may decrease fitness, but this may not be the case in naturally occurring *D. transversa* populations. The mussels may decrease the fitness of some individuals, but not have a large effect on the populations of *D. transversa* as a whole.

Even though we still achieved statistically significant results, our choice to limit the time given to larvae to right themselves to only 20 seconds proved to be a poor one. As best exemplified by Figure 6 limiting this time to 20 seconds prevented a smooth continuity in our results. We believe that the accuracy of our regression lines was significantly skewed by these faulty data points and averages taken for flip times were not entirely accurate when including 20 second righting times. It is entirely possible that some larvae would have righted themselves shortly after the 20-second limit given to them, meaning inaccurate data. However, it was observed that in many cases, the larvae simply were not capable of righting themselves when they expressed high amounts of attachment. At some point we had to conclude that a given larva was not going to right itself, 20 seconds was probably just a little too short. Given this problem, we feel strongly that a longer time limit would have actually produced even stronger results suggesting attachment hinders mobility. If some of the data points for individuals with large

burdens were increased to longer times, which likely would have been the case given the general trend, the regression line would have actually been steeper, or even exponential, as proposed in Figure 8. Thus, while our results are somewhat biased, they are most likely overly conservative, as opposed to the possibility that the data are overestimating the effects of mussel attachment.

A heavy load of *D. polymorpha* on *D. transversa* and *H. brevistylus* may result in decreased fitness resulting from high energy costs, and potentially longer exposure to predation. This may not have a large effect on naturally occurring populations, as the larvae in our study were artificially induced with *D. polymorpha* in numbers not usually found in Douglas Lake. Further tests could look at trends in population densities and emergence predation of the two Anisopteran species to get a better idea of the extent of zebra mussel colonization. Studying the effects of zebra mussels on Anisoptera larvae can lead to a better understanding of ecological impacts in aquatic as well as terrestrial systems, as the Anisoptera move from one to the other. As terrestrial predators, they prey upon insect pests of humans, such as mosquitoes. Dragonfly larvae are usually the top invertebrate predators in the shallow littoral zone of lakes, and may even be the top predators in the absence of insectivorous fish (Corbet 1999). Because many aquatic systems are controlled top-down, a change in the dragonfly larvae population size will affect all the trophic levels below them, which can alter lake dynamics (Dodds 2002).

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