Appendix W
Laboratory-Based Attachment Study for Colonization Potential of Dry Cargo Residue in the Great Lakes by Zebra Mussels (*Dreissena polymorpha*) and Quagga Mussels (*Dreissena bugensis*): Phase IV—Postveliger Colonization

Laboratory Based Attachment Study for Colonization Potential of Dry Cargo Residue in the Great Lakes by Zebra Mussels (Dreissena polymorpha) and Quagga Mussels (Dreissena bugensis): Phase IV: Post-Veliger Colonization

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Introduction

Concern over dry cargo residue (DCR) as potential substrates for the colonization of the invasive species *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) within the Great Lakes has prompted an investigation into their attachment onto DCR. Invasion of the Great Lakes by dreissenids has caused both environmental and economic concerns and provided additional habitat for their proliferation that may increase their expansion into other areas of the lakes.

Attachment of adult zebra and quagga mussels to hard substrates is well documented in the literature and in previous studies conducted by the authors (Phase I through III of the Attachment Studies). Mellina and Rasmussen (1994) indicted that zebra mussels were found on hard substrates, particularly rocky surfaces, but were usually absent on softer substrates. Quagga mussels on the other hand appear to be able to colonize both hard and soft substrates. The bottom of Lake Erie has extensive colonies of quagga mussels on soft sediment (Dermott and Munawar 1993; Dermott and Keroc 1997; Roe and MacIsaac 1997).

The attachment of zebra and quagga mussels to hard substrates is a process that occurs with the adult which can be actively mobile and by dressenid post-veliger larvae searching for their initial home for attachment.

As the adults reproduce their early life history unfolds with the zygote, trochophore, D-larva, veliger, and post-veliger stages. The veligers are photopositive, active swimmers by use of the ciliated velum (derived from the prototroch of the trocophore larva). After about 10-15 days, the veligers metamorphose to the first post-veliger stage, the pediveliger, it becomes photonegative and settles to the benthic zone in search of a suitable substrate for attachment. Under normal conditions, 99%+ of veligers do not reach a successful attachment stage. The pediveliger has both a velum and a ciliated foot and both are used in substrate exploration. The pediveliger is the critical life stage that explores available substrates in search of optimal attachment conditions (i.e., hard substrate, current, chemistry, etc.). The pediveliger will swim a few millimeters off the substrate, then will hover stationary over the

substrate using its velum before settling and then using its foot to investigate the substrate. If it is a hard substrate the pediveliger may stay. If the substrate is soft sediment (and the pediveliger is within about a week's time window for substrate selection) it will rise off the substrate and continue to search for optimal conditions. If optimal (or near optimal) conditions are not found within about a week's time, then the pediveliger will attempt colonization of marginal attachment conditions (i.e., soft substrate, etc). Once development proceeds to the next post-veliger stage, the plantigrade, it loses its velum and thus it's swimming capability. It is the pediveliger that is the primary life stage involved in substrate selection. The plantigrade is subordinate to substrate selection, although it is active in 'fine tuning' its initial attachment location.

This study investigated pediveliger selection and plantigrade attachment success on cargo sweepings (limestone, taconite, and coal) and on native soft sediment. Two experimental designs were used: 1) bulk settlement and attachment success on clean DCR material and native sediment and 2) bulk settlement and attachment on DRC material covered with 1 mm and 3 mm's of native sediment. The study attempted to answer the following questions:

- Will post-veligers attach to uncovered DCR?, and
- Will post-veligers attach to DCR covered with 1mm and 3 mm of native sediment?

Phase IVa: Post-Veliger 14-Day Colonization Study: Bulk Settlement and Attachment on DCR Material and Native Sediment

Methodology and Results

The bulk settlement study used five replicate 700 ml chambers (12 x12 cm) for each DCR material (limestone, taconite, and coal) and a native sediment control. Post-veliger larvae were collected from the Milwaukee Lake Michigan inner harbor located at the Great Lakes WATER Institute, The University of Wisconsin-Milwaukee. A distinction was not made between zebra versus quagga veligers, however it is known that the distribution of adults, where the veligers were collected, was approximately 50/50%. Samples were concentrated as needed based on harbor *in situ* abundance densities. The density of post-veliger larvae used for the bulk settlement and attachment studies was approximately 665.5 larvae/chamber. 700 ml of raw harbor water was used in each chamber, to a depth of 6.5 cm, to cover the DCR. The post-veliger larvae were allowed to settle for a total period of 14 days. Periodic observations were made about every four days to gauge settling, and general success or failure of the experiment.

The post-veliger 14 day colonization study was conducted from June 30 through July 14, 2008. The results are listed in Table 1. The results indicate that post-veligers (pedivelgers, plantigrade veligers) will attach to DCR. An analysis of the results indicated a clear preference for the DCR material as opposed to the native sediment (Tables 2 and 3). The analysis shows that the percent attachment for each DCR type was significantly greater than the percent attachment on native sediment. The analysis also shows that the percent attachment for limestone was significantly greater than the percent attachment to coal, but not for taconite. The percent attachment between taconite and coal was not significantly

different. The data suggests a potential attachment preference of the sequence limestone>taconite>coal>native sediment (Figure 1).

TABLE 1
Results of bulk settlement and attachment by Post-Veligers on DCR material

Sample	Limestone		Taconite		Coal		Sediment*	
	No. Attached	%	No. Attached	%	No. Attached	%	No. Attached	%
1	42	6.3	49	7.4	18	3.5	0	0.0
2	51	7.7	18	2.7	2	0.3	0	0.0
3	47	7.1	31	4.7	10	1.5	0	0.0
4	34	5.1	5	0.8	11	1.7	1	0.2
5	29	4.4	26	3.9	23	3.5	0	0.0
Average	**40.6**	**6.1**	**25.8**	**3.9**	**12.8**	**2.1**	**0.2**	**0.03**

* **10 mm sediment depth**

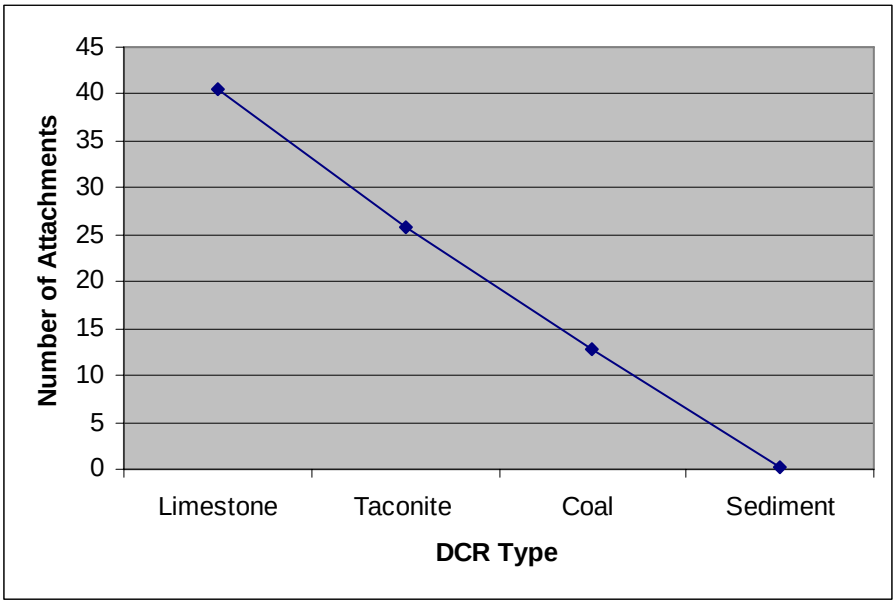
TABLE 2
Results of ANOVA Test on the Bulk Settlement and Attachment Data (log x+1 transformed data)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments (between columns)	3	6.860	2.287
Residuals (within columns)	16	1.086	0.06786
Total	**19**	**7.946**	
F = 33.699 = (MStreatment/MSresidual)			

TABLE 3
Results of Tukey-Kramer Multiple Comparison Test on the bulk settlement and attachment data. If the value of q is greater than 4.046 then the P value is less than 0.05

Comparison	Mean Difference	q	P value
Limestone vs Taconite	0.2719	2.334	ns p>0.05
Limestone vs Coal	0.5590	4.799	* p<0.05
Limestone vs Sediment	1.550	13.307	*** p<0.001
Taconite vs Coal	0.2871	2.465	ns
Taconite vs Sediment	1.278	10.973	*** p<0.001
Coal vs Sediment	0.9911	8.508	*** p<0.001

FIGURE 1
Post-veliger attachment preference based on the above data



Phase IVb: Post-Veliger 14-Day Colonization Study: Sediment Penetration onto DCR Material

The sediment penetration study used five replicate 700 ml chambers, 12x12cm, for each DCR material (limestone, taconite, and coal) and a native sediment control. Two test conditions were assessed, one with 1 mm depth of native sediment covering the DCR material , and one with 3 mm. Post-Veliger larvae were again collected from the Milwaukee Lake Michigan inner harbor located at the Great Lakes WATER Institute, The University of Wisconsin-Milwaukee. Samples were concentrated as needed based on harbor *in situ* abundance densities. The density of post-veligers for the sediment penetration studies was approximately 630.7 larvae/chamber. 700 ml of harbor water was used in each chamber, at a depth of 6.5 cm, to cover the DCR. The post-veligers were allowed to settle for a total period of 14 days. Observations were again made about every four days to gauge settling, and general success or failure of the experiment.

The results are presented in Tables 4 and 5. The data indicate that a minimum 1mm cover of DCR material by native sediment will curtail attachment by post-veligers to DCR. An analysis of the data indicated no significant difference (critical p-value of 0.05) in attachments between each type of DCR material and native sediment (Tables 6 and 7).

TABLE 4
Results of sediment penetration study by post-veligers on DCR material. Overlying sediment Depth: 1mm

Sample	Limestone		Taconite		Coal		Sediment*	
	No. Attached	%	No. Attached	%	No. Attached	%	No. Attached	%
1	0	0.0	1	0.2	2	0.3	0	0.0
2	2	0.3	0	0.0	0	0.0	0	0.0
3	1	0.2	1	0.2	0	0.0	0	0.0
4	0	0.0	0	0.0	1	0.2	0	0.0
5	0	0.0	0	0.0	0	0.0	0	0.0
Average	**0.6**	**0.1**	**0.4**	**0.1**	**0.6**	**0.1**	**0**	**0.0**

TABLE 5
Results of sediment penetration study by post-veligers on DCR material. Overlying sediment depth: 3mm

Sample	Limestone		Taconite		Coal		Sediment*	
	No. Attached	%	No. Attached	%	No. Attached	%	No. Attached	%
1	0	0.0	1	0.2	2	0.3	0	0.0
2	0	0.0	0	0.0	0	0.0	0	0.0
3	0	0.0	1	0.2	0	0.0	0	0.0
4	1	0.2	0	0.0	1	0.2	0	0.0
5	0	0.0	0	0.0	0	0.0	0	0.0
Average	**0.2**	**0.04**	**0.4**	**0.08**	**0.6**	**0.1**	**0**	**0.0**

TABLE 6
Results of ANOVA test on the sediment penetration, 1 mm depth (log x+1 transformed data)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments (between columns)	3	0.08178	0.02726
Residuals (within columns)	16	0.5031	0.03144
Total	**19**	**0.5848**	
F = 0.8670 = (MStreatment/MSresidual)			

TABLE 7
Results of ANOVA test on the sediment penetration, 3 mm depth (log x+1 transformed data)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments (between columns)	3	0.01359	0.004531
Residuals (within columns)	16	0.2175	0.01359
Total	**19**	**0.2311**	
F = 0.3333 = (MStreatment/MSresidual)			

Conclusions

The phase IV study was developed to answer the following questions:

- Will post-veligers attach to uncovered DCR?, and
- Will post-veligers attach to DCR covered with 1 and 3 mm of native sediment?

Based on the results it appears that the post-veligers will attach to DCR material . In addition it appears that at least in this experiment there was a distinct attachment preference by post-veligers for limestone, followed by taconite and coal. The second part of the study indicated that by covering the DCR with a layer of native sediment to a depth of 1mm or more will curtain post-veliger attachment onto DCR. The addition of these data to what has been developed by the other experiments further supports the premise that Zebra Mussels (*Dreissena polymorpha*) and Quagga Mussels (*Dreissena bugensis*) will attached to exposed DCR including the post-veliger stages. In addition, if the mussels are covered with native sediment, attachment to DCR will be curtailed depending on the depth to which the material is buried. In the case of post-veligers, as little as 1 mm of sediment appears to prevent almost all attachment. It should be kept in mind that given the high reproductive capacity of adult female dressinids (500,000 veligers/mussel), even a 0.2% attachment rate would equate to 1000 attachments.

References

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