

Kari, I thought this summary may be of some interest to you. Comments or questions... just call. Take care, Cliff. Starliper 1996

March 8, 1996.

Summary of a preliminary study to evaluate a protocol to isolate bacteria from freshwater mussels.

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INTRODUCTION

During October, 1995 a study was developed to evaluate the potential for mutual bacterial pathogen transfer between freshwater mussels and salmonid fishes. The purpose for development of this plan was to lend support to the growing effort to save native freshwater mussels in the Ohio River from an inevitable onslaught of the zebra mussel. Salmonid rearing facilities are playing an important role in this effort by maintaining and rearing native mussels until an appropriate time that they may be used to restock the Ohio River. The idea for this study is a result of a lack of information about the bacteriology of freshwater mussels and the lack of information concerning the potential of pathogen transfer between mussels and salmonids given the fact that they will be reared on the same facility.

This study is to begin during the sampling season of 1996. However, because of our lack of experience of working with these animals and the relatively warm weather in November 1995, we felt a pilot study with a few animals would be a tremendous benefit. The summary provided here summarizes the results of this pilot study.

PROCEDURE

Patti Morrison of the Ohio River Islands NWR coordinated collection of the animals. On November 2, 1996 120 animals were transported to the Leetown facility. Six species were represented: Amblema plicata, Quadrula metanerva, Q. pestulosa, Q. quadrula, Pleurobema cordatum, and Obliquaria reflexa. They were hauled in a fish transport tank in Ohio River water and kept in this water overnight with aeration and a bubbling of oxygen. The next day, while they were in their native water, 40 animals were sacrificed and bacteriological isolation done. The remaining animals were quarantined by placing them in six aquaria (one species each) supplied with Leetown reservoir water. After ten days, another 15 animals representing all species were sacrificed for bacterial analysis. Another 15 animals were sacrificed at 30 days post collection.

BACTERIAL ISOLATION

The attached tables summarize the data we collected from the sampling. Length, width and weight was measured for each mussel. The mussels were opened and tissues excised. Effort was made to collect the tissues aseptically, however, we felt that the difficulty getting the shells open would provide much opportunity for contamination. Two tissue samples were collected, 1) gut and 2) all remaining tissues. Each tissue was weighed, diluted 1:2 with PBS, homogenized in a stomacher and diluted ten-fold through the

1:2000 dilution. Dilutions of each tissue were drop plated onto blood agar and cytophaga agar. Plates were incubated at room temperature and evaluated after 48 hr. Total bacterial counts were quantified. Also, individual colony types were quantified and isolated for identification.

SIGNIFICANT ACCOMPLISHMENTS OF THE PILOT STUDY

* we had hoped only to become familiar with working with mussels and iron out any experimental design bugs, but to our surprise the study turned out better than expected and we feel we already have some useful data

* dealing with these animals was an educational experience in that the anatomy was tremendously different from what we are familiar with in fish

* there was very little lost data due to fungal contamination and the similarity of the total counts within each sample was an indication that our tissue collection techniques were appropriate

* total bacterial counts decreased significantly at sometime after the mussels were placed in Leetown water, especially the H₂S producing bacteria *

* the tissue dilution series selected for use was appropriate

* we were able to maintain the animals, with no mortality, for the duration of the study

* motile Aeromonads were the predominate bacteria isolated. It was determined that the battery of tests routinely used to identify motile aeromonads from fish did not suffice for those from mussels. A new battery was developed and utilized that performed great. Assuming we isolate numerous motile aeromonad during the 1996 sampling season, having known this ahead of time will prevent a lot of frustration and lost time

* preliminary results show the predominate bacteria to be motile aeromonads and nonfermenters, generally not significant fish pathogens but, opportunists *

* if we can assume to expect a similar bacterial population during 1996, we have a good idea of what ~~the~~ bacteria to expect and can plan accordingly

STUDIES ARE CONTINUING

Due to the furlough and facility closure, the chance for a more specific bacterial identification was lost. Attempts were made to keep the bacteria going in the basement of one researcher, but after nearly a month this was a lost cause. The isolates from the first sampling were placed in stock culture agar and refrigerated prior to the furlough. Attempts will be made to recover as many as possible. Those that are recovered will have more complete biochemical studies. Also, motile aeromonads from mussels and fish

will be compared biochemically and for their virulence to fish. The remaining mussels will be subjected to bacterial challenges to determine if they are susceptible to an experimental challenge.

Data collected from samples of 40 mussels from their native Ohio River water on November 3, 1995.

Species	Number	Mean length/width		Mean weight	Mean gut tissue weight	Mean weight other tissues
<u>A. plicata</u>	22	102.3	76.2	240.6	11.44	18
<u>Q. metanerva</u>	4	75.5	63.8	138.2	7.13	8.13
<u>P. cordatum</u>	2	75	66.5	130.7	7.85	7.82
<u>Q. pestulosa</u>	4	54	51.3	72.4	2.86	4.16
<u>Q. quadrula</u>	4	80	65	145.7	5	7.2
<u>O. reflexa</u>	4	55	47.3	69.1	3.3	4.7

Data collected from samples of 15 mussels from Leetown water on November 13, 1995.

Species	Number	Mean length/width		Mean weight	Mean gut tissue weight	Mean weight other tissues
<i>A. plicata</i>	3	114	83.3	339.8	21.9	21
<i>Q. metanerva</i>	3	78	59	151.7	8.02	9.5
<i>Q. pestulosa</i>	3	58.7	58.3	114.6	4.52	7.64
<i>Q. quadrula</i>	3	72.3	59	131.8	4.3	6.5
<i>Q. reflexa</i>	3	43	34	32.9	.94	2.35

Data collected from samples of 15 mussels from Leetown water on December 4, 1995.

Species	Number	Mean length/width		Mean weight	Mean gut tissue weight	Mean weight other tissues
<u>A. plicata</u>	3	121	80	468	23.7	28.8
<u>Q. metanerva</u>	3	70	58	127	7.14	7.41
<u>Q. pestulosa</u>	3	48	47.3	59.6	2.95	3.97
<u>Q. quadrula</u>	3	75	60	151.2	3.84	6.82
<u>Q. reflexa</u>	3	44.7	34.3	37.92	1.12	2.74

Mean total bacterial counts (cfu/g tissue) for the November 3, 1995 sampling. BAP = blood agar plates; cyto = cytophaga agar plates.

Species	Gut		Other tissues	
	BAP	Cyto	BAP	Cyto
<i>A. plicata</i>	6.35e5	3.8e5	1.68e5	1.42e5
<i>Q. metanerva</i>	1.04e5	1.02e5	6.02e4	1.35e5
<i>P. cordatum</i>	6e5	3.2e5	1.23e5	1.96e5
<i>Q. pestulosa</i>	5.6e4	7.6e4	2.22e5	2.5e5
<i>Q. quadrula</i>	7.24e5	5.1e5	3.4e4	4e4
<i>Q. reflexa</i>	1.16e4	1.18e4	1.72e5	3.3e5

Mean total bacterial counts (cfu/g tissue) for the November 13, 1995 sampling. BAP = blood agar plates; cyto = cytophaga agar plates.

Species	Gut		Other tissues	
	BAP	Cyto	BAP	Cyto
<i>A. plicata</i>	6.99e4	4.5e5	1.41e4	6.4e4
<i>Q. metanerva</i>	4.53e3	6.13e3	2.08e4	3.79e4
<i>Q. pestulosa</i>	3.31e3	2.6e4	1.71e4	1.6e5
<i>Q. guandrula</i>	1.41e5	1.39e5	5.73e5	2.11e6
<i>Q. reflexa</i>	8.4e2	2e3	5.6e3	9.6e3

Mean total bacterial counts (cfu/g tissue) for the December 4, 1995 sampling. BAP = blood agar plates; cyto = cytophaga agar plates.

Species	Gut		Other tissues	
	BAP	Cyto	BAP	Cyto
<i>A. plicata</i>	1.68e3	2.48e3	7.73e3	9.87e3
<i>Q. metanerva</i>	6.8e3	1.17e4	1.04e4	1.44e3
<i>Q. pestulosa</i>	4.4e3	1.32e4	8.8e3	1.45e5
<i>Q. quadrula</i>	5.06e4	8.27e4	1.52e4	2.19e4
<i>Q. reflexa</i>	2.4e3	1.07e4	4.11e3	4.8e3

Presumptive identification of bacterial colony types isolated from freshwater mussels. Values are percent of total colony types.

November 3, 1995 sampling

Tissue and media	Motile aeromonads	Non-fermenters	Others
Gut BAP	45	45	10.6
Gut Cyto	55	41	3.4
Other tissues BAP	20.2	68.1	11.6
Other tissues Cyto	26.1	39.1	34.8

November 13, 1995 sampling

Tissue and media	Motile aeromonads	Non-fermenters	Others
Gut BAP	41.4	27.6	31
Gut Cyto	30.8	42.3	26.9
Other tissues BAP	8.8	64.7	26.5
Other tissues Cyto	23.1	57.7	19.2

December 4, 1995 sampling

Tissue and media	Fermenters	Non-fermenters
Gut BAP	68.8	31.3
Gut Cyto	71.4	28.6
Other tissues BAP	59.4	40.6
Other tissues Cyto	66.7	33.3