TECHNICAL REPORT

Report Title: Assessing the Impact of Continuous Bacterial Treatments Over a Two-Week Period on Zebra Mussel Mortality

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* This "quarterly" report covers a nine-month period, and it is the first report to be submitted for this project. Although this project had an official start date of 1 October 2000, research was only initiated in spring 2001.

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ABSTRACT:

These experiments indicated that in waters at 23°C the window of opportunity to kill zebra mussels with bacterial strain CL0145A is limited to the first two days of treatment. Treatments beyond two days will not increase mortality.

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EXECUTIVE SUMMARY:

Subtask 2.3 of the Statement of Work (SOW) requires that the Contractor shall determine optimal exposure time in relation to the concentration of bacterial strain CL0145A. To address this subtask, 2 experiments were conducted to assess the impact of continuous bacterial treatment on zebra mussel mortality over a two-week period. In these experiments, a concentration of bacteria (i.e., 25 ppm) was used that preliminary trials (author, unpublished data) indicated would cause ca. 50% mortality when mussels were treated for 2 days. In the experiments presented herein, however, mussels were treated continuously with bacterial strain CL0145A of *Pseudomonas fluorescens* for 2 weeks. These experiments indicated that in waters at 23°C the window of opportunity to kill zebra mussels with bacterial strain CL0145A was during the first two days of treatment. Treatments beyond 2 days did not raise mortality. Thus, additional experiments must more thoroughly examine this initial 48 hr period of treatment to better define this window. Future tests, for example, will be run to assess the impact of treatment durations varying from 3 hr to 48 hr. These tests will define the maximum effective treatment duration, and then this duration will be used to treat pipes at bacterial concentrations sufficient to achieve high mussel kill (>95% mortality).

Subtask 2.1 of the SOW requires that the Contractor shall continue to confirm prior test results, which have indicated that mussels in the size range ca. 1-30 mm appear equally susceptible to treatment. Data in these present tests provided such evidence since mussels averaging 9.4 mm and 15.0 mm had the same mean mortality.

EXPERIMENTAL MATERIALS AND METHODS:**Production of Bacteria - Culturing of Strain CL0145A:**

Under static conditions, 10-ml tubes containing 6 ml of tryptic soy broth (TSB) (Difco) were each inoculated with 0.1 ml stock frozen bacterial culture. The tubes were then incubated at 26°C under static conditions for ca. 42 hr. Tubes were subsequently used to inoculate 250-ml flasks (1 tube per flask) containing 100-ml TSB and incubated at 26°C under static conditions for ca. 72 hr.

Obtaining the Cell Fraction (CF):

Final whole cultures were centrifuged (30 min at 827 x g) in 50-ml batches, supernatants were discarded, and cell pellets were resuspended in dilution water (80 ppm KH_2PO_4 , 405.5 ppm $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in deionized water). The optical density of the CF inoculum was determined by taking one absorbance reading (spectrophotometer, $\lambda = 660 \text{ nm}$) from each of 3 separate samples of the CF. Based on an absorbance equation developed during previous trials (author, unpublished data), the optical density of the CF was then used to calculate the volume of CF inoculum required to treat the mussels at the 25 ppm concentration. In order to treat at 25 ppm each day, equal portions of CF were placed into 50-ml centrifuge tubes which were stored on dry ice (-78°C). The amount of CF in each tube was the quantity needed to obtain the 25-ppm concentration when diluted in 3.5 L of Mohawk River water.

Treatment of Zebra Mussels with Bacterial Cells:

Zebra mussels from the Mohawk River were used in the tests, and mean mussel length ($\pm\text{SD}$) used in Experiment #1 and #2 were, respectively, $9.4 \pm 1.2 \text{ mm}$ and $15.0 \pm 1.5 \text{ mm}$. At the beginning of each experiment, 500 ml of Mohawk River water (23°C) containing strain CL0145A at a 25 ppm concentration were placed into a glass jar holding 25 mussels (6 replicate jars). Water within jars was aerated throughout the 16-day (Experiment #1) and 14-day (Experiment #2) treatment periods. At 24 hr intervals mussel mortality was scored, dead mussels removed, and fresh Mohawk River water (23°C) containing bacteria at a 25 ppm concentration again placed into each glass jar. Control jars of mussels (6 replicates) were also maintained and received fresh untreated Mohawk River water each day. Mortality data were statistically analyzed following angular transformation (Sokal and Rohlf 1995).

RESULTS AND DISCUSSION:**Experiment #1**

Mussel mortality increased gradually within the first week and then leveled off (Fig. 1). Maximum mortality was recorded on the 5th day of treatment (Fig. 2). The final mortality achieved (44.8%, Table 1) was very similar to ca. 50% mortality achieved at the same concentration (25 ppm) in prior 2-day exposures (author, unpublished data). Therefore, these results suggest that there is no advantage to treating mussels for more than 2 days. One hypothesis to explain the relative ineffectiveness of the treatments that occurred after 2 days was that the frozen stored inoculum lost its toxicity during storage. This was highly unlikely since separate "quality control" tests were also run with fresh mussels treated with frozen inoculum that had been stored for 1 and 7 days, and mussel mortalities resulting from CF stored on dry ice for 1 and 7 days were not statistically different ($P=0.67$, t -test). Thus, inoculum used daily throughout at least the first week was of uniform toxicity.

Fig. 1. Cumulative mortality over time in Experiment #1.

Fig. 2. Individual daily mussel mortality in Experiment #1.

Experiment #2

As in the first experiment, mussel mortality increased gradually within the first week and then leveled off (Fig. 3). The final mortality achieved (44.8%, Table 1) was identical to that achieved in the first experiment. Maximum mortality was recorded on the 3rd day of treatment (Fig. 4). These results again suggested that mussel exposure in the initial 2 days of treatment was the critical exposure period. As in the first experiment, separate "quality control" tests were also run with fresh mussels, and mussel mortalities resulting from CF stored on dry ice for 1 and 7 days were not statistically different ($P=0.67$, t -test). Thus, as in the first experiment, inoculum used daily throughout at least the first week was of uniform toxicity.

Fig. 3. Cumulative mortality over time in Experiment #2.

Fig. 4. Individual daily mussel mortality in Experiment #2.

Table 1: Zebra mussel mortality following two weeks of continuous bacterial treatment at 25 ppm (23°C).

Experiment*	Treatment	Mortality (Mean $\pm$ SD)
#1	Control (no bacterial treatment)	8.8 $\pm$ 5.2%
	Treated	44.8 $\pm$ 13.9%
#2	Control (no bacterial treatment)	8.0 $\pm$ 2.8%
	Treated	44.8 $\pm$ 9.1%

* Duration of treatments were 16 days in experiment #1 and 14 days in experiment #2.

Mussel Size and Susceptibility

Subtask 2.1 of the SOW requires that the Contractor shall continue to confirm prior test results, which have indicated that mussels in the size range ca. 1-30 mm appear equally susceptible to treatment. Data in these present tests provided such evidence: mean mussel mortalities ($\pm$ SD) in Experiment #1 and #2 were not statistically different (respectively, 44.8 $\pm$ 9.1% versus 44.8 $\pm$ 13.9%, $P=0.82$, t -test), yet mean mussel lengths ($\pm$ SD) were statistically different (respectively, 9.4 $\pm$ 1.2 mm and 15.0 $\pm$ 1.5 mm, $P<0.001$, t -test).

CONCLUSIONS:

These experiments indicated that in 23°C waters the window of opportunity to kill zebra mussels with bacterial strain CL0145A was during the first two days of treatment. Treatments beyond two days did not raise mortality. Thus, additional experiments must be conducted to examine this initial 48 hr period of treatment to better define this window. Future tests, for example, will be run to assess the impact of treatment durations varying from 3 hr to 48 hr. These tests will define the maximum effective treatment duration, and then this duration will be used to treat pipes at bacterial concentrations sufficient to achieve high mussel kill (>95% mortality).

REFERENCES:

Sokal, R. R. and Rohlf, F. J. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. Third edition. W. H. Freeman and Company, New York. 887 pp.