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Efficacy of *Pseudomonas fluorescens* Strain CL145A Spray Dried Powder for Controlling Zebra Mussels Adhering to Native Unionid Mussels Within Field Enclosures

By James A. Luoma, Kerry L. Weber, Todd J. Severson, and Denise A. Mayer

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
micrometer (µm)	3.937×10^{-5}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
nanometer (nm)	3.937×10^{-8}	inch (in.)
	Area	
square meter (m^2)	10.76	square foot (ft^2)
	Volume	
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Conductivity is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.

Abbreviations

AI	active ingredient
$CaCO_3$	calcium carbonate
DO	dissolved oxygen
MBI	Marrone Bio Innovations
mE	meters East
mN	meters North
NH_3	un-ionized ammonia
OR	odds ratio
SAS	Statistical Analysis System
SD	standard deviation
SDP	spray dried powder
TAN	total ammonia nitrogen
Pf-CL145A	*Pseudomonas fluorescens* strain CL145A
USGS	U.S. Geological Survey
UTM	Universal Transverse Mercator
w/w	weight to weight ratio

Efficacy of *Pseudomonas fluorescens* Strain CL145A Spray Dried Powder for Controlling Zebra Mussels Adhering to Native Unionid Mussels Within Field Enclosures

By James A. Luoma,[1] Kerry L. Weber,[1] Todd J. Severson,[1] and Denise A. Mayer[2]

Abstract

The efficacy of a commercially prepared spray dried powder (SDP) formulation of *Pseudomonas fluorescens* (strain CL145A) was evaluated for removing zebra mussels (*Dreissena polymorpha*) adhering to a population of unionid mussels in Lake Darling (Alexandria, Minnesota). Two groups of unionid mussels were used in the study. Unionid mussels were collected near the test area, weighed, photographed, individually tagged, and randomly allocated to one of nine test enclosures in equal proportions and then divided into two groups. The first group of unionid mussels (Group 1, $n = 5$ per test enclosure) were indiscriminately selected from each test enclosure and used to estimate the number of zebra mussels adhering to unionid mussels prior to exposure. The second group of unionid mussels (Group 2, $n = 22$ per test enclosure) were used to evaluate the efficacy of SDP for removal of adhering zebra mussels. Both Group 1 and Group 2 mussels were used to evaluate the effects of SDP exposure on unionid mussel survival.

Treatment was assigned to each test enclosure by using a randomized block design. The three treatment groups were tested in triplicate and included an untreated control group and groups that received a single application of 50 or 100 milligrams per liter (mg/L) of SDP based on active ingredient. All treatment concentrations are reported as active ingredient of SDP. Test enclosures were removed at the 8-hour exposure termination. Both Group 1 and Group 2 mussels remained in their assigned exposure location during the postexposure holding period. The number of zebra mussels adhering to Group 2 mussels (live and dead) was assessed 18 to 20 days postexposure in addition to assessing the survival of Group 1 and Group 2 unionid mussels.

SDP, administered as a single treatment, significantly ($p < 0.01$) reduced the number of adhering zebra mussels when compared to the untreated controls. The number of zebra mussels adhering to unionid mussels (Group 2) was reduced 53 percent in the 50-mg/L treatment group and 68 percent in the 100-mg/L treatment group. The number of adhering zebra mussels did not differ ($p = 0.79$) between the 50- and 100-mg/L treatment groups after exposure. When standardized to the amount of SDP applied per square meter, each gram (g) of SDP applied in the 50-mg/L treatment reduced the number of adhering zebra mussel 59.8 percent more than the 100-mg/L treatment group.

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Group 1 mussel survival did not differ between treatment groups ($p > 0.05$); however, a difference was detected ($p < 0.01$) in the survival of Group 2 mussels. The survival of Group 2 mussels did not differ ($p > 0.23$) between control and treated groups. A difference in Group 2 mussel survival was detected ($p = 0.03$; odds ratio [OR] = 0.290) between the 50- and 100-mg/L treatment groups (that is, the survival was highest in the 50-mg/L treatment group and lowest in the 100-mg/L treatment group), however, the biological significance of the difference is indeterminate.

Introduction

Freshwater mussel populations of North America were historically considered the most diverse in the world, but diversity is declining rapidly in response to a variety of anthropogenic influences such as habitat degradation and alteration, pollution, and overharvest (Williams and others, 1993; Neves and others, 1997). Master (1990) found that 55 percent of North American mussel species were either extinct or imperiled. At least 127 imperiled mussel species are predicted to reach extinction within the next 100 years; however, the 6.4 percent decadal extinction rate does not factor in extirpations related to invasions by dreissenid mussels (zebra, *Dreissena polymorpha* and quagga, *D. rostriformis bugensis*) (Ricciardi and Rasmussen, 1999).

Because of their high reproductive capacity and their planktonic lifestage, dreissenid mussels can quickly disperse and inundate aquatic environments (Mackie, 1991; Birnbaum, 2011). Since their introduction into the United States and establishment in the Great Lakes in the 1980s, zebra mussels have been identified in 680 lakes, not including impoundments and connected waterways, within 27 states (Benson and others, 2015). In a power plant canal in Lake Erie, Schloesser and Kovalak (1991) estimated zebra mussel colonization as high as approximately 10,700 zebra mussels per unionid mussel, with a mean estimated density of approximately 6,800 zebra mussels per unionid mussel. Adhering zebra mussel colonies may contain in excess of 10,000 individuals, which could weigh two to five times as much as the unionid mussel (Hebert and others, 1991; Mackie, 1991). Prediction models estimate that a colonization density of as few as 100 zebra mussels per unionid can result in unionid mussel mortality (Ricciardi and others, 1995).

Colonization by zebra mussels may interfere with unionid mussel locomotion, feeding, reproduction, and respiration (Mackie, 1991; Schloesser and Kovalak, 1991). Heavy zebra mussel colonization may contribute to starvation of the unionid through reduced intake of food and increased metabolic costs (Baker and Hornbach, 1997; Strayer and Malcom, 2007). Heavily colonized unionid mussels may lack the energy stores required to survive winter, and their ability to burrow into the sediment to avoid winter or other environmental stressors is impeded (Nalepa, 1994; Schloesser and Nalepa, 1994). Colonization by zebra mussels causes declines in unionid mussel populations and is dependent upon zebra mussel density, biomass, time since invasion, and substrate type (Burlakova and others, 2000).

Federal and state management agencies have implemented recovery and propagation programs for threatened and endangered unionid species coinciding with legislation and programs for control and removal of introduced and invasive species such as zebra mussels (Neves, 2004; Nalepa and Schloesser, 2014). However, there is currently a lack of access to environmentally safe and effective tools to control dreissenid mussel populations in open-water environments. A potential tool for limited open-water control of dreissenid mussels is a commercially formulated spray dried powder (SDP) product, which contains the killed cells of a specific strain (CL145A) of the common soil bacterium *Pseudomonas fluorescens*. The goal of this study was to determine the potential use of SDP in unionid mussel recovery and restoration efforts by controlling zebra mussel populations. The study objectives were (1) to determine the efficacy of SDP to control zebra mussels adhering to unionid mussels, and (2) to

evaluate the effects of SDP exposure on survival of unionid mussels compromised by zebra mussel colonization. This report summarizes a single field trial completed from July 18, 2013, to November 18, 2013. The exposures were completed on July 26, 2013, and the postexposure assessments were completed August 13–15, 2013.

Materials and Methods

The protocol for this study entitled "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures" is contained in appendix 1, item 1. All methods and materials follow the written protocol except those instances that were identified in a note to file (appendix 1, item 2) and in study deviations (appendix 2, items 1–5).

Experimental Design

The study was done in Lake Darling (Alexandria, Minnesota) and consisted of a twofold assessment to evaluate the reduction in the number of zebra mussels adhering to unionid mussels and to evaluate unionid mussel survival following exposure to SDP. Zebra mussel reduction was assessed by comparing the estimated number of zebra mussels adhering to unionid mussels prior to SDP exposure to the enumerated number of zebra mussels adhering to mussels after SDP exposure. Unionid mussel survival was assessed after SDP exposure by observing foot movement or resistance to valve pressure.

Unionid mussels were collected, individually tagged, and randomly assigned (appendix 3, item 4) to test enclosures in equal proportions (n = 243; 27 per test enclosure). A group of unionid mussels (Group 1; n = 45; 5 per test enclosure) were removed and used to estimate the number of adhering zebra mussels prior to SDP exposure (that is, adhering zebra mussels were removed and enumerated). After zebra mussel removal, Group 1 unionid mussels were returned to their assigned treatment enclosure for the remainder of the study. The undisturbed unionid mussels (Group 2; n = 198; 22 per test enclosure) remained in the assigned test enclosure area for the entire study duration and were used to evaluate the efficacy of SDP for removal of adhering zebra mussels. Both groups were exposed concurrently in the test enclosures.

Treatments were administered in triplicate as a single SDP exposure according to a randomized block design (appendix 3, item 1). The treatment levels were (1) an untreated control group, (2) a group that received an application of 50 milligrams per liter (mg/L) of SDP active ingredient (AI) for 8 hours, and (3) a group that received an application of 100 mg/L of SDP AI for 8 hours. The experimental unit for the trial was the individual test enclosure.

Test Enclosure Area

Nine test enclosure areas were identified near the north shore of Lake Darling (fig. 1), and the Universal Transverse Mercator (UTM) coordinates (test area centroid) were recorded (table 1). Each test area was characterized as predominately sandy substrate with minimal algae and macrophyte growth. The areas selected were approximately 1.4 meter (m) deep and were spaced at least 6 m apart (by center point). Unionid mussel retention barriers (fig. 2) were placed in each test enclosure area approximately 48 hours prior to exposure. The expanded metal 1-square-meter (m^2) retention barriers were used to confine the unionid mussels to the test enclosure area for the duration of the study. After unionid mussel allocation, a 2.25-m^2 test enclosure (fig. 2) was placed around each retention barrier and secured to fenceposts driven into the lakebed. The bottom sealing flaps of each test enclosure were secured to the lakebed with sandbags.

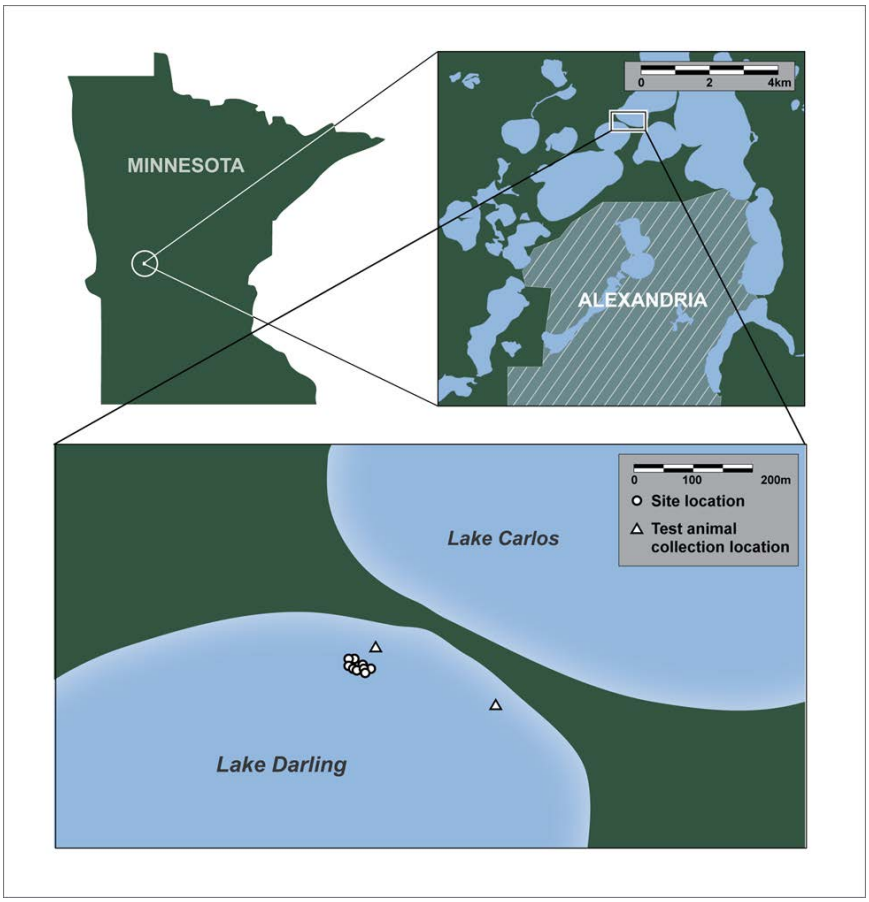

Figure 1. Test enclosure site and test animal collection locations.

Figure 2. Example of 1-square-meter (m^2) expanded metal mussel retention barrier (left) and 2.25-m^2 impermeable test enclosure (right).

Table 1. Universal Transverse Mercator (UTM) centroid location of each test enclosure.
[mg/L, milligrams per liter; mE, meters East; mN, meters North]

Enclosure	Treatment group (mg/L)	Zone	Row	Easting (mE)	Northing (mN)
1	100	15	T	315318	5089217
2	50	15	T	315305	5089224
3	0	15	T	315292	5089230
4	50	15	T	315281	5089231
5	50	15	T	315279	5089221
6	100	15	T	315290	5089218
7	0	15	T	315294	5089215
8	0	15	T	315302	5089211
9	100	15	T	315307	5089205

Test Article

The test article was a commercially prepared SDP formulation of *Pseudomonas fluorescens*, strain CL145A containing 50 percent AI (weight to weight ratio [w/w] *P. fluorescens,* strain CL145A). The test article (lot number TR 4669-4-3) was obtained from Marrone Bio Innovations, Inc. (MBI; Davis, California; Certificate of Analysis, appendix 4, item 2). Test article use was documented in the test chemical logbooks (appendix 4, items 5 and 6). Verification of test article biological activity was determined on a sample of test article by the New York State Museum Field Research Laboratory (Cambridge, New York), using their standard dreissenid mussel bioassay (appendix 4, item 7). Biological activity was confirmed as demonstrated by a mean zebra mussel mortality in the treated group of 96.0 ± 6.9 percent compared to 1.3 ± 2.2 percent in the untreated group.

Test Animals

Adult fatmucket unionid mussels (*Lampsilis siliquoidea*) with adhering zebra mussels (fig. 3) were collected 3 days prior to exposure from an existing population located within 300 m of the test enclosures. An incidental number of fragile papershell unionid mussels (*Leptodea fragilis, n* = 0–3 per enclosure) were inadvertently included. The *L. fragilis* were treated and analyzed the same as *L. siliquoidea*. Unionid mussels were confined within a 1-m^2 retention barrier in approximately 0.75 m of water until distributed to the test enclosures. A Hallprint® shellfish tag with a unique alphanumeric code was fixed to each mussel shell with cyanoacrylate glue, and an initial wet weight with adhering zebra mussels was obtained for each unionid mussel.

Groups of unionid mussels (*n* = 4–5) with adhering zebra mussels were randomly allocated to each test enclosure (appendix 3, item 4) and placed within a retention barrier until each test enclosure/retention barrier received a total of 27 unionid mussels.

At exposure termination, the test enclosures were removed and both Group 1 and Group 2 unionid mussels remained in the retention barriers at the treatment location for a postexposure holding period. At 18–20 days postexposure, the unionid mussels were removed from the retention barriers, and

wet weights were measured before and after removal of adhering zebra mussels. Additionally, each unionid mussel was photographed, assessed for survival, and measured for shell length. Unionid mussels were euthanized according to permit requirements (appendix 5, items 1–3). The unionid mussel shells were retained and used for confirmative species identification. All zebra mussels removed were sorted into categories of dead or alive and were enumerated; a subsample from each group was measured for shell length.

Figure 3. Representative unionid mussel with adhering zebra mussels preexposure (top left), postexposure (top right) and with zebra mussels removed (bottom).

Reduction of Adhering Zebra Mussels

Estimation of Initial Number of Adhering Zebra Mussels (Group 1)

After distribution, five unionid mussels (Group 1) were indiscriminately removed from each test enclosure and used to estimate the initial preexposure biomass of adhering zebra mussels for each test enclosure (that is, the initial number of adhering zebra mussels per gram of zebra mussels [equation 1]):

$$en = \frac{n_{zm}}{[W_1 - W_2]} \quad (1)$$

where en is the estimated number of adhering zebra mussels per gram of zebra mussels,
n_{zm} is the number of live adhering zebra mussels removed from Group 1 mussels (preexposure),
W_1 is the group 1 mussel wet weight with adhering zebra mussels, in grams (g), and

W_2 is the group 1 mussel wet weight after zebra mussel removal (g).

The average number of adhering zebra mussels, per gram, from each test enclosure was used to estimate the initial number of adhering zebra mussels on Group 2 mussels (equation 2).

Estimation of the Number of Adhering Zebra Mussels (Group 2)

For each test enclosure, the number of zebra mussel adhering to Group 2 mussels prior to exposure was estimated (equation 2) by using the mean estimated number ($\bar{x}_{en}$) of adhering zebra mussels, per gram, of Group 1 mussels (derived from equation 1). Initial Group 2 mussel wet weight with adhering zebra mussels before exposure and Group 2 mussel wet weight after zebra mussel removal were used to determine the weight of adhering zebra mussels (equation 2);

$$en_2 = [W_1 - W_2] \times \bar{x}_{en} \quad (2)$$

where en_2 is the estimated number of zebra mussels adhering to Group 2 mussel prior to exposure,

W_1 is the preexposure wet weight of Group 2 mussel with adhering zebra mussels (g),

W_2 is the postexposure wet weight Group 2 mussel after zebra mussel removal (g), and

$\bar{x}_{en}$ is the mean estimated number of adhering zebra mussels per gram of zebra mussels for each test enclosure (derived from equation 1).

Calculation of Zebra Mussel Body Weight Burden (Group 2)

The percent body weight burden of adhering zebra mussels was calculated for each individual unionid mussel by comparing the estimated weight of adhering zebra mussels to the weight of the unionid mussel without zebra mussels (equation 3). A mean percent body weight burden was calculated for each treatment group:

$$ZM\ Burden\ (\text{percent}) = (W_1 / W_2) \times 100 \quad (3)$$

where

ZM Burden is the adhering zebra mussel burden expressed as percent of unionid mussel body weight,

W_1 is the estimated preexposure wet weight of zebra mussels (g), and

W_2 is the postexposure wet weight unionid mussel without zebra mussels (g).

Calculation of Adhering Zebra Mussels Reduction (Group 2)

For each test enclosure, the reduction in the number of zebra mussels adhering to Group 2 mussels was estimated by using the mean estimated number ($\bar{x}_{en_2}$) of adhering zebra mussels (derived from equation 2) and the number of adhering zebra mussels postexposure (equation 4). A mean percent reduction was calculated for each treatment group:

$$Percent\ \Delta = \left(\left[\frac{\{\bar{x}_{en_2} - n_{zm}\}}{\bar{x}_{en_2}} \right] \times 100 \right) \quad (4)$$

where Δ is the percent reduction in the number of adhering zebra mussels on Group 2 unionids,

$\bar{x}_{en_2}$ is the mean estimated number of zebra mussels adhering to Group 2 mussel prior to exposure (derived from equation 2),

n_{zm} is the number of live zebra mussels removed from Group 2 mussels (postexposure).

Calculation of SDP Reduction Coefficient and Efficiency (Group 2)

A reduction coefficient of SDP was calculated for each test enclosure by comparing the reduction in the number of adhering zebra mussels to the amount of SDP applied to the test enclosure area (equation 5). A mean reduction coefficient was calculated for each treatment group:

$$RC = \left(\frac{\Delta}{[P/A]}\right) \quad (5)$$

where RC (reduction coefficient) is the percent reduction of adhering zebra mussels per gram of SDP applied,

Δ is the percent reduction in the number of adhering zebra mussels on Group 2 unionids (derived from equation 4),

P is the amount of SDP applied (g), and

A is the area treated (=2.25 m^2).

Unionid Mussel Survival

Each unionid mussels was assessed for survival 18–20 days after SDP exposure. Survival was defined as foot or valve movement in response to tactile stimuli or resistance to valve pressure by adductor muscle contraction. Unrecovered unionid mussels were treated as mortalities within the data analysis.

Stock-Solution Preparation and Dosing

A separate SDP stock solution was prepared and immediately applied to each test enclosure. The appropriate amount of SDP required to treat each test enclosure (appendix 4, item 4) was added to approximately 10 liters (L) of unfiltered lake water and mixed. Immediately after mixing, the stock was poured through a strainer, and clumps of test article were macerated with a pestle and rinsed into the stock solution with unfiltered lake water, bringing the final stock volume to approximately 15 L.

The prepared stock solution was transported in a 19.4-L screw-top bucket to the assigned test enclosure. Treatments were individually applied in the following sequence: control, 50-, and 100-mg/L SDP. The 50- and 100-mg/L SDP treatments were applied by adding the stock solution to four areas of the test enclosure. The water and stock solution within the test enclosure was then mixed by using a boat paddle. The control treatments involved the same application technique used for the SDP-treated groups, except that untreated Lake Darling water was applied in place of SDP stock solution.

Test Enclosure Water Sample Collection

Water samples were collected by submersing a 1-L screw-top container below the surface of each test enclosure. The water samples were used to verify exposure concentration and to measure water-quality parameters (that is, hardness, alkalinity, conductivity, dissolved oxygen, temperature, pH, and ammonia).

Concentration Verification

Exposure water SDP concentrations were determined at 1, 4, and 8 hours by comparing surface water samples collected from each test enclosure to a linear regression curve created from known concentrations (25, 50, 100 and 200 mg/L) and absorbance of the test article. Sample absorbance was measured on a Barnstead-Turner SP-830 Plus (model SM110215) spectrophotometer at 660 nanometers (nm). Linear regression equations were fit by using the SAS® software Proc Reg procedure (SAS®

version 9.3; SAS Institute Inc., Cary, North Carolina), and the exposure sample concentrations were predicted from the regression analysis (appendix 7, items 2 and 3).

Water Chemistry

Hardness, alkalinity, and conductivity were measured in surface water samples collected from each test enclosure before administering the test article. Dissolved oxygen, pH, and temperature were measured in surface water samples collected from each test enclosure prior to exposure as well as 4 and 8 hours after exposure initiation. Immediately before exposure period termination, water samples were collected, filtered (0.45 micrometer [µm]), acidified with 10 percent sulfuric acid to ≤ pH 2.5, and stored at approximately 4 degrees Celsius (°C) until analyzed for total ammonia nitrogen (TAN) content by using the automated phenate method (Standard Method 4500G; American Public Health Association and others, 2012). The un-ionized ammonia fractions were calculated by using the sample pH and temperatures measured at the time of sample collection according to the formula identified by Emerson and others (1975). Temperature loggers (Onset, Bourne, Massachusetts, HOBO® Pendent Temperature/Light Data Logger) were attached to the retention barriers and used to measure water temperature every 3 hours during the postexposure period. Dissolved oxygen, pH, and temperature of the lake water were measured daily during the assessment period (18–20 days postexposure) near the retention barriers.

Data Analysis

Water chemistry data analyses were limited to simple descriptive statistics; comparative statistics were not generated. Statistical comparisons of zebra mussel density, zebra mussel length, and unionid mussel survival were performed by using SAS® software version 9.3 (SAS Institute, Inc., Cary, N.C.). Significance for all analyses was declared at $p \leq 0.05$. Exposure concentrations were determined by using SAS® software version 9.3.

Reduction of Adhering Zebra Mussels

A general linear model created with the SAS® software Proc GLM procedure was used to analyze the number of zebra mussels adhering to unionid mussels before and after exposure (appendix 10, item 5). The mean number of zebra mussels adhering to unionid mussels in each treatment group before and after exposure was modeled with the “no intercept” and “solution” options specified. The assumptions of normal distribution and homogeneity of variance were assessed by using the univariate procedure with the “normal” option specified and the Bartlett’s test. The mean number of zebra mussels adhering to unionid mussels of each treatment group (before and after exposure) was individually compared to the number of zebra mussels adhering to unionid mussels of the untreated control group by using a two-sided means comparison test.

By using the procedures previously described (SAS® software Proc GLM; appendix 10, item 8), the mean length of zebra mussels removed from unionid mussels collected from each treatment replicate before (Group 1) and after (Group 2) SDP exposure was analyzed to determine whether a correlation existed between zebra mussel length and zebra mussel survival within each treatment assignment. The analysis of test animal length was limited to adhering live zebra mussels because dead zebra mussel shells could not be retained.

Unionid Mussel Survival

A generalized linear mixed model (SAS® software Proc GLIMMIX) was used to analyze the survival of unionid mussels in each treatment (appendix 10, item 2). The proportion of mortalities (number of dead unionid mussels compared to the total number unionid mussels) was modeled with a binomial distribution and a logit link function. A scale parameter was added to the model using the "random_residual" statement. Unionid mussel survival of each treatment group was individually compared to the survival in the untreated control group by using a two-sided means comparison test with a Tukey post hoc adjustment.

Exposure Concentration

The mean exposure concentrations were determined for individual test enclosures, for each sampling time, and for each treatment group using the SAS® software Proc Means statement (appendix 7, item 3).

Results

Adverse Events

There were no observable adverse effects observed in the unionid mussels or zebra mussels within the test enclosures. There was a significant algal bloom within Lake Darling (dominated by *Mougeotia* and *Spirogyra* species) during the postexposure period. The study was terminated approximately 10 days earlier than planned because of concerns that the algae bloom may impact native or zebra mussel survival (appendix 2, items 1 and 6).

Reduction of Adhering Zebra Mussels

Statistical analysis output can be found in appendix 10 (item 4), and the data summary can be found in appendix 8 (item 1). The number of zebra mussels adhering to unionid mussels before and after treatment is summarized in table 2. No difference ($p > 0.74$) was detected in the number of zebra mussels adhering to unionid mussels in each treatment group prior to exposure. The observed percent body weight burden of adhering zebra mussels from each treatment group ranged from 125.9 to 130.0 percent of unionid mussel body weight. The mean number of adhering zebra mussels before and after exposure differed in the 50- and 100-mg/L treatment groups (160 versus 74 [$p < 0.01$] and 180 versus 59 [$p < 0.01$], respectively). The number of adhering zebra mussels was reduced on average by 53 and 68 percent in the 50- and 100-mg/L treatment groups, respectively. The number of adhering zebra mussels did not differ ($p = 0.79$) between the 50- and 100-mg/L treatment groups after exposure. When standardized to the amount of SDP applied per square meter, the 50-mg/L treatment was more efficient than the 100-mg/L treatment at reducing the number of adhering zebra mussels (0.44 percent reduction per gram applied versus 0.28 percent reduction per gram applied, respectively).

Statistical analysis output can be found in appendix 10 (item 7), and the data summaries can be found in appendix 5 (items 6–7). Mean length of adhering zebra mussels is summarized in table 3. Mean length did not differ ($p > 0.89$), indicating that zebra mussel length was not a predictor of mortality.

Table 2. Mean (standard deviation) number of adhering zebra mussels per unionid mussel before and after exposure, percent change of zebra mussel reduction, zebra mussel burden (as percent unionid body weight), and spray dried powder (SDP) reduction coefficient for number of adhering zebra mussels for each treatment group.
[mg/L, milligrams per liter; ZM, zebra mussel; SDP, spray dried powder; NA, not applicable. Means with the same letter are not significantly different ($p > 0.05$).]

Treatment group (mg/L)	Zebra mussels per unionid mussel			ZM burden[1]	SDP reduction coefficient[2] (percent)
	Preexposure	Postexposure	Colonization change (percent)		
Control (0)	165[a] (15)	179[a] (9)	9 (9)	130.0 (4.4)	NA
50	160[a] (11)	74[b] (9)	-53 (8)	125.9 (12.5)	0.441 (0.043)
100	180[a] (13)	59[b] (23)	-68 (10)	127.1 (11.7)	0.276 (0.033)

[1]Expressed as a percentage of unionid mussel body weight.

[2]Expressed as percent reduction in the number of adhering zebra mussels per gram of SDP applied.

Table 3. Mean (standard deviation; number measured) length of adhering zebra mussels (live) by treatment group.
[mg/L, milligrams per liter; mm, millimeters. Means with the same letter are not significantly different ($p > 0.05$)]

Treatment group (mg/L)	Length (mm)	
	Preexposure	Postexposure
Control (0)	12.0[a] (0.1; 1,453)	11.2[a] (2.3; 1,366)
50	12.1[a] (0.5; 1,266)	11.8[a] (0.5; 590)
100	11.2[a] (0.4; 1,359)	12.0[a] (0.5; 584)

Unionid Mussel Survival

Statistical analysis output can be found in appendix 10 (item 1), and the data summary can be found in appendix 9 (item 1). Survival of unionid mussels is summarized in table 4. Treatment did not significantly affect Group 1 mussel survival ($p > 0.05$) but did significantly affect Group 2 mussel survival ($p < 0.01$) and Group 1 and Group 2 combined mussel survival ($p < 0.01$). No difference ($p > 0.23$) was detected in survival between the Group 2 control group and the 50- or 100-mg/L treatment groups. A difference ($p = 0.03$) was detected in the mean survival between the 50- and the 100-mg/L treatment groups (96.8 and 85.1 percent, respectively), however, the biological significance of the difference is indeterminate. The log odds of unionid mussel survival in the 100-mg/L treatment was 0.290 times the odds of survival of unionid mussels assigned to the 50-mg/L treatment.

Table 4. Mean (standard deviation) percent survival of Group 1 and 2 unionid mussels.
[mg/L, milligrams per liter. Means within columns with the same letter are not significantly different ($p > 0.05$)]

Treatment group (mg/L)	Survival (percent)	
	Group 1	Group 2
Control (0)	100.0[a] (0.0)	89.4[ab] (6.9)
50	100.0[a] (0.0)	96.8[a] (3.9)
100	80.0[a] (20.0)	85.1[b] (13.8)

Dose Verification

The linear regression, exposure concentrations, and data summary can be found in appendix 7 (items 1–2). Exposure concentrations for each treatment group are summarized in table 5. The measured exposure concentrations were lower than the target concentration. The mean SDP exposure concentrations measured throughout the exposure period were 35.3 ± 3.3 mg/L and 74.2 ± 3.4 mg/L for the 50- and 100-mg/L treatment groups, respectively.

Table 5. Mean (standard deviation) spray dried powder exposure concentration in surface water samples collected from test enclosures during the exposure period.
[mg/L, milligrams per liter; ND, not detectable/below detection limit]

Treatment group (mg/L)	Time (hours)		
	1	4	8
Control (0)	ND	ND	ND
50	38.6 (1.7)	35.5 (2.7)	31.9 (1.0)
100	76.9 (1.3)	75.5 (2.4)	70.3 (1.3)

Water Chemistry

The water-chemistry data summaries are presented in appendix 6 (items 1–5). Water-chemistry parameters (dissolved oxygen, pH, and temperature) in the test enclosures are summarized in table 6. Dissolved oxygen concentration remained above the minimum level recommended (4 mg/L) in the ASTM International guide for conducting laboratory tests with freshwater mussels (ASTM International, 2013). Hardness, alkalinity, conductivity, and ammonia are summarized in table 7. The alkalinity ranged from 174 to 176 mg/L as calcium carbonate ($CaCO_3$), hardness from 189 to 193 mg/L as $CaCO_3$, and conductivity from 322 to 324 microsiemens per centimeter (µS/cm) (automatic temperature corrected to 25 °C); un-ionized ammonia was ≤ 0.01 mg/L. Data collected by the temperature data loggers during the postexposure period indicated a water temperature range of 22.3 °C to 23.9 °C. Mean (standard deviation [SD]) water-chemistry parameters measured 18–20 days postexposure were dissolved oxygen, 8.60 mg/L (0.40); temperature, 21.9 °C (0.3); and pH range, 8.54–8.63.

Table 6. Mean (standard deviation) water chemistry (dissolved oxygen, temperature) and pH range of surface water samples collected from each treatment group during the study period.
[mg/L, milligrams per liter; °C; degrees Celsius]

Sample time	pH	Dissolved oxygen (mg/L)	Temperature (°C)
Control (0 mg/L)			
Preexposure	8.45 – 8.58	7.03 (0.12)	20.6 (0.5)
4 hours	8.45 – 8.50	6.49 (0.12)	22.9 (0.2)
8 hours	8.40 – 8.49	6.36 (0.39)	23.0 (0.0)
50 mg/L			
Preexposure	8.47 – 8.56	7.22 (0.48)	20.4 (0.2)
4 hours	8.35 – 8.38	5.95 (0.17)	23.1 (0.1)
8 hours	8.25 – 8.36	5.63 (0.06)	23.1 (0.1)
100 mg/L			
Preexposure	8.53 – 8.56	6.86 (0.24)	19.6 (0.6)
4 hours	8.16 – 8.28	5.85 (0.19)	23.0 (0.2)
8 hours	8.18 – 8.23	5.36 (0.17)	23.1 (0.2)

Table 7. Mean (standard deviation) alkalinity, hardness, conductivity, and ammonia content of surface water samples collected from each treatment group during the study period.
[mg/L, milligrams per liter; µS/cm, microsiemens per centimeter; TAN, total ammonia nitrogen; mg NH_3-N/L, milligrams un-ionized ammonia nitrogen per liter; NH_3, un-ionized ammonia; <, less-than; $CaCO_3$, calcium carbonate; °C, degrees Celsius]

Treatment group (mg/L)	Preexposure			Exposure termination	
	Alkalinity (mg/L)[1]	Hardness (mg/L)[1]	Conductivity (µS/cm)[2]	TAN (mg NH_3-N/L)	NH_3 (mg/L)
Control (0)	175 (0)	189 (1)	323 (5)	0.04 (0.02)	<0.01 (<0.01)
50	174 (2)	190 (2)	322 (3)	0.10 (0.02)	<0.01 (<0.01)
100	176 (3)	193 (4)	324 (4)	0.13 (0.01)	0.01 (<0.01)

[1]Reported as milligrams per liter $CaCO_3$.

[2]Temperature compensated to 25 °C.

Conclusions

The application of a single dose of spray dried powder (SDP) formulation of *Pseudomonas fluorescens* (strain CL145A, 50 or 100 milligrams per liter [mg/L]) for 8 hours to open-water test enclosures significantly reduced the number of zebra mussels adhering to unionid mussels. The number of adhering zebra mussels after exposure did not differ ($p = 0.79$) between the 50-mg/L and the 100-mg/L treatment groups. When standardized to the amount of SDP applied, the 50-mg/L treatment

was more efficient than the 100-mg/L treatment at reducing the number of zebra mussels adhering to zebra mussels (0.44 percent reduction per gram of SDP applied versus 0.28 percent reduction per gram of SDP applied, respectively). Multiple 50-mg/L applications may be more efficient at reducing zebra mussels than a single 100-mg/L application of SDP. The use of a subsurface injection application technique to deliver SDP only to the benthic zone may further reduce the amount required to achieve control. Treatment success was not dependent upon zebra mussel length ($p > 0.89$). No difference was detected in unionid mussel survival between the treated and control groups. A difference ($p = 0.03$; OR = 0.290) in unionid mussel survival was detected between the Group 2 50-mg/L and Group 2 100-mg/L treatment groups. Though statistically different, the biological significance of the slight difference in observed mortality between the treatment groups is indeterminate. Unionid mussel mortality may have been related to poor initial condition, as indicated by the high initial burden of adhering zebra mussels (> 125 percent of unionid mussel body weight).

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Appendix 1. Study Protocol With Data Forms

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Protocol Title:

Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures

Study Number: AEH-13-PSEUDO-05

Test Facilities and Study Sponsor
Upper Midwest Environmental Sciences Center (UMESC)
US Geological Survey
2630 Fanta Reed Rd.
La Crosse, Wisconsin 54603

Field Study Location
Lake Darling, Douglas County, MN

Proposed Experimental Start Date: July 2013
Proposed Experimental Termination Date: March 2014

Protocol Approval
Reviewed by:

[redacted] 7-19-13
Mark P. Gaikowski, M.A. Date
Supervisory Biologist

[redacted] 7/19/13
Michael D. Jawson, Ph.D. Date
Center Director

Approved by:

[redacted] 7/19/13
James A. Luoma, B.A. Date
Study Director

File Folder: 3

Item Number: 1

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1. INTRODUCTION:

Native freshwater mussel populations of North America were historically considered the most diverse in the world with about 297 recognized taxa consisting of 281 species and 16 subspecies (Williams et al., 1993). Mussels are largely sedentary in nature, relying on movement of host fish during glochidial attachment as means of transport. They are thus particularly vulnerable to a variety of anthropogenic influences including habitat degradation and alteration, pollution and overharvest. A Nature Conservancy survey (Master, 1990) found 55% of North America's mussels as extinct or imperiled compared to 7% of terrestrial species, even though terrestrial species traditionally receive far greater attention. Projections in 1999 (Ricciadi and Rasmussen, 1999) suggested that at least 127 imperiled mussel species will be lost in the next 100 years – a conservative extinction rate of 6.4% per decade given it did not take into account extirpations caused by invasive dreissenid mussels (zebra mussel *Dreissena polymorpha* and quaqqa mussel *D. bugensis*).

Concerns for native mussels in the Southeast are potentially even greater given that only 25% of the 269 species historically present are reported as stable compared to the 13% presumed extinct and the 28, 14 and 18% listed, respectively, as endangered, threatened or of special concern. (Neves et al., 1997)

Many unionid mussels in North America were imperiled prior to epizoic colonization by zebra and quaqqa mussels though the introduction of dreissenid mussels have dramatically heightened concerns for the continued survival of native mussels. Zebra mussels were reported to be responsible for the extirpation of unionids from waters in Europe as early as 1937 (Sebestyen, 1937). Severe declines in unionid abundance in Europe (Karatayev and Burlakova, 1995; Burlakova, 1998) and North America (Haag et al., 1993; Nalepa, 1994; Ricciardi et al., 1996) have since been well documented in the literature.

The 1973 Endangered Species Act (ESA) brought forth the need to recognize, protect and recover rare mussels in the United States. The United States Fish and Wildlife Service (USFWS) develops recovery plans for threatened and endangered species which utilize a range of tools to promote recovery of the species including restoring and acquiring critical habitat, removing introduced or invasive species and captive propagation and release into historic ranges.

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control techniques since 1991. They discovered that cell components of a strain of common bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) are capable of causing mortality when ingested by dreissenid mussels by degrading the epithelial cells lining the dreissenid digestive system. The NYSM also demonstrated that that efficacy for *Pf*-CL145A to induce dreissenid mussel mortality was retained when using dead vs. live cells.

Marrone Bio Innovations (MBI; Davis, CA) has developed a dead cell, spray dried formulation (SDP) of this bacterium called Zequanox®. Zequanox® is currently registered with the USEPA for use within closed systems such as power generating plant cooling systems. The NYSM, USFWS (Genoa NFH) and United States Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) were awarded a grant from the US Environmental Protection Agency Great Lakes Restoration Initiative entitled "Safe *Dreissena* Control: Promise for Unionid Restoration". The goal of this grant is to determine the suitability of this product for limited open water zebra mussel control applications including treatment of native mussel propagation cages or native mussel beds. Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the United States government. Throughout the remainder of this document the formulated *Pf*-CL145A product (Zequanox®) will be referred to as sprayed dried powder (SDP).

Naturally occurring surface waters may be unique in their chemical and biological properties which may affect the efficacy of applied control agents such as SDP. The research to be completed according to this protocol will assess the efficacy as well as the non-target unionid mussel safety of SDP treatments for controlling settled zebra mussels (*D. polymorpha*) in open water enclosures.

2. PROTOCOL OBJECTIVE:

To assess the efficacy and non-target unionid mussel safety of two exposure concentrations of SDP for controlling settled zebra mussels in open water enclosures.

3. STUDY SCHEDULE:

3.1 Proposed initiation: July 2013

3.2 Schedule of events: A proposed schedule of events is provided in Table 1.

3.3 Proposed completion date: March 2014

Table 1. Proposed Schedule of Events

Date	Activity
July 2013	SDP field exposures
August 2013	Efficacy/safety assessment
March 2014	final report submission

4. STUDY DESIGN:

4.1 General Description:

Native mussels with attached zebra mussels will be collected from Lake Darling, MN individually tagged and assessed for zebra mussel colonization. The mussels will then be placed into retention barriers situated in areas within the lake that will be enclosed

(enclosure area) by an impermeable membrane barrier (enclosures) during a SDP treatment period (exposure period). One of three SDP treatment levels (untreated control, 50 mg/L A.I. and 100 mg/L A.I) will be applied to each enclosure for an 8-h exposure period. After the exposure period the enclosures will be removed. Approximately 30-d after exposure, the native mussels will be assessed for survival and zebra mussel colonization.

4.2 Experimental Design:

Major study activities are summarized in Figure 1. Approximate locations for the nine enclosure areas were previously identified in Lake Darling, WI. Final enclosure area selection will be completed upon arrival at the test location. The enclosure areas will be <2 m deep and will have sandy to a sandy/silt substrate, be similar in substrate composition and be spatially separated as far as feasibly possible within approximately 50 m of shoreline. Each enclosure area selected will be marked with a numbered buoy and a perforated metal retention barrier (1-m^2) will be installed at each location to contain the test animals for the duration of the study. The 35-cm high retention barrier will be driven into the lake bed approximately 10 cm and the coordinates of each barrier will be determined using a GPS.

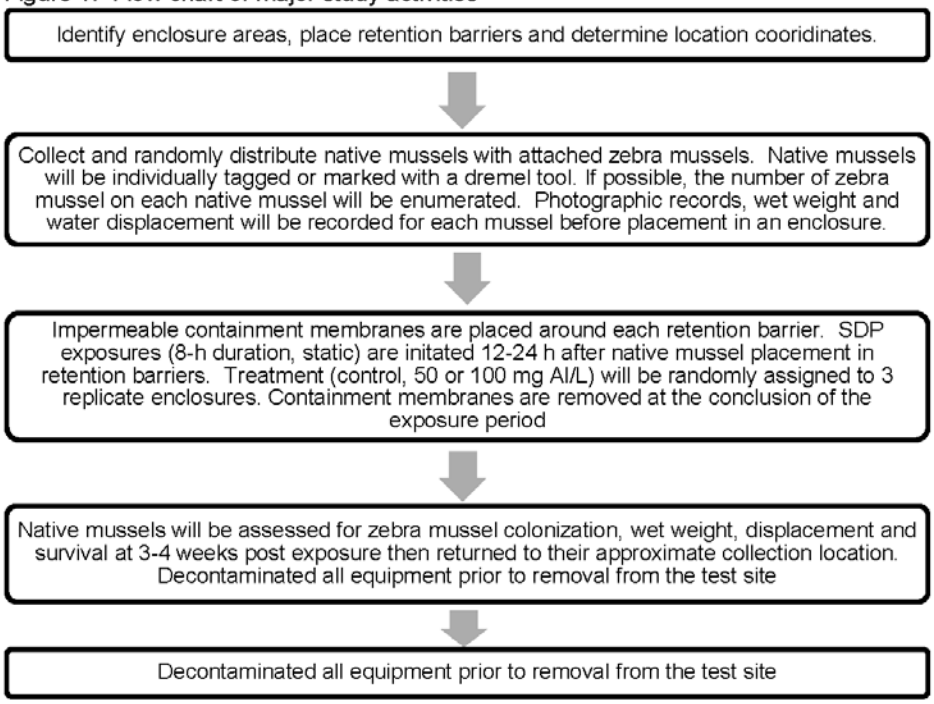
Native mussels with attached zebra mussels will be collected from Lake Darling, MN by wading or SCUBA. A minimum of 8 mussels and up to 25 mussels of each species will be placed in each enclosure for a total of 72 to 225 mussels per species (8 mussels x 9 enclosures = 72; 25 mussels x 9 enclosures = 225). If a single species of native mussels is collected, 25 mussels will be placed in each enclosure, if available. If two species of native mussels are collected, 15 mussels of each species will be placed in each enclosure, if available. If multiple species are used, they will be distributed to the enclosure areas in balanced numbers (i.e. 15 of each species) or equal ratios (i.e. 10 mussels of species X; 15 mussels of species Y, etc.) as determined by the number of animals collected. The exact number of animals collected and distributed to the enclosure areas will be documented in the study records. Native mussels will be consolidated in large coolers with frequent (< 1 h) water exchanges until distributed to the enclosures. Prior to distribution, individual mussels will be tagged with a Hallprint tag, photographed on each side, weighed and their water displacement measured to assist in individual tracking and estimating pre-treatment zebra mussel colonization.

Initial zebra mussel colonization on native mussels will be determined prior to treatment on 3 to 5 mussels per species selected at random from each enclosure. Individual native mussel weight and water displacement will be measured pre- and post-zebra mussel removal on the native mussels selected for initial zebra mussel colonization determination and the zebra mussels removed from each individual mussel will be enumerated. Estimates of initial zebra mussel colonization for the remaining native mussels will be completed by comparison to data collected from the initial colonization determinations (using individual weight and water displacement data collected prior to

treatment and at the termination of the study). Exact measures used to assess pre-treatment zebra mussels colonization will be documented in the study records.

If a single species of native mussels are collected, up to 25 mussels (if available) will be randomly distributed to each enclosure area in groups of 5 mussels in five distributions rounds (ie: 5 mussels placed in a bucket and transferred to a randomly selected enclosure area). If multiple species of native mussels are collected, equal ratios of mussels will be equally distributed to each enclosure area in multiple distribution rounds with up to 5 mussels of each species per round. The exact procedures used to distribute the mussels will be recorded in the study records.

Figure 1. Flow chart of major study activities



Enclosures containing native mussels with attached zebra mussels will be assigned to one of three treatments, a non-exposed control, a 50 mg/L A.I./L treatment and a 100 mg/L A.I./L treatment. There will be 3 replicates per treatment level and enclosures will be assigned a treatment according to a completely randomized assignment scheme. An exposure duration of 8 hours will be assigned for all treatment groups. The treatment exposure period will be initiated by the addition of an appropriate amount of SDP stock solution. The treatment concentration will be verified by comparison to a standard curve

prepared from a known mass of SDP. Exposures will be terminated 8-h after treatment application by removal of the enclosure membranes.

Approximately 30 days following treatment, the native mussels will be assessed for survival and for zebra mussel colonization by the measuring individual weight and water displacement pre- and post-zebra mussel removal. All zebra mussels removed will be preserved for enumeration to determine the number zebra mussels attached to each native mussel. Native mussel survival and the change in weight and water displacement along with the number of zebra mussels removed from each mussel will be compared between treatment groups to determine treatment related effects.

After assessment, all native mussels will be returned to their approximate collection location.

5. STUDY PROCEDURES

5.1 Test Animals

5.1.1 Description:

5.1.1.1 Age – Native mussels > 2 yrs

Zebra Mussels < 2yrs

5.1.1.2 Sex – Test animals will be used without regard to sex.

5.1.1.3 Species – Fatmucket, (*Lampsilis siliquoidea*); Fragile Papershell (Leptodea fragilis); Giant floater, (*Pyganodon grandis*); zebra mussel, (*Dreissena polymorpha*) The exact native unionid species will be determined at the time of collection. If multiple species are used, native species will be equally distributed in equal ratios to the treatment enclosures. During the final assessment, the mussels will be cleaned of any remaining zebra mussels and photographed for identification confirmation by an experienced USGS, USFWS or DNR malacologist.

5.1.2 Number of animals: No more than 360 native mussels will be used and no more than 225 of a single species will be used. This number is consistent with the objective of the study and contemporary scientific standards. Approximately 9,000 to 36,000 zebra mussels will be used (360 native mussels X 25-100 attached zebra mussels on each native = 9,000-36,000).

5.1.3 Source of animals: Animals will be collected from Lake Darling, Douglas County, MN.

5.1.4 Inclusion criterion: Native mussels with ≥ 25 attached zebra mussels.

5.1.5 Acclimation: Mussels will be held in the assigned retention barrier ≥12 h prior to exposure initiation.

5.1.6 Distribution to enclosure area: Up to 25 mussels will be distributed to each enclosure area according to a predetermined randomization scheme. Five mussels will be placed in each enclosure replicate in each distribution round.

5.1.7 Feeding: Mussels (native and zebra mussels) will have access to natural food in the water column supplemental feeds will not be provided.

5.2 Water Chemistry

5.2.1 Dissolved oxygen: Dissolved oxygen will be measured (UMESC SOP AEH 394 or equivalent) and recorded in each enclosure replicate during the acclimation and exposure periods. Dissolved oxygen will be measured and recorded at least once during the pre-exposure period and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination. The location of the measurements will be documented in the study records.

5.2.2 Temperature: Temperature will be monitored throughout the pre-exposure, exposure and post-exposure periods using submersible HOBO® data loggers placed in each enclosure. Additionally, temperature will be measured and recorded at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination using a handheld digital thermometer. The location of the measurements will be documented in the study records.

5.2.3 pH: pH will be measured (UMESC SOP AEH 335 or equivalent) and recorded in each enclosure replicate during the pre-exposure and exposure periods. pH will be measured and recorded at least once during the pre-exposure period and at least twice during the exposure with the last measurement observed ≤ 30 minutes prior to exposure termination. The location of the measurements will be documented in the study records.

5.2.4 Hardness: Hardness will be measured (UMESC SOP AEH 712) and recorded prior to exposure initiation.

5.2.5 Alkalinity: Alkalinity will be measured (UMESC SOP AEH 706) and recorded prior to exposure initiation.

5.2.6 Conductivity: Conductivity will be measured (UMESC SOP AEH 188 or equivalent) and recorded prior to exposure initiation.

5.2.7 Ammonia: Samples for total ammonia-nitrogen will be collected in 50-mL centrifuge tubes from the surface of each enclosure at the termination of the exposure period for each exposure replicate. Ammonia samples will be filtered through a 0.45-µm syringe filter, acidified (~pH 2.5) with sulfuric acid and then stored at ~4°C until analyzed by the UMESC Long Term Resources Monitoring (LTRM) Water Quality Laboratory using the automated phenate method.

5.3 Disposal: All live native mussels at the end of the post-exposure observation period will be euthanized by MS-222 overdose and disposed of as required by MN-DNR permit.

5.4 Study facilities:

5.4.1 Test Facility

U.S. Geological Survey, Upper Midwest Environmental Sciences Center

2630 Fanta Reed Rd

La Crosse, Wisconsin 54603

5.4.1.1 Test location: Lake Darling, Douglas County, MN

5.4.1.2 Exposure system: The test system consists of nine replicate 1.5 m^2 enclosures positioned in <2 m of water. The enclosures will be spatially placed are far as feasibly possible along ~ 50m of shoreline to reduce cross contamination. Each enclosure area location will have similar substrate and water depth. Each enclosure area will be uniquely identified and coding procedures and the treatment assignment will be documented in the study records.

5.4.1.3 Aeration: Supplemental aeration will not be provided.

5.4.1.4 Test Water: Exposures will be conducted using natural lake water. Exposures will be conducted within an impermeable membrane suspended around the mussel retention barrier.

5.4.1.5 Treated water discharge: The SDP will be immediately mixed with raw lake water at the termination of the exposure period when the impermeable membranes are withdrawn. The SDP concentrations are expected to rapidly decrease during mixing with the lake water.

5.4.1.6 Lighting: Native mussels will be exposed to a natural photoperiod with no manipulation of the photoperiod.

5.4.1.7 Permits: This study has been verified exempt and does not require an EPA or State of Minnesota Experimental Use Permit.

5.5 Observations:

5.5.1 Mortality: Mussels that are gapping and do not respond to tactile stimuli by shell closure will be coded as a mortality.

5.6 Exposure administration:

5.6.1 Treatment: There will be three treatment groups: 0 [control], 50 or 100 mg/L A.I. A single exposure duration (8-h) will be applied. Each treatment group will have three replicate enclosures.

5.6.2 Route of administration: Exposure will be initiated by addition of an appropriate amount of SDP stock solution followed by a gently mixing to uniformly distribute the SDP.

5.6.3 Concentration verification: Concentration will be determined spectrophotometrically. A standard curve will be prepared using a known mass of SDP. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Barnstead/Thermolyne Corporation Model Turner SP-830 Plus Beckman spectrophotometer (UMESC SOP AEH 302) or equivalent.

6. DATA ANALYSIS

6.1 Experimental unit: The experimental unit will be the enclosure.

6.2 Number of exposures and replicates: There will be a total of 3 treatment levels (0 [control], 50 and 100 mg SDP/L) and 1 treatment duration (8-h). There will be a total of 3 enclosures for each treatment level which will serve as the replicates. All treatment levels and replicates will be run concurrently.

6.3 Statistical methodology:

Post treatment zebra mussel density associations with treatments types will be analyzed using a count model that addresses nesting of density data within enclosures. Pairwise comparison tests will be completed to compare each treatment group to the control group. Count models will be fitted using a log link and SAS® generalized linear mixed modeling procedure (PROC GLIMMIX; SAS® 9.3).

Native Mussel survival data will be analyzed using a binary assumption that addresses nesting of the enclosures. Models will be fitted using SAS®' generalized linear mixed modeling procedure (PROC GLIMMIX; SAS® 9.3). Pairwise comparison tests will be completed to compare each treatment group to the control group.

Change in weight and displacement pre- and post-treatment with treatment types will be analyzed using pairwise comparison tests to compare each treatment group to the control group. Models will be fitted using SAS®' generalized linear mixed modeling procedure (PROC GLIMMIX; SAS® 9.3).

6.4 Statistical significance: Statistical significance will be declared at $p < 0.05$.

6.5 Other data analyses: Statistical methods for other study data collected will include calculation of means, standard deviations and coefficients of variation. The statistical procedures used will be described in detail in the final study report.

7. PERSONNEL

7.1 Study Director: James A. Luoma, B.A.

7.1.1 Address: Upper Midwest Environmental Sciences Center, US Geological Survey, 2630 Fanta Reed Rd., La Crosse, Wisconsin 54603

7.1.2 Contact: Tel: (608) 781-6391, Fax: (608) 783-6066; jluoma@usgs.gov

7.1.3 Training and experience: CV on file at UMESC.

7.2 Other personnel involved in study: Technical staff involved in the study will be identified in the study raw data to include study function. UMESC technical staff training and experience will be documented in CVs included in the study raw data.

8. DISPOSITION/STORAGE

8.1 Study Records: All data generated in the study will be recorded in bound laboratory notebooks, electronic files or kept in file folders. All data sheets, file folders, laboratory notebooks and computer disks will be encoded with the study number when the data are generated. Raw data, laboratory notebooks and electronic files (including a CD-ROM containing the annotated SAS program used for the statistical analysis, the data

files, SAS log and SAS output files) generated by UMESC and contract laboratory reports will be filed in the UMESC archives (SOP No. GEN 007) of the Upper Midwest Environmental Sciences Center, La Crosse Wisconsin, before the final report is signed by the Study Director. The final report will then be signed and archived.

9. AMENDMENT/DEVIATIONS TO THE PROTOCOL

9.1 Protocol amendments: A signed copy of the Study Protocol will be retained on-site. Proposed amendments to the protocol shall be brought to the attention of UMESC Management. When the Study Director and Management agree verbally, the study can proceed with the change. As soon as possible, the Study Director will then prepare a written protocol amendment that is signed by the Study Director and Branch Chief. The amendment then becomes an official part of the protocol.

9.2 Protocol deviations: All deviations from this approved protocol will be documented and reviewed by the Study Director. The Study Director will make a judgment on the impact of the deviations. The Study Director will notify Management, as soon as possible, of any deviations to the protocol, including their impact on the study.

10. INVESTIGATIONAL TEST ARTICLE

10.1 Test Substance(s): *Pseudomonas fluorescens* (*Pf*-CL145A) SDP formulation

10.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

10.1.2 Trade name: Zequanox

10.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A), 50% active ingredient (A.I.) by weight.

10.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

10.1.5 Lot number: Lot number(s) will be included in the test chemical log books, lab notebook, and study files.

10.1.6 Expiration date: As determined by the manufacturer. An aliquot of each lot tested will be returned to the NYSM or MBI at the conclusion of exposures for post-exposure zebra mussel bioassay tests (the standard testing protocol to assess *Pseudomonas fluorescens* [*Pf*-CL145A] formulation activity). Results of these confirmation bioassays will be used to validate the retention of activity of the *Pseudomonas fluorescens* (*Pf*-CL145A) SDP and will be included in the study files when available.

10.1.7 Storage during study: The test chemical will be stored refrigerated. Test material will be transported in a cooler with ice packs to maintain proper storage temperature (4-10 °C)

10.1.8 Safety: A NIOSH approved respirator will be used when preparing stock solutions to avoid inhalation. Protective eyewear, gloves and lab coats will be worn at all times when working with the test substance.

11. ADVERSE EVENTS: Any adverse event will be recorded in the study logbook and the Study Director will be notified.

12. BIOSECURITY PROCEDURES: All personnel involved in the study will review the UMESC biosecurity (UMESC SOP APP 075) and project HACCP plans. Testing will be conducted in a field setting. All equipment will be thoroughly cleaned and disinfected prior to return to UMESC. Waste generated will be placed in plastic garbage bags and disposed of in accordance with local and state regulations. See Appendix 1 for the HACCP plan for this project.

13. STANDARD OPERATING PROCEDURES

A complete list of the standard operating procedures used in the study will be included in the study guide. The follow SOP's were cited in this protocol:

UMESC SOP APP 075 – Procedures to Minimize the Risk of Transfer of Pathogens and Invasive Species
UMESC SOP AEH 188 – Accumet Portable Waterproof Conductivity meter Model # AP75
UMESC SOP AEH 302 – Instrument Operating Procedure: Barnstead/Thermolyne Corporation Model: Turner SP-830 Plus Beckman spectrophotometer Serial # 1365070560781
UMESC SOP AEH 335 – Beckman Portable pH/mV Meter, Model 250
UMESC SOP AEH 394 – YSI Handheld Dissolved Oxygen Meter, Model 55/12FT, Serials 94C17261 & 97F0837AG
UMESC SOP AEH 706 – Determination of Total Alkalinity by the Titrimetric (pH 4.5) Method
UMESC SOP AEH 712 – Determination of Total Hardness

14. REFERENCES.

Burlakova, L.E., 1998. Ecology of *Dreissena polymorpha* (PALLAS) and its role in the structure and function of aquatic ecosystems. Candidate dissertation, Zoology Institute of the Academy of Science Republic Belarus, 168 p. (in Russian) in Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000. The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Haag, W.R., D.L. Berg, D.W. Garton, and J.L. Farris, 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (Dreissena polymorpha) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 50(1):13-19.

Karatayev, A.Y. and L.E. Burlakova, 1995. Present and future patterns in Dreissena population development in the Narochanskaya lakes system. Vestisi Akad. Navuk Belarusi. Ser. Biyol. Navuk 3: 95098 (in Belarussian) in Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000.

The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Master, L., 1990. The imperiled status of North American aquatic animals. Biodiversity Network News 3(3):5-8.

Napela, T.F., 1994. Decline of native unionid bivalves in Lake St. Clair after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51:2227-2233.

Neves R. J., 2004. Propagation of endangered freshwater mussels in North America. Journal of Conchology, special publication 3:69-80.

Neves, R.J., A.E. Bogan, J.D. Williams, S.A. Ahlstedt, and P.W. Hartfield, 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. Pages 43-85 in G.W. Benz and D.E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Southeastern Aquatic Research Institute, Lenz Design and communications, Decatur, Georgia.

Ricciardi, A. F.G. Whoriskey, and J.B. Rasamussen, 1996. Impact of the *Dreissena* invasion on native unionid bivalves in the upper St. Lawerance River. Can. J. Fish. Aquat. Sci. 53:1434-1444.

Ricciardi A., and J.B. Rasmussen, 1999. Extinction rates of North American freshwater fauna. Conservation Biology. Vol. 13 (5):1220-1222.

Sebestyn, O., 1937. Colonization of two new fauna-elements of Pontus-origin (*Dreissena polymorpha* Pall. And *Corophuim curvispinum* G.O. Sars forma devium Wundsch) in Lake Balaton, Verh. Int. Ver. Theor. Angew. Limnol. 8:169-182 in
Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000. The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Williams, J.D., M.L. Warren Jr., K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6-22.

Zequanox: EPA Reg. No.: 84059-15. MSDS [Online]: Marrone Bio Innovations: Davis, CA. http://www.marronebioinnovations.com/products/labelsmsds/zequanox-labelsmsds (accessed Jan. 3, 2013).

15. APPENDIX.

15.1 Appendix 1. HACCP PLAN for the study Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures

Step 1 – Activity Description

Facility: US Geological Survey-Upper Midwest Environmental Sciences Center	Site: Various – Keyes Lake, Florence, WI
Site Coordinator: Jim Luoma	Activity: Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures
Site Manager: Mark Gaikowski	
Address: 2630 Fanta Reed Road La Crosse WI, 54601	
Phone: 608-781-6322	

Project Description
The objective of this study is to assess the efficacy and non-target native mussel safety of two exposure concentrations of SDP for controlling settled zebra mussels (*D. polymorpha*) in open water enclosures

Step 2 – Potential Hazards: Species which may potentially be moved/introduced

Vertebrates: Multiple species of freshwater fish, eggs and gametes found in the Great Lakes Basin.
Invertebrates: Zebra mussel (*Dreissena polymorpha*) Multiple endemic species found in the Great Lakes Basin
Plants: Multiple endemic species found in the Great Lakes Basin
Other biologicals (disease, pathogen, parasite): Largemouth Bass Virus Spring Viremia of Carp Virus Bluegill Virus Infectious Pancreatic Necrosis Virus Viral Hemorrhagic Septicemia Furunculosis *Aeromonas salmonicida* Enteric Redmouth Disease *Yersinia ruckeri* Bacterial Kidney Disease *Renibacterium salmoninarum* Other Assorted parasites/pathogens found in the Great Lakes Basin
Other: NA

Step 3 – Flow Diagram

Flow diagram outlining sequential tasks to complete activity/project

Task 1	Enclosure test sites identified and retention barriers placed in Keyes Lake, WI
↓	
Task 2	Native mussels with attached zebra mussels are obtained via SCUBA or wading from Keyes Lake, WI
↓	
Task 3	Native mussels are place in enclosures and acclimated to test conditions with impermeable barriers lowered
↓	
Task 4	Native mussels are exposed to test article for 8-h
↓	
Task 5	Impermeable barriers are removed and native mussels left undisturbed for post-exposure holding
↓	
Task 6	Equipment is decontaminated and returned to UMESC
↓	
Task 7	Native mussels are removed from enclosures and assessed for zebra mussel colonization and survival
↓	
Task 8	Native mussels are cleaned of zebra mussels and returned to the approximate collection location
↓	
Task 9	All remaining equipment, retention barriers, etc. are decontaminated and returned to UMESC

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards probable? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 1 Enclosure test sites identified and retention barriers placed in Lake Darling, MN	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Invertebrates	yes	Surface water contains multiple invertebrate species include AIS	Assure the thorough decontamination all equipment with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no
	Plants	yes	Surface water contains multiple plant species including AIS	Assure the thorough decontamination all equipment with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted. Do not proceed if any fish disease outbreak (i.e.: VHS) is documented in the waterbody	yes

Task 2 Native mussels with attached zebra mussels are obtained via SCUBA or wading from Keyes Lake, WI	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted. Do not proceed if any fish disease outbreak (i.e.: VHS) is documented in the waterbody	no

Task 3 Native mussels are place in enclosures and acclimated to test conditions with impermeable barriers lowered	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

Task 4 Native mussels are exposed to test article for 8-h	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No

Task 5 Impermeable barriers are removed and native mussels left undisturbed for post-exposure holding	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	No

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again. Inspect all equipment prior to set up and repeat decontamination if warranted.	no

Task 6 Equipment is decontaminated and returned to UMESC	Vertebrates	yes	Surface water contains multiple vertebrate species	Physical removal of all visible vertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes
	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Physical removal of all visible invertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes

	Plants	yes	Surface water contains multiple plant species and may include AIS	Physical removal of all visible plants. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes
	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes

Task 7 Native mussels are removed from enclosures and assessed for zebra mussel colonization and survival	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no
	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no

Task 8 Native mussels are cleaned of zebra mussels and returned to their approximate place of collection	Vertebrates	yes	Surface water contains multiple vertebrate species	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no
	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no
	Plants	yes	Surface water contains multiple plant species and may include AIS	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no

	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	no

Task 9 All remaining equipment, retention barriers, etc. are decontaminated and returned to UMESC	Vertebrates	yes	Surface water contains multiple vertebrate species	Physical removal of all visible vertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed aga Physical removal of all visible vertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes

	Invertebrates	yes	Surface water contains multiple invertebrate species and may include AIS	Physical removal of all visible invertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes
	Plants	yes	Surface water contains multiple plant species and may include AIS	Physical removal of all visible plants. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	Yes
	Others	yes	Surface water has potential to transfer fish diseases	Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location, unless impractical, then decontamination shall be done as soon as possible and must be done before equipment is deployed again.	yes

HACCP Plan Form (all CCP's or "yes's" from column 6 of HACCP Step 4 – Hazard Analysis Worksheet)								
			Monitoring					
Critical Control Point (CCP)	**Significant Hazard(s)**	**Limits for each Control Measure**	**What**	**How**	**Frequency**	**Who**	**Evaluation & Corrective Action(s) (if needed)**	**Supporting Documentation (if any)**
Tasks 1, 2, 3,6,7,8 & 9	Transfer of endemic and AIS including vertebrates, invertebrates, plants and pathogens	Transfer of vertebrates, invertebrates, plants and pathogens must not occur. All equipment must be thoroughly inspected and disinfected as soon as possible and inspected and/or re-decontaminated prior to setup at new location.	Equipment disinfection	Mechanical cleaning/removal, pressure washing, steam cleaning, chemosterilant or other approved methods.	Prior to equipment arrival, upon deployment, prior to movement from location or asap	Technicians/lead field supervisor	Supervisor and staff are responsible for methodical decontamination using established procedures. Corrective actions required to complete decontamination must be performed prior to any equipment movement from test location or as soon as feasible. Decontamination and inspection must be completed asap	Records in log books all procedures used for decontamination

Facility: Upper Midwest Environmental Sciences Center Mobile Research Laboratory	**Activity:** Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures
Address: 2630 Fanta Reed Road, La Crosse, WI 54601	
Signature: HACCP Plan was followed.	**Date:**

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

Date: February 19, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Note To File # 1; Study number AEH-13-PSEUDO-05

This note is to document and clarify foot biopsy collection and resulting data use.

A 10-mg foot sample was collected from each unionid test animal using a biopsy needle during the post-exposure assessments conducted August 13-15, 2013. The biopsy samples were placed in individually labeled cryogenic vials and then stored in liquid nitrogen. After the assessment, the unionids were consolidated into retention barriers for an additional holding period of 26 days. A second foot biopsy sample was taken from each surviving mussel unionid on September 10, 2013.

Pending funding, the foot biopsy samples will be analyzed for glycogen content and will be compared to unionid survival and treatment. If completed, a separate report will be generated for these data.

Due to confound factors (i.e. additional handling stress, biopsy stress) the data collected at the termination of the second holding period (i.e. survival) will be retained but not used during analysis.

[redacted] 14 FEB 2014
Date

Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted]d by 2/14/2014
Date

James A. Luoma, B.A.
Study Director, UMESC

File Folder: 3

Item Number: 2

Study Number: AEH-13-PSEUDO-05 Reviewed by: ___ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Test Chemical Stock Preparation

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: _____ Date Rec'd: _____ Exp. Date: _____

Test Location: _____ Instruments Used: _____

Weights of Chemical Samples:

Sample ID	Sample Weight	Comments	Date	Initials

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

Stock Solution Preparation:

Sample ID	Dilution Volume (mL)	Dilution Time	Use	Exposure Time	Date	Initials

FF # 12
Item No. 1
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
1	C379				
1	C358				
1	C414				
1	C237				
1	C221				
1	C312				
1	C292				
1	C350				
1	C380				
1	C233				
1	C377				
1	C424				
1	C314				
1	C263				
1	C363				
1	C309				
1	C208				
1	C228				
1	C266				
1	C195				
1	C360				
1	C359				
1	C302				
1	C207				
1	C345				
1	C245				
1	C329				

FF # 17
Item No. 2
Pg 1 of 9

Study Number: AEH-13-PSEUDO-C5 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
2	C321				
2	C276				
2	C249				
2	C310				
2	C242				
2	C406				
2	C375				
2	C232				
2	C436				
2	C293				
2	C419				
2	C342				
2	C304				
2	C187				
2	C332				
2	C217				
2	C203				
2	C331				
2	C298				
2	C317				
2	C408				
2	C188				
2	C395				
2	C410				
2	C278				
2	C219				
2	C235				

Study Number: AEH-13-PSEUDO-05
File Folder: _____
Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 | Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A | Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
3	C434				
3	C374				
3	C197				
3	C382				
3	C418				
3	C194				
3	C210				
3	C325				
3	C218				
3	C243				
3	C405				
3	C354				
3	C437				
3	C311				
3	C427				
3	C326				
3	C364				
3	C193				
3	C204				
3	C384				
3	C393				
3	C231				
3	C254				
3	C373				
3	C390				
3	C246				
3	C215				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
4	C282				
4	C255				
4	C296				
4	C412				
4	C239				
4	C283				
4	C285				
4	C225				
4	C351				
4	C388				
4	C339				
4	C429				
4	C403				
4	C404				
4	C270				
4	C279				
4	C286				
4	C421				
4	C353				
4	C398				
4	C333				
4	C348				
4	C223				
4	C271				
4	C303				
4	C389				
4	C334				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
5	C256				
5	C407				
5	C190				
5	C284				
5	C238				
5	C240				
5	C244				
5	C253				
5	C335				
5	C300				
5	C352				
5	C294				
5	C320				
5	C433				
5	C330				
5	C368				
5	C392				
5	C236				
5	C397				
5	C259				
5	C365				
5	C290				
5	C425				
5	C343				
5	C214				
5	C431				
5	C265				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013		Test Location: Lake Darling, Alexandria, MN			
Test Chemical: Pf-CL145A		Lot #: TR4669-4-(3)			
Enclosure ID	**Tag ID**	**Mussel Species**	**Comments**	**Date**	**Initials**
6	C297				
6	C362				
6	C385				
6	C250				
6	C280				
6	C423				
6	C356				
6	C200				
6	C396				
6	C281				
6	C322				
6	C251				
6	C313				
6	C252				
6	C308				
6	C277				
6	C336				
6	C402				
6	C315				
6	C213				
6	C205				
6	C426				
6	C378				
6	C383				
6	C372				
6	C307				
6	C316				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
7	C357				
7	C299				
7	C273				
7	C257				
7	C301				
7	C349				
7	C355				
7	C361				
7	C401				
7	C248				
7	C260				
7	C413				
7	C306				
7	C275				
7	C268				
7	C247				
7	C261				
7	C394				
7	C272				
7	C234				
7	C319				
7	C417				
7	C212				
7	C370				
7	C347				
7	C289				
7	C192				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
8	C369				
8	C189				
8	C415				
8	C269				
8	C274				
8	C191				
8	C186				
8	C211				
8	C337				
8	C399				
8	C366				
8	C435				
8	C196				
8	C428				
8	C222				
8	C391				
8	C198				
8	C230				
8	C199				
8	C216				
8	C264				
8	C241				
8	C324				
8	C387				
8	C340				
8	C209				
8	C220				

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
9	C327				
9	C338				
9	C323				
9	C305				
9	C227				
9	C229				
9	C376				
9	C206				
9	C411				
9	C262				
9	C226				
9	C344				
9	C346				
9	C422				
9	C420				
9	C202				
9	C430				
9	C409				
9	C295				
9	C287				
9	C258				
9	C367				
9	C386				
9	C328				
9	C416				
9	C381				
9	C432				

FF # 17
Item No. 2
Pg 9 of 9

Study Number: AEH-13-PSEUDO-05

File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____

Verified by: _____ Date: _____

Mussel Distribution Data

Exposure Date: _____ Mussel Species: _____ Test Location: _____

Test Chemical: Pf-CL145A SDP Lot #:

Enclosure ID	Distribution Round 1	Distribution Round 2	Distribution Round 3	Distribution Round 4	Distribution Round 5	Date & Initials
1						
2						
3						
4						
5						
6						
7						
8						
9						

FF # 11
Item No. 2
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

Water Quality

Exposure Start Time/Date: _____
Test Chemical: Pf-CL145A SDP
Sampling Time: _____

Test Location: _____
Lot Number: _____
Instruments Used: _____

Enclosure ID	Treatment Level (mg/L)	Enclosure Depth (m)	Enclosure Volume (L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Date	Initials
3	0							
7	0							
8	0							
4	50							
5	50							
2	50							
1	100							
9	100							
6	100							

Comments: _____

FF # 17
Item No. 4
Pg 1 of 1

Study Number AEH-13-PSEUDO-05

Reviewed by: _____ Date: _____

Verified by: _____ Date: _____

Exposure Date: _____ Instruments: _____

Test Chemical: Pf-CL145A SDP Lot Number: _____ Test Location: _____

Alkalinity

Enclosure ID	Treatment Level (mg/L)	Initial pH	Initial Temp (°C)	mL of 0.02 N H_2SO_4	Multiplication Factor	Alkalinity[1] (mg/L $CaCO_3$)	Sample Time	Date	Initials
3	0				10				
7	0				10				
8	0				10				
4	50				10				
5	50				10				
2	50				10				
1	100				10				
9	100				10				
6	100				10				

[1] Alkalinity in mg/L CaCO3 = (mL 0.02N H2SO4 used) x (Multiplication Factor of 10)

Alkalinity Sample volume = 100 mL

Comments: _____

FF # 17
Item No. 5
Pg 1 of 1

Study Number AEH-13-PSEUDO-05

Reviewed by: _____ Date: _____

Verified by: _____ Date: _____

Exposure Date: _____ Instruments: _____

Test Chemical: Pf-CL145A SDP Lot Number: _____ Test Location: _____

Conductivity and Hardness

Enclosure ID	Treatment Level (mg/L)	Conductivity (µS/cm)	mL of 0.01 M EDTA	Multiplication Factor	Hardness[1] (mg/L $CaCO_3$)	Sample Time	Date	Initials
3	0			20				
7	0			20				
8	0			20				
4	50			20				
5	50			20				
2	50			20				
1	100			20				
9	100			20				
6	100			20				

[1]Hardness in mg/L $CaCO_3$ = (mL of 0.01 M Na_2EDTA titrant added to the sample) x (multiplication factor of 20).

Hardness Sample volume = 50 mL

Comments: _____

FF # 17
Item No. 6
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

HOBO Data

Exposure Start Time/Date: _____ Test Location: _____

Test Chemical: Pf-CL145A SDP Lot Number: _____

Sampling Time: _____ Instruments Used: _____

Enclosure ID	Treatment Level (mg/L)	Enclosure Depth (m)	Enclosure Volume (L)	HOBO S/N	Date	Initials
3	0					
7	0					
8	0					
4	50					
5	50					
2	50					
1	100					
9	100					
6	100					

Comments: _____

FF # 12
Item No. 3
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folcer: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Spectrophotometric Data

Exposure Start Time/Date: _____	Test Location: _____
Test Chemical: Pf-CL145A SDP	Lot Number: _____
Sampling Time: _____	Instruments Used: _____
Wavelength: _____	Analyst: _____

Calculation of Regression Coefficient for a standard curve

Std Conc. (mg/L)	Spec abs
25	
25	
25	
50	
50	
50	
100	
100	
100	
200	
200	
200	

Pf-CL145A SDP Treatment Concenteration Verification

Sample ID	Enclosure Number	Treatment Level (mg/L)	Sample Time (h)	Sample Location	Observed Spec abs	Date & Initials

FF # 17
Item No. 8
Pg 1 of 2

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Spectrophotometric Data

Exposure Start Time/Date: _____	Test Location: _____
Test Chemical: Pf-CL145A SDP	Lot Number: _____
Sampling Time: _____	Instruments Used: _____
Wavelength: _____	Analyst: _____

Pf-CL145A SDP Treatment Concenteration Verification

Sample ID	Enclosure Number	Treatment Level (mg/L)	Sample Time (h)	Sample Location	Observed Spec abs	Date & Initials

FF # 17
Item No. 6
Pg 2 of 2

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Initial Assessment of Native and Zebra Mussels

Exposure Date: _____ Mussel Species: _____ Test Location: _____

Test Chemical: Pf-CL145A SDP Lot #: _____

Tag ID[1]	Enc. ID	Treat Level (mg/L)	with Zebra Mussels			without Zebra Mussels				Number of Zebra Mussels	Date & Initials
			Weight (g)	Initial Headspace (mL)	Final Headspace (mL)	Shell Length (mm)	Weight (g)	Initial Headpace (mL)	Final Headspace (ml)		

[1] - Tag Prefix Identification: A = White tag; B = Yellow tag; C = Green tag; D = Blue tag

Comments: _____

FF # 17 Item No. 2 Pg 1 of 1

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Mussel Initial Assessment

Exposure Date: _____ Test Location: _____

Test Chemical: Pf-CL145A SDP Lot #: _____

Mussel Species	Tag ID[1]	Initial Weight (g)	Initial Water Displaced (mL)	Comments	Date & Initials

[1] - Tag Prefix Identification: A = White tag; B = Yellow tag; C = Green tag; D = Blue tag

FF # 17
Item No. 10
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

Mussel Weight and Length Assessment

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: *Pf*-CL145A Lot #: TR4669-4-(3) Assessment Date: _____

Mussel Species	Tag ID[1]	Enclosure ID	Treatment Level (mg/L)	Weight w/Zebra Mussels (g)	Weight w/o Zebra Mussels (g)	Shell Length (mm)	Comments	Date & Initials

[1] - Tag Prefix Identification: A = White tag; B = Yellow tag; C = Green tag; D = Blue tag

FF # 11
Item No. 11
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

Mussel Survival Assessment

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: *Pf*-CL145A Lot #: TR4669-4-(3) Assessment Date:

Mussel Species	Tag ID[1]	Enclosure ID	Treat Level (mg/L)	Native Mussel Status[1]	Live Zebra Count	Dead Zebra Count	Date	Initials

[1] - Status is: Alive/Dead/Unrecovered

Comments:

FF # 17-
Item No. 12
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Verified by: _____ Date: _____

Lab book/pgs: _____

Mussel Final Survival Assessment

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Final Assessment: Date: September 9 to 11, 2013

Tag ID	Native Mussel Status[1]	Number of Translocated Zebra Mussels	Comments	Date	Initials

[1] - Status is: Alive or Dead

FF # 17
Item No. 13
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Zebra Mussel Lengths

Test Organism: Zebra Mussels Exposure Date: 26 JUL 2013

Test Chemical: Pf-CL145A SDP Lot #: TR4669-4-3 (run 4; sample 3)

Test Location: Lake Darling, Alexandria, MN

Mussel Tag ID	Enclosure ID	Treatment Level (mg/L)	Zebra Mussel Number	Shell Length (mm)	Date	Initials

FF # 17
Item No. 14
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: __________ Lab book/pgs: __________
Reviewed by: __________ Date: __________
Verified by: __________ Date: __________

Assessment Zebra Mussel Lengths

Test Organism: Zebra Mussels Exposure Date: 26 JUL 2013
Test Chemical: PF-CL145A SDP Lot #: TR4669-4-3 (run 4; sample 3)
Test Location: Lake Darling, Alexandria, MN

Mussel Tag ID	Enclosure ID	Treatment Level (mg/L)	Status (Alive/Dead)	Zebra Mussel Number	Shell Length (mm)	Date	Initials

FF # 17
Item No. 15
Pg 1 of 1

Study Number: AEH-13-PSEUDO-05
File Folder: _____ Lab book/pgs: _____

Reviewed by: _____ Date: _____
Verified by: _____ Date: _____

Mussel Volume Displacement Assessment

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: *Pf*-CL145A Lot #: TR4669-4-(3) Assessment Date: _____

Mussel Species	Tag ID[1]	Enclosure ID	Treat Level (mg/L)	With Zebra Mussels		Without Zebra Mussels		Comments	Date & Initials
				Initial Headspace (mL)	Final Headspace (mL)	Initial Headspace (mL)	Final Headspace (mL)		

[1] - Tag Prefix Identification: A = White tag; B = Yellow tag; C = Green tag; D = Blue tag

FF # 13
Item No. 15
Pg 1 of 1

Appendix 2. Deviations From the Study Protocol

Item number	Item description	Number of pages	Report page number
1	Deviation 1: Early termination of holding period	1	76
2	Deviation 2: Unrecovered unionid mussels during post-exposure assessment	1	77
3	Deviation 3: Mussel #C331 assessed twice	1	78
4	Deviation 4: Discontinuation of volume displacement	1	79
5	Deviation 5: Unequal ratios of Fragile Papershell (*Leptodea fragilis*) mussels in the study	2	80
6	Filamentous algae report from John Manier	5	82

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 18, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Deviation 1 to study AEH-13-PSEUDO-05

Deviation #1 – Early termination of holding period

Sections 3.3, 4.1, and 4.2 of study protocol (study number AEH-13-PSEUDO-05; study title "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures") state that ~30 d after exposure, the native mussels will be assessed for survival and zebra mussel colonization.

Twelve days post-exposure (August 7, 2013), the status of the mussel retention barriers was assessed due to concerns with filamentous algae within Lake Darling. Heavy infestations of filamentous algae were observed and subsequently removed from each retention barrier. Due to potential for suffocation and poor water quality related to the filamentous algae bloom, the study was terminated early at ~18 days post-exposure on August 13-15, 2013.

Samples of the algae were collected between August 13 and 15, 2013 and were characterized as being dominated by *Mougeotia* and *Spirogyra*. Other genera were present, but to a lesser extent, including *Oedogonium* , *Zygnema*, and *Lyngbya*. A copy of the report can be found in the study records (FF 4: Correspondence).

The effects of the filamentous algae bloom are indeterminate, however, zebra mussel survival in the controls was excellent (> 91.4%) indicating that observed zebra mussel mortality was likely treatment related. The potential impacts to the study will be evaluated and any impacts to the study will be noted in the final report.

[redacted] 18 FEB 2014
Date

Kerry L. Weber, M.S.
Principal Investigator,
UMESC

[redacted] 2/18/14
Date

James A. Luoma, B.A.
Study Director,
UMESC

File Folder: 3

Item Number: 3

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 18, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Deviation 2 to study AEH-13-PSEUDO-05

Deviation #2 – Unrecovered unionid mussels during post-exposure assessment

During the post-exposure assessment, unionid mussels from each treatment replicate (i.e. enclosure) were assessed for survival and zebra mussel colonization. Two unionid mussels (C356 from enclosure 6 [100 mg/L]; C323 from enclosure 9 [100 mg/L]) were not recovered.

Mussels C356 and C323 may have been inadvertently removed from the enclosures during algae removal on August 7, 2013 (see study deviation #1). The unionid mussels will be coded as mortalities within the survival analysis. The initial zebra mussel colonization data may be removed from analysis. The exact details of how these data are treated will be noted in the data summaries.

No adverse impacts to the study are anticipated as a result of this deviation. Any impacts to the study will be noted in the final report.

[redacted] 18 FEB 2014
Date

Kerry L. Weber, M.S.
Principal Investigator,
UMESC

[redacted] by 2/18/14
Date

James A. Luoma, B.A.
Study Director,
UMESC

File Folder: 3

Item Number: 4

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 18, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Deviation 3 to study AEH-13-PSEUDO-05

Deviation #3 – Mussel #C331 assessed twice

During the post-exposure unionid mussel assessment, native mussel C331 (Enclosure 2 [50 mg/L]) was assessed for survival and zebra mussel colonization following the procedure outlined in the laboratory notebook on August 13, 2013. Mussel C331 was inadvertently left in a retention barrier used to temporarily hold the mussels prior to assessment and mussels from Enclosure 9 (100 mg/L) were placed in the same retention barrier as mussel C331. As a result, mussel C331was assessed for a second time with the mussels from Enclosure 9. The error was discovered during data analysis and data collected for mussel C331 during the Enclosure 9 assessment will be removed from analysis.

This error also clarifies the logbook entry made on August 15, 2013, which indicated that a single mussel from the retention barrier containing the native mussels assessed on August 13, 2013 was not recovered before being placed into the new holding area.

There are no adverse impacts to the study as the necessary data was collected for mussel C331.

[redacted] 18 FEB 2014
Date

Kelly L. Weber, M.S.
Principal Investigator,
UMESC

[redacted]d by 2/18/14
Date

James A. Luoma, B.A.
Study Director,
UMESC

File Folder: 3

Item Number: 5

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 18, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Deviation 4 to study AEH-13-PSEUDO-05

Deviation #4 – Discontinuation of volume displacement

Section 4.2 of the study protocol (study number AEH-13-PSEUDO-05) states that individual mussels will be tagged, photographed on each side, weighed and their water displacement measured to assist with individual tracking and estimating pre- and post-treatment zebra mussel colonization.

The apparatus constructed and initially used to measure the water displacement of the unionids leaked and was found to be inconsistent. Therefore, during the post-exposure assessment, water displacement measurements were discontinued. The data will be retained but will not be used during analysis.

There are no adverse impacts to the study anticipated from the discontinuation of water displacement as the wet weights will be used to estimate the pre-exposure zebra mussel colonization. Any impacts to the study will be noted in the final report.

[redacted] 18FEB2014
Date

Kerry L. Weber, M.S.
Principal Investigator,
UMESC

[redacted]d by 2/18/14
Date

James A. Luoma, B.A.
Study Director,
UMESC

File Folder: 3

Item Number: 6

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

MEMORANDUM

Date: February 18, 2014
To: The Record Study Number AEH-13-PSEUDO-05

Subject: Deviation 5 to study AEH-13-PSEUDO-05

Deviation #5 – Unequal ratios of Fragile Papershell (*Leptodea fragilis*) mussels in the study

Sections 4.2 and 5.1.1.3 of the protocol states that "if multiple species are used, native species will be equally distributed in equal ratios to the treatment enclosures". A total of 243 unionid mussels were included in the study. During collection and tagging of test animals for inclusion in the study, fatmucket (*Lampsilis siliquoidea*) was the dominate species, however, positive identification to species was difficult due to heavy zebra mussel colonization. Therefore, the mussels were equally distributed to enclosures regardless of species.

At the conclusion of the study, the unionid mussel shells (with their associated identification tag) were retained for species confirmation. Species confirmation was completed based on the structure of the pseudocardinal teeth, umbo, and shell thickness. Fatmucket (FAM) mussels were the dominate species while fragile papershell (FPS) mussels were rare (i.e., 231 FAM versus 12 FPS). Enclosure, treatment level and tag numbers of fragile papershell mussels test animals are summarized in Table 1. Data collected for fragile papershell will be used for analysis.

File Folder: 3

Item Number: 7

Table 1. Fragile papershell mussels present in the study

Enc.	Treatment Level (mg/L)	Mussel Tag
1	100	C363
		C207
2	50	C342
		C331
4	50	C398
5	50	C335
6	100	C362
		C336
		C307
7	Control	C394
9	100	C344
		C386

There are no adverse impacts to the study anticipated as the primary study objective was to determine the efficacy of formulated *Pf*-CL145a for removal of zebra mussels adhering to unionid mussels. Any impacts to the study will be noted in the final report.

[redacted] 18 FEB 2014
Date

Kerry L. Weber, M.S.
Principal Investigator,
UMESC

[redacted] by 2/18/14
Date

[redacted] Luoma, B.A.
Study Director,
UMESC

File Folder: 3

Item Number: 7

Lab Technician: John Manier

Sample ID: AEH-13-PSUEDO-05

AEH-13-PSEUDO-05

Sample Date: August 13th and 15th, 2013

Location: Lake Darling

Preservation: Two samples (1000 mL) were preserved with Lugol's Solution; two samples (1000 mL) were un-preserved but refrigerated.

Method: A wet mount was created using a sub-sample (approx. 1 mL). The sub-sample was then analyzed using a Nikon Eclipse E600 microscope with attached camera (Q Imaging, Micropublisher 3.3 RTV). The microscope was also equipped with 10, 20, and 40X magnifications, as well as phase contrast.

Results: This is a typical periphyton community, consisting of filamentous green algae, attached diatoms, and protozoa. The algal community was dominated by *Mougeotia* and *Spirogyra* (Figures 1-2). Other genera were present, but to a lesser extent, including *Oedogonium* , *Zygnema*, and *Lyngbya* (Figures 3-6). The diatoms commonly seen were *Gomphonema* and *Cymbell*a (Figures 7-8).

FF # 4
Item No. 4
Pg 1 of 5

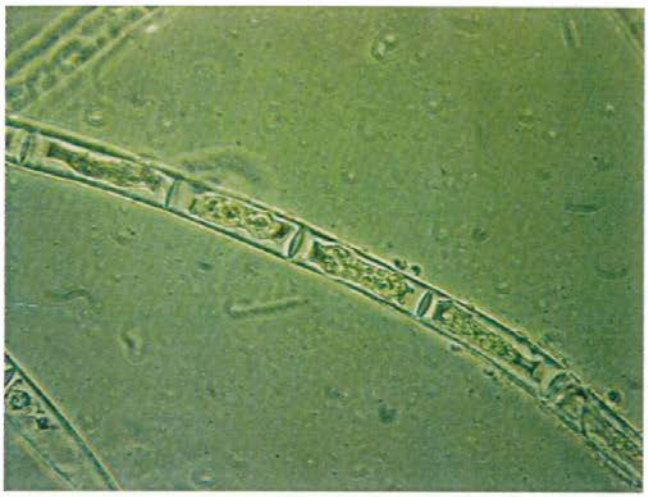


Figure 1. *Mougeotia*- Contains a plate-like chloroplast, which rotates within the cell to gain better access to light. Widely known to create large, nuisance growths, especially in waters affected by acidification.

Figure 2. *Spirogyra*- Identified by the spiral-shaped chloroplast. They are very common within periphytic mats.

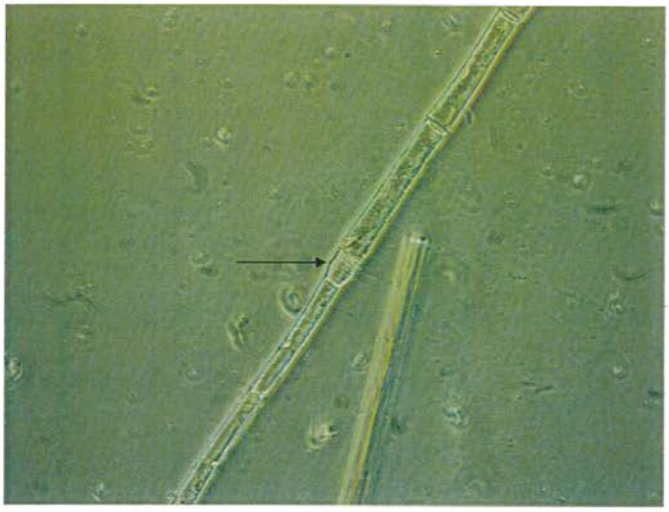


Figure 3. *Oedogonium*- Distinguished by the annular "rings", which denote the number of times it has reproduced.

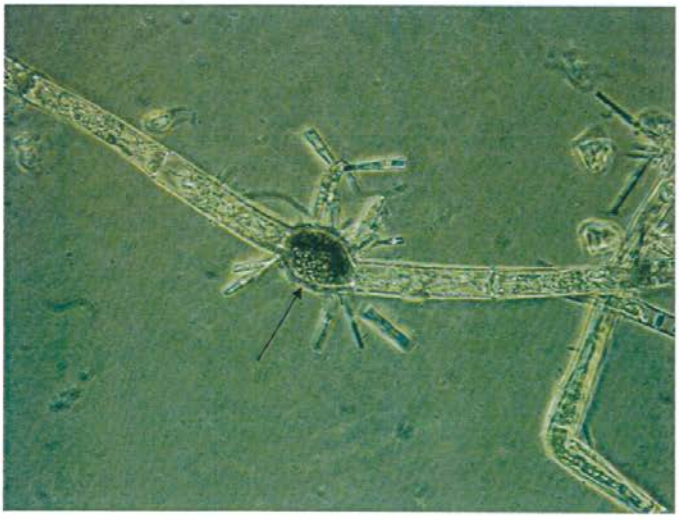


Figure 4. *Oedogonium* filament with attached zygote, formed during reproduction. The zygote is covered with stalked diatoms.

Figure 5. *Zygnema*- Distinguished by the axial, stellate, and paired chloroplasts.

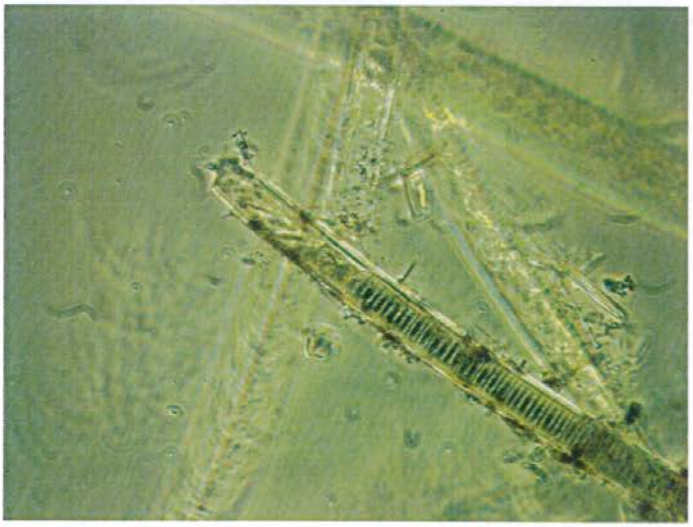


Figure 6. *Lyngbya*- Members of this genus have long, unbranching filaments with a mucilage sheath. They are widely known to create large, benthic mats.

AEH-13-PSEUDO-05

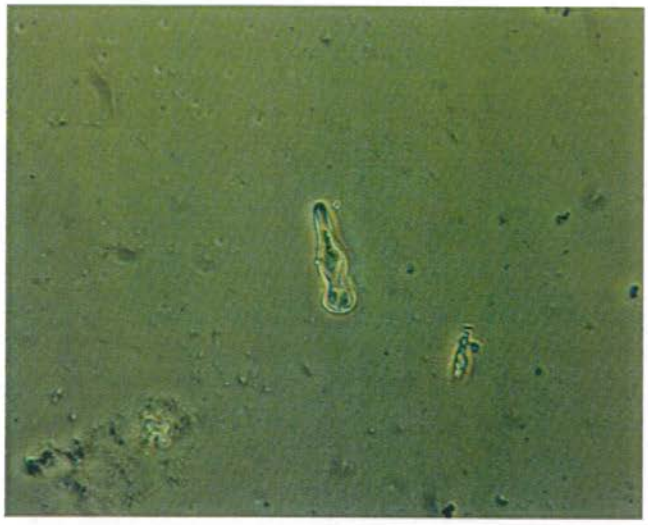


Figure 7. *Gomphonema*- All species are symmetrical to the apical axis and asymmetrical to the transapical axis. Many attach themselves to the periphyton using a stalk.

Figure 8. *Cymbella*- Members of this genus usually grow in benthic habitats via an attached stalk.

FF # 4
Item No. 4
Pg 5 of 5

Appendix 3. Randomization Assignments

Item number	Item description	Number of pages	Report page number
1	SAS output for random assignment of treatment to experimental enclosures	1	88
2	SAS program for random assignment of treatment to experimental enclosures	2	89
3	SAS log for random assignment of treatment to experimental enclosures	2	91
4	SAS output for random assignment of mussels to enclosures	2	93
5	SAS program for random assignment of mussels to enclosures	1	95
6	SAS log for random assignment of mussels to enclosures	2	96

Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling zebra mussels within field enclosures
AEH-13-PSUEDO-05
Random assignment of treatment to experimental enclosures
Lake Darling, Alexandria, MN

7/12/13
Jo

Obs	block	tank	x	tankn	trt
1	1	3	0.02019	Enclosure 3	control
2	1	7	0.10071	Enclosure 7	control
3	1	8	0.13853	Enclosure 8	control
4	1	4	0.28485	Enclosure 4	50
5	1	5	0.34767	Enclosure 5	50
6	1	2	0.44693	Enclosure 2	50
7	1	1	0.79889	Enclosure 1	100
8	1	9	0.85987	Enclosure 9	100
9	1	6	0.88438	Enclosure 6	100

AEH-13-PSEUDO-05

Analysis performed by J. Luoma SAS version 9.3 12:28 12JUL13

FF # 9
Item No. 1
Pg 1 of 1

```
/*********************************************************************
*  Study Number  : AEH-13-PSUEDO-05
*  Study Director: Jim Luoma
*  date created  : July 12, 2013 - JAL
*  Verified by:  _____ (Date:_____)
*  Random allocation of treatment to enclosure.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental enclosure*/
/*AEH-PSEUDO-05; Zequanox native mussel enclosure treatment, Lake Darling, Alexandria, MN*/
data MUS;
do block = 1 to 1 by 1;
do tank = 1 to 9 by 1;
     x = ranuni(-1);
   output;
  end;
 end;
run;
data MUS2; set MUS;
 if block = 1 and tank = 1 then tankn = 'Enclosure 1';
  if block = 1 and tank = 2 then tankn = 'Enclosure 2';
   if block = 1 and tank = 3 then tankn = 'Enclosure 3';
    if block = 1 and tank = 4 then tankn = 'Enclosure 4';
     if block = 1 and tank = 5 then tankn = 'Enclosure 5';
      if block = 1 and tank = 6 then tankn = 'Enclosure 6';
       if block = 1 and tank = 7 then tankn = 'Enclosure 7';
        if block = 1 and tank = 8 then tankn = 'Enclosure 8';
         if block = 1 and tank = 9 then tankn = 'Enclosure 9';

if block = 2 and tank = 1 then tankn = 'Enclosure 1';
  if block = 2 and tank = 2 then tankn = 'Enclosure 2';
   if block = 2 and tank = 3 then tankn = 'Enclosure 3';
    if block = 2 and tank = 4 then tankn = 'Enclosure 4';
     if block = 2 and tank = 5 then tankn = 'Enclosure 5';
      if block = 2 and tank = 6 then tankn = 'Enclosure 6';
       if block = 2 and tank = 7 then tankn = 'Enclosure 7';
        if block = 2 and tank = 8 then tankn = 'Enclosure 8';
         if block = 2 and tank = 9 then tankn = 'Enclosure 9';

if block = 3 and tank = 1 then tankn = 'Enclosure 1';
  if block = 3 and tank = 2 then tankn = 'Enclosure 2';
   if block = 3 and tank = 3 then tankn = 'Enclosure 3';
    if block = 3 and tank = 4 then tankn = 'Enclosure 4';
     if block = 3 and tank = 5 then tankn = 'Enclosure 5';
      if block = 3 and tank = 6 then tankn = 'Enclosure 6';
       if block = 3 and tank = 7 then tankn = 'Enclosure 7';
        if block = 3 and tank = 8 then tankn = 'Enclosure 8';
         if block = 3 and tank = 9 then tankn = 'Enclosure 9';

    run;
proc sort data=MUS2;
```

AEH-13-PSEUDO-05

page 1 of 2

FF # 9
Item No. 2
Pg 1 of 2

```
 by x;
run;

data assign_trt_MUS; set MUS2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = 'control';
   if _n_ = 3 then trt = 'control';
    if _n_ = 4 then trt = '50';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '50';
   if _n_ = 7 then trt = '100';
    if _n_ = 8 then trt = '100';
     if _n_ = 9 then trt = '100';
    run;
proc print data= assign_trt_MUS;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling zebra mussels within 1
title2 h=1.5 'AEH-13-PSUEDO-05';
title3 h=1 'Random assignment of treatment to experimental enclosures';
title4 h=1 'Lake Darling, Alexandria, MN';
run;
```

SM 7-12-11

Page 2 of 2

AEH-13-PSEUDO-05

FF # 9
Item No. 2
Pg 2 of 2

```
156  *  date created  : July 12, 2013 - JAL
157  *  Verified by:  _____ (Date:_____)                    page 1 of 2
158  *  Random allocation of treatment to enclosure.sas
159  ************************************************************************/    AEH-13-PSEUDO-05
160  DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
161
162  FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.
163
164  options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;
165
166  /*Random assignment of treatment to experimental enclosure*/
167  /*AEH-PSEUDO-05; Zequanox native mussel enclosure treatment, Lake Darling, Alexandria, MN*/
168  data MUS;
169  do block = 1 to 1 by 1;
170  do tank = 1 to 9 by 1;
171       x = ranuni(-1);
172     output;
173    end;
174   end;
175  run;

NOTE: The data set WORK.MUS has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


176  data MUS2; set MUS;
177   if block = 1 and tank = 1 then tankn = 'Enclosure 1';
178    if block = 1 and tank = 2 then tankn = 'Enclosure 2';
179     if block = 1 and tank = 3 then tankn = 'Enclosure 3';
180      if block = 1 and tank = 4 then tankn = 'Enclosure 4';
181       if block = 1 and tank = 5 then tankn = 'Enclosure 5';
182        if block = 1 and tank = 6 then tankn = 'Enclosure 6';
183         if block = 1 and tank = 7 then tankn = 'Enclosure 7';
184          if block = 1 and tank = 8 then tankn = 'Enclosure 8';
185           if block = 1 and tank = 9 then tankn = 'Enclosure 9';
186
187  if block = 2 and tank = 1 then tankn = 'Enclosure 1';
188    if block = 2 and tank = 2 then tankn = 'Enclosure 2';
189     if block = 2 and tank = 3 then tankn = 'Enclosure 3';
190      if block = 2 and tank = 4 then tankn = 'Enclosure 4';
191       if block = 2 and tank = 5 then tankn = 'Enclosure 5';
192        if block = 2 and tank = 6 then tankn =  Enclosure 6';
193         if block = 2 and tank = 7 then tankn = 'Enclosure 7';
194          if block = 2 and tank = 8 then tankn = 'Enclosure 8';
195           if block = 2 and tank = 9 then tankn = 'Enclosure 9';
196
197  if block = 3 and tank = 1 then tankn = 'Enclosure 1';
198    if block = 3 and tank = 2 then tankn = 'Enclosure 2';
199     if block = 3 and tank = 3 then tankn = 'Enclosure 3';
200      if block = 3 and tank = 4 then tankn = 'Enclosure 4';
201       if block = 3 and tank = 5 then tankn = 'Enclosure 5';
202        if block = 3 and tank = 6 then tankn = 'Enclosure 6';
203         if block = 3 and tank = 7 then tankn = 'Enclosure 7';
```

FF # 9
Item No. 3
Pg 1 of 2

```
204          if block = 3 and tank = 8 then tankn = 'Enclosure 8';
205           if block = 3 and tank = 9 then tankn = 'Enclosure 9';
206
207      run;

NOTE: There were 9 observations read from the data set WORK.MUS.
NOTE: The data set WORK.MUS2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.04 seconds


208  proc sort data=MUS2;
209   by x;
210  run;

NOTE: There were 9 observations read from the data set WORK.MUS2.
NOTE: The data set WORK.MUS2 has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


211
212  data assign_trt_MUS; set MUS2;
213   if _n_ = 1 then trt = 'control';
214    if _n_ = 2 then trt = 'control';
215     if _n_ = 3 then trt = 'control';
216      if _n_ = 4 then trt = '50';
217   if _n_ = 5 then trt = '50';
218    if _n_ = 6 then trt = '50';
219     if _n_ = 7 then trt = '100';
220      if _n_ = 8 then trt = '100';
221       if _n_ = 9 then trt = '100';
222      run;

NOTE: There were 9 observations read from the data set WORK.MUS2.
NOTE: The data set WORK.ASSIGN_TRT_MUS has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


223  proc print data= assign_trt_MUS;
NOTE: Writing HTML Body file: sashtml1.htm
224  title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling zebra mussels
224!  within field enclosures';
225  title2 h=1.5 'AEH-13-PSUEDO-05';
226  title3 h=1 'Random assignment of treatment to experimental enclosures';
227  title4 h=1 'Lake Darling, Alexandria, MN';
228  run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_MUS.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.36 seconds
      cpu time            0.25 seconds
```

JR 7-12-13 Page 2 of 2

AEH-13-PSEUDO-05

FF # 9
Item No. 3
Pg 2 of 2

AEH-12-PSUEDO-05: Efficacy of Pseudomonas fluorescens (PF-CL145A) SDP for controlling zebra mussels within field enclosures
Random assignment of mussels to enclosures
Up to 5 rounds and 5 mussels per round

7/12/13
JM

Obs	round	block	tank	x	tankn
1	1	1	3	0.09327	Enclosure 3
2	1	1	2	0.11567	Enclosure 2
3	1	1	1	0.35032	Enclosure 1
4	1	1	5	0.39641	Enclosure 5
5	1	1	8	0.43169	Enclosure 8
6	1	1	7	0.46482	Enclosure 7
7	1	1	4	0.65898	Enclosure 4
8	1	1	9	0.73791	Enclosure 9
9	1	1	6	0.79402	Enclosure 6
10	2	1	7	0.15148	Enclosure 7
11	2	1	1	0.20408	Enclosure 1
12	2	1	2	0.41424	Enclosure 2
13	2	1	3	0.45513	Enclosure 3
14	2	1	5	0.46805	Enclosure 5
15	2	1	8	0.61298	Enclosure 8
16	2	1	4	0.68416	Enclosure 4
17	2	1	9	0.83429	Enclosure 9
18	2	1	6	0.95673	Enclosure 6
19	3	1	6	0.01246	Enclosure 6
20	3	1	7	0.12053	Enclosure 7
21	3	1	5	0.19480	Enclosure 5
22	3	1	9	0.20606	Enclosure 9
23	3	1	1	0.53144	Enclosure 1
24	3	1	2	0.55787	Enclosure 2
25	3	1	3	0.68940	Enclosure 3
26	3	1	8	0.74438	Enclosure 8
27	3	1	4	0.79054	Enclosure 4
28	4	1	5	0.06805	Enclosure 5
29	4	1	7	0.12602	Enclosure 7
30	4	1	3	0.55437	Enclosure 3
31	4	1	2	0.63499	Enclosure 2

① If more than 1 species are used up to 5 mussels of each species will be distributed per round.
7/12/13
JM

AEH-13-PSEUDO-05

FF # 9
Item No. 4
Pg 1 of 2

32	4	1	8	0.67644	Enclosure 8
33	4	1	4	0.68682	Enclosure 4
34	4	1	9	0.70878	Enclosure 9
35	4	1	1	0.78054	Enclosure 1
36	4	1	6	0.87768	Enclosure 6
37	5	1	9	0.02835	Enclosure 9
38	5	1	6	0.03358	Enclosure 6
39	5	1	1	0.10315	Enclosure 1
40	5	1	8	0.12591	Enclosure 8
41	5	1	2	0.27132	Enclosure 2
42	5	1	5	0.41851	Enclosure 5
43	5	1	3	0.42010	Enclosure 3
44	5	1	4	0.88894	Enclosure 4
45	5	1	7	0.97724	Enclosure 7

Analysis performed by J. Luoma SAS version 9.3 13:53 12JUL13

AEH-13-PSEUDO-05

FF # 9
Item No. 4
Pg 2 of 2

```
/*********************************************************************
*  Study Number  : AEH-12-PSUEDO-05
*  Study Director: Jim Luoma
*  date created  : 12 July 2013 - JAL
*  Verified by:  _____ (Date:_____)
*  Random allocation of fish to chamber.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=58 formdlim='-'  pageno = 1 nocenter nodate nosource2;

/*Random distribution of mussels to experimental enclosures*/
/* Place groups of up to 5 mussels in a bucket and transfer to assigned enclosure during each round *,

/*****************************************************************************************************

/*mussels to enclosures*/
data mussel;
 do round = 1 to 5 by 1;
 do block = 1 to 1 by 1;
   do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
run;
data musseldist; set mussel;
if block = 1 and tank = 1 then tankn = ' Enclosure 1';
  if block = 1 and tank = 2 then tankn = ' Enclosure 2';
   if block = 1 and tank = 3 then tankn = 'Enclosure 3';
    if block = 1 and tank = 4 then tankn = 'Enclosure 4';
     if block = 1 and tank = 5 then tankn = 'Enclosure 5';
      if block = 1 and tank = 6 then tankn = 'Enclosure 6';
       if block = 1 and tank = 7 then tankn = 'Enclosure 7';
        if block = 1 and tank = 8 then tankn = 'Enclosure 8';
         if block = 1 and tank = 9 then tankn = 'Enclosure 9';


run;


proc sort data= musseldist;
 by round x;
 run;
proc print data = musseldist;
title1 h=1 'AEH-12-PSUEDO-05: Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling zebr
title2 h=1 'Random assignment of mussels to enclosures';
title3 h=1 'Up to 5 rounds and 5 mussels per round';
run;
```

page 1 of 1

AEH-13-PSEUDO-05

FF # 9
Item No. 5
Pg 1 of 1

```
4     *  date created  : 12 July 2013 - JAL
5     *  Verified by:  _____ (Date:_____)                    page 1 of 2
6     *  Random allocation of fish to chamber.sas
7     ***************************************************************/ AEH-13-PSEUDO-05
8     DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
9
10    FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.
11
12    options ls=97 ps=58 formdlim='-'  pageno = 1 nocenter nodate nosource2;
13
14    /*Random distribution of mussels to experimental enclosures*/
15    /* Place groups of up to 5 mussels in a bucket and transfer to assigned enclosure during
15 !  each round */
16
17    /*************************************************************************************************
17 !  **********/
18
19    /*mussels to enclosures*/
20    data mussel;
21     do round = 1 to 5 by 1;
22     do block = 1 to 1 by 1;
23       do tank = 1 to 9 by 1;
24       x = ranuni(-1);
25       output;
26      end;
27      end;
28     end;
29    run;

NOTE: The data set WORK.MUSSEL has 45 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


30    data musseldist; set mussel;                                    FF # 9
31    if block = 1 and tank = 1 then tankn = ' Enclosure 1';          Item No. 6
32      if block = 1 and tank = 2 then tankn = ' Enclosure 2';        Pg 1 of 2
33       if block = 1 and tank = 3 then tankn = 'Enclosure 3';
34        if block = 1 and tank = 4 then tankn = 'Enclosure 4';
35         if block = 1 and tank = 5 then tankn = 'Enclosure 5';
36          if block = 1 and tank = 6 then tankn = 'Enclosure 6';
37           if block = 1 and tank = 7 then tankn = 'Enclosure 7';
38            if block = 1 and tank = 8 then tankn = 'Enclosure 8';
39             if block = 1 and tank = 9 then tankn = 'Enclosure 9';
40
41
42    run;

NOTE: There were 45 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSELDIST has 45 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds
```

```
43
44
45   proc sort data= musseldist;
46    by round x;
47    run;

NOTE: There were 45 observations read from the data set WORK.MUSSELDIST.
NOTE: The data set WORK.MUSSELDIST has 45 observations and 5 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


48   proc print data = musseldist;
NOTE: Writing HTML Body file: sashtml.htm
49   title1 h=1 'AEH-12-PSUEDO-05: Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for
49 ! controlling zebra mussels within field enclosures  ';
50   title2 h=1 'Random assignment of mussels to enclosures';
51   title3 h=1 'Up to 5 rounds and 5 mussels per round';
52   run;

NOTE: There were 45 observations read from the data set WORK.MUSSELDIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.34 seconds
      cpu time            0.25 seconds
```

5a
7-12-13
Page 282

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FF # 9
Item No. 6
Pg 2 of 2

Appendix 4. Test Article Information

Item number	Item description	Number of pages	Report page number
1	Material Safety Data Sheet: MBI-401 Spray Dried Powder	2	99
2	MBI-401 SDP (lot # TR-4669-4-3) Test Article: Certificate of Analysis	1	101
3	Test Material Requirements for Enclosure treatments	1	102
4	Test Chemical Stock Preparation	1	103
5	Copy of test article information from test article log book for MBI-401 SDP; lot number TR4669-4-3; Container 1 of 2 – Used for enclosure treatment at Lake Darling	6	104
6	Copy of test article information from test article log book for MBI-401 SDP; lot number TR4669-4-3; Container 2 of 2 – Used for enclosure treatment at Lake Darling	6	110
7	Post-treatment product validation assay for test article (lot # TR4669-4-3)	2	116
8	MBI-401 SDP (lot # 401P120724C) Test Article: Certificate of Analysis – Not Used for Treatment	1	118
9	Test Chemical Stock Preparation – Not Used for Treatment	1	119
10	Pre-treatment product validation assay for test article (lot # 401P120724C) – FAILED	2	120
11	Copy of test article information from test article log book for MBI-401 SDP; lot number 401P120724C; Container 1 of 1 – Not used in dosing (FAILED pre-test validation)	4	122

AEH-13-PSEUDO-05

MATERIAL SAFETY DATA SHEET

Product Name: MBI-401 SDP

Contact: Marrone Bio Innovations, 2121 Second Street, Suite B-107, Davis, CA 95618
Phone (Business hours): 530-750-2800
www.marronebioinnovations.com
For emergencies such as leaks or spills call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300

Trade names/ Synonyms: MBI-401 SDP
EPA Registration Number: None, Experimental
PMRA Research Authorization #: 0030-RP-10
Primary Hazards: Inhalation

SECTION 1: MATERIAL IDENTIFICATION

INGREDIENT 1

Common Name:	CL145A strain of *Pseudomonas fluorescens*
Chemical Name:	Not applicable
Molecular Formula:	Not applicable
CAS Number:	Not applicable
Percent:	50%

OTHER INGREDIENTS: inert, non-reactive

SECTION 2: PHYSICAL DATA

Boiling Point:	Not applicable
Melting Point:	Not applicable
Bulk Density:	0.78 g/ml
Solubility in Water:	Dispersible in water
Appearance:	Powder
Color:	Tan
Odor:	Sweet, musty

SECTION 3: FIRE AND EXPLOSION DATA

Flash Point:	Not flammable
Method:	Not applicable
Extinguishing Media:	Use extinguishing media appropriate for the surrounding fire
Special Fire Fighting Procedures:	None
Unusual Fire and Explosion Hazards:	None

SECTION 4: REACTIVITY

Stability:	Material is non-reactive
Hazardous Polymerization:	Does not occur
Incompatibility:	None known
Hazardous Decomposition Products:	None known
Conditions to avoid:	None known

SECTION 5: HEALTH HAZARDS

Primary Route of Entry:	Skin contact, Eye, Inhalation
Exposure Limit:	Not established
Corrosive:	Not corrosive
Inhalation:	May be irritating to respiratory tract for some individuals. Avoid breathing dust.
Skin/ Eye Irritation:	May be irritating to skin and eyes for some individuals.
Effects of Overexposure:	If product comes in contact with eyes or skin, irritation may occur.
Toxicity:	None of the components of this product are listed as carcinogenic by NTP, IARC, OSHA

Acute studies:

Acute Oral LD_{50} (Rat):	>5,000 mg/kg (very low toxicity)
Acute Dermal LD_{50} (Rabbit)	>2,000 mg/kg (non-irritating, mild or slight irritation)
Primary Dermal Irritation	Slight Irritation, Class 4
Eye Irritation	Minimal Irritation, Class 4
Inhalation:	>2.25 g/ml, Class 4

SECTION 6: FIRST AID

Emergency First Aid Procedures:

If in eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center of doctor for treatment advice.
If inhaled:	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
If on skin:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for further treatment advice.
If swallowed:	Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to by a poison control center or doctor. Do not give anything by mouth to an unconscious person.

FF # 7A
Item No. 1
Pg 1 of 2

AEH-13-PSEUDO-05

MATERIAL SAFETY DATA SHEET

SECTION 7: SPILL, LEAK AND DISPOSAL PROCEDURES

Steps to be taken in case material is released or spilled:

Wear suitable protective clothing such as long-sleeved shirt, pants, waterproof gloves and shoes with socks. Carefully mop or sweep up spill and place in a closed container for disposal.

Waste disposal method: Dispose of in accordance with all applicable federal, state, and local environmental regulations.

For emergencies such as leaks or spills, call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300.

SECTION 8: SPECIAL HANDLING

Respiratory:	Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product.
Protective gloves:	Wear gloves made of Latex or other impervious material.
Eye protection:	Safety goggles or safety glasses with side shields recommended.
Other protective clothes:	Clothing to prevent prolonged skin contact as needed such as long-sleeved shirt, long pants and shoes with socks.

SECTION 9: SPECIAL PRECAUTIONS

Precautions to be taken in handling and storing:

Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product.

Store in a dry area inaccessible to children. Store in original containers only. Keep container closed when not in use.

Empty container completely and dispose of in accordance with all applicable federal, state, and local environmental regulations.

Wash any contamination from skin or eyes immediately. Wash hands and exposed skin before eating, drinking, smoking after work or using the toilet.

SECTION 10: SHIPPING REGULATIONS

Proper shipping name:	None
DOT Label (s) Required:	None
Freight Classification:	Insecticides, Fungicides N.O.I., Other Than Poisons. NMFC 102120 Class 60

SARA Title III Hazard Classification:

Immediate (acute) Health:	None
Delayed (chronic) Health:	None
Fire:	None
Sudden Release of Pressure:	None
Reactivity:	None

National Fire Protection Association Rating:

Health:	None
Flammability:	None
Reactivity:	None

This document set forth is based on information that Marrone Bio Innovations, Inc. (MBI) believes to be accurate. No warranty, expressed or implied, is intended. The information is provided solely for your information and consideration and MBI assumes no legal responsibility for use or reliance thereon.

Marrone™ Bio Innovations

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100

Marrone™ Bio Innovations

CERTIFICATE OF ANALYSIS

AEH-13-PSEUDO-05

Name of Product:	MBI-401 SDP
Active Ingredient:	100% *Pseudomonas fluorescens* strain CL145A cells and spent fermentation media
Percent Active Ingredient:	50% by weight
Viable Cfu/g:	0 cfu/g *Pseudomonas fluorescens* strain CL145A
Lot Number:	TR 4669-4-(3)
Mussel Bioassay:	Pass
Appearance:	Tan powder
Storage Conditions:	4 °C, protected from light
Date of Manufacture:	08 February 2012
Expiration Date:	08 November 2013

I hereby certify that the above information is true and correct.

Quality Control: [redacted] Date: 28 August 2013

Tamara Nicholson, Quality Control Supervisor

FF # 7a
Item No. 2
Pg 1 of 1

2121 Second Street, Suite B-107 • Davis, CA 95618 • Phone: 530-750-2800

Study Number AEH-13-PSEUDO-05

Test Material Requirements for Enclosure treatments

Date 7/25/13 Initials JDL

Location L. Darling Exp. Date 7/26/13

Product Lot Number TR4669-4-3 (run 4 sample 3)

1" = 0.0254 meters

Enclosures = 56" square = 2.02322 square meters

Enclosure ID	Treatment mg/L	Depth (inches)	Depth (meters)	Enclosure Vol. (m3)	Enc. Vol (L)	Active Req. (g)	Product Req. (g)
1	100	53	1.35	2.72	2723.66	272.37	544.73
2	50	51	1.30	2.62	2620.88	131.04	262.09
3	0	54	1.37	2.78	2775.05	0.00	0.00
4	50	54	1.37	2.78	2775.05	138.75	277.51
5	50	54	1.37	2.78	2775.05	138.75	277.51
6	100	53	1.35	2.72	2723.66	272.37	544.73
7	0	53	1.35	2.72	2723.66	0.00	0.00
8	0	54	1.37	2.78	2775.05	0.00	0.00
9	100	55	1.40	2.83	2826.44	282.64	565.29

FF # 7a
Item No. 3
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Study Number: AEH-13-PSEUDO-05 Reviewed by: KW Date: 18FEB2014
File Folder: 7a Lab book/pgs: e-log page 9,11,13,14 Verified by: JH Date: 2/19/14

Test Chemical Stock Preparation

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: TR4669-4-3 Date Rec'd: 24JUL13 Exp. Date: 24JUL13 M 26JUL13 KW 26JUL13

Test Location: Lake Darling, Alexandria, MN Instruments Used: BAL 3; BAL 4

Weights of Chemical Samples:

Sample ID	Sample Weight	Comments	Date	Initials
Analytical #1	2.00	Bag 1 of 2	25JUL13	KW
Analytical #2	2.00	Bag 1 of 2	↑	↑
Stock 1	277.53	Bag 1 of 2; Enclosure 4		
Stock 2	279.59	Bag 1 of 2; Enclosure 5		
Stock 3	262.09	Bag 1 of 2; Enclosure 2		
Stock 4	544.73	Bag 1 of 2; Enclosure 1		
Stock 5	563.27	Bag 1 of 2; Enclosure 9	↓	↓
Stock 6	544.61	Bag 2 of 2; Enclosure 6	25JUL13	KW

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

Stock Solution Preparation:

Sample ID	Dilution Volume (mL)	Dilution Time	Use	Exposure Time	Date	Initials
Analytical #1	500	0500	Standard curve	—	26JUL13	KW
Stock 3	~15L	0600	Enclosure 2	0625	7/26/13	JH
Stock 6	~15L	0645	Enclosure 6	0650	7/26/13	JH
Stock 1	~15L	0705	Enclosure 4	0715	7/26/13	JH
Stock 4	~15L	0735	Enclosure 1	0740	7/26/13	JH
Stock 2	~15L	0750	Enclosure 5	0800	7/26/13	JH
Stock 5	~15L	0810	Enclosure 9	0820	7/26/13	JH
Enclosure #3	- Control enclosure		mixed at	6:20 am	7/26/13	JH
Enclosure #7	- Control enclosure		mixed at	6:55 am	7/26/13	JH
Enclosure 8	- Control enclosure		mixed at	7:45 am	7/26/13	JH

Wrote wrong control enclosure 7/26/13 JH

FF # 7a
Item No. 4
Pg 1 of 1

AEH-13-PSEUDO-05

CHEMICAL LOG BOOK

MBI-401 SDP

Marrone Bio Innovations
Davis, California

LOT NUMBER: TR4669-4-3
(Run 4; Sample 3)

Container 1 of 2

FF # 18
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AEH-13-PSEUDO-05

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry Lubber	[redacted]	KLL	12/6/13

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on_____ by_____."
Date Quality Assurance Unit

ORIGINAL

Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) Pf-CLI45A; MBI-401 SDP

Circle one: (Test Article) Control Article

Trade Name of Test Chemical (Synonyms) Pf-CLI45A

Source of Test Chemical (Manufacturer) Marine Bio Innovations

Storage Location Cooler with ice and fridge in hotel room when out in field
Refrigerator in Room 2 at UMES.

Date Received 24JUL13 Date Opened 25JUL13 Expiration Date _____
(5 years unless otherwise stated)

Test Chemical Lot Number TR 4669-4-3 (run 4, Sample 3) Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~2 kg

Initial Mass (with cover on) of Test Chemical and Container 2046.77

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes (2) No __

Additional Comments about the Test Chemical:
(1) Incorrect test chemical name Rec'd 1 Aug 2013
(2) A COA will be placed in study records when received from MBI. Rec'd 6 Aug 2013

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location N/A Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 01 Aug 2013

Study Number: AEH-13-PSEUDO-05

Study Title: Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures

Test Chemical Lot/Use:

Zequanox test chemical (lot #401P120724C)was brought to the test location for use in the study based on preliminary results from pre-test activity tests conducted by the New York State Museum Field Research Laboratory (NYSM-FRL). While at the field test site (Lake Darling; Alexandria, MN) on 23 JUL 2013, NYSM-FRL staff called and indicated the test chemical lot #401P120724C did not pass initial efficacy testing and it was therefore not used for the study. Marrone Bio Innovations shipped 4kg of replacement test chemical (lot TR4660-4-3; run 4, sample 3) to the field location via overnight UPS. The new lot of test chemical was weighed based on the calculated amounts needed for the proper treatment level. The data sheet with individual samples weights can be found in File Folder 7. A copy of the data sheet is bound in the chemical logbooks and the data were transcribed to the chemical logbooks that were created upon returning to UMESC. Three analytical aliquots were weighed. The first analytical aliquot was spilled and therefore discarded. A sample of the test material will be shipped overnight to NYSM-FRL to verify post-exposure activity.

[redacted]
Kerry L. Weber, Fisheries Biologist

01 Aug 2013
Date

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____
File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Test Chemical Stock Preparation

Test Chemical: *Pseudomonas fluroescens* strain 145A
Test Chemical Lot #: TR4669-4-3 Date Rec'd: 24JUL13 Exp. Date: _____
Test Location: Lake Darling, Alexandria, MN Instruments Used: BAL 3; BAL 4

Weights of Chemical Samples:

Sample ID	Sample Weight	Comments	Date	Initials
Analytical #1	2.00	Bag 1 of 2	25JUL13	KW
Analytical #2	2.00	Bag 1 of 2	↑	↑
Stock 1	277.53	Bag 1 of 2; Enclosure 4		
Stock 2	279.59	Bag 1 of 2; Enclosure 5		
Stock 3	262.09	Bag 1 of 2; Enclosure 2		
Stock 4	544.73	Bag 1 of 2; Enclosure 1		
Stock 5	563.27	Bag 1 of 2; Enclosure 9	↓	↓
Stock 6	544.61	Bag 2 of 2; Enclosure 6	25JUL13	KW

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

Stock Solution Preparation:

Sample ID	Dilution Volume (mL)	Dilution Time	Use	Exposure Time	Date	Initials
Analytical #1	500	0500	Standard curve	—	26JUL13	KW
stock 3	~15L	0600	Enclosure 2	0625	7/26/13	JW
stock 6	~15L	0645	Enclosure 6	0650	7/26/13	JW
stock 1	~15L	0705	Enclosure 4	0715	7/26/13	JW
stock 4	~15L	0735	Enclosure 1	0740	7/26/13	JW
stock 2	~15L	0750	Enclosure 5	0800	7/26/13	JW
stock 5	~15L	0810	Enclosure 9	0820	7/26/13	JW
Enclosure #3	– Control	enclosure	mixed at	6:20 am	7/26/13	JW
Enclosure #7	– control	enclosure	mixed at	6:55 am	7/26/13	JW
Enclosure 8	– Cntl	enclosure	mixed at	7:45 am	7/26/13	JW

Diluted using control enclosure 7/26/13 JW

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ORIGINAL

Form GEN 012.3b
Page 1 of 1

TEST CHEMICAL USE LOG

Test Chemical: MBI-401 SDP	Manufacturer: Marrone Bio Innovations	Lot or Batch #: TR-4069-4-3 (run 4; sample 3)

A Initial mass of test chemical & container with cap/ lid on (g)	B Amount removed (g)	C Mass of test chemical & container after removal, with cap/lid on (g)	D Study Number	E Purpose and Other Comments	F Date	G Initials
*1 A sample was not archived due to this not being a regulated study.	*	*		*Archived Sample	1AUG13	KW
2046.77	2.00	—	AEH-13-PSEUDO-05	Analytical Sample was spilled and discarded		
—	2.00	—		Analytical #1 for standard curve		
—	2.00	—		Analytical #2 (back-up)		
—	277.53	—		Stat 1 for Enclosure 4		
—	277.59	—		Stat 2 for Enclosure 5		
—	262.09	—		Stat 3 for Enclosure 2		
—	544.73	—		Stat 4 for Enclosure 1		
—	563.27	111.68	AEH-13-PSEUDO-05	Stat 5 for Enclosure 9	1AUG13	KW
Remaining amount of test product was shipped to MBI overnight via FedEx on 05AUG2013. KW 06Aug13						

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

AEH-13-PSEUDO-05

CHEMICAL LOG BOOK

AEH-13-PSEUDO-05

MBI-401 SDP

Marrone Bio Innovations
Davis, California

LOT NUMBER: TR4669-4-3
(Run 4; Sample 3)

Container 2 of 2

FF # 18
Item No. 2
Pg 1 of 6

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry L. Weber	[redacted]	KLW	1Aug13

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on__________________by________________________."
Date Quality Assurance Unit

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ORIGINAL

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401SDP

Circle one: (Test Article) Control Article

Trade Name of Test Chemical (Synonyms) Pf CL145A

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Cooler with ice and fridge in hotel room when out in field
refrigerator in Room 2 at UMESC

Date Received 24 JUL 2013 Date Opened 25JUL13 Expiration Date ______
(5 years unless otherwise stated)

Test Chemical Lot Number JR-4669-4-3 (run 4; sample 3) Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~2kg

Initial Mass (with cover on) of Test Chemical and Container 2095.39

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes(1) No __

Additional Comments about the Test Chemical:
(1) The COA will be placed in study records when received by MBI. [illegible] 01Aug2013

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location N/A Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signatu[redacted] Date 01Aug2013

Page 3 of 6

Study Number: AEH-13-PSEUDO-05

Study Title: Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling zebra mussels within field enclosures

Test Chemical Lot/Use:

Zequanox test chemical (lot #401P120724C)was brought to the test location for use in the study based on preliminary results from pre-test activity tests conducted by the New York State Museum Field Research Laboratory (NYSM-FRL). While at the field test site (Lake Darling; Alexandria, MN) on 23 JUL 2013, NYSM-FRL staff called and indicated the test chemical lot #401P120724C did not pass initial efficacy testing and it was therefore not used for the study. Marrone Bio Innovations shipped 4kg of replacement test chemical (lot TR4660-4-3; run 4, sample 3) to the field location via overnight UPS. The new lot of test chemical was weighed based on the calculated amounts needed for the proper treatment level. The data sheet with individual samples weights can be found in File Folder 7. A copy of the data sheet is bound in the chemical logbooks and the data were transcribed to the chemical logbooks that were created upon returning to UMESC. Three analytical aliquots were weighed. The first analytical aliquot was spilled and therefore discarded. A sample of the test material will be shipped overnight to NYSM-FRL to verify post-exposure activity.

[redacted]
Kerry L. Weber, Fisheries Biologist

01 AUG 2013
Date

Study Number: AEH-13-PSEUDO-05 Reviewed by: _____ Date: _____

File Folder: _____ Lab book/pgs: _____ Verified by: _____ Date: _____

Test Chemical Stock Preparation

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: TR4669-4-3 Date Rec'd: 24JUL13 Exp. Date: _____

Test Location: Lake Darling, Alexandria, MN Instruments Used: BAL 3; BAL 4

Weights of Chemical Samples:

Sample ID	Sample Weight	Comments	Date	Initials
Analytical #1	2.00	Bag 1 of 2	25JUL13	KW
Analytical #2	2.00	Bag 1 of 2	↑	↑
Stock 1	277.53	Bag 1 of 2; Enclosure 4		
Stock 2	279.59	Bag 1 of 2; Enclosure 5		
Stock 3	262.09	Bag 1 of 2; Enclosure 2		
Stock 4	544.73	Bag 1 of 2; Enclosure 1		
Stock 5	563.27	Bag 1 of 2; Enclosure 9	↓	↓
Stock 6	544.61	Bag 2 of 2; Enclosure 6	25JUL13	KW

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

Stock Solution Preparation:

Sample ID	Dilution Volume (mL)	Dilution Time	Use	Exposure Time	Date	Initials
Analytical #1	500	0500	Standard curve	—	26JUL13	KW
Stock 3	~15L	0600	Enclosure 2	0625	7/26/13	JM
Stock 6	~15L	0645	Enclosure 6	0650	7/26/13	JM
Stock 1	~15L	0705	Enclosure 4	0715	7/26/13	JM
Stock 4	~15L	0735	Enclosure 1	0740	7/26/13	JM
Stock 2	~15L	0750	Enclosure 5	0800	7/26/13	JM
Stock 5	~15L	0810	Enclosure 9	0820	7/26/13	JM
Enclosure #3	– Control	enclosure	mixed at	6:20 am	7/26/13	JM
Enclosure #7	– Control	enclosure	mixed at	6:55 am	7/26/13	JM
Enclosure 8	– Control	enclosure	mixed at	7:45 am	7/26/13	JM

Wrote wrong control enclosure 7/26/13 JM

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ORIGINAL

Form GEN 012.3b
Page 1 of 1

TEST CHEMICAL USE LOG

Test Chemical: MBI-49 SDP	Manufacturer: Manore Bio Innovations	Lot or Batch #: TP4669-4-3 (run 4; sample 3)

A Initial mass of test chemical & container with cap/ lid on (g)	B Amount removed (g)	C Mass of test chemical & container after removal, with cap/lid on (g)	D Study Number	E Purpose and Other Comments	F Date	G Initials
*1 No sample was archived due to this study not being negotiated	*	*		*Archived Sample	1AUG 2013	KLW
2045.39	544.61	1500.36	AEH-13-PSEUDO-05	Stock 6 for Enclosure 6	1 AUG 2013	KLW
1503.0	100.17	1402.5	AEH-13-PSEUDO-05	Sample to NYSM for activity verification	05AUG13	KLW
Remaining amount of test product was shipped to MBI overnight via FedEx on 05AUG2013				Ad archive	KLW 06AUG2013	

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

AEH-13-PSEUDO-05

NYSM Post-Treatment Product Validation Assay
MBI-401 SDP TR-4669-4-(3)
(USGS Study #AEH-13-PSEUDO-05 Field trials at Darling Lake in 2013)

Date product received from USGS: 2013/08/08
Date of start of test: 2013/08/08

AEH-13-PSEUDO-05

BACKGROUND: As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity post-treatment.

- MBI-401 SDP TR-4669-4-(3)
  - o USGS Study #AEH-13- PSEUDO-05 Field trials at Darling Lake in 2013

PURPOSE: Post-test product validation of MBI-401 SDP TR-4669-4-(3) used in AEH-13-PSEUDO-05 Field Trials at Darling Lake in 2013.

MATERIALS AND METHODS:

Preparation of product for testing:

Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare treatment stock of MBI-401 formulated product:

MBI-401 SDP TR-4669-4-(3) (SDP – 50% active ingredient): 3 g of the powder was added slowly to a beaker with dilution water with stirring on a stir plate for even suspension and then the total volume was adjusted to 30 ml with dilution water. The suspension was transferred to a 50 ml centrifuge and stored in refrigerator until ready to use. The suspension, when evenly dispersed was 100 mg product/ml or 50 mg a.i./ml. For 200 ppm a.i. treatments in testing jars, 2 ml were added to each testing jar (500 ml).

Cambridge CF (Standard for Positive Controls):

As an efficacy standard, we used *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, a positive control suspension was produced on 06/18/2012 from the frozen blocks described above and dispensed into multiple 50-ml centrifuge tubes for single-use treatment of bioassays. The dry weight of the material was 68.15 mg/ml; therefore 1.5 ml was added to each testing jar to treat at 200 ppm.

Mussel collections:

Mussels were scraped from substrates (rocks) in the field and brought back to the lab in coolers. Mussels were placed in pint-sized jars and placed in aquaria filled with unchlorinated tap water with aeration and filtration (Whisper filters) in the laboratory at 20°C. One day prior to treatment, mussels were carefully examined and placed into testing jars and allowed to attach overnight.

FF # 7a
Item No. 5
Pg 1 of 2

Mussel collection and handling:

Species	Collection site	Collection date	Date in lab (20°C)	Picked for test
Zebra mussels	Hedges Lake (Washington County)	07/09/2013	07/09/2013	08/07/2013

Experimental design:
For validation of efficacy the following treatments were set up:

Zebra mussels (25 mussels/jar):
3 – Untreated Control (A, B, C)
3 – 200 ppm (a.i.) Cambridge CF Positive Control (*Pf*-CL145A killed cells) (A, B, C)
3 – 200 ppm (a.i.) MBI-401 SDP TR-4669-4-(3) (A, B, C)

Total should be 9 jars.

Testing jar bioassay protocol:
On the day prior to treatment (08/07/2013) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (08/08/2013), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\ nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 6 days (7 days total). On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

Results:

Mussel length: Zebra mussels 16.63 ± 3.80 mm.

Optical density of treatments:

Treatment	Mean (±SD) OD ($A_{660\ nm}$)
Untreated Control	0.002 ± 0.002
Cambridge CF (Positive Control)	0.198 ± 0.009
Field trial product MBI-401 SDP TR4669-4-(3)	0.212± 0.008

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 7 days.

Treatment	Mean % mortality (±SD)
Untreated Control	1.3 ± 2.2%
Cambridge CF (Positive Control)	100.0 ± 0.0%
Field trial product MBI-401 SDP TR4669-4-(3)	96.0 ± 6.9%

MBI-401 SDP TR4669-4-(3) PASSED the post-test bioassay validation (96% mortality). Untreated control mortality was 1%.

FF # 7a
Item No. 5
Pg 2 of 2

Marrone™ Bio Innovations

CERTIFICATE OF ANALYSIS AEH-13-PSEUDO-05

Name of Product:	MBI-401 SDP
Active Ingredient:	100% *Pseudomonas fluorescens* strain CL145A cells and spent fermentation media
Percent Active Ingredient:	50% by weight
Viable Cfu/g:	0 cfu/g *Pseudomonas fluorescens* strain CL145A
Lot Number:	401P120724C
Mussel Bioassay:	Pass
Appearance:	Tan powder
Storage Conditions:	4 °C, protected from light
Date of Manufacture:	02 August 2012
Expiration Date:	02 August 2013

I hereby certify that the above information is true and correct.

Quality Control: [redacted] Date: 18 March 2013

Tamara Nicholson, Quality Control Supervisor

FF # 7a
Item No. 6
Pg 1 of 1

2121 Second Street, Suite B-107 • Davis, CA 95618 • Phone: 530-750-2800

Study Number: AEH-13-PSEUDO-05
File Folder: 7a   Lab book/pgs: e-log page 8,9

Reviewed by: KW   Date: 18FEB2014
Verified by: [illegible]   Date: 2/19/14

Test Chemical Stock Preparation

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: 401P120724C   Date Rec'd: 18 JUL 2013   Exp. Date: 02 AUG 2013

Test Location: Lake Darling, Alexandria, MN   Instruments Used: BAL 1 - Sartorius Top loader Model LC3400P (S/N 30303920)
BAL 2 - Sartorius Analytical Model R200D (S/N) 20903620 write over S/N 20903620 KW 18FEB2014

Weights of Chemical Samples:

Sample ID	Sample Weight	Comments	Date	Initials
Analytical #1	2.00013g	NONE	19JUL13	KW
Analytical #2	2.00009g	NONE	19JUL13	KW
*- This stock of test product was determined to be below acceptable activity levels. Therefore, this lot of test product will not be used for dosing. A new lot will be used. KW 25JUL13				

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

Stock Solution Preparation:

Sample ID	Dilution Volume (mL)	Dilution Time	Use	Exposure Time	Date	Initials

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NYSM Pre-Treatment Product Validation Assay
MBI-401 SDP P120724C (FAILED)
(USGS Study #AEH-13-PSEUDO-05 Field trials at Darling Lake in 2013)

Date product received from MBI: 2013/07/10
Date of start of test: 2013/07/10

AEH-13-PSEUDO-05

BACKGROUND: As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity pre-treatment.

- MBI-401 SDP P120724C
  - USGS Study #AEH-13- PSEUDO-05 Field trials at Darling Lake in 2013

PURPOSE: Pre-test product validation of MBI-401 SDP P120724C to be used in AEH-13-PSEUDO-05 Field Trials at Darling Lake in 2013.

MATERIALS AND METHODS:

Preparation of product for testing:

Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare treatment stock of MBI-401 formulated product:

MBI-401 SDP P120724C (SDP – 50% active ingredient): 3 g of the powder was added slowly to a beaker with dilution water with stirring on a stir plate for even suspension and then the total volume was adjusted to 30 ml with dilution water. The suspension was transferred to a 50 ml centrifuge and stored in refrigerator until ready to use. The suspension, when evenly dispersed was 100 mg product/ml or 50 mg a.i./ml. For 200 ppm a.i. treatments in testing jars, 2 ml were added to each testing jar (500 ml).

Cambridge CF (Standard for Positive Controls):

As an efficacy standard, we used *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, a positive control suspension was produced on 06/18/2012 from the frozen blocks described above and dispensed into multiple 50-ml centrifuge tubes for single-use treatment of bioassays. The dry weight of the material was 68.15 mg/ml; therefore 1.5 ml was added to each testing jar to treat at 200 ppm.

Mussel collections:

Mussels were scraped from substrates (rocks) in the field and brought back to the lab in coolers. One day prior to treatment, mussels were carefully examined and placed into testing jars and allowed to attach overnight.

Mussel collection and handling:

Species	Collection site	Collection date	Date in lab (20°C)	Picked for test
Zebra mussels	Hedges Lake (Washington County)	07/09/2013	07/09/2013	07/09/2013

① Correct lot # is 401P120724C per EMAIL 2014

FF # 79
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Pg 1 of 2

Experimental design:
For validation of efficacy the following treatments were set up: AEH-13-PSEUDO-05

Zebra mussels (25 mussels/jar):
3 – Untreated Control (A, B, C)
3 – 200 ppm (a.i.) Cambridge CF Positive Control (*Pf*-CL145A killed cells) (A, B, C)
3 – 200 ppm (a.i.) MBI-401 SDP P120724C (A, B, C)
Total should be 9 jars.

Testing jar bioassay protocol:
On the day prior to treatment (07/09/2013) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (07/10/2013), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\ nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 14 days (15 days total). This bioassay was terminated after >10% mortality was achieved in the untreated controls. On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

Results:

Mussel length: Zebra mussels 18.59 ± 3.05 mm.

Optical density of treatments:

Treatment	Mean (±SD) OD ($A_{660\ nm}$)
Untreated Control	0.003 ± 0.004
Cambridge CF (Positive Control)	0.200 ± 0.003
Field trial product MBI-401 SDP P120724C	0.246± 0.003

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 15 days.

Treatment	Mean % mortality (±SD)
Untreated Control	10.6 ± 8.4%
Cambridge CF (Positive Control)	100.0 ± 0.0%
Field trial product MBI-401 SDP P120724C	68.0 ± 8.0%

MBI-401 SDP P120724C FAILED the pre-test validation bioassay (68% mortality). Untreated control mortality was 11%. Subsequent to this product's failure, MBI sent us a new lot (TR4669-4-(3)) for use in the Darling Lake field trials. Lot TR4669-4-(3) passed validation.

Correct lot number is 401P120724C.tw 6MAR2014

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AEH-13-PSEUDO-05

CHEMICAL LOG BOOK

MBI-401 SDP

Marrone Bio Innovations
Davis, California

***LOT NUMBER:* 401P120724C**

Container 1 of 1

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SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

PRINTED NAME	SIGNATURE	INITIALS	DATE
Kerry L. Weber	[redacted]	KLW	18JUL13

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on July 18, 2013 by [redacted] QAO."
Date   Quality Assurance Unit

Page 2 **of** 4

ORIGINAL

Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: (Test Article) Control Article

Trade Name of Test Chemical (Synonyms) PF-CC145A

Source of Test Chemical (Manufacturer) Marrow Bio Innovations

Storage Location Locked refrigerator Room 2

Date Received 18 JUL 2013 Date Opened _____ Expiration Date 2 AUG 2013
(5 years unless otherwise stated)

Test Chemical Lot Number 401P120724C Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~5 kg

Initial Mass (with cover on) of Test Chemical and Container 5150.3g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location — Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 18 JUL 13

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ORIGINAL

Form GEN 012.3b
Page 1 of 1

TEST CHEMICAL USE LOG

Test Chemical: MBI-401 SDF	Manufacturer: MBI	Lot or Batch #: 401 PI207241

A Initial mass of test chemical & container with cap/ lid on (g)	B Amount removed (g)	C Mass of test chemical & container after removal, with cap/lid on (g)	D Study Number	E Purpose and Other Comments	F Date	G Initials
*1 No sample was archived. This is	*	*	not a regulated	*Archived Sample Study (AEH-13-PSEUDO-05).	18 JUL 13	KMW
5150.5	4.0022g	5146.6	AEH-13-PSEUDO05	Analytical # 1=2.00013g Analytical # 2: 2.0002g	19JUL13	KMW
#This lot of test chemical did not	pass initial efficacy testing. Therefore it was not used					
for study number AEH-13-PSEUDO-05. The containers of test chemical was returned to						
MBI (shipped overnight via FedEx) on 05 AUG 2013 A new lot (#TR4669-4-3) was						
received in the field from MBI, which was used for treatmen. KMW					06 AUG 2013	

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

AEH-13-PSEUDO-05

Appendix 5. Test Animal Information

Item number	Item description	Number of pages	Report page number
1	Special permit (No. 18804) for taking and possession of mussels	2	127
2	Prohibited Invasive Species Permit (No. 290)	1	129
3	Collection Report for Special Permit 18804	2	130
4	Mussels Species Identification datasheet	9	132
5	Unionid Mussel Weight and Length – Data Summary	6	141
6	Zebra Mussel Lengths Pre-exposure Assessment – Data Summary	10	147
7	Zebra Mussel Lengths Post-exposure Assessment – Data Summary	11	157

STATE OF MINNESOTA
DEPARTMENT OF NATURAL RESOURCES
DIVISION OF ECOLOGICAL AND WATER RESOURCES
500 LAFAYETTE ROAD, BOX 25
ST. PAUL, MN 55155

SPECIAL PERMIT NO. 18804
(Taking and possession of mussels)
July 22, 2013

AEH-13-PSEUDO-05

TO WHOM IT MAY CONCERN:

Under the authority of Minn. Statutes, Section 97A.401 and Minn. Rules, Part 6212.1400, permission is hereby granted to:

James Luoma
U.S. Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
LaCrosse, WI 54603

to collect and possess up to 250 individuals of each of the following species: Fatmucket (*Lampsilis siliquoidea*), Fragile Papershell (*Leptodea fragilis*), and Giant Floater (*Pyganodon grandis*) for the purpose of research, subject to the following conditions:

1. Kerry Weber, Jeremy Wise, Hugh McMath, and Denise Mayer are considered Subpermittees under this permit. The Permittee and Subpermittees must possess a copy of this permit while conducting permitted activities. Assistants may carry out activities allowed by this permit under the direct on-site supervision of the Permittee or Subpermittees.
2. Collection of mussels will be limited to Lake Darling, Douglas County. Collection will be conducted only by hand via. wading, snorkeling, or scuba diving.
3. Any mussels retained under this permit will be destroyed upon completion of research. No mussels will be returned to the wild. Humane methods will be used in taking tissue samples and/or euthanizing the mussels.
4. Before conducting work under this permit in state waters, the Permittee must decontaminate any equipment that has been used in any waters in Minnesota or other locations that are infested with aquatic invasive species. When leaving any waters, the Permittee must remove any aquatic plants and animals (e.g., snails, zooplankton) from equipment, and drain water from watercraft and all equipment used. If permitted activities require live transport, the Permittee must either bring uninfested surface water or ground water to the collection site for specimen transportation, or obtain a Permit to Appropriate/Transport Infested Waters. If permitted activities include the collection of any prohibited invasive species, the Permittee must obtain a Prohibited Invasive Species Permit. See the attached for further information on aquatic invasive species and related permits.
5. It is the Permittee's responsibility to acquire any additional permits that may be required related to the collection, possession, or transportation of mussels under this permit, including any permits required to transport specimens across state or international borders. This permit does not allow collection of any endangered or threatened species.
6. A report must be submitted by mail or email to the Minnesota Endangered Species Coordinator (Richard J. Baker, Division of Ecological and Water Resources, Box 25, 500 Lafayette Road, St. Paul, MN 55155; 651/259-5073; richard.baker@state.mn.us) by January 31, 2014 describing the results of work authorized under this permit. Copies of other reports and publications resulting from permitted activities shall be submitted as they become available.

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Item No. 1
Pg 1 of 2

This permit is effective immediately, and through December 31, 2013, but may be revoked at any time. Failure to submit reports or deliver specimens as required may be grounds for denial of future permit requests.

[redacted]

James Japs [redacted]
EMPS Section Manager, Division of Ecological and Water Resources

7/22/2013
Date

I hereby certify that I have read and understand the provision of this permit and understand that this permit is not valid unless it is signed by me.

[redacted]

Permittee

7/22/13
Date

C: Richard J. Baker, Endangered Species Coordinator, Div. Ecological and Water Resources
Peter Buesseler, Regional Manager, Div. Ecological and Water Resources
Stacey Sharp, Regional Manager , Div. Enforcement
Christine Herwig, Regional Nongame Specialist, Div. Ecological and Water Resources
Mike Davis, Malacologist, Div. Ecological and Water Resources
Bernard Sietman, Malacologist, Div. Ecological and Water Resources
Don Pereira, Research Manager, Div. Fish and Wildlife
Gary Montz, Research Scientist, Div. Ecological and Water Resources
Permit Clerk, Div. Fish and Wildlife

FF # 4
Item No. 1
Pg 2 of 2

Prohibited Invasive Species Permit

MN Department of Natural Resources, Division of Ecological and Water Resources
1200 Warner Road, St. Paul Minnesota 55106-6793

Permittee: James A. Luoma and Designees
USGS-Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, WI 54603

Permit number: 290

Daytime Phone: 608-781-6391

Location: Lake Darling (DOW# 21008000)

AEH-13-PSEUDO-05

Species: Zebra mussel (*Dreissena polymorpha*)

Scope: Permission is hereby granted to possess and transport zebra mussels that may be part of a research project on Lake Darling in Douglas County. Zebra mussels can be used to calculate populations that are adhering to native mussels. The permittee and all designees shall have a copy of this permit in their possession while conducting authorized activities.

Conditions:

Confinement and Disposal: The permittee shall take all reasonable measures to prevent the spread of zebra mussels to other waters during the activities authorized by this permit. All materials potentially containing zebra mussels shall be transported over the road in covered, water-tight containers to a laboratory setting.

Decontamination of Equipment: All equipment used in movement of potentially contaminated materials shall be decontaminated at an onsite location as outlined in the permit application. When not transporting materials between worksites, equipment shall be clean and free of infested material prior to leaving the site.

Inspections: Facilities for holding prohibited invasive species of wild animals and aquatic plants for research, exhibition, education, control, or disposal purposes identified in this permit are subject to inspection at any reasonable time by the commissioner of natural resources or a designated employee.

Revocation: Permits issued under this part may be revoked by the commissioner if the permittee does not comply with the conditions of this permit or Minnesota rules 6216.0100 to 6216.0600, or if revocation is necessary to protect the interest of the public, to protect native plant and animal populations in the state, or to otherwise protect the state's natural resources.

Transferability: This permit is not transferable.

Disclaimer of Liability: No liability is assumed by the State or any of its officers, agents, or employees by issuance of this prohibited invasive species permit, or for any act or omission of the permittee, or by any prohibited species in possession of the permittee.

Expiration date: This permit is valid from July 22, 2013 through September 15, 2013

Issued by: [redacted] 7/22/2013

Barry Stratton, EWR Region 1 South District Manger

I hereby certify that I have read and understand the provisions of this permit and understand this permit is not valid unless signed by permittee.

[redacted] Permittee Signature | Date 7/22/13

cc: DNR Division of Enforcement — Joe Elsterhold; Capt. Greg Salo; Mark Ranweiler; Ann Pierce; Jim Japs; Barry Stratton

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Item No. 3
Pg 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

November 18, 2013

Richard Baker, Minnesota Endangered Species Coordinator
Minnesota Department of Natural Resources
Division of Ecological and Water Resources
Box 25
500 Lafayette Road
St. Paul, MN 55144

AEH-13-PSEUDO-05

RE: Collection Report for Special Permit 18804

Dear Mr. Baker:

Below you will find a collection and disposition report of activities conducted under special permit number 18804, issued to the James Luoma, USGS Upper Midwest Environmental Sciences Center on July 22, 2013.

If you need additional information regarding this report, please contact myself at 608.781.6391 or (jluoma@usgs.gov). Thank you for your assistance with this permit process.

Sincerely,

[redacted]

James A Luoma
Research Fisheries Biologist

FF # 4
Item No. 3
Pg 1 of 2

cc: Randy Hines

Collection, Use and Disposition Report. Special Permit # 18804

Special Permit # 18804, Report of collection, use and disposition U.S. Geological Survey Upper Midwest Environmental Sciences Center 2630 Fanta Reed Road LaCrosse, WI 54603				
Species	**Source**	**Number of Animals**	**Use**	**Disposition**
L. siliquoidea	Lake Darling	229	L. Darling Zequanox exposures	Euthanized after assessment
L. siliquoidea	Lake Darling	1	L. Darling Zequanox exposures	Unrecovered, identification from photograph
L. fragilis	Lake Darling	12	L. Darling Zequanox exposures	Euthanized after assessment
L. fragilis	Lake Darling	1	L. Darling Zequanox exposures	Unrecovered, identification from photograph

AEH-13-PSEUDO-05

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Item No. 3
Pg 2 of 2

Study Number: AEH-13-PSEUDO-05
File Folder: 7b
Lab book/pgs: e-log page 21

Reviewed by: KW Date: 18FEB 2014
Verified by: JAC Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
1	C379	FAM		18NOV13	KW
1	C358	FAM			
1	C414	PAM			
1	C237	PAM			
1	C221	FPM	Dead during assessment		
1	C312	FAM	Dead during assessment		
1	C292	FAM			
1	C350	FAM			
1	C380	FAM			
1	C233	PAM			
1	C377	FAM			
1	C424	FAM			
1	C314	FAM			
1	C263	FAM			
1	C363	FPS			
1	C309	FAM			
1	C208	FAM			
1	C228	FAM			
1	C266	FAM			
1	C195	PAM			
1	C360	PAM			
1	C359	FAM	Dead during assessment		
1	C302	FAM			
1	C207	FPS			
1	C345	PAM			
1	C245	FAM			
1	C329	PAM		18NOV13	KW

FF # 7b
Item No. 1
Pg 1 of 9

Study Number: AEH-13-PSEUDO-05
File Folder: 76
Lab book/pgs: e-log page 31

Reviewed by: KW Date: 18FEB2014
Verified by: JM Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
2	C321	FAM		18NOV13	KW
2	C276	FAM		↑	↑
2	C249	FAM			
2	C310	FAM			
2	C242	FAM			
2	C406	FAM			
2	C375	FAM			
2	C232	FAM			
2	C436	FAM	Dead during assessment		
2	C293	FAM			
2	C419	FAM			
2	C342	FPS			
2	C304	FAM			
2	C187	FAM			
2	C332	FAM			
2	C217	FAM			
2	C203	FAM			
2	C331	FPS			
2	C298	FAM			
2	C317	FAM			
2	C408	FAM			
2	C188	FAM			
2	C395	FAM	Dead during assessment		
2	C410	FAM			
2	C278	FAM			
2	C219	FAM		↓	↓
2	C235	FAM		18NOV13	KW

Study Number: AEH-13-PSEUDO-05
File Folder: 7b
Lab book/pgs: e-log page 21

Reviewed by: KW Date: 18 Feb 2014
Verified by: JAM Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
3	C434	FAM		18NOV13	KW
3	C374	FAM			
3	C197	FAM			
3	C382	FAM			
3	C418	FAM			
3	C194	FAM			
3	C210	FAM			
3	C325	FAM			
3	C218	FAM			
3	C243	FAM			
3	C405	FAM			
3	C354	FAM			
3	C437	FAM			
3	C311	FAM			
3	C427	FAM			
3	C326	FAM			
3	C364	FAM			
3	C193	FAM	Dead during assessment		
3	C204	FAM			
3	C384	FAM			
3	C393	FAM			
3	C231	FAM			
3	C254	FAM			
3	C373	FAM			
3	C390	FAM			
3	C246	FAM			
3	C215	FAM		18NOV13	KW

Study Number: AEII-13-PSEUDO-05 Reviewed by: KMW Date: 18Feb2014
File Folder: 76 Verified by: SM Date: 2/19/14
Lab book/pgs: e-log page 21

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
4	C282	FAM		18Nov13	KMW
4	C255	FAM			
4	C296	FAM			
4	C412	FAM			
4	C239	FAM			
4	C283	FAM			
4	C285	FAM			
4	C225	FAM			
4	C351	FAM			
4	C388	FAM			
4	C339	FAM			
4	C429	FAM			
4	C403	FAM			
4	C404	FAM			
4	C270	FAM			
4	C279	FAM			
4	C286	FAM			
4	C421	FAM			
4	C353	FAM			
4	C398	FPS			
4	C333	FAM			
4	C348	FAM			
4	C223	FAM			
4	C271	FAM	Dead during assessment		
4	C303	FAM			
4	C389	FAM			
4	C334	FPS FAM	Dead during assessment	18Nov13	KMW

① Wrong Species ID. Correct ID is FAM. KMW 18Nov13

Study Number: AEH-13-PSEUDO-05 Reviewed by: [illegible] Date: 1/8/2014

File Folder: 7b Verified by: [illegible] Date: 2/19/14

Lab book/pgs: E-log page 21

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN

Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
5	C256	FAM		18 NOV 13	[illegible]
5	C407	FAM		↑	↑
5	C190	FAM			
5	C284	FAM			
5	C238	FAM			
5	C240	FAM			
5	C244	FAM			
5	C253	FAM	Dead during assessment		
5	C335	FAM			
5	C300	FAM			
5	C352	FAM			
5	C294	FAM			
5	C320	FAM			
5	C433	FAM			
5	C330	FAM			
5	C368	FAM			
5	C392	FAM			
5	C236	FAM			
5	C397	FAM			
5	C259	FAM			
5	C365	FAM			
5	C290	FAM			
5	C425	FAM			
5	C343	FAM			
5	C214	FAM			
5	C431	FAM		↓	↓
5	C265	FAM		18 NOV 13	[illegible]

Study Number: AEH-13-PSEUDO-05 Reviewed by: MW Date: 18FEB2014
File Folder: 76 Verified by: JM Date: 2/18/14
Lab book/pgs: e-log page 21

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
6	C297	FAM		18NOV13	MW
6	C362	FPS		↑	↑
6	C385	FAM			
6	C250	FAM			
6	C280	FAM	Dead during assessment		
6	C423	FAM			
6	C356	Unknown	NM recovered		
6	C200	FAM			
6	C396	FAM	Dead during assessment		
6	C281	FAM			
6	C322	FAM			
6	C251	FAM	Dead during assessment		
6	C313	FAM			
6	C252	FAM			
6	C308	FAM			
6	C277	FAM			
6	C336	FPS			
6	C402	FAM			
6	C315	FAM			
6	C213	FAM	Dead during assessment		
6	C205	FAM			
6	C426	FAM			
6	C378	FAM			
6	C383	FAM			
6	C372	FAM			
6	C307	FPS	Dead during assessment	↓	↓
6	C316	FAM		18NOV13	MW

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Study Number: AEH-13-PSEUDO-05
File Folder: Th
Lab book/pgs: E-log page 21

Reviewed by: KW Date: 18FEB2014
Verified by: JM Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
7	C357	FAM		18NOV13	KW
7	C299	FAM	Dead during assessment	↑	↑
7	C273	FAM			
7	C257	FAM			
7	C301	FAM	~~Dead during assessment~~ ①		
7	C349	FAM			
7	C355	FAM			
7	C361	FAM			
7	C401	FAM			
7	C248	FAM	Dead during assessment		
7	C260	FAM			
7	C413	FAM			
7	C306	FAM			
7	C275	FAM			
7	C268	FAM			
7	C247	FAM			
7	C261	FAM			
7	C394	FPS			
7	C272	FAM			
7	C234	FAM			
7	C319	FAM			
7	C417	FAM			
7	C212	FAM			
7	C370	FAM			
7	C347	FAM			
7	C289	FAM	Dead during assessment	↓	↓
7	C192	FAM		18NOV13	KW

① NL Dead during assessment KW 18NOV2013

Study Number: AEH-13-PSEUDO-05
File Folder: 76
Lab book/pgs: e-log page 21

Reviewed by: KW Date: 18FEB2014
Verified by: SA Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
8	C369	FAM		18NOV13	KW
8	C189	FAM		↑	↑
8	C415	FAM			
8	C269	FAM			
8	C274	FAM			
8	C191	FAM			
8	C186	FAM			
8	C211	FAM			
8	C337	FAM			
8	C399	FAM			
8	C366	FAM			
8	C435	FAM			
8	C196	FAM			
8	C428	FAM			
8	C222	FAM			
8	C391	FAM			
8	C198	FAM			
8	C230	FAM			
8	C199	FAM			
8	C216	FAM			
8	C264	FAM			
8	C241	FAM			
8	C324	FAM			
8	C387	FAM	Dead during assessment		
8	C340	FAM	Dead during assessment		
8	C209	FAM		↓	↓
8	C220	FAM		18NOV13	KW

Study Number: AEH-13-PSEUDO-05
File Folder: 7b
Lab book/pgs: e-log page 31

Reviewed by: KW Date: 18 FEB 2014
Verified by: JA Date: 2/19/14

Mussel Species Identification

Exposure Date: 26 JUL 2013 Test Location: Lake Darling, Alexandria, MN
Test Chemical: Pf-CL145A Lot #: TR4669-4-(3)

Enclosure ID	Tag ID	Mussel Species	Comments	Date	Initials
9	C327	FAM		18NOV13	KW
9	C338	FAM		↑	↑
9	C323	Unknown	Not recovered		
9	C305	FAM			
9	C227	FAM			
9	C229	FAM			
9	C376	FAM			
9	C206	FAM			
9	C411	FAM	Dead during assessment		
9	C262	FAM			
9	C226	FAM	Dead during assessment		
9	C344	FPS			
9	C346	FAM			
9	C422	FAM			
9	C420	FAM			
9	C202	FAM	Dead during assessment		
9	C430	FAM	Dead during assessment		
9	C409	FAM			
9	C295	FAM			
9	C287	FAM			
9	C258	FAM	Dead during assessment		
9	C367	FAM			
9	C386	FPS			
9	C328	FAM			
9	C416	FAM	Dead during assessment		
9	C381	FAM		↓	↓
9	C432	FAM		18NOV13	KW

FF # 7b
Item No. 1
Pg 9 of 9

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (pages 15 to 17 & 21)	Created......	7-Mar-14	KLW
Data Source: Form: "Mussel Weight and Length Assessment" (File Folder 13c)	Revised......	10-Mar-14	KLW
"Mussel Species Identification" (File Folder 7b)	Reviewed...	10 MAR 2014	
	Certified...	3/10/2014	

File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Unionid Mussel Weight and Length.xlsx]Unionid Weight-Length Cover

Coversheet
Page 1 of 2

Unionid Mussel Weight and Length

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:

Each of the 9 enclosures contained 22 unionid mussels with adhering zebra mussels and 5 unionid mussels with zebra mussels removed (i.e., used to assess initial zebra mussel density). During the post-exposure assessment (August 13-15, 2013), the unionid mussels were weighed before and after zebra mussel removal. Shell length was measured on each unionid mussel, and species confirmed from shell morphology. Fatmucket (*Lampsilis siliquoidea* ; FAM) was the dominate species while fragile papershell (*Leptodea fragilis* ; FPS) was rare (i.e., 231 FAM versus 12 FPS). Weight and length data for fragile papershell mussels were analyzed separately. Data exclusions, including weights of unionid mortalities and weight and length of unrecovered unionids can be found on page 2 of this data summary.

Tag ID = Hallprint shellfish tag glued to the native mussel
Weight (g) = Weight of unionid mussel after zebra mussel removal during the post-exposure assessment (August 13-15, 2013)
Shell Length (mm) = Length of unionid mussel after zebra mussel removal during the post-exposure assessment (August 13-15, 2013)

Data Summary:

Fatmucket Unionid Mussels

	Control Treatment			50 mg/L Treatment			100 mg/L Treatment		
	Enclosure 3	Enclosure 7	Enclosure 8	Enclosure 2	Enclosure 4	Enclosure 5	Enclosure 1	Enclosure 6	Enclosure 9
Mean Weight (g)	38.1	32.8	36.4	38.0	32.3	41.9	34.1	37.3	39.5
(SD)	(12.6)	(7.8)	(11.4)	(12.5)	(12.4)	(12.4)	(10.0)	(6.9)	(10.8)
Min	19.2	20.3	17.4	18.4	12.6	23.3	19.3	25.0	22.5
Max	74.9	46.9	67.9	65.4	62.4	72.5	54.5	50.5	60.1
Grand Mean		35.8			37.4			37.0	
(SD)		(2.2)			(3.9)			(2.2)	
Mean SL (mm)	65.74	62.73	65.33	65.97	62.57	68.69	62.32	64.81	66.97
(SD)	(7.39)	(5.03)	(7.03)	(7.62)	(7.12)	(6.20)	(8.26)	(5.54)	(6.83)
Min	47.10	52.34	53.29	48.65	48.94	56.66	34.42	48.54	55.56
Max	84.75	75.11	78.73	78.04	74.77	77.76	77.03	73.64	78.91
Grand Mean		64.60			65.74			64.70	
(SD)		(1.33)			(2.50)			(1.90)	

SL = Shell length

Fragile Papershell Unionid Mussels

	Weight (g)	SL (mm)
Mean	30.3	65.90
(SD)	(18.9)	(11.62)
Min	12.6	52.28
Max	83.0	95.64

SL = Shell length

File Folder 7b
Item Number 2
Page 1 of 6

Unionid Mussel Weight and Length

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A (SDP)); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Exclusions:
The following unionid mussels were mortalities from the assessment (August 13 to 15, 2013). The weight data for these mussels will not be used within the unionid weight analysis and will be denoted with "." within the dataset. The length data for these mussels will be used within the unionid length analysis:

Test Enclosure 1 (100 mg/L):	Test Enclosure 6 (100 mg/L):	Test Enclosure 8 (0 mg/L):
C312	C213	C340
C359	C251	C387
Test Enclosure 2 (50 mg/L):	C280	Test Enclosure 9 (100 mg/L):
C436	C307	C202
Test Enclosure 3 (0 mg/L):	C396	C226
C193	Test Enclosure 7 (0 mg/L):	C258
Test Enclosure 4 (50 mg/L):	C248	C430
C271	C289	C411
C334	C299	C416
Test Enclosure 5 (50 mg/L):	C301	
C253		

The following mussels were not recovered for the assessment (August 13 to 15, 2013) and were removed from the unionid mussel weight and length analysis:

Test Enclosure 6 (100 mg/L):	Test Enclosure 9 (100 mg/L):
C356	C323

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 15 to 17 & 21)
Data Source: Form: "Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Species Identification" (File Folder 7b)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Unionid Mussel Weight and Length
Post-exposure - Fatmucket (*Lampsilis siliquoidea*)
Control Treatments

Enclosure 3

Tag ID	Weight (g)	Shell Length (mm)
C193	.	70.41
C194	39.3	63.44
C197	30.6	63.48
C204	45.9	70.76
C210	29.9	62.79
C215	27.7	64.02
C218	28.1	60.34
C231	35.4	66.40
C243	41.9	47.10
C246	31.5	61.62
C254	63.0	79.81
C311	46.2	71.70
C325	45.4	69.48
C326	51.6	71.93
C354	45.6	72.08
C364	25.4	57.03
C373	29.8	66.93
C374	27.7	57.19
C382	42.3	67.40
C384	48.8	67.81
C390	19.4	58.26
C393	38.8	67.64
C405	19.2	57.13
C418	41.7	69.84
C427	31.2	62.43
C434	74.9	84.75
C437	29.4	63.12

Enclosure 7

Tag ID	Weight (g)	Shell Length (mm)
C192	29.6	57.30
C212	36.8	66.18
C234	26.5	59.53
C247	29.4	61.13
C248	.	65.73
C257	32.0	59.33
C260	21.5	56.92
C261	31.0	61.19
C268	40.7	62.09
C272	36.7	65.83
C273	20.3	52.34
C275	33.5	67.55
C289	.	64.83
C299	.	58.19
C301	.	69.72
C306	44.7	70.41
C319	23.8	56.14
C347	31.1	62.29
C349	36.5	62.80
C355	44.3	66.09
C357	30.1	66.67
C361	23.9	56.79
C370	26.8	60.47
C401	46.3	75.11
C413	29.6	61.06
C417	46.9	65.37

Enclosure 8

Tag ID	Weight (g)	Shell Length (mm)
C186	31.4	62.28
C189	40.1	68.67
C191	34.0	67.17
C196	48.1	73.50
C198	22.3	56.43
C199	44.5	74.82
C209	39.2	67.78
C211	37.0	65.06
C216	20.9	53.29
C220	23.8	59.94
C222	45.7	68.56
C230	24.9	57.72
C241	44.9	71.94
C264	27.4	56.28
C269	47.1	69.35
C274	33.8	66.29
C324	35.1	66.99
C337	46.7	73.46
C340	.	67.79
C366	48.0	74.40
C369	17.4	56.25
C387	.	56.36
C391	30.4	60.40
C399	45.5	72.78
C415	26.8	55.77
C428	27.9	61.89
C435	67.9	78.73

	Enclosure 3	Enclosure 7	Enclosure 8
Mean Weight (g)	38.1	32.8	36.4
(SD)	(12.6)	(7.8)	(11.4)
Min	19.2	20.3	17.4
Max	74.9	46.9	67.9
Grand Mean		35.8	
(SD)		(2.2)	
Mean Shell Length (mm)	65.74	62.73	65.33
(SD)	(7.39)	(5.03)	(7.03)
Min	47.10	52.34	53.29
Max	84.75	75.11	78.73
Grand Mean		64.60	
(SD)		(1.33)	

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 15 to 17 & 21)
Data Source: Form: "Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Species Identification" (File Folder 7b)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Unionid Mussel Weight and Length
Post-exposure - Fatmucket (*Lampsilis siliquoidea*)
50 mg/L Treatments

Enclosure 2

Tag ID	Weight (g)	Shell Length (mm)
C187	54.7	75.57
C188	43.4	70.61
C203	37.8	64.93
C217	49.0	72.52
C219	38.9	68.21
C232	19.8	53.53
C235	27.8	60.43
C242	18.4	48.65
C249	39.1	62.28
C276	32.6	64.72
C278	36.6	64.58
C293	36.8	67.13
C298	24.9	61.48
C304	65.4	77.91
C310	24.2	55.93
C317	48.9	70.75
C321	32.4	64.54
C332	59.3	71.92
C375	41.8	71.25
C395	20.4	55.59
C406	32.4	65.29
C408	40.0	72.67
C410	29.7	60.21
C419	56.5	75.39
C436	.	78.04

Enclosure 4

Tag ID	Weight (g)	Shell Length (mm)
C223	36.4	64.95
C225	26.1	60.96
C239	24.0	57.69
C255	21.6	54.40
C270	34.2	60.38
C271	.	61.77
C279	56.3	73.09
C282	36.3	62.02
C283	41.6	69.03
C285	24.9	59.85
C286	12.6	48.94
C296	28.2	66.28
C303	53.2	73.17
C333	18.0	52.16
C334	.	58.27
C339	51.1	73.33
C348	62.4	74.77
C351	23.5	55.71
C353	28.2	65.93
C388	26.1	58.51
C389	32.8	63.31
C403	33.8	72.69
C404	28.1	60.13
C412	17.5	51.38
C421	25.1	59.79
C429	33.9	68.22

Enclosure 5

Tag ID	Weight (g)	Shell Length (mm)
C190	25.2	61.09
C214	56.0	75.12
C236	52.9	74.35
C238	36.0	64.42
C240	26.8	58.83
C244	23.3	56.66
C253	.	69.87
C256	55.9	72.27
C259	40.1	67.50
C265	61.0	75.61
C284	49.4	77.25
C290	44.7	68.53
C294	34.4	70.80
C300	72.5	77.76
C320	40.4	68.45
C330	30.0	58.92
C343	41.3	68.21
C352	40.1	65.10
C365	62.9	75.88
C368	49.6	75.58
C392	34.2	73.62
C397	34.4	60.17
C407	28.1	70.46
C425	31.0	62.63
C431	33.5	63.86
C433	39.9	72.87

	Enclosure 2	Enclosure 4	Enclosure 5
Mean Weight (g)	38.0	32.3	41.9
(SD)	(12.5)	(12.4)	(12.4)
Min	18.4	12.6	23.3
Max	65.4	62.4	72.5
Grand Mean		37.4	
(SD)		(3.9)	
Mean Shell Length (mm)	65.97	62.57	68.69
(SD)	(7.62)	(7.17)	(6.20)
Min	48.65	48.94	56.66
Max	78.04	74.77	77.76
Grand Mean		65.74	
(SD)		(2.50)	

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 15 to 17 & 21)
Data Source: Form: "Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Species Identification" (File Folder 7b)

Test Article: MBI-401 SDP
Lot #: TR4665-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Unionid Mussel Weight and Length
Post-exposure - Fatmucket (*Lampsilis siliquoidea*)
100 mg/L Treatments

Enclosure 1

Tag ID	Weight (g)	Shell Length (mm)
C195	43.7	65.32
C208	46.0	69.70
C221	29.6	59.19
C228	35.5	68.89
C233	28.6	60.74
C237	31.3	34.42
C245	30.7	61.57
C263	50.3	71.10
C266	24.4	56.91
C292	49.4	68.80
C302	38.2	68.53
C309	20.5	55.42
C312	.	63.26
C314	25.8	59.01
C329	41.4	67.91
C345	36.5	59.00
C350	34.8	66.17
C358	26.0	57.80
C359	.	62.39
C360	29.4	64.78
C377	19.3	53.63
C379	54.5	77.03
C380	24.5	56.67
C414	21.2	56.61
C424	42.9	73.30

Enclosure 6

Tag ID	Weight (g)	Shell Length (mm)
C200	48.5	71.87
C205	26.6	56.79
C213	.	62.24
C250	44.5	68.94
C251	.	60.01
C252	40.7	64.82
C277	44.4	67.79
C280	.	63.19
C281	42.6	71.65
C297	36.5	68.83
C308	36.1	65.42
C313	36.4	71.34
C315	25.0	59.56
C316	31.9	63.91
C322	31.8	66.10
C372	50.5	73.64
C378	38.2	65.80
C383	37.6	66.13
C385	38.9	64.96
C396	.	48.54
C402	39.2	67.83
C423	32.0	58.62
C426	27.8	62.72

Enclosure 9

Tag ID	Weight (g)	Shell Length (mm)
C202	.	57.77
C206	43.1	72.13
C226	.	61.74
C227	32.0	63.69
C229	55.3	72.81
C258	.	75.85
C262	46.3	73.54
C287	56.7	76.39
C295	40.1	68.10
C305	36.4	71.70
C327	42.6	66.46
C328	25.5	60.45
C338	25.0	59.02
C346	28.5	65.48
C367	60.1	78.91
C376	43.6	70.54
C381	22.5	55.56
C409	29.9	63.83
C411	.	76.49
C416	.	56.11
C420	35.2	61.86
C422	44.9	66.90
C430	.	60.38
C432	43.4	71.56

	Enclosure 1	Enclosure 6	Enclosure 9
Mean Weight (g)	34.1	37.3	39.5
(SD)	(10.0)	(6.9)	(10.8)
Min	19.3	25.0	22.5
Max	54.5	50.5	60.1
Grand Mean		37.0	
(SD)		(2.2)	
Mean Shell Length (mm)	62.32	64.81	66.97
(SD)	(8.26)	(5.54)	(6.83)
Min	34.42	48.54	55.56
Max	77.03	73.64	78.91
Grand Mean		64.70	
(SD)		(1.90)	

Stucy Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 15 to 17 & 21)
Data Source: Form: "Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Species Identification" (File Folder 7b)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessmen: Dates: August 13-15, 2013

Unionid Mussel Weight and Length
Post-exposure - Fragile papershell (*Leptodea fragilis*)
All Treatments

Enclosure ID	Treatment Level (mg/L)	Tag ID	Weight (g)	Shell Length (mm)
7	0	C394	27.5	66.90
2	50	C331	23.2	63.74
2	50	C342	40.5	74.08
4	50	C398	15.7	54.72
5	50	C335	12.6	53.34
1	100	C207	83.0	95.64
1	100	C363	31.7	72.23
6	100	C307	.	60.81
6	100	C336	18.2	52.28
6	100	C362	39.1	74.40
9	100	C344	25.5	63.94
9	100	C386	16.1	58.71
		Mean	30.3	65.90
		(SD)	(18.9)	(11.62)
		Min	12.6	52.28
		Max	83.0	95.64

File Folder 7b
Item Number 2
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Study Number: AEH-13-PSEUDO-05 Electronic Lab Notebook (pages 18 & 20) Data Source: File Folder: 14 "Zebra Mussel Lengths" Datasheets	Action	Date	Initials
	Created.....	13-Feb-14	KLW
	Revised.....	12-Mar-14	KLW
	Reviewed...	3/12/14	
	Certified...	3/12/14	
File Name: See filenames as stated below			

Zebra Mussel Lengths
Pre-exposure Assessment

Test Article: MBI-401 SDP (*Pseudomonas fluorescens Pf*-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Overall Data Summary:

	Control			50 mg/L			100 mg/L		
	Enc 3	Enc 7	Enc 8	Enc 2	Enc 4	Enc 5	Enc 1	Enc 6	Enc 9
Grand Mean	12.04	11.95	12.11	12.63	11.65	11.90	11.32	11.53	10.82
(SD)	(0.88)	(0.76)	(0.02)	(0.17)	(0.40)	(0.74)	(0.15)	(0.33)	(0.41)
Min	6.03	6.11	6.08	6.07	6.01	6.19	6.01	6.00	6.01
Max	27.43	25.70	26.49	19.72	25.28	27.82	23.56	27.95	17.38

Data Explanation:
Five unionid mussels with adhering zebra mussels were collected from each test enclosure for use in estimating the pre-exposure zebra mussel colonization density. All zebra mussels were removed, sorted into live/dead and preserved in 70% ethanol for enumeration and length measurement. Live zebra mussels removed from 3 unionids from each treatment replicate were measured for length; mussels < 6 mm were removed from analysis.

Pre-exposure Control (0 mg/L) Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 3
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 7
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 8

Pre-exposure 50 mg/L Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 2
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 4
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 5

Pre-exposure Benthic Injection Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 1
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 6
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Pre-exp Enc 9

Data anomalies and deviations:
NONE

File Folder 14
Item Number 1
Page 1 of 10

Study Number: AEH-13-PSEUDO 05
Electronic Lab Notebook (page 18)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MB-401 SDF
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 1 (100 mg/L)

Mussel Tag ID	N	Lengths (mm)																				Mean (SD)	Grand Mean (SD)
C377	195	7.53	16.62	15.87	10.76	11.85	13.76	14.77	13.47	8.99	12.45	6.17	10.49	8.66	15.70	10.32	10.90	6.73	8.91	13.51	9.10	11.11 (2.47)	11.32 (0.15)
		13.93	13.55	12.91	9.20	7.70	14.46	7.57	12.90	8.41	9.48	16.25	15.45	11.98	10.92	11.13	8.00	13.22	13.68	8.75	10.84		
		9.34	11.66	14.25	10.41	15.00	8.62	6.11	12.72	13.48	13.08	9.49	8.81	6.85	11.71	8.84	10.15	12.24	10.33	8.62	13.80		
		13.68	7.50	7.78	12.03	7.67	13.06	14.73	10.71	10.91	10.32	7.32	9.25	11.45	10.79	12.55	7.54	11.44	10.50	12.75	13.87		
		8.80	11.11	12.10	7.90	12.09	10.19	14.37	9.55	13.70	12.69	16.04	11.84	10.15	11.27	13.28	13.49	9.34	10.50	6.40	11.18		
		8.78	11.30	10.24	7.64	9.54	6.38	13.26	15.47	10.82	12.38	6.83	15.85	12.09	10.05	14.81	9.90	12.61	12.12	8.68	13.18		
		12.52	13.04	12.80	10.55	12.08	7.56	13.51	11.51	8.91	12.21	12.74	11.84	12.82	14.45	9.94	6.45	12.26	10.03	9.45	15.00		
		11.58	13.49	10.10	11.08	10.85	6.95	9.37	12.53	9.80	11.56	6.86	10.02	12.15	11.69	15.23	11.96	12.68	10.04	8.80	11.28		
		10.68	8.00	16.39	7.49	12.81	11.22	9.48	14.32	13.20	13.62	8.38	13.61	9.90	10.98	14.33	12.76	7.40	6.58	11.54	11.87		
		14.94	10.96	13.97	10.58	12.81	7.23	11.40	12.69	12.45	8.36	11.21	9.39	8.28	9.64	11.68	7.48						
C358	188	11.82	10.68	14.21	14.08	14.04	13.53	13.34	11.77	13.44	11.04	15.70	13.14	12.80	10.73	8.55	11.01	10.59	12.37	13.13	14.82	11.43 (2.24)	
		10.30	11.00	11.03	11.27	14.27	13.33	13.40	10.37	11.87	12.08	10.36	14.89	10.32	9.32	12.90	12.17	12.85	9.40	13.67	13.04		
		7.06	15.51	12.30	11.66	8.60	14.52	10.93	15.19	11.18	9.37	10.96	14.94	11.79	14.13	13.71	12.81	13.11	9.18	9.67	11.45		
		8.67	7.92	15.91	13.17	10.70	12.88	10.63	9.86	14.57	7.99	14.58	13.22	10.44	10.38	9.53	11.66	10.71	11.22	13.66	9.03		
		9.04	13.41	7.71	13.61	10.75	14.73	14.33	10.78	7.60	7.37	9.37	11.63	17.10	14.28	15.57	13.57	13.96	8.90	12.43	8.76		
		12.49	12.31	11.47	6.85	12.48	9.34	14.83	9.26	6.88	12.60	8.83	15.43	10.43	10.36	9.96	11.96	11.63	13.31	12.82	13.61		
		7.79	8.98	9.13	8.86	13.24	9.58	12.38	8.45	12.96	9.43	12.19	11.16	11.66	15.52	9.81	10.90	10.98	11.62	11.91	10.42		
		10.94	10.43	12.69	10.68	11.06	12.94	10.10	12.86	12.76	10.44	15.50	11.97	11.81	11.82	11.28	7.76	9.51	7.73	8.81	7.14		
		12.79	8.85	13.17	6.18	7.70	10.07	9.66	13.11	11.17	14.96	6.81	7.83	9.03	11.09	9.55	11.79	12.50	9.94	9.15	14.69		
		13.96	11.12	14.71	11.89	11.41	9.99	9.47	8.36														
C263	201	8.67	11.85	9.62	11.60	13.88	10.99	11.58	12.89	9.66	12.70	7.78	11.67	7.02	8.81	9.20	11.11	10.94	10.22	11.43	11.29	11.42 (2.54)	
		8.60	12.77	9.60	14.28	8.69	11.33	12.00	12.90	9.17	14.41	8.69	11.89	12.91	8.94	17.55	12.00	15.16	10.31	8.81	9.64		
		8.42	12.22	12.67	11.44	6.52	9.88	11.07	8.94	11.41	10.33	9.40	12.44	12.92	14.59	11.61	14.11	13.39	9.57	10.80	10.25		
		13.08	11.41	9.49	10.56	14.42	8.90	7.17	11.41	10.85	9.38	14.48	9.74	13.71	8.42	8.90	12.97	14.40	13.76	8.12	10.40		
		6.85	12.85	10.32	12.56	13.25	15.38	12.21	6.03	8.69	8.76	11.80	12.63	12.55	13.40	11.66	12.39	14.35	15.92	16.94	11.34		
		12.10	12.18	9.58	10.29	13.54	8.39	10.87	23.56	8.66	11.75	7.61	11.06	6.50	8.99	11.79	10.80	13.73	10.76	11.54	10.24		
		10.61	9.41	8.24	10.21	7.67	12.58	16.10	9.50	10.86	10.19	6.85	11.37	8.94	12.47	9.87	8.37	14.38	8.96	11.64	13.65		
		10.47	8.80	13.31	13.86	10.69	10.80	12.71	14.96	14.58	9.60	6.38	7.93	8.92	9.82	9.44	14.08	14.35	13.19	11.99	10.91		
		8.97	11.68	11.28	12.46	7.58	15.31	11.34	13.82	14.53	9.40	12.55	14.06	12.22	13.41	10.72	14.96	10.51	9.62	10.13	14.17		
		17.04	13.83	11.85	9.92	15.36	13.08	14.69	12.54	10.41	10.54	14.87	16.26	6.01	12.25	8.39	14.96	9.07	11.64	8.77	16.63		
		14.27	15.84																				

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (page 18)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SDP
Lot #: TR4669-4-4
Exposure Date: July 28, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 2 (50 mg/L)

Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
C382	181	17.68	17.13	17.38	17.26	17.93	15.60	15.36	15.51	18.91	17.36	15.05	16.54	15.53	18.41	17.26	15.40	16.06	15.77	16.84	16.57	12.48 (2.91)	12.63 (0.17)
		16.07	15.62	17.74	14.97	14.45	15.19	14.34	16.99	13.54	14.88	12.67	15.11	15.42	16.02	15.03	15.56	13.70	7.06	14.56	12.20		
		12.88	14.56	14.40	13.42	12.86	14.90	12.12	14.03	14.19	13.74	7.47	14.15	14.55	10.33	9.23	11.50	10.68	10.03	14.43	11.84		
		11.95	11.37	12.23	14.13	9.29	10.82	14.81	10.43	9.59	11.38	16.87	13.50	12.01	11.29	12.80	8.99	10.52	8.05	10.52	7.09		
		6.93	7.91	6.38	8.26	7.71	8.28	7.68	8.89	10.34	8.80	8.48	9.45	11.82	10.22	13.82	15.27	11.82	10.08	9.22	12.74		
		13.78	11.10	14.65	13.26	12.14	14.97	13.71	13.31	13.30	13.54	8.42	7.21	8.31	14.77	12.97	12.07	14.88	15.83	14.94	12.43		
		14.41	16.10	12.30	15.47	14.30	10.69	13.28	12.43	14.55	12.96	12.91	9.34	12.24	12.31	13.03	10.71	12.49	12.59	11.73	13.81		
		14.05	11.62	14.83	12.53	15.56	9.56	6.36	13.93	12.57	9.84	12.21	12.65	9.57	9.74	10.01	10.05	9.62	10.72	12.06	11.49		
		7.02	7.33	14.53	10.40	10.72	9.41	9.57	11.67	9.36	7.12	9.42	13.36	8.39	11.28	13.33	10.51	9.43	8.91	8.50	9.42		
		9.99																					
C310	114	8.33	16.17	18.29	16.78	18.56	16.76	18.12	15.62	17.83	15.30	16.06	17.16	14.49	15.55	13.98	13.72	12.90	16.88	14.40	13.21	12.85 (2.54)	
		14.90	6.24	14.18	8.72	11.62	15.62	13.90	11.81	14.09	14.08	7.74	10.30	8.79	13.15	10.82	13.79	13.44	10.88	14.81	10.74		
		14.00	16.15	16.40	14.02	13.70	14.45	15.42	14.76	16.66	13.67	15.43	13.82	14.44	14.84	13.33	15.20	13.87	14.58	14.12	15.05		
		12.78	14.07	11.80	11.76	12.91	12.55	13.24	10.51	10.12	12.64	9.74	10.34	13.54	12.22	10.80	9.11	11.71	10.83	10.72	11.05		
		9.57	12.45	9.43	14.55	12.12	10.84	12.73	10.05	13.56	15.16	9.28	10.39	12.96	11.60	13.90	11.42	11.54	13.59	11.00	10.87		
		10.00	10.40	9.25	11.00	9.23	11.59	11.87	10.03	11.62	12.17	14.41	12.27	10.00	7.01								
C410	97	19.72	18.04	15.69	18.41	15.69	17.01	17.46	15.91	15.45	15.75	14.69	15.99	15.10	14.44	16.69	14.82	15.76	14.44	14.46	13.98	12.61 (2.98)	
		14.14	13.81	15.24	13.91	16.02	13.26	13.97	15.84	14.65	15.23	14.59	10.43	12.11	13.48	14.53	13.98	13.91	8.20	14.89	13.07		
		12.04	11.71	15.77	12.90	13.58	12.72	12.71	16.86	8.90	12.67	12.11	15.47	10.79	10.59	9.86	8.45	12.69	12.62	16.34	13.56		
		10.56	13.19	12.57	13.05	7.78	9.85	12.32	12.43	11.31	12.74	15.00	12.79	11.85	11.76	10.60	12.75	8.81	10.53	10.27	11.00		
		8.89	10.70	9.18	6.79	9.20	7.46	10.23	6.12	9.17	10.98	8.41	8.89	6.07	10.13	7.77	8.82	6.59					

Study Number: ABH-13-PSEJDO-05
Electronic Lab Notebook (page 18)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MRI-401 SDP
Lot #: TR4669-4-9
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 3 (Control)

Mussel Tag ID	N	Lengths (mm)																				Mean (SD)	Grand Mean (SD)
C197	201	8.86	10.58	12.13	13.40	16.20	17.92	14.46	15.21	24.04	9.96	14.20	10.04	11.32	11.82	17.05	11.53	13.69	13.53	7.10	13.95	12.15 (3.16)	12.04 (0.88)
		11.64	17.05	13.66	11.12	14.29	9.61	13.30	10.71	15.26	14.57	8.65	8.19	11.52	9.49	13.74	11.88	7.64	12.85	15.26	12.61		
		12.75	15.28	15.62	6.08	15.28	23.93	15.04	16.82	10.81	12.69	9.80	11.51	16.22	12.19	10.07	9.44	14.01	10.21	12.09	12.97		
		10.02	15.66	12.83	13.47	10.80	9.80	11.35	10.77	8.87	7.93	9.28	11.07	12.63	11.17	12.51	16.00	7.55	16.57	12.99	10.19		
		16.84	9.04	9.59	27.43	10.05	9.75	9.45	11.50	11.12	15.17	13.80	10.72	11.19	9.01	9.52	12.38	14.20	12.48	11.74	13.26		
		12.72	9.37	14.41	7.46	12.47	14.19	10.24	12.85	11.93	13.64	14.93	7.44	7.29	11.50	8.31	9.88	10.41	9.17	9.58	9.45		
		10.79	23.50	14.49	10.48	14.94	12.60	16.89	15.61	16.97	15.64	13.04	10.74	13.64	10.37	9.49	8.89	8.75	7.34	8.96	14.28		
		14.03	12.63	14.06	11.82	11.86	13.73	8.74	10.61	11.97	12.63	15.32	12.54	9.21	15.54	11.44	9.20	6.13	11.65	14.69	11.86		
		10.83	10.90	9.52	8.91	10.25	9.88	9.43	14.48	10.84	14.10	11.45	25.37	11.30	10.53	12.82	13.00	14.74	9.25	11.17	14.97		
		14.59	14.67	9.26	11.84	11.55	9.62	7.78	11.48	10.58	13.74	9.55	8.62	11.03	10.74	9.79	10.55	12.51	13.90	14.75	11.25		
		9.61																					
C427	168	8.76	10.05	10.25	12.90	12.41	11.43	8.82	12.87	11.10	9.83	10.17	10.31	10.49	10.60	8.40	10.29	10.92	9.25	12.49	9.04	10.91 (1.77)	
		9.27	11.03	13.11	10.85	9.84	12.58	9.04	8.91	13.04	13.99	9.16	13.75	9.59	9.74	12.82	7.61	14.17	7.30	11.67	10.64		
		12.70	12.25	13.54	12.76	15.83	9.70	7.92	13.02	13.44	11.43	12.21	10.14	12.11	13.01	10.26	11.26	12.96	10.10	10.26	11.04		
		7.82	9.99	9.76	8.02	13.54	14.71	9.13	11.27	10.51	12.95	11.36	10.55	7.95	11.36	10.71	8.10	8.72	12.09	14.23	11.22		
		12.93	10.24	8.32	8.54	9.74	12.78	13.53	11.54	13.70	9.51	10.54	10.47	9.66	6.95	11.32	11.84	11.22	9.38	10.14	13.35		
		11.34	9.70	9.80	9.13	11.08	9.95	7.14	11.64	10.39	11.21	11.49	11.52	14.97	13.95	11.56	11.37	12.34	10.45	13.86	6.26		
		9.18	10.14	10.22	13.34	10.15	13.43	10.42	11.65	12.61	5.13	13.72	11.04	9.50	12.75	8.01	9.45	13.51	11.74	10.74	11.57		
		15.20	11.87	12.08	12.24	9.66	12.32	11.68	9.45	8.05	11.19	11.13	8.67	10.74	12.50	11.10	8.27	8.55	10.00	9.58	9.62		
		11.48	10.60	11.80	9.38	11.30	9.72	11.14	10.59														
C382	164	11.08	14.66	15.69	8.56	12.63	13.91	14.60	15.77	16.43	12.26	15.11	15.79	12.04	14.71	13.60	12.20	12.94	13.16	8.53	12.48	13.06 (2.30)	
		8.76	15.13	13.80	14.88	12.31	16.07	13.02	14.87	13.11	8.46	12.16	11.20	12.82	13.97	11.53	12.87	15.67	11.35	18.16	12.92		
		18.21	12.94	11.63	10.98	11.75	8.00	16.17	10.45	12.34	12.44	14.57	9.83	11.08	11.93	14.47	14.32	14.86	13.13	13.54	13.79		
		10.86	11.91	6.48	11.89	13.71	9.59	9.79	13.87	12.10	12.27	14.79	13.25	15.10	8.18	13.99	15.43	15.88	13.84	10.17	12.17		
		14.35	16.40	15.49	12.56	12.69	12.71	13.17	12.70	14.99	14.52	11.83	12.63	13.48	15.67	13.03	14.72	16.38	14.88	15.91	16.51		
		16.21	11.04	11.82	9.17	16.73	10.87	14.63	11.68	13.10	10.29	11.96	10.24	15.28	17.81	15.05	15.82	11.08	12.51	11.38	10.82		
		11.02	14.53	15.39	11.00	13.47	13.68	11.54	9.93	15.22	14.96	13.91	12.95	12.10	11.70	11.62	13.81	8.66	14.31	14.04	14.20		
		15.03	14.00	16.31	9.80	12.42	10.83	14.63	13.65	15.65	9.89	9.11	13.14	11.77	11.52	15.63	8.56	11.60	17.95	11.34	14.22		
		8.62	12.37	17.62	14.27																		

Study Number: AEU-13-PSEUDO-05
Electronic Lab Notebook (pages 18 & 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 4 (50 mg/L)

Mussel Tag ID	N	Lengths (mm)																			Mean (SD)	Grand Mean (SD)
C239	257	9.58	9.74	10.24	9.15	10.16	12.85	15.69	12.54	11.33	12.43	12.98	11.96	7.88	10.23	12.44	6.88	11.11	11.91	9.26	12.02	11.15 (2.47)	11.65 (0.40)
		9.09	12.22	13.85	9.44	9.05	13.21	12.09	12.66	11.44	8.93	8.54	11.38	15.22	12.69	12.76	13.81	11.08	11.20	7.92	8.54		
		12.07	8.43	8.70	11.67	9.84	9.85	13.12	8.04	11.74	13.44	9.57	10.47	13.03	11.13	11.58	22.61	11.10	10.98	8.15	20.59		
		10.23	13.45	12.35	13.63	6.69	10.67	12.21	6.43	8.52	9.85	12.26	11.80	12.28	8.77	8.71	11.56	9.72	12.16	13.00	7.83		
		15.41	9.34	8.67	12.98	15.57	16.42	6.54	14.00	13.91	12.68	11.14	12.09	9.27	7.83	13.89	12.65	12.43	11.22	7.26	12.94		
		10.98	6.83	7.41	7.00	13.12	9.52	14.28	11.27	7.94	9.65	11.13	7.33	10.61	11.90	8.61	10.29	9.73	13.05	10.28	9.38		
		11.65	5.65	14.54	8.24	10.59	13.13	9.85	12.46	12.80	6.74	12.96	10.36	11.76	13.90	16.88	9.26	9.25	8.95	11.38	8.13		
		7.61	13.13	14.82	9.60	12.71	11.30	13.84	11.41	12.41	8.49	10.22	10.20	11.82	12.13	10.90	10.87	15.08	12.89	9.43	15.12		
		12.76	10.72	9.88	10.86	13.79	9.41	12.60	13.70	11.30	13.43	10.23	8.97	9.73	9.97	10.17	8.80	10.99	15.41	12.70	8.51		
		8.43	10.97	6.97	9.44	10.14	16.33	11.57	9.16	14.48	10.98	11.12	11.18	11.24	8.51	11.56	13.70	10.56	14.41	9.61	13.95		
		12.19	10.47	12.43	12.20	10.29	9.52	14.70	8.33	10.57	11.08	7.97	12.07	7.47	10.48	9.19	12.75	11.31	9.19	12.05	9.46		
		12.83	14.11	8.95	10.34	9.68	12.12	7.64	11.53	12.96	10.23	9.66	14.44	12.01	9.50	9.55	6.88	14.39	11.17	8.44	12.52		
		9.15	13.11	14.88	10.95	13.05	12.82	6.66	12.86	10.12	13.15	8.06	14.87	12.76	10.51	9.14	21.43	9.74					
C225	108	12.36	9.44	12.98	11.25	14.02	6.58	9.17	7.87	11.14	12.04	12.87	14.89	15.28	11.24	12.38	11.24	7.12	11.42	11.58	16.19	12.12 (2.73)	11.65 (0.40)
		11.21	11.62	8.01	10.24	12.76	8.16	12.29	16.07	7.85	11.86	11.46	11.74	15.05	11.63	12.41	10.82	12.00	9.00	13.96	12.07		
		10.69	12.93	11.52	13.45	10.58	11.51	14.33	18.89	11.13	16.66	7.75	12.53	11.21	9.79	10.47	18.17	10.42	11.72	12.52	8.55		
		11.55	14.07	14.01	8.71	14.41	9.86	8.99	13.71	12.18	9.10	19.08	9.85	9.56	11.98	11.71	12.26	14.89	17.30	13.72	12.06		
		13.95	11.40	15.38	9.50	16.83	13.28	10.32	16.68	13.55	10.48	13.25	17.62	10.46	10.11	7.57	8.78	14.98	14.33	12.90	6.96		
		7.83	15.99	12.02	11.96	12.83	11.69	15.59	15.78														
C333	126	10.98	15.51	24.41	13.84	13.19	13.26	15.50	11.55	16.60	12.14	13.84	17.08	7.46	13.38	13.84	13.94	15.80	14.01	13.30	13.57	11.67 (3.05)	11.65 (0.40)
		7.41	10.21	10.98	13.66	9.72	13.26	15.96	9.58	9.64	8.28	6.01	10.87	7.11	12.72	11.57	7.90	11.90	13.55	25.28	12.88		
		14.71	12.87	10.44	6.51	12.06	11.95	11.89	10.47	10.27	12.87	14.46	13.76	12.67	12.37	12.83	12.12	5.56	12.20	9.85	8.73		
		11.03	13.44	11.07	12.43	13.41	14.28	12.08	7.06	11.79	12.19	10.66	6.80	9.90	13.76	13.34	13.01	12.04	12.63	15.47	13.44		
		12.98	9.49	10.19	9.79	12.70	14.39	13.88	15.58	11.94	14.53	12.49	14.95	12.22	7.01	14.13	13.45	9.83	10.58	13.41	13.73		
		12.33	11.65	11.60	11.73	9.88	8.35	6.68	7.44	7.99	6.87	5.52	7.64	7.69	9.56	7.52	10.79	11.96	9.81	8.81	7.92		
		7.88	11.44	11.19	8.55	11.36	10.60																

Study Number: AEH-33-PSEUDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 5 (50 mg/L)

Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
C256	156	15.88	10.76	9.91	10.00	21.44	11.65	7.25	7.53	8.95	11.22	13.21	8.64	7.21	10.81	9.14	8.39	11.25	12.36	11.96	9.67	10.90 (2.45)	11.90 (0.74)
		12.52	15.48	10.91	14.62	10.77	10.89	14.11	12.88	13.64	10.91	14.85	12.25	12.12	8.51	10.84	11.10	11.62	9.90	9.65	9.52		
		15.18	14.26	10.64	11.63	13.67	14.11	13.12	15.28	13.50	11.22	11.17	14.17	10.30	12.19	11.51	12.88	13.91	14.24	13.26	12.48		
		10.48	11.17	12.41	11.22	14.05	9.70	11.29	14.44	12.10	11.80	8.47	12.63	14.14	11.50	8.56	11.22	10.53	11.75	12.06	13.77		
		9.32	13.85	9.51	14.94	13.18	7.47	11.91	10.09	13.51	14.52	14.98	11.91	13.26	12.33	12.51	12.47	11.07	10.47	10.85	12.36		
		11.37	10.91	11.54	9.83	10.04	9.79	11.24	7.05	11.09	12.35	10.65	6.71	6.28	6.53	8.13	9.74	6.19	9.26	9.44	7.28		
		11.82	7.52	9.89	12.68	6.53	10.30	9.47	8.04	8.11	7.22	9.78	8.89	10.98	9.43	8.81	10.82	9.45	7.84	7.22	7.89		
		6.93	11.39	9.13	8.62	6.65	11.62	10.11	10.66	9.72	9.03	7.59	7.91	8.40	7.92	6.30	13.94						
C259	114	12.60	12.13	11.92	12.75	10.39	16.74	14.19	13.01	14.96	13.37	15.36	13.55	11.84	15.70	8.52	8.48	10.70	13.17	7.54	13.94	12.14 (2.54)	
		17.55	10.29	14.56	8.57	14.31	13.80	12.92	11.46	11.51	15.48	14.74	11.55	13.41	17.97	15.54	8.73	8.72	12.42	14.34	14.00		
		10.42	15.01	14.27	13.21	15.61	15.08	13.50	10.33	13.50	12.75	11.08	7.79	7.81	11.94	13.44	11.94	12.46	12.44	10.01	10.01		
		9.03	9.52	11.22	13.41	8.13	9.37	11.82	17.09	9.42	7.97	6.88	7.17	14.73	15.27	13.85	11.22	9.46	11.39	13.58	9.71		
		10.25	12.27	16.09	12.06	11.48	8.45	13.46	14.82	15.84	15.78	13.35	13.00	14.15	10.74	15.35	15.48	13.62	13.20	11.81	12.81		
		12.00	8.31	9.72	10.01	9.54	8.28	11.38	13.05	13.37	13.01	10.84	9.85	10.16	9.16								
C240	113	11.68	12.02	18.08	22.29	17.90	11.10	12.15	14.96	15.20	14.91	7.11	8.51	10.34	14.57	15.26	12.24	27.82	17.12	16.86	15.01	12.66 (3.54)	
		13.91	14.00	12.57	9.52	9.72	8.84	7.01	8.18	14.70	13.36	17.56	14.29	16.64	13.10	16.44	14.70	14.91	11.01	12.75	12.35		
		12.28	11.91	13.41	9.44	10.37	13.65	14.46	14.44	13.81	12.49	12.02	12.84	12.27	11.64	11.14	17.00	14.75	13.66	13.03	7.69		
		9.87	14.76	12.14	13.90	9.00	8.69	13.03	9.16	15.89	24.41	11.82	8.03	12.28	6.30	12.97	11.66	11.78	11.25	9.76	9.49		
		11.53	12.05	8.30	11.40	14.56	13.93	7.35	10.79	15.04	10.11	11.13	13.75	12.73	15.05	12.48	9.33	8.60	8.15	12.09	11.98		
		11.07	9.98	13.91	11.91	17.89	8.73	12.18	11.07	13.59	8.24	11.13	12.84	13.46									

Study Number: AEH 13 PSEUDO 05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SDP
Lot #: TR4969-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 6 (100 mg/L)

Mussel Tag ID	N	Length (mm)																				Mean (SD)	Grand Mean (SD)
C250	124	10.44	13.94	14.25	9.46	7.58	10.56	13.15	7.14	15.28	13.01	11.98	13.06	13.23	12.45	12.83	10.43	12.93	12.22	12.21	14.71	11.47 (2.38)	11.53 (0.23)
		13.71	9.22	8.29	10.44	13.90	11.23	10.82	10.46	8.74	14.40	11.25	14.03	15.60	12.96	15.58	6.46	8.25	10.65	9.85	12.80		
		15.02	11.90	10.71	13.92	13.03	12.09	11.97	17.19	14.86	15.66	10.86	13.06	8.92	12.53	13.59	13.84	11.33	8.15	14.11	14.19		
		10.95	12.49	7.33	12.38	12.76	14.42	6.45	10.21	14.43	15.81	13.19	12.33	10.74	8.13	7.78	7.74	7.61	8.44	10.35	10.04		
		9.25	12.05	8.79	8.67	13.21	11.93	9.75	14.26	10.73	12.09	12.23	13.48	12.46	11.77	11.57	15.18	9.77	10.55	8.54	14.00		
		13.48	11.55	13.12	10.84	14.77	8.90	11.58	8.04	12.36	10.43	9.41	13.97	9.81	12.11	11.74	10.82	9.04	9.45	8.27	10.87		
		9.09	6.39	10.11	9.85																		
C426	140	27.95	17.45	23.34	14.19	8.03	11.93	25.56	13.58	13.35	14.69	12.62	14.56	26.51	16.27	14.00	8.03	12.52	12.16	9.09	13.26	11.96 (3.40)	
		14.08	9.32	9.71	14.45	14.53	12.58	15.15	13.70	9.92	11.90	14.78	11.22	15.94	13.87	12.63	8.83	14.90	13.07	10.31	13.14		
		15.12	13.08	13.82	13.77	16.59	12.79	13.50	14.95	13.50	14.73	13.41	8.71	14.19	11.92	12.83	10.98	11.26	9.56	11.07	9.34		
		8.94	10.10	7.06	8.11	18.26	12.94	11.05	12.41	11.33	10.88	10.33	13.98	14.65	11.71	11.14	9.59	10.82	11.09	11.04	12.54		
		13.25	8.15	10.94	10.78	10.68	10.80	11.15	12.91	8.64	12.22	9.99	8.13	11.40	7.20	8.92	10.74	11.41	10.46	13.42	7.94		
		12.24	11.40	12.57	13.96	10.80	8.43	9.93	15.38	11.19	11.20	15.65	8.58	12.72	7.34	8.95	12.68	7.22	13.19	10.76	8.21		
		9.40	8.34	15.41	13.97	9.26	10.90	11.08	8.70	9.37	6.19	13.66	11.21	13.24	11.74	13.02	8.84	7.70	9.30	6.31	6.66		
C200	156	13.17	16.84	8.60	6.41	11.58	13.19	12.22	11.56	11.98	11.24	10.09	11.42	11.02	12.05	9.95	9.93	12.76	10.68	15.51	10.64	11.15 (2.41)	
		15.77	10.49	8.56	11.63	6.32	16.75	11.26	9.29	11.30	6.28	6.50	15.67	9.44	14.48	12.09	11.09	13.93	12.90	10.58	8.68		
		11.42	7.91	14.58	6.00	13.12	14.03	7.56	14.02	11.47	13.59	12.20	13.56	11.32	10.68	12.47	12.11	12.04	8.24	10.70	9.49		
		10.89	9.61	10.13	12.35	10.79	7.71	6.21	13.27	10.19	14.57	7.26	9.95	11.71	13.90	9.24	12.38	14.24	11.47	11.32	11.20		
		10.37	8.34	7.46	10.03	15.71	15.62	15.03	14.68	10.44	12.80	15.07	11.82	13.24	9.92	11.16	10.64	12.00	12.78	12.95	11.72		
		12.67	12.15	12.18	14.05	10.12	11.08	11.64	11.32	13.77	12.81	11.62	10.87	8.19	13.20	10.11	7.80	12.71	10.22	10.38	11.51		
		11.78	8.47	9.89	10.19	11.34	8.86	10.16	6.80	7.99	6.88	8.86	10.14	6.09	12.42	12.60	9.02	12.16	8.35	9.54	9.88		
		12.32	10.89	8.95	11.29	12.27	13.19	13.87	15.15	9.18	15.08	13.08	10.95	7.31	6.27	13.96	6.78						

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-421 SDP
Lot #: TR4668-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 7 (Control)

Mussel Tag ID	N	Lengths (mm)																			Mean (SD)	Grand Mean (SD)
C417	145	15.43	16.50	13.29	8.54	12.15	7.97	8.90	12.08	9.05	14.95	13.87	9.22	16.52	15.08	12.12	15.30	16.50	12.43	14.88	11.02	12.02 (2.95)	
		11.70	12.62	12.81	12.02	12.46	23.72	17.02	15.84	19.11	16.84	13.18	14.62	15.45	14.80	15.20	11.77	8.75	13.75	10.71	8.62		
		9.68	13.68	17.84	10.00	17.27	17.85	15.38	14.92	14.57	13.95	11.26	7.56	11.70	8.23	10.52	7.58	13.44	15.70	15.79	10.55		
		13.95	13.60	14.70	12.14	15.38	15.92	12.93	10.29	11.31	13.34	11.54	10.68	11.28	11.50	11.11	10.53	11.58	13.70	11.56	11.25		
		12.94	8.36	10.73	12.85	11.63	9.23	9.24	10.27	12.86	10.43	7.98	15.50	9.52	13.22	14.04	14.43	14.20	15.91	10.88	13.09		
		13.64	16.25	12.12	10.82	10.10	11.16	8.99	8.80	13.50	17.55	11.44	11.85	12.03	9.56	6.98	7.73	11.00	10.88	13.37	7.42		
		13.51	9.95	8.50	6.91	7.80	11.74	8.64	9.37	9.08	8.88	11.94	6.52	10.60	10.67	11.87	6.16	10.28	10.03	12.19	13.25		
		10.41	7.69	9.84	11.13	11.97	9.88	16.49	8.78	6.11													
C192	118	13.51	11.88	15.41	17.64	9.56	13.87	10.67	13.16	14.18	15.75	16.44	14.80	16.37	14.21	14.49	28.85	8.72	25.70	14.11	24.04	12.84 (3.42)	11.95 (0.76)
		14.01	7.20	9.99	7.50	13.14	15.36	14.65	17.68	17.27	14.55	14.85	15.33	12.64	13.23	13.73	15.97	15.21	9.85	10.66	7.86		
		16.47	15.12	12.86	12.81	14.11	11.84	8.88	9.09	9.92	11.32	12.48	12.12	13.93	16.40	8.75	17.67	10.38	11.11	12.19	14.08		
		18.77	11.91	13.56	7.00	8.99	12.86	16.87	15.23	11.91	16.15	9.25	16.23	13.56	14.70	13.92	13.52	17.68	13.08	14.05	14.54		
		10.41	10.64	12.67	13.70	13.90	7.23	15.00	12.53	17.09	12.16	10.65	8.19	6.52	12.23	13.00	13.08	11.55	14.77	7.46	8.62		
		12.97	10.85	8.37	11.79	14.61	12.74	8.04	7.80	11.87	10.07	10.49	9.46	10.15	10.07	9.66	11.38	8.38	13.64				
C334	133	18.48	11.26	12.01	10.92	13.51	11.78	15.10	11.76	9.75	12.24	12.11	14.57	9.22	14.04	8.79	8.98	11.35	10.46	14.06	14.55	10.98 (2.28)	
		11.96	12.03	12.97	7.16	10.92	13.47	10.49	10.83	12.92	11.67	13.84	9.74	14.48	6.73	15.28	8.95	6.40	10.18	8.56	11.95		
		10.27	10.97	12.19	9.72	11.89	11.12	10.96	12.17	12.11	11.24	9.71	17.00	10.31	9.38	10.56	12.12	10.06	7.99	7.13	12.70		
		11.13	8.21	13.98	12.85	10.09	10.57	10.35	8.64	16.53	13.15	11.70	12.99	11.30	10.29	8.74	10.06	14.50	8.95	11.07	11.38		
		10.39	15.74	8.06	13.54	10.50	10.81	11.10	14.05	11.33	9.67	12.31	14.28	8.30	15.85	11.18	6.96	12.25	9.55	8.52	9.62		
		9.45	8.29	10.97	8.80	7.59	8.23	9.95	10.84	12.59	9.98	7.68	11.46	10.85	13.48	10.27	7.61	7.49	10.82	12.29	9.92		
		10.25	8.15	9.26	9.33	10.62	11.21	9.80	7.33	12.57	12.05	10.67	10.11	7.13									

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SOP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 8 (Control)

Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
C139	146	9.11	12.16	10.97	10.72	12.78	7.90	7.04	11.04	14.04	8.54	9.44	15.92	11.33	16.04	12.49	9.11	8.16	10.43	17.61	11.50	12.09 (2.62)	
		16.97	10.24	9.14	17.07	14.80	14.36	16.98	15.25	11.39	9.91	11.22	16.72	14.66	12.92	17.51	12.27	15.40	12.51	13.63	8.23		
		12.20	11.94	14.54	15.13	9.20	12.49	10.77	11.33	12.07	15.75	10.86	12.34	14.90	8.73	11.53	14.38	11.60	15.61	11.30	9.43		
		9.32	10.03	8.75	14.51	16.80	10.74	13.76	8.34	15.44	10.32	12.25	12.82	10.66	12.73	18.06	13.74	14.06	12.10	11.04	12.08		
		11.93	12.83	12.00	15.47	17.50	14.00	12.28	14.48	9.38	11.71	9.87	10.71	7.80	13.53	13.25	8.26	11.18	11.51	9.41	11.58		
		11.84	14.17	12.58	15.81	16.94	13.69	9.86	8.18	9.94	8.74	8.45	10.22	12.87	10.29	10.37	12.32	11.07	13.68	14.38	12.62		
		14.54	14.04	13.86	15.71	10.68	17.01	11.67	13.43	8.03	9.14	10.86	11.59	13.86	10.16	10.12	16.74	14.49	7.41	12.47	11.40		
		9.35	8.47	8.91	7.79	9.46	8.31																
C209	182	12.92	11.77	12.97	13.07	8.99	8.24	12.80	13.24	9.07	7.28	14.15	8.03	9.45	9.77	10.93	7.94	11.47	11.51	14.15	13.58	12.11 (2.78)	12.11 (0.02)
		16.25	12.76	9.78	8.55	18.11	10.83	12.43	14.27	14.75	13.11	13.34	12.68	13.25	15.47	21.60	15.48	14.49	17.01	14.68	14.84		
		14.35	13.61	13.30	9.64	17.58	15.49	9.80	10.84	15.36	11.95	11.18	11.33	12.13	15.33	13.04	8.65	9.59	15.05	13.17	14.82		
		12.86	16.11	8.07	14.00	14.43	10.04	12.55	11.91	11.96	7.91	9.35	11.24	12.75	8.44	10.41	10.68	13.22	11.21	11.10	11.12		
		14.83	13.84	11.16	12.54	9.50	13.25	15.55	9.33	12.28	9.95	9.03	9.91	13.95	10.07	14.16	14.40	14.02	15.44	13.52	12.83		
		13.98	12.09	13.17	16.28	8.66	12.90	13.62	12.14	14.67	14.38	11.25	11.65	10.98	9.68	12.68	13.28	10.98	10.63	13.34	14.35		
		17.42	9.74	10.80	8.58	12.76	10.79	11.07	8.59	11.83	6.62	15.64	13.45	8.25	10.98	9.46	6.51	9.54	14.91	9.57	10.09		
		8.61	10.15	14.80	7.28	11.90	10.16	16.39	12.61	11.88	8.59	11.48	10.44	11.59	11.79	12.26	13.21	14.50	6.08	10.46	12.16		
		10.89	10.12	8.17	12.77	13.57	6.69	12.45	12.68	11.27	10.63	15.83	10.89	15.72	8.83	24.58	6.48	11.16	11.56	14.01	15.44		
		16.15																					
C220	193	19.58	19.86	20.30	11.28	12.91	13.99	15.49	6.82	7.06	7.75	8.50	7.83	10.96	12.19	9.65	13.33	12.42	6.38	26.49	14.90	12.14 (5.12)	
		15.06	13.38	8.41	7.10	10.73	10.77	15.44	15.42	15.19	13.31	12.18	23.48	15.64	17.78	11.91	6.92	10.91	10.93	12.07	24.20		
		16.39	10.99	11.71	11.86	12.12	11.64	12.46	11.68	12.46	10.04	13.72	9.40	10.16	9.70	11.19	11.33	11.51	8.68	13.73	13.03		
		13.14	11.82	11.33	12.66	11.45	13.93	12.97	14.67	12.31	10.30	12.82	10.91	14.13	13.90	6.95	9.79	9.13	13.45	10.70	7.47		
		11.25	13.47	16.56	15.51	12.65	7.88	9.41	13.33	12.15	14.92	15.07	14.85	13.65	6.92	9.86	11.82	11.07	12.17	12.97	13.52		
		12.21	6.12	13.68	15.23	12.77	9.60	9.29	10.91	9.97	10.71	11.82	10.15	14.81	9.45	12.45	12.24	8.06	9.89	15.91	14.22		
		13.78	15.80	10.15	11.52	8.04	10.12	14.59	11.08	8.03	11.01	9.54	9.21	9.35	16.08	9.98	7.51	14.49	10.49	10.70	12.22		
		14.46	8.09	14.37	16.91	14.34	16.16	15.66	14.04	14.35	7.07	7.20	11.46	10.23	12.19	12.29	15.15	12.42	11.62	14.91	13.22		
		10.51	12.20	15.42	10.82	16.40	17.10	9.98	10.59	9.06	13.11	11.55	8.75	11.83	9.76	9.88	10.64	12.32	15.17	10.51	10.56		
		9.08	13.21	13.51	10.83	9.37	16.10	11.83	14.91	9.19	10.90	9.44	9.55	11.61									

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 14
"Zebra Mussel Lengths" Datasheets

Test Article: MBI-401 SDP
Lot #: TR4668-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Pre-Exposure Zebra Mussel Lengths
Test Enclosure 9 (100 mg/L)

Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
C41G	149	8.47	8.34	7.05	11.71	8.82	7.14	8.15	13.98	10.93	11.64	10.75	12.96	10.48	8.90	14.23	15.38	16.59	11.76	13.57	6.08	10.50 (2.63)	10.82 (0.41)
		7.74	9.32	10.60	12.12	11.70	7.08	14.83	11.01	8.81	10.83	15.54	12.85	12.88	16.84	8.04	14.90	7.55	9.68	16.58	10.48		
		13.12	15.40	10.37	13.13	10.88	10.03	12.19	14.97	13.54	14.58	14.17	12.48	13.74	7.84	9.64	7.96	10.96	9.71	7.82	9.35		
		6.71	8.23	9.95	10.41	7.56	10.81	12.86	10.48	7.37	11.99	12.38	7.78	10.37	9.92	8.84	7.93	12.07	10.10	10.21	9.77		
		8.51	11.96	12.94	12.47	11.04	7.47	8.93	8.79	11.61	10.65	7.45	7.10	12.62	9.20	10.16	10.47	13.24	7.56	12.43	6.89		
		7.93	9.84	8.72	11.74	6.62	16.10	7.48	6.67	11.84	6.73	9.63	11.68	10.98	9.02	7.75	9.97	8.23	12.37	15.99	6.83		
		10.02	14.93	9.79	13.14	9.65	15.14	6.42	8.81	8.88	10.71	6.91	15.64	8.44	13.06	8.73	13.06	9.82	15.63	6.83	8.47		
		7.46	6.53	10.51	11.06	10.58	9.37	11.52	8.60	7.24													
C295	114	12.16	7.85	8.55	9.95	13.53	9.36	11.54	7.98	11.38	9.12	9.67	14.09	15.19	13.14	15.59	13.67	10.36	16.56	13.48	7.86	11.40 (2.28)	
		15.89	14.54	12.50	12.63	15.40	9.52	7.46	6.39	12.44	11.80	14.48	11.39	13.57	12.96	14.21	6.20	13.04	12.34	13.21	11.83		
		11.40	11.32	9.09	9.15	9.53	14.52	12.17	13.84	11.21	10.70	11.06	13.25	12.62	13.12	12.11	8.40	13.09	13.80	13.70	10.68		
		11.58	7.74	12.27	9.57	11.86	12.01	11.53	8.81	11.28	11.23	11.65	9.87	11.80	12.74	10.60	10.05	13.35	11.95	14.08	10.90		
		9.86	12.95	7.93	14.39	11.18	13.53	9.48	9.48	12.98	11.32	8.29	10.91	9.04	13.85	11.50	9.19	7.20	9.75	9.92	10.25		
		14.27	12.84	9.50	14.58	14.74	8.14	10.52	9.98	12.87	10.49	7.66	12.72	12.19	6.01								
C305	90	8.18	8.96	8.47	10.11	11.18	8.36	14.90	17.38	9.48	13.89	12.22	14.93	11.55	11.22	10.61	10.79	7.90	9.27	7.68	14.04	10.54 (2.30)	
		11.77	7.86	11.85	12.88	11.59	13.87	10.29	8.74	13.31	10.65	10.87	15.46	10.45	12.47	11.32	13.24	14.54	9.51	9.49	10.40		
		8.82	10.11	11.50	14.01	9.11	11.64	10.59	11.92	8.00	8.60	8.46	11.46	10.38	12.61	6.54	12.62	9.28	9.82	9.85	10.39		
		8.87	9.33	8.42	10.38	10.97	7.88	9.85	11.20	8.05	10.03	11.35	8.65	9.56	11.97	6.79	6.46	15.04	10.35	9.76	12.54		
		14.29	8.89	6.11	7.34	13.06	8.64	10.50	8.69	11.94	7.80												

File Folder 14
Item Number 1
Page 10 of 10

Study Number: AEH-13-PSUEDO-05
Electronic Lab Notebook (pages 18 to 20)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

File Name: See filenames as stated below

Action	Date	Initials
Created.....	13-Feb-14	KLW
Revised.....	12-Mar-14	KLW
Reviewed...	12 MAR 14	
Certified...	3/12/14	

Zebra Mussel Lengths
Post-exposure Assessment

Test Article: MBI-401 SDP (*Pseudomonas fluorescens Pf*-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Overall Data Summary:

		Control			50 mg/L			100 mg/L		
		Enc 3	Enc 7	Enc 8	Enc 2	Enc 4	Enc 5	Enc 1	Enc 6	Enc 9
Alive	Grand	12.58	12.46	8.61	11.63	11.39	12.29	12.33	11.44	12.10
	(SD)	(0.36)	(1.00)	(0.36)	(0.89)	(0.45)	(0.74)	(0.69)	(0.23)	(0.18)
	Min	6.20	6.07	6.08	6.03	6.35	6.18	6.36	6.01	6.09
	Max	25.89	26.91	21.29	25.00	25.35	28.19	22.10	24.59	18.43
Dead	Grand	9.97	10.02	11.98	12.50	12.24	11.69	12.84	12.19	12.20
	(SD)	(3.35)	(2.17)	(2.41)	(1.93)	(0.92)	(1.04)	(0.29)	(1.25)	(1.10)
	Min	6.73	7.14	8.37	6.61	6.24	6.04	6.03	6.02	6.26
	Max	15.05	12.52	17.74	25.48	26.52	27.19	23.75	22.33	26.27

Data Explanation:
All unionid mussels from each test enclosure (5 used in pre-exposure assessment and 22 with adhering with zebra mussels) were assessed for survival and zebra mussel colonization 18-20 days post-exposure. The zebra mussels removed from 3 unionids from each test enclosure were sorted into live/dead, enumerated and measured for length. Mussels measuring < 6 mm was removed from analysis.

Post-exposure Control (0 mg/L) Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 3
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 7
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 8

Post-exposure 50 mg/L Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 2
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 4
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 5

Post-exposure 100 mg/L Test Enclosure Zebra Mussel Length Data:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 1
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 6
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Post-exp Enc 9

Data Imported to SAS for analysis:
I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Lengths.xlsx]Lengths for SAS

enc = Test Enclosure ID (1 through 9)
treat = Treatment Level
0 = 0 mg/L (Control)
50 = 50 mg/L
100 = 150 mg/L
time = Sampling Time
1 = Pre-exposure
2 = Post-exposure
status = Survival status of mussel
L = Alive
D = Dead
length = Grand Mean of Mussel Length for Test Enclosure (mm)

Data anomalies and deviations:
NONE

File Folder 15
Item Number 1
Page 1 of 11

Study Number: ADH-13-PSUEDO-05
Electronic Lab Notebook (page 18)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 1 (100 mg/L)

Alive/ Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C379	31	15.05	15.47	11.09	12.46	9.29	10.25	15.93	13.77	12.38	10.66	8.54	15.82	13.08	13.13	12.32	11.80	12.77	18.22	13.83	17.51	12.60 (2.76)	12.83 (0.69)
			12.66	14.90	8.46	10.90	6.45	11.76	14.98	14.48	9.08	13.74	12.98	12.80	13.17	14.20	11.46	8.17	9.45	14.81	12.50	14.01		
			16.01	6.36	8.66	14.02	18.00	16.35	12.52	13.12	16.10	22.10	15.15	16.85	11.02	8.92	12.00	10.94	10.77	11.14	11.90	11.88		
			10.76	10.77	15.26	14.11	10.97	15.38	7.51	11.48	14.89	12.94	14.21	10.41	12.07	13.54	13.46	13.65	9.17	12.56	9.57	9.56		
			11.78																					
	C360	65	15.64	10.41	15.32	12.99	13.55	11.97	9.10	13.97	18.93	15.69	11.54	15.05	13.67	14.51	16.32	9.02	15.79	13.14	13.92	14.84	12.84 (2.98)	
			14.91	11.65	16.77	15.49	16.15	15.18	16.37	15.25	16.57	12.95	13.61	11.54	12.60	13.14	8.12	8.90	8.16	10.83	14.92	9.82		
			12.38	16.58	13.10	15.17	15.29	12.59	15.50	17.00	6.98	11.68	11.08	15.87	16.40	8.83	13.63	10.94	8.76	11.66	11.91	12.87		
			12.22	9.36	8.32	8.98	8.06	7.20																
	C414	61	7.04	8.88	15.76	13.30	11.01	12.49	14.48	9.08	13.57	7.07	13.59	9.61	17.87	8.93	6.63	11.48	8.67	12.72	9.58	10.58	11.55 (2.68)	
			15.82	12.39	8.91	11.21	12.41	9.08	6.76	14.04	9.54	13.82	11.55	11.91	10.95	10.85	10.54	13.67	13.51	8.13	11.15	16.23		
			14.85	13.46	12.32	8.48	15.12	12.47	10.69	10.42	7.10	13.42	12.48	10.87	14.30	8.90	12.32	11.55	10.63	9.48	10.61	19.95		
			12.46																					
Dead	C379	78	12.26	15.03	14.27	9.98	13.30	18.73	9.31	13.81	18.50	13.50	12.38	13.69	6.03	13.80	16.35	13.93	11.68	10.68	13.00	15.98	12.60 (2.51)	12.84 (0.29)
			13.31	9.38	13.76	12.12	12.77	10.57	9.75	12.34	8.56	13.63	10.94	15.71	14.78	13.05	13.79	14.31	11.59	13.34	14.45	15.19		
			9.46	11.32	10.25	13.63	15.08	11.62	8.19	12.83	13.97	10.17	13.33	14.13	14.13	14.97	13.11	10.14	10.96	6.79	11.76	15.32		
			8.73	12.78	9.04	11.54	16.01	6.35	11.63	14.63	13.89	15.68	13.20	16.11	13.19	16.19	8.92	13.81	10.02	12.20				
	C360	51	9.44	12.65	14.72	16.98	9.73	13.18	11.14	14.96	14.48	7.89	12.79	13.81	16.28	14.58	9.93	15.56	16.25	13.66	12.72	9.69	13.16 (2.50)	
			14.20	11.21	12.93	11.30	15.61	10.90	16.44	14.46	13.34	15.10	15.52	15.71	8.05	16.69	13.64	12.53	11.45	15.71	13.73	10.26		
			15.39	12.51	13.56	13.53	13.22	17.97	15.37	10.27	9.32	14.27	8.39											
	C414	39	13.12	12.50	10.82	19.20	10.49	13.08	13.49	14.11	9.84	14.96	17.66	12.19	15.05	8.46	17.21	8.86	17.40	14.80	14.71	16.22	12.77 (2.68)	
			10.65	13.75	12.93	10.08	23.76	12.06	15.26	11.92	10.48	13.65	12.67	13.39	11.50	10.81	16.00	13.47	13.33	9.81	17.36	13.46		
			9.39	6.13	13.29	8.96	10.56	13.71	10.94	11.15	12.52	11.62	7.82	14.90	12.10	13.10	12.69	16.41	8.80	12.49	9.22	12.05		
			14.45	10.72	12.51	14.96	14.04	13.15	13.24	11.89	12.00	12.57	15.93	11.34	10.75	13.31	13.15	15.65	10.57	10.74	12.89	16.06		
			9.91	10.17	16.83	12.02	13.63	10.98	13.06	12.82	13.17													

Study Number: AEH-13-PSUEDO-05
Electronic Lab Notebook (page 19)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SOP
lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 2 (50 mg/L)

Alive/ Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C304	67	10.50	10.22	17.03	13.63	13.42	11.49	6.87	8.80	7.20	10.19	9.97	8.15	7.21	15.07	11.51	11.61	15.00	12.70	11.83	7.95		
			11.25	7.85	7.96	16.31	15.58	11.48	16.99	17.69	11.50	10.01	11.17	7.84	16.34	12.10	14.91	17.21	17.03	7.79	15.06	18.00	12.26	
			12.89	15.32	15.56	10.79	13.47	13.41	7.89	9.80	13.70	15.35	16.41	12.99	9.51	12.61	10.54	14.78	7.60	9.53	13.68	17.23	(3.15)	
			12.96	7.08	16.17	10.40	12.01	13.43	11.68															
	C342	76	20.16	9.78	24.78	13.87	12.04	9.89	15.58	11.56	10.19	14.36	8.40	9.68	8.85	25.00	14.23	11.15	12.75	9.01	7.91	14.10		
			10.67	12.88	11.40	11.61	9.93	16.59	11.48	13.28	10.21	10.67	6.70	9.96	12.98	15.03	13.68	13.06	16.59	16.10	14.01	14.68	12.01	11.63
			11.06	11.77	13.77	12.58	11.55	11.43	12.91	11.73	12.83	11.58	11.73	10.86	7.28	10.21	9.92	8.17	11.49	12.88	12.63	12.59	(3.24)	(0.89)
			12.73	10.04	8.70	13.14	8.83	9.51	13.05	11.19	7.51	10.31	10.52	9.36	10.68	10.15	14.64	8.60						
	C219	45	9.93	10.88	12.76	10.47	13.09	9.70	13.20	9.44	13.25	7.99	10.67	10.83	11.25	11.66	11.21	12.10	10.62	13.50	11.77	9.39	10.61	
			9.98	10.72	13.69	6.84	9.63	10.58	6.03	12.66	10.21	10.99	14.87	8.99	13.30	12.94	11.28	6.39	9.31	8.75	8.03	11.50	(1.98)	
			11.14	8.31	9.36	9.54	8.22																	
Dead	C304	52	15.70	15.88	11.93	16.81	14.42	13.51	14.38	14.68	15.55	13.80	11.65	25.48	19.45	15.75	16.51	15.00	12.30	9.85	18.89	15.73	14.64	
			14.39	14.57	14.59	8.59	12.62	13.30	12.59	15.79	13.70	14.62	14.26	18.10	13.26	15.64	11.47	17.74	16.58	10.74	14.36	16.18	(2.58)	
			16.87	14.05	15.58	14.19	13.42	14.72	12.04	14.45	14.60	14.61	12.39	14.06										
	C342	64	23.75	13.73	10.23	9.45	10.46	14.17	9.34	13.12	12.57	11.54	11.43	10.85	13.30	10.29	11.07	14.18	10.38	10.57	10.33	13.17		
			10.89	13.33	9.73	9.95	9.81	8.38	13.63	11.98	12.07	11.13	13.26	14.59	14.63	9.85	12.15	13.76	12.64	10.69	10.85	11.99	11.94	12.50
			13.38	8.80	12.42	18.58	14.08	12.11	14.17	12.93	21.67	12.60	13.40	8.04	7.38	9.19	13.04	11.65	7.17	15.35	8.69	12.45	(2.88)	(1.93)
			8.16	12.20	8.54	10.19																		
	C219	53	7.99	11.93	11.44	12.10	6.71	11.82	8.98	11.41	15.22	15.53	14.33	15.10	12.88	12.48	13.77	11.69	12.96	15.81	11.86	12.23	10.61	
			11.78	12.41	13.20	10.52	7.87	6.61	10.38	10.58	9.61	10.60	7.69	7.94	12.73	11.01	10.60	12.46	9.35	10.27	8.93	9.02	(2.20)	
			10.27	8.94	7.90	11.72	12.68	9.57	9.28	8.54	9.54	11.50	10.27	10.40	9.51									

Study Number: AEH-13-PSUEDO-05
Electronic Lab Notebook (page 19)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4699-4-1
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 3 (Control)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C390	91	13.59	8.97	6.32	13.99	9.53	15.31	24.64	11.46	8.56	13.68	10.64	7.05	14.24	12.03	15.66	13.44	13.70	14.88	10.70	12.89	11.16 (2.72)	12.58 (3.36)
			9.02	11.38	15.24	10.15	14.85	11.99	11.84	13.00	15.45	13.90	8.36	13.75	12.04	7.49	11.81	12.47	17.67	13.21	10.57	14.46		
			11.64	16.88	10.74	11.98	6.20	15.03	15.72	9.98	10.97	11.17	11.80	11.73	12.13	12.16	11.84	8.89	10.33	16.44	9.70	12.06		
			8.95	14.83	15.28	14.22	14.01	10.21	12.67	11.80	10.62	9.58	12.22	10.78	9.04	14.17	10.30	14.13	14.23	12.31	12.60	14.43		
			11.69	10.46	7.44	12.62	10.72	12.29	13.71	11.26	13.60	11.87	9.04											
	C311	119	8.69	15.37	13.72	18.20	16.47	18.91	15.61	13.06	16.38	13.82	13.05	17.19	12.94	12.84	16.30	10.97	17.14	15.88	14.36	12.49	12.84 (3.05)	
			15.72	11.97	14.55	12.56	14.22	8.89	12.54	13.94	15.13	11.79	11.14	14.89	16.32	7.84	13.24	16.59	15.24	11.90	15.59	10.16		
			7.63	13.80	14.14	13.10	14.69	25.89	13.65	11.58	10.48	13.18	14.18	15.61	14.00	18.01	15.39	13.74	15.50	9.06	14.35	8.11		
			14.85	9.35	15.29	16.19	10.40	7.89	12.55	15.43	11.38	11.15	10.10	10.07	11.41	9.93	16.08	10.51	12.31	16.39	13.21	11.76		
			13.04	7.33	9.01	6.33	10.87	14.71	13.36	15.71	7.11	14.04	9.35	12.37	13.88	10.68	14.48	15.39	13.76	13.37	11.97	8.25		
			16.12	12.65	15.22	6.54	7.81	8.96	11.54	14.44	11.21	13.24	7.09	10.29	11.96	13.39	11.51	11.28	11.24	8.23	10.19			
	C210	123	11.78	15.90	12.53	9.23	14.19	14.92	13.76	17.09	13.87	13.21	13.83	12.31	16.60	12.99	16.48	10.39	16.02	13.60	17.12	11.81	12.73 (2.70)	
			13.68	10.24	15.45	13.60	10.80	7.76	11.87	14.31	12.84	14.58	10.23	9.05	15.04	15.23	9.35	15.15	15.49	16.07	13.25	17.06		
			14.09	10.38	15.10	13.69	17.53	13.11	15.54	13.76	12.73	9.19	12.07	11.15	8.65	8.16	16.45	14.65	14.35	11.85	11.85	8.69		
			12.37	11.32	12.71	13.38	15.33	12.23	15.00	9.04	13.99	16.56	8.88	16.18	12.56	8.76	8.53	16.04	10.79	13.75	13.11	11.57		
			13.07	10.71	10.12	10.27	11.41	15.99	16.08	14.22	8.87	9.42	11.14	14.00	10.52	14.49	12.67	15.20	10.81	13.87	14.47	11.01		
			13.87	10.81	15.72	13.99	12.53	7.29	14.51	14.40	8.59	15.11	7.71	14.23	9.85	11.69	10.42	13.75	11.11	11.76	13.16	9.62		
			10.92	9.51	6.79																			
Dead	C390	1	6.73																		6.73 N/A	9.97 (3.35)
	C311	2	11.35	8.19																	9.77 (1.58)	
	C210	2	15.05	11.78																	13.42 (1.64)	

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Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4669 4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 4 (50 mg/L)

Alive/ Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C302	69	11.59	10.65	10.89	10.88	11.52	10.05	14.95	13.25	12.11	8.23	14.61	15.54	8.57	14.56	12.18	13.28	10.73	10.07	11.71	10.93	11.30 (2.41)	11.39 (0.45)
			9.68	8.48	9.68	7.08	11.15	11.91	10.17	9.14	12.86	12.33	14.23	16.08	9.54	12.62	11.55	13.60	9.28	9.74	16.39	8.77		
			14.41	11.89	11.30	12.88	10.94	7.91	11.35	15.43	12.67	14.56	10.38	12.84	7.10	7.69	12.60	11.90	13.39	8.13	11.69	15.17		
			8.78	9.00	13.48	13.78	9.14	9.36	8.89	6.86	6.76													
	C339	68	9.42	10.16	11.37	14.79	12.47	21.39	23.55	14.27	15.14	12.07	11.00	10.08	11.98	10.53	11.72	10.51	11.02	11.35	12.37	15.05	10.99 (2.94)	
			11.67	12.48	10.86	13.06	13.59	11.48	10.85	7.07	6.39	12.51	12.27	12.80	14.35	14.12	9.82	11.76	9.51	9.30	7.54	11.60		
			11.99	12.00	9.87	10.20	11.21	13.55	7.00	7.44	9.88	8.05	5.24	8.48	8.64	12.42	9.91	6.36	10.53	9.45	9.80	9.09		
			9.72	11.55	8.45	7.56	9.59	8.27	8.64	7.92														
	C279	63	25.85	17.48	18.24	14.32	16.15	15.08	15.00	16.12	12.99	13.17	15.86	13.26	14.38	14.20	14.57	14.00	13.29	13.77	12.26	12.75	11.87 (3.17)	
			12.76	13.71	14.42	11.48	12.60	12.64	6.35	7.40	6.50	6.89	7.64	7.47	7.40	8.19	8.90	9.72	10.37	10.61	9.69	13.00		
			9.52	10.18	11.73	10.21	9.71	13.21	11.46	10.76	9.93	12.72	10.09	10.05	12.06	9.97	11.04	10.07	10.82	12.21	10.11	10.95		
			10.03	12.88	8.55																			
Dead	C308	82	12.07	12.60	11.85	11.14	12.98	13.30	12.20	13.41	12.81	11.95	12.85	11.71	13.20	7.73	11.08	12.03	13.55	12.27	13.20	13.13	13.07 (3.10)	12.24 (0.92)
			12.15	11.46	9.30	11.96	11.32	10.91	14.43	12.39	13.53	12.38	11.37	10.91	12.26	9.97	11.97	9.87	14.24	12.12	13.09	10.38		
			12.99	10.70	10.52	9.27	8.65	10.36	9.06	12.01	9.38	11.48	7.62	9.23	9.46	8.99	12.23	26.52	15.58	17.08	25.63	15.11		
			13.85	17.33	16.21	17.01	15.43	16.37	16.25	15.96	18.30	13.77	14.82	14.95	14.38	16.35	13.11	14.98	16.45	13.64	15.12	13.82		
			14.69	13.91																				
	C339	79	13.91	21.46	13.55	14.04	14.29	13.13	14.10	12.95	12.19	11.24	14.02	14.20	12.96	13.54	10.50	13.02	14.38	11.54	10.87	13.60	11.25 (2.32)	
			13.25	10.01	13.71	11.13	10.73	12.16	11.20	10.56	13.91	11.38	10.33	9.05	10.46	11.10	10.49	12.04	10.94	12.70	12.12	11.80		
			12.48	10.16	14.74	10.09	13.53	11.02	10.54	9.96	9.04	12.06	7.42	12.16	10.92	10.38	9.78	12.18	9.55	10.64	10.07	10.67		
			10.23	9.49	10.89	6.64	10.46	12.39	8.00	8.48	10.62	8.77	9.13	8.09	7.06	6.83	6.24	8.14	9.53	13.21	9.42			
	C279	56	7.57	9.11	14.64	24.61	16.18	16.25	14.09	16.76	16.54	16.05	17.23	13.82	14.62	13.12	13.00	13.85	11.16	12.56	13.98	8.70	12.39 (2.98)	
			12.25	9.63	7.16	10.07	12.36	10.50	12.42	13.42	9.92	8.88	13.59	12.59	13.41	9.32	13.13	12.17	11.39	10.96	12.83	9.40		
			9.57	13.12	14.45	13.22	11.93	11.81	9.61	6.71	9.68	15.01	14.15	12.09	13.56	10.46	9.76	12.82						

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"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 5 (50 mg/L)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																			Mean (SD)	Grand Mean (SD)
Alive	C433	59	13.42	9.74	11.22	9.57	13.05	12.28	8.75	14.06	7.09	9.32	6.68	13.49	12.53	10.17	10.24	10.57	7.59	15.53	10.84	12.02	11.44 (2.86)	12.29 (0.74)
			12.25	10.59	28.56	9.56	11.08	11.75	9.37	10.52	8.57	14.62	10.89	14.75	11.84	6.57	11.57	13.38	12.65	6.84	11.82	8.77		
			9.98	11.54	9.94	15.58	14.45	12.24	9.33	10.26	7.02	11.98	13.45	13.33	11.87	11.05	18.04	11.44	11.34	12.86	12.14			
	C407	75	16.11	7.97	6.84	7.85	13.55	9.60	16.03	12.50	12.24	14.10	12.77	12.47	13.64	10.08	13.30	14.61	15.99	13.84	9.32	13.38	12.74 (3.86)	
			10.03	15.82	13.91	17.16	13.20	16.74	13.36	11.48	14.61	7.19	15.55	9.49	9.58	11.41	18.17	11.91	15.43	12.14	9.46	12.98		
			14.11	9.73	12.41	15.57	16.32	11.21	14.05	8.20	28.19	7.98	10.56	11.18	9.09	13.20	13.51	6.18	12.07	12.51	14.80	14.80		
			11.75	15.81	13.64	9.03	13.78	7.80	11.38	14.29	8.67	11.52	15.01	12.65	18.63	16.47	14.55							
	C352	68	21.38	13.78	11.48	10.55	16.24	9.89	7.95	16.20	13.62	17.37	14.54	8.48	13.39	10.67	9.51	9.17	12.64	14.41	15.84	12.11	12.70 (2.89)	
			15.42	17.01	18.59	9.89	13.48	13.73	17.68	11.53	15.38	14.59	9.98	13.34	11.11	10.15	10.72	10.71	15.99	12.86	16.54	15.13		
			14.18	13.25	15.18	14.35	14.32	12.66	11.51	12.40	11.98	16.15	10.49	9.99	8.15	11.49	10.98	6.82	9.21	7.16	12.15	10.71		
			13.07	12.44	8.51	13.49	11.62	14.71	13.88	10.60														
Dead	C433	66	8.58	11.72	9.24	9.55	9.81	15.69	13.92	10.09	12.05	10.80	12.36	11.19	13.05	12.09	7.32	9.32	11.69	11.59	7.57	11.03	10.50 (2.26)	11.69 (1.04)
			13.09	6.06	12.07	9.71	11.17	6.51	10.90	12.07	12.54	10.02	10.89	8.66	10.67	9.18	9.62	7.38	13.58	9.18	13.14	7.47		
			7.40	6.37	16.65	7.65	12.00	10.15	13.71	9.96	9.86	12.45	9.39	9.91	14.33	11.21	15.29	9.95	7.75	6.80	8.51	11.13		
			9.87	9.97	10.79	9.94	11.26	8.96																
	407	47	13.91	8.77	12.94	13.44	14.59	10.00	11.65	13.36	27.19	9.53	11.23	15.92	14.24	14.90	13.48	9.01	12.85	7.10	8.64	14.39	12.14 (3.53)	
			16.04	14.36	16.23	8.54	15.22	12.64	11.35	8.43	14.75	10.55	14.01	10.95	6.91	8.91	11.41	13.88	15.17	12.43	12.49	15.42		
			7.11	11.52	9.98	9.06	7.04	12.05	7.20															
	C352	54	13.83	13.06	10.85	14.62	13.85	6.63	12.29	10.76	15.77	13.08	13.40	11.17	9.58	11.22	12.54	10.08	11.11	12.62	8.05	15.94	12.42 (2.68)	
			11.06	13.89	10.69	13.49	8.87	8.87	15.39	6.04	18.99	10.21	12.92	14.69	15.47	14.64	13.22	12.69	14.45	16.24	14.84	15.62		
			14.56	12.78	9.58	6.81	13.04	13.73	16.30	10.52	13.86	8.57	12.50	12.59	13.58	10.78								

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Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 6 (100 mg/L)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C277	65	9.55	7.91	11.93	12.15	8.32	8.84	8.79	6.01	6.60	13.37	12.87	11.53	11.30	15.52	10.71	10.75	13.16	17.18	7.60	15.14	11.53 (2.86)	11.44 (0.23)
			16.49	12.92	13.11	12.87	9.17	9.01	10.98	16.21	16.73	14.58	12.16	14.49	15.08	12.04	8.35	13.58	7.34	11.60	8.07	6.31		
			12.26	14.20	11.51	12.01	10.51	12.33	10.81	8.88	9.59	14.80	13.96	7.07	8.67	12.12	15.33	15.66	12.52	10.42	9.70	15.62		
			14.08	10.30	8.71	9.75	8.15																	
	C322	33	12.16	8.20	8.57	8.98	10.81	24.59	11.25	13.10	17.55	10.67	12.00	7.82	10.86	10.94	12.07	11.64	6.94	11.65	6.35	11.39	11.17 (3.44)	
			10.97	6.02	8.83	11.36	12.91	11.95	11.33	8.18	11.03	12.04	14.30	6.95	15.28									
	C336	53	10.21	11.55	8.75	12.96	14.44	14.71	14.32	13.32	11.64	6.97	14.65	10.78	17.52	11.34	7.49	10.23	13.46	8.41	15.40	11.91	11.61 (2.42)	
			12.34	12.36	13.68	8.73	10.46	8.42	13.35	11.12	10.50	12.72	11.73	15.12	13.63	5.96	7.19	12.01	13.80	12.65	7.99	14.06		
			8.28	10.20	14.05	11.50	12.50	12.62	10.80	10.51	11.42	9.42	13.53	10.29	8.73									
Dead	C277	110	12.80	18.56	11.04	21.47	13.17	8.72	18.12	13.47	14.00	15.58	15.18	14.37	11.04	15.66	14.57	9.60	18.66	13.07	14.70	15.74	13.63 (2.62)	12.19 (1.25)
			14.18	15.86	12.57	15.87	9.40	14.61	17.86	14.33	12.18	15.28	16.78	12.90	15.76	8.59	12.53	10.78	12.28	15.91	13.06	19.71		
			13.68	16.61	14.62	8.73	10.57	16.31	14.51	9.93	17.48	14.66	16.60	13.50	13.33	12.04	15.46	13.21	13.38	13.43	13.15	14.58		
			11.25	11.83	15.56	14.61	13.86	18.03	22.33	11.47	11.09	13.94	13.26	15.20	12.59	10.52	11.88	14.03	12.65	12.06	13.40	14.17		
			13.15	11.92	11.58	14.47	11.80	13.98	17.33	13.76	13.33	15.25	11.86	12.68	11.96	14.77	13.67	10.84	19.63	11.97	10.85	11.40		
			12.45	11.68	14.98	9.26	13.66	14.54	13.09	11.75	11.03	6.06												
	C322	43	6.29	11.85	11.38	7.28	13.15	12.84	14.85	10.85	16.12	7.74	12.71	6.85	7.08	12.10	7.16	17.58	11.91	6.97	11.08	14.71	11.51 (2.69)	
			10.48	10.91	10.73	11.47	11.21	11.36	14.84	10.79	14.24	11.85	11.23	12.55	11.69	9.52	11.03	12.97	13.34	12.33	15.31	12.90		
			6.73	14.82	12.85																			
	C336	50	12.23	12.90	8.09	9.71	9.94	6.02	18.02	12.77	13.09	12.40	11.46	7.99	8.19	13.34	13.65	10.69	10.48	10.97	11.70	8.79	11.42 (2.22)	
			12.36	10.82	7.30	10.42	15.59	13.62	12.51	13.84	12.87	12.58	10.86	9.18	7.48	15.18	11.48	14.00	13.07	9.14	11.88	9.05		
			11.36	15.28	10.22	12.11	9.52	11.22	10.64	12.46	13.36	11.60												

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Data Source: File Folder: 13
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4869-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 7 (Control)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																			Mean (SD)	Grand Mean (SD)
Alive	C234	144	11.05	15.18	12.84	8.32	11.27	11.05	11.09	14.10	16.85	10.49	11.14	16.80	9.31	10.12	10.85	7.51	6.20	9.25	12.89	7.80	11.36 (2.79)	12.46 (1.00)
			10.38	10.32	8.35	12.63	9.73	16.09	9.93	12.36	8.15	13.21	9.71	9.02	16.12	11.78	12.66	15.27	6.67	6.85	10.38	14.28		
			6.85	12.45	12.34	16.34	10.53	15.18	8.52	11.91	13.18	8.99	7.50	15.29	12.26	9.59	13.73	8.25	8.61	8.89	9.74	8.49		
			11.27	6.70	8.76	9.82	12.44	14.16	13.00	13.27	9.23	18.05	10.91	9.81	10.71	6.47	11.23	10.26	13.93	12.02	18.37	10.93		
			21.45	15.25	12.03	10.39	9.64	8.25	13.89	14.69	14.20	10.35	14.15	13.34	11.50	12.25	10.54	12.95	11.82	12.56	7.99	15.50		
			14.20	6.08	11.02	9.47	12.88	10.57	7.13	12.80	7.20	6.22	16.40	9.57	8.56	17.11	11.59	9.34	14.18	14.10	14.60	9.34		
			8.42	9.76	13.91	9.73	9.43	12.53	11.91	9.23	11.87	8.50	10.86	11.00	7.94	8.20	10.48	13.55	11.21	11.42	11.44	12.60		
			11.05	15.42	14.95	13.13																		
	C355	151	16.15	7.62	14.99	14.66	11.96	14.32	17.13	11.58	10.90	12.31	11.48	12.66	14.86	8.64	14.55	15.99	9.12	13.76	15.60	11.98	13.31 (2.99)	
			15.73	9.77	11.62	16.48	9.11	8.92	11.95	10.18	14.06	8.45	15.46	10.94	12.58	13.78	7.44	13.21	13.24	12.27	18.52	19.44		
			11.88	11.53	15.93	12.36	12.03	8.44	11.84	10.76	13.22	18.77	15.95	6.80	13.41	7.37	11.87	14.38	10.58	13.58	14.07	13.59		
			12.11	11.11	13.50	14.50	16.06	5.07	15.14	16.99	14.04	25.38	16.33	15.46	12.46	15.15	12.97	15.03	14.70	16.95	17.58	15.23		
			11.48	12.07	11.18	16.25	14.04	18.24	18.05	13.74	8.26	13.74	8.84	12.49	15.73	11.92	12.91	11.87	11.85	14.81	15.85	16.38		
			18.32	10.69	9.83	15.38	10.91	15.13	17.01	15.10	7.03	18.53	12.33	14.48	8.90	12.80	13.42	6.78	14.86	11.06	12.02	10.86		
			16.16	13.84	14.05	15.65	12.68	9.74	14.44	18.03	16.07	15.89	7.04	12.84	14.47	12.74	14.57	15.91	15.09	16.78	11.20	15.34		
			16.76	15.44	3.57	10.84	15.91	13.65	16.26	10.84	16.74	13.68	11.57											
	C212	182	14.48	13.87	8.57	14.85	14.02	13.90	24.04	17.58	11.90	10.58	26.91	16.07	21.02	15.62	14.77	12.12	11.16	11.94	12.94	14.66	12.70 (2.74)	
			16.89	13.79	11.95	10.60	12.08	16.02	12.22	23.12	14.05	8.13	11.13	10.65	9.05	13.96	12.19	14.86	16.22	12.54	15.34	10.31		
			13.32	12.07	13.38	11.99	10.95	8.10	9.59	14.07	13.01	14.19	11.03	14.29	13.13	9.85	7.92	9.32	14.17	15.30	14.72	12.61		
			11.14	10.68	15.02	12.76	13.21	11.66	12.11	12.76	6.53	13.75	11.33	12.15	13.22	12.85	13.81	17.26	15.44	14.91	12.09	14.67		
			10.69	14.60	10.59	13.77	8.44	9.77	11.40	10.66	13.96	13.46	12.15	8.86	12.29	12.45	6.77	11.32	10.88	14.04	14.23	14.64		
			11.50	15.92	11.86	14.80	14.72	16.52	8.43	11.03	9.73	13.25	14.95	10.33	16.71	13.39	14.65	14.33	10.99	12.48	12.39	14.27		
			13.73	13.78	12.07	12.76	8.49	13.20	10.34	12.69	12.04	12.84	9.89	12.16	14.29	7.71	11.97	9.63	11.39	15.75	12.29	10.96		
			10.40	14.05	11.08	12.44	13.26	13.12	9.13	11.94	12.94	12.56	13.03	12.23	10.72	13.72	10.94	14.73	9.39	9.86	8.47	8.73		
			13.01	17.15	14.69	12.27	13.16	10.56	11.35	14.53	13.33	12.88	14.56	13.78	13.22	10.15	9.19	11.76	13.17	14.00	11.24	13.62		
			13.78	11.49																				
Dead	C234	2	10.51	7.14																		8.83 (1.69)	10.02 (2.17)	
	C355	1	12.52																			12.52 N/A		
	C212	2	7.36	10.07																		8.72 (1.36)		

Study Number: AEH-13-PSUEDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SOP
Lot #: TR4589-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Test Enclosure 8 (Control)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C415	178	10.24	13.79	16.95	8.75	11.81	8.10	11.44	10.65	10.47	10.41	11.30	12.79	13.81	13.96	11.93	12.42	14.20	13.38	9.21	14.29	11.09 (2.64)	8.61 (0.36)
			10.18	11.85	9.85	12.38	6.10	10.28	13.63	10.46	10.19	13.54	11.45	16.12	15.47	14.13	6.88	9.63	12.65	8.01	10.50	14.89		
			6.08	13.04	10.40	9.65	8.81	12.29	14.84	9.76	15.84	9.26	14.05	11.54	15.04	12.13	10.70	14.94	12.26	12.12	16.78	10.43		
			11.50	17.09	12.38	9.86	11.78	10.59	12.97	9.82	8.60	12.04	9.24	9.25	13.45	14.67	11.06	6.83	8.03	14.31	10.93	9.45		
			6.41	6.32	6.67	13.96	14.17	6.85	10.81	11.47	7.57	7.30	12.52	8.58	12.80	8.62	14.37	13.25	16.71	9.51	16.16	9.59		
			9.66	10.95	6.87	7.83	10.06	15.55	9.56	11.38	8.57	6.94	15.62	12.16	9.31	11.01	13.21	10.38	8.96	13.52	15.39	6.20		
			12.61	8.36	12.31	9.99	7.13	11.73	9.36	8.12	11.36	12.78	12.91	9.39	7.87	6.36	7.35	11.64	6.34	13.34	10.02	14.66		
			13.06	11.04	10.97	12.27	11.11	11.23	10.92	13.66	13.08	10.51	7.01	11.62	8.84	14.10	15.38	14.14	7.04	15.38	10.92	7.09		
			9.60	7.52	6.75	13.02	8.49	10.58	11.16	9.61	11.91	12.70	9.84	11.49	9.59	13.81	11.70	9.94	6.87	9.44				
	C391	182	15.75	6.94	14.42	15.28	7.32	10.43	10.16	10.44	6.25	15.33	10.24	14.87	15.69	16.99	15.28	16.24	12.80	16.83	15.50	10.88	11.80 (2.71)	
			12.70	10.21	13.74	13.32	10.15	10.82	17.15	13.54	13.82	11.88	12.15	7.35	10.43	10.46	10.68	9.59	12.73	9.77	11.50	7.05		
			6.40	14.98	13.97	11.91	8.94	13.41	10.31	12.10	14.72	13.65	11.41	12.94	15.67	15.61	14.02	13.43	8.79	7.13	6.19	13.53		
			10.18	11.18	13.24	12.18	11.07	14.26	16.26	10.71	10.78	9.97	11.42	10.51	9.36	9.65	12.35	13.69	11.30	12.26	12.43	11.47		
			12.24	13.65	7.73	12.64	18.02	14.31	12.92	14.62	10.74	7.99	12.60	11.42	11.65	17.41	15.71	12.94	9.30	13.07	12.80	16.28		
			6.16	9.49	9.60	12.04	11.66	12.85	13.74	11.29	15.90	11.16	11.08	11.57	7.94	14.64	12.61	9.67	9.40	14.66	13.13	11.44		
			14.37	12.09	12.60	15.48	8.80	10.70	10.22	11.28	15.52	10.79	10.71	11.73	12.76	13.06	7.68	15.62	11.30	7.67	10.36	9.01		
			8.46	16.09	11.49	13.96	6.09	6.57	7.91	14.70	9.49	13.35	11.99	16.54	9.47	13.78	11.22	9.46	13.39	8.08	8.59	15.33		
			10.33	11.14	10.88	14.83	6.76	9.96	16.81	12.81	12.36	14.40	7.60	13.08	12.21	10.25	11.00	13.96	17.92	11.15	7.75	11.20		
			11.84	11.21	7.68	8.65	12.69	12.79	10.57	11.19	11.73	6.82	11.33	10.59										
	C269	186	14.61	11.98	15.94	10.03	9.89	9.86	9.87	8.24	7.09	14.70	11.67	12.28	12.06	11.81	8.55	7.41	7.86	11.56	12.33	15.81	11.55 (2.51)	
			11.68	13.22	10.33	15.42	14.29	12.64	16.74	12.26	11.19	11.65	7.42	11.32	15.96	14.26	10.79	10.97	11.94	7.22	12.26	6.95		
			12.67	9.75	10.31	15.65	12.76	14.76	13.79	12.75	8.45	9.49	10.17	15.62	6.90	12.07	8.75	11.91	12.29	9.40	13.15	13.56		
			14.39	11.85	7.87	9.54	14.84	10.59	11.04	13.76	11.52	8.40	13.85	13.80	10.69	10.51	6.48	8.87	15.00	11.56	10.21	13.72		
			8.68	14.44	8.34	15.48	7.44	7.88	14.43	13.80	12.74	11.65	9.87	15.11	6.80	12.70	13.88	14.02	12.24	14.26	6.10	11.85		
			12.37	14.02	15.78	8.04	14.70	7.55	13.67	12.71	12.59	11.27	13.31	12.83	12.90	8.09	14.09	7.55	9.39	10.67	6.60	9.47		
			9.90	12.28	11.47	13.08	8.29	14.51	10.88	15.91	9.11	10.65	10.27	11.90	10.67	12.64	8.78	13.17	21.29	9.62	10.31	12.59		
			9.26	12.13	14.83	11.59	9.93	10.75	13.77	13.89	10.00	13.67	14.33	12.66	13.77	10.60	11.11	12.60	6.57	13.39	10.64	11.87		
			15.55	8.57	11.08	12.76	12.88	15.37	12.70	9.44	12.43	9.45	11.06	10.38	8.03	10.04	10.66	9.96	12.28	12.86	14.99	13.91		
			12.94	9.06	10.07	9.07	9.40	10.61																
Dead	C415	1	9.25																		9.25 N/A	11.98 (2.41)
	C391	7	17.74	15.95	17.42	10.89	8.37	10.43	9.49												12.90 (3.69)	
	C269	1	13.79																		13.79 N/A	

Study Number: AEH-13-PSUEDO-05
Electronic Lab Notebook (page 20)
Data Source: File Folder: 15
"Assessment Zebra Mussel Lengths" Datasheet

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Post-exposure Zebra Mussel Lengths
Enclosure 9 (100 mg/L)

Alive / Dead	Mussel Tag ID	N	Lengths (mm)																		Mean (SD)	Grand Mean (SD)
Alive	C409	85	9.27	7.19	15.19	15.21	17.28	11.99	12.50	14.89	8.42	11.42	8.74	16.70	13.57	11.50	15.14	12.79	13.41	16.65	15.12	14.69	11.95 (2.54)	12.10 (0.18)
			13.11	14.61	15.64	8.81	12.93	14.08	12.93	12.52	6.28	8.47	10.60	10.67	6.86	12.85	13.40	12.18	14.24	13.53	9.74	14.46		
			13.10	7.20	12.20	7.71	6.78	9.35	12.68	10.91	12.28	13.48	10.01	10.72	9.94	13.39	10.17	12.79	10.76	13.01	13.60	10.60		
			12.48	10.64	17.27	12.94	9.23	9.21	9.92	10.16	12.87	12.14	11.60	17.27	10.09	12.95	11.21	10.23	11.72	10.30	13.81	8.95		
			11.94	11.07	10.02	14.09	14.80																	
	C206	48	9.12	7.91	9.30	13.55	11.59	7.20	8.69	16.19	8.01	14.48	12.80	14.55	13.70	14.76	10.60	12.38	11.34	14.14	7.18	10.19	12.04 (2.63)	
			11.84	13.47	13.20	14.22	16.62	12.18	10.41	12.92	6.11	11.60	13.25	12.48	6.91	13.45	13.26	16.50	13.20	14.57	16.04	15.35		
			12.61	10.20	10.79	13.37	12.80	9.86	11.79	9.33														
	C262	92	15.73	8.84	11.91	9.88	9.44	13.48	15.71	7.34	10.90	7.06	11.80	13.73	15.83	15.73	13.89	15.15	15.55	15.82	12.88	14.99	12.90 (2.73)	
			14.89	18.48	14.82	9.35	13.45	13.06	6.09	13.03	14.04	9.65	10.26	15.55	9.95	8.57	12.31	12.05	6.45	12.05	10.29	10.70		
			9.49	11.31	13.42	10.96	13.74	12.34	16.07	8.02	13.82	14.44	18.97	13.14	12.71	14.41	15.06	13.07	12.22	8.66	14.82	12.23		
			14.20	15.10	14.32	14.94	13.13	10.10	11.29	13.10	15.76	11.11	8.08	16.60	9.83	14.66	11.41	15.16	11.94	11.08	10.28	10.68		
			8.30	7.90	13.04	10.40	11.87	12.78	10.36	11.92	5.75	11.88	13.37	9.41										
Dead	C409	82	10.76	10.49	6.26	6.62	13.88	12.01	26.27	6.71	13.80	16.18	11.02	8.63	9.73	13.46	13.20	6.68	12.23	12.64	13.36	10.16	10.93 (5.22)	12.20 (1.10)
			10.63	14.71	13.05	7.08	13.58	10.87	9.84	11.22	11.31	9.64	9.93	8.23	6.53	9.34	13.51	9.80	13.84	7.64	13.78	7.02		
			6.96	12.08	8.17	8.12	17.26	9.56	10.14	14.78	11.94	13.57	12.56	7.50	10.06	6.37	10.80	13.94	10.68	9.55	13.61	10.02		
			9.82	16.31	9.47	6.27	9.91	9.54	14.61	9.69	9.30	13.16	6.96	7.47	10.58	14.15	13.87	10.10	16.99	9.26	10.19	11.41		
			6.38	7.58																				
	C206	75	14.22	12.56	18.47	15.43	14.78	12.33	16.85	14.86	10.04	13.72	10.96	12.14	10.94	14.53	7.42	24.39	11.27	12.61	8.04	13.79	12.90 (2.85)	
			14.47	8.96	12.07	11.39	14.69	7.35	12.83	16.13	14.13	13.12	13.91	16.63	11.84	7.17	17.08	13.11	13.20	9.83	12.05	9.34		
			16.06	15.65	10.77	16.63	12.30	14.76	10.36	9.20	11.75	16.08	11.85	9.91	12.46	10.26	13.60	14.84	13.02	14.03	13.39	12.81		
			13.81	9.91	11.85	12.54	13.52	7.17	11.56	12.77	17.09	11.32	16.70	11.91	11.94	13.53	11.94							
	C262	79	9.60	11.48	12.20	14.88	13.49	16.65	15.00	9.12	13.78	8.90	13.80	11.07	13.51	16.40	14.86	14.45	13.76	15.09	15.86	13.60	12.76 (2.25)	
			11.34	12.75	8.92	12.26	14.88	10.23	9.01	11.86	13.27	11.96	15.38	15.56	12.85	12.65	15.45	13.87	8.54	15.30	14.94	11.65		
			10.39	9.15	13.89	11.03	14.92	12.73	9.46	14.52	14.66	12.44	9.67	18.66	10.87	11.11	11.35	9.42	17.12	11.37	12.12	9.96		
			14.08	11.65	11.01	15.59	11.85	13.71	10.48	13.37	12.72	14.18	10.84	12.36	14.29	11.62	14.16	9.52	13.11	16.60	12.30			

enc	treat	time	length
1	100	1	11.32
2	50	1	12.63
3	0	1	12.04
4	50	1	11.65
5	50	1	11.90
6	100	1	11.53
7	0	1	11.95
8	0	1	12.11
9	100	1	10.82
1	100	2	12.33
2	50	2	11.63
3	0	2	12.58
4	50	2	11.39
5	50	2	12.29
6	100	2	11.44
7	0	2	12.46
8	0	2	8.61
9	100	2	12.10

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Item No. 1
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Appendix 6. Water Quality

Item number	Item description	Number of pages	Report page number
1	Pre-Exposure Period Water Chemistry – Data Summary	2	169
2	Exposure Period Water Chemistry – Data Summary	2	171
3	Ammonia – Data Summary	3	173
4	Temperature Data Loggers (HOBOs) – Data Summary	4	176
5	Report of Analysis – Ammonia Report from Water Quality Laboratory at UMESC	1	180

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (page 12)	Created.....	5-Sep-13	KLW
Data Source: File Folder: 11	Revised.....	19-Feb-14	KLW
Forms: "Water Quality" datasheet (pre-exposure for DO, pH, temperature); "Alkalinity" datasheet; "Conductivity and Hardness datasheet	Reviewed...	19 FEB 14	
	Certified...	2/19/14	
File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Water Chem.xlsx]Pre-Exposure Water Chem			

Pre-Exposure Period Water Chemistry

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:
Pre-Exposure Period Water Chemistry - Dissolved oxygen, pH, and temperature were measured prior to exposure initiation in each test enclosure. Alkalinity, hardness, and conductivity were measured from water samples collected in the vicinity of each test enclosure.

Data anomalies and deviations
NONE

File Folder 11
Item Number 1
Page 1 of 2

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (page 12)
Data Source: File Folder: 11
Forms: "Water Quality" datasheet (pre-exposure for DO, pH, temperature)
"Alkalinity" datasheet
"Conductivity and Hardness datasheet

Test Article: MBI 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Pre-Exposure Period Water Chemistry

Treatment Group (mg/L)	Test Enclosure ID	pH	DO (mg/L)	Temp (°C)
Control	3	8.45	7.07	21.0
	7	8.45	6.87	20.8
	8	8.58	7.16	19.9
50	4	8.54	7.85	20.5
	5	8.56	7.12	20.5
	2	8.47	6.58	20.1
100	1	8.53	6.58	20.3
	9	8.56	7.20	18.9
	6	8.54	6.69	19.5
Mean		N/A	7.04	20.2
(SD)		N/A	0.35	0.6
Min		8.45	6.58	18.9
Max		8.58	7.85	21.0

Treatment Group (mg/L)	Test Enclosure ID	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (µS)
Control	3	175	188	320
	7	175	190	330
	8	175	188	318
50	4	175	192	324
	5	173	190	318
	2	173	188	323
100	1	174	188	322
	9	174	192	321
	6	181	198	329
Mean		175	190	323
(SD)		2	3	4
Min		173	188	318
Max		181	198	330

File Folder 11
Item Number 1
Page 2 of 2

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (page 12)	Created.....	5-Sep-13	KLW
Data Source: File Folder: 11	Revised.....	19-Feb-14	KLW
Forms: "Water Quality" datasheet (4h and 8h)	Reviewed..	2/19/14	
	Certified...	2/19/14	
File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Water Chem.xlsx]Exposure Water Chem			

Exposure Period Water Chemistry

Test Article: M81-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:
Exposure Period Water Chemistry - Dissolved oxygen, pH and temperature measurements from the center of all test enclosures at 4 and 8h.

Data anomalies and deviations
NONE

File Folder 11
Item Number 12
Page 1 of 2

Study Number: AEH-13-95FUDO-05
Electronic Lab Notebook (page 12)
Data Source: File Folder: 11
Forms: "Water Quality" datasheet (4h and 8h)

Test Article: M8I 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Exposure Water Chemistry

Treatment Group (mg/L)	Test Enclosure ID	4 Hour			8 Hour		
		DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)
Control	3	6.48	8.50	23.2	5.83	8.40	23.0
	7	6.34	8.46	22.6	6.49	8.46	23.0
	8	6.64	8.45	22.9	6.77	8.49	22.9
Mean		6.49	N/A	22.9	6.36	N/A	23.0
(SD)		0.12	N/A	0.2	0.39	N/A	0.0
Min		6.34	8.45	22.6	5.83	8.40	22.9
Max		6.64	8.50	23.2	6.77	8.49	23.0
50	4	5.96	8.38	23.3	5.67	8.35	23.0
	5	6.15	8.36	23.1	5.68	8.36	23.2
	2	5.73	8.35	23.0	5.54	8.25	23.1
Mean		5.95	N/A	23.1	5.63	N/A	23.1
(SD)		0.17	N/A	0.1	0.06	N/A	0.1
Min		5.73	8.35	23.0	5.54	8.25	23.0
Max		6.15	8.38	23.3	5.68	8.36	23.2
100	1	5.68	8.16	23.1	5.24	8.20	23.2
	9	6.12	8.28	23.1	5.60	8.23	22.8
	6	5.75	8.24	22.7	5.24	8.18	23.2
Mean		5.85	N/A	23.0	5.36	N/A	23.1
(SD)		0.19	N/A	0.2	0.17	N/A	0.2
Min		5.68	8.16	22.7	5.24	8.18	22.8
Max		6.12	8.28	23.1	5.60	8.23	23.2
Grand Mean		6.09	N/A	23.0	5.78	N/A	23.0
(SD)		0.33	N/A	0.2	0.49	N/A	0.1
Min		5.68	8.16	22.6	5.24	8.18	22.8
Max		6.64	8.50	23.3	6.77	8.49	23.2

File Folder 11
Item Number 2
Page 2 of 2

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (page 12)	Created.....	11-Feb-14	KLW
TAN Data Source: LTRMP Report (File Folder 11)	Revised.....	19-Feb-14	KLW
pH and Temperature Data Source: 8 h Water Quality Datasheet (File Folder 11)	Reviewed...	19FEB14	KLW
	Certified.....	2/19/14	SAC

File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Ammonia Data.xlsx]Ammonia Data

Ammonia

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:
1) Water samples were collected upon exposure termination, acidified with 10% sulfuric acid, and analyzed for total ammonia nitrogen (TAN) by the 4500-NH_3 G. Automated Phenate Method (Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005) using a Technicon Autoanalyzer II.
2) Un-ionized ammonia fractions were calculated according to the formula in Emerson et al. (1975) using pH and temperature observed at the time of sample collection.

Data Anomalies and Deviations:
NONE

File Folder 11
Item Number 3
Page 1 of 3

Study Number: AEH-13-PSEUDO-05

Electronic Lab Notebook (page 12)

TAN Data Source: LTRMP Report (File Folder 11)

pH and Temperature Data Source: 8 h Water Quality Datasheet (File Folder 11)

Test Article: MBI-401 SDP

Lot #: TR4669-4-3

Exposure Date: July 26, 2013

Assessment Dates: August 13-15, 2013

Ammonia

Treatment Level	Test Enclosure ID	pH	Temperature (°C)	TAN as NH_3-N (mg/L)	Un-ionized Fraction[1] (f)	NH_3 (mg/L)[2]	Mean NH_3 (mg/L)	SD
Control	3	8.40	23.0	0.077	0.1100	0.008	0.005	0.002
	7	8.46	23.0	0.030	0.1242	0.004		
	8	8.49	22.9	0.026	0.1311	0.003		
50	4	8.35	23.0	0.103	0.0992	0.010	0.009	0.001
	5	8.36	23.2	0.074	0.1026	0.008		
	2	8.25	23.1	0.120	0.0810	0.010		
100	1	8.20	23.2	0.141	0.0733	0.010	0.010	0.001
	9	8.23	22.8	0.113	0.0761	0.009		
	6	8.18	23.2	0.145	0.0702	0.010		

[1] Un-ionized fraction (f) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15 + T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018 + \left(\frac{2729.92}{(273.15+T_c)}\right)\right) - pH}\right] + 1\right\}}$

[2] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia = f * TAN (mg/L)

	A	B	C	D	E	F	G	H	I
1	Study Number: AEH-13-PSEUDO-05							Test Article: MBI-401 SDP	
2	Electronic Lab Notebook (page 12)							Lot #: TR4669-4-3	
3	TAN Data Source: LTRMP Report (File Folder 11)							Exposure Date: July 26, 2013	
4	pH and Temperature Data Source: 9 h Water Quality Datasheet (File Folder 11)							Assessment Dates: August 13-15, 2013	
5									
6									
7					**Ammonia**				
8									
9									
10	Treatment	Test Enclosure	pH	Temperature	TAN	Un-Ionized Fraction[1]	NH_3	Mean NH_3	SD
11	Level	ID		(°C)	as NH_3-N (mg/L)	(f)	(mg/L)[2]	(mg/L)	
12		3	8.40	23.0	0.077	x=1/(10^(0.09018+(2729.92/(273.15+E12))-D12)+1)	x=G12*F12		
13	Control	7	8.46	23.0	0.030	x=1/(10^(0.09018+(2729.92/(273.15+E13))-D13)+1)	x=G13*F13	x=AVERAGE(H12:H14)	x=STDEV.P(H12:H14)
14		8	8.49	22.9	0.026	x=1/(10^(0.09018+(2729.92/(273.15+E14))-D14)+1)	x=G14*F14		
15		4	8.35	23.0	0.103	x=1/(10^(0.09018+(2729.92/(273.15+E15))-D15)+1)	x=G15*F15		
16	50	5	8.36	23.2	0.074	x=1/(10^(0.09018+(2729.92/(273.15+E16))-D16)+1)	x=G15*F16	x=AVERAGE(H15:H17)	x=STDEV.P(H15:H17)
17		2	8.25	23.1	0.120	x=1/(10^(0.09018+(2729.92/(273.15+E17))-D17)+1)	x=G17*F17		
18		1	8.20	23.2	0.141	x=1/(10^(0.09018+(2729.92/(273.15+E18))-D18)+1)	x=G18*F18		
19	100	9	8.23	22.8	0.113	x=1/(10^(0.09018+(2729.92/(273.15+E19))-D19)+1)	x=G19*F19	x=AVERAGE(H18:H20)	x=STDEV.P(H18:H20)
20		6	8.18	23.2	0.145	x=1/(10^(0.09018+(2729.92/(273.15+E20))-D20)+1)	x=G20*F20		
21									
22	[1] Un-ionized fraction (f) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$								
23	$T_k = T_c + 273.15$; $pKa = 0.09018 - \frac{2729.92}{(273.15+T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018-\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$								
24									
25									
26									
27	[2] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia = f * TAN (mg/L)								
28									
29									
30									
31									
32									
33									
34									
35									
36									
37						File Folder 11			
38						Item Number 3			
39						Page 3 of 3			

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (pages 11, 16 & 17)	Created.....	7-Feb-14	KLW
Data Source: File Folder: 11	Revised.....	5-Mar-14	KLW
Forms: Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 11)	Reviewed...	5/16/14	
(I:\AEH-13-PSEUDO-05\HOBO Data)	Certified...	5/16/14	
File Names: Week 1: I:\AEH-13-PSEUDO-05\Data Summaries\[HOBO Data.xlsx]Temp Data (Week 1)			
Week 2: I:\AEH-13-PSEUDO-05\Data Summaries\[HOBO Data.xlsx]Temp Data (Week 2)			
Week 3: I:\AEH-13-PSEUDO-05\Data Summaries\[HOBO Data.xlsx]Temp Data (Week 3)			

Temperature Data Loggers (HOBOs)

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP)); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Data Explanation:
On July 26, 2013 a HOBO temperature loggers were programmed to record water temperature every three hours (eight times a day) and attached to the retention barriers. The HOBO temperature loggers were removed and temperature data were offloaded during the post-exposure unionid mussel assessment (August 13, 2013 for test enclosures 1, 2 and 3; August 14, 2013 for test enclosures 4, 6 and 7; August 15, 2013 for test enclosures 5, 8 and 9). Mean daily temperature (°C) and standard deviation were calculated from daily temperature measurements from all HOBO temperature loggers.

Data Logger Number	Serial Number	Test Enclosure ID	Treatment Level (mg/L)	Deployment Date	Termination Date
1	10020132	1	100	25-Jul-13	13-Aug-13
2	10020138	2	50	25-Jul-13	13-Aug-13
3	10020139	3	Control	25-Jul-13	13-Aug-13
4	10020141	4	50	25-Jul-13	14-Aug-13
5	10020142	5	50	25-Jul-13	15-Aug-13
6	10020133	6	100	25-Jul-13	14-Aug-13
7	10020131	7	Control	25-Jul-13	14-Aug-13
8	10020134	8	Control	25-Jul-13	15-Aug-13
9	10020136	9	100	25-Jul-13	15-Aug-13

Average Daily Temperature (°C)

Date	Mean (SD)	N	Date	Mean (SD)	N
7/26/2013	23.86 (0.35)	9	8/5/2013	22.69 (0.29)	9
7/27/2013	22.96 (0.32)	9	8/6/2013	22.59 (0.55)	9
7/28/2013	22.26 (0.33)	9	8/7/2013	22.77 (0.62)	9
7/29/2013	22.35 (0.50)	9	8/8/2013	23.03 (0.57)	9
7/30/2013	22.54 (0.76)	9	8/9/2013	22.73 (0.33)	9
7/31/2013	22.69 (0.35)	9	8/10/2013	23.04 (0.86)	9
8/1/2013	22.89 (0.63)	9	8/11/2013	23.39 (0.54)	9
8/2/2013	23.00 (0.45)	9	8/12/2013	23.33 (0.52)	9
8/3/2013	22.82 (0.53)	9	8/13/2013	23.34 (0.64)	6
8/4/2013	22.78 (0.78)	9	8/14/2013	23.51 (0.72)	3

Data anomalies and deviations:
Temperature readings from the first and last days of recorder deployment (July 25, 2013 for all test enclosures and after August 12, 2013 for test enclosures 1, 2 and 3, after August 13, 2013 for test enclosures 4, 6 and 7 and after August 14, 2013 for test enclosures 5, 8 and 9) were omitted from data analysis. The temperature recorders may have sampled ambient air temperature during these time points. These entire days will be omitted as diurnal temperature fluctuation skew the mean daily temperature. The mean temperature for August 13, 2013 was calculated using 6 data loggers (#4, 5, 6, 7, 8 and 9). The mean temperature for August 14, 2013 was calculated using only 3 data loggers (#5, 8 and 9).

File Folder 11
Item Number 4
Page 1 of 4

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 11, 16 & 17)
Data Source: File Folder: 11
Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 11)
Location: I:\AEH-13-PSEUDO-05\HOBO Data

Test Article: MBI 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Exposure Period and Post-exposure Holding Period Water Temperature
Week 1

Date	Data Logger Number									Mean
Time (GMT-05:00)	1	2	3	4	5	6	7	8	9	(SD)
	Temperature (°C)									
07/26/2013 0:00:00	24.5	24.5	24.5	24.4	24.4	24.5	24.4	24.4	24.5	
07/26/2013 3:00:00	24.1	24.1	24.2	24.0	24.1	24.2	24.0	24.0	24.2	
07/26/2013 6:00:00	23.7	23.7	23.8	23.6	23.8	23.8	23.6	23.6	23.8	
07/26/2013 9:00:00	23.6	23.6	23.6	23.5	23.6	23.6	23.5	23.5	23.6	23.9
07/26/2013 12:00:00	23.6	23.7	23.8	23.6	23.7	23.7	23.7	23.7	23.7	(0.4)
07/26/2013 15:00:00	23.8	24.1	24.2	23.6	23.7	24.2	24.0	23.8	23.8	
07/26/2013 18:00:00	24.3	24.1	24.2	23.8	24.0	24.4	24.1	24.4	24.1	
07/26/2013 21:00:00	23.5	23.4	23.4	23.3	23.4	23.5	23.3	23.4	23.5	
07/27/2013 0:00:00	23.3	23.3	23.2	23.1	23.2	23.3	23.1	23.2	23.2	
07/27/2013 3:00:00	23.0	23.1	23.0	22.9	23.0	23.1	22.9	23.0	23.0	
07/27/2013 6:00:00	22.8	22.8	22.8	22.6	22.8	22.8	22.7	22.8	22.8	
07/27/2013 9:00:00	23.3	23.1	23.4	22.7	22.9	23.3	23.2	23.0	22.9	23.0
07/27/2013 12:00:00	23.5	23.2	23.3	22.6	22.9	24.1	23.2	23.1	23.1	(0.3)
07/27/2013 15:00:00	23.2	23.0	23.1	22.7	22.9	23.1	23.1	23.0	23.0	
07/27/2013 18:00:00	23.2	23.0	23.1	22.7	22.9	23.3	23.0	23.3	23.0	
07/27/2013 21:00:00	22.4	22.3	22.4	22.2	22.3	22.3	22.2	22.3	22.3	
07/28/2013 0:00:00	22.1	22.1	22.1	22.0	22.1	22.1	22.0	22.0	22.1	
07/28/2013 3:00:00	22.0	22.0	22.0	21.9	22.0	22.0	21.9	22.0	22.0	
07/28/2013 6:00:00	21.9	21.9	21.9	21.7	21.9	21.9	21.8	21.9	21.9	
07/28/2013 9:00:00	22.4	22.2	22.4	21.9	22.0	22.4	22.4	22.1	22.1	22.3
07/28/2013 12:00:00	22.9	22.6	22.7	22.0	22.2	23.2	22.5	22.4	22.4	(0.3)
07/28/2013 15:00:00	22.8	22.6	22.6	22.1	22.2	22.4	22.6	22.8	22.4	
07/28/2013 18:00:00	22.9	22.6	22.7	22.2	22.4	22.8	22.6	22.9	22.6	
07/28/2013 21:00:00	22.2	22.2	22.2	22.0	22.2	22.2	22.1	22.2	22.2	
07/29/2013 0:00:00	22.1	22.1	22.1	22.0	22.1	22.1	22.0	22.0	22.1	
07/29/2013 3:00:00	22.0	22.0	22.0	21.8	22.0	22.0	21.9	21.9	22.0	
07/29/2013 6:00:00	21.8	21.7	21.8	21.6	21.8	21.7	21.6	21.7	21.7	
07/29/2013 9:00:00	22.6	22.1	22.5	21.8	22.0	22.4	22.5	22.2	22.0	22.3
07/29/2013 12:00:00	23.3	23.2	23.6	22.6	22.9	23.9	23.3	23.1	23.0	(0.5)
07/29/2013 15:00:00	23.1	23.0	23.0	22.6	22.8	22.9	22.8	22.8	22.9	
07/29/2013 18:00:00	22.6	22.4	22.6	22.3	22.4	22.5	22.3	22.4	22.5	
07/29/2013 21:00:00	22.3	22.3	22.3	22.1	22.2	22.3	22.1	22.2	22.2	
07/30/2013 0:00:00	22.1	22.0	22.0	21.8	22.0	22.0	21.9	22.0	22.0	
07/30/2013 3:00:00	22.0	22.0	22.0	21.8	21.9	22.0	21.9	21.9	22.0	
07/30/2013 6:00:00	21.9	21.9	21.9	21.7	21.8	21.9	21.8	21.9	21.9	
07/30/2013 9:00:00	22.0	22.0	22.0	21.8	21.9	22.0	21.9	22.0	22.0	22.5
07/30/2013 12:00:00	22.9	22.3	22.4	21.9	22.0	22.4	22.4	22.3	22.5	(0.8)
07/30/2013 15:00:00	24.3	23.7	24.2	23.2	23.2	23.6	23.8	23.5	23.4	
07/30/2013 18:00:00	23.7	23.6	23.7	23.4	23.5	23.6	23.5	23.6	23.6	
07/30/2013 21:00:00	23.2	23.2	23.1	23.0	23.1	23.1	23.0	23.1	23.2	
07/31/2013 0:00:00	22.8	22.8	22.8	22.6	22.8	22.8	22.7	22.7	22.9	
07/31/2013 3:00:00	22.6	22.6	22.5	22.4	22.6	22.6	22.4	22.5	22.6	
07/31/2013 6:00:00	22.3	22.3	22.2	22.1	22.3	22.2	22.1	22.2	22.3	
07/31/2013 9:00:00	22.6	22.3	22.4	22.0	22.2	22.4	22.3	22.3	22.3	22.7
07/31/2013 12:00:00	23.7	23.3	23.4	22.7	22.9	23.5	23.3	23.2	23.1	(0.3)
07/31/2013 15:00:00	23.3	23.1	23.0	22.6	22.8	22.9	23.0	23.1	22.9	
07/31/2013 18:00:00	23.1	22.9	22.9	22.5	22.7	23.0	22.8	23.3	22.8	
07/31/2013 21:00:00	22.5	22.6	22.5	22.4	22.5	22.5	22.4	22.5	22.5	
08/01/2013 0:00:00	22.2	22.3	22.3	22.1	22.3	22.3	22.2	22.2	22.2	
08/01/2013 3:00:00	22.2	22.2	22.2	22.0	22.2	22.3	22.1	22.2	22.3	
08/01/2013 6:00:00	22.1	22.2	22.1	22.0	22.1	22.2	22.0	22.1	22.1	
08/01/2013 9:00:00	23.0	22.6	22.8	22.2	22.4	22.8	22.8	22.6	22.4	22.9
08/01/2013 12:00:00	24.0	23.5	23.5	22.9	23.2	23.8	23.4	23.2	23.2	(0.6)
08/01/2013 15:00:00	24.0	23.7	23.5	23.2	23.4	23.5	23.5	23.6	23.5	
08/01/2013 18:00:00	24.1	23.9	23.8	23.4	23.7	23.9	23.7	24.0	23.7	
08/01/2013 21:00:00	23.3	23.2	23.1	22.9	23.1	23.1	23.0	23.1	23.2	

Study Number: AEH-13-PSEUDO-05
Electron c Lab Notebook (pages 11, 16 & 17)
Data Source: File Folder: 11
Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 11)
Location: I:\AEH-13-PSEUDO-05\HOBO Data

Test Article: MBI 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Exposure Period and Post-exposure Holding Period Water Temperature
Week 2

	Data logger Number									
Date	1	2	3	4	5	6	7	8	9	Mean
Time (GMT-05:00)	Temperature (°C)									(SD)
08/02/2013 0:00:00	22.8	22.9	22.8	22.6	22.8	22.8	22.7	22.8	22.9	
08/02/2013 3:00:00	22.8	22.8	22.7	22.5	22.7	22.8	22.7	22.7	22.8	
08/02/2013 6:00:00	22.4	22.5	22.4	22.3	22.4	22.4	22.3	22.3	22.4	
08/02/2013 9:00:00	23.1	22.8	23.0	22.5	22.5	23.0	22.9	22.8	22.6	23.0
08/02/2013 12:00:00	23.7	23.4	23.8	22.9	23.0	23.6	23.3	23.2	23.2	(0.4)
08/02/2013 15:00:00	24.2	23.9	23.7	23.1	23.3	23.6	23.7	23.8	23.6	
08/02/2013 18:00:00	24.0	23.8	23.6	23.0	23.3	23.7	23.5	23.6	23.6	
08/02/2013 21:00:00	22.8	22.8	22.7	22.6	22.7	22.8	22.6	22.7	22.8	
08/03/2013 0:00:00	22.6	22.7	22.6	22.4	22.5	22.6	22.4	22.5	22.5	
08/03/2013 3:00:00	22.4	22.5	22.4	22.2	22.4	22.4	22.3	22.4	22.4	
08/03/2013 6:00:00	22.2	22.3	22.2	22.0	22.2	22.2	22.1	22.2	22.2	
08/03/2013 9:00:00	22.5	22.5	22.4	22.0	22.3	22.4	22.3	22.3	22.3	22.8
08/03/2013 12:00:00	24.1	23.6	23.6	22.9	23.1	23.7	23.6	23.4	23.4	(0.5)
08/03/2013 15:00:00	24.0	23.5	23.4	22.8	23.1	23.3	23.4	23.8	23.5	
08/03/2013 18:00:00	23.7	23.4	23.4	22.9	23.2	23.6	23.3	23.7	23.5	
08/03/2013 21:00:00	22.8	22.8	22.7	22.5	22.7	22.7	22.6	22.6	22.7	
08/04/2013 0:00:00	22.5	22.5	22.5	22.3	22.4	22.5	22.4	22.4	22.5	
08/04/2013 3:00:00	22.3	22.3	22.2	22.1	22.2	22.2	22.1	22.1	22.2	
08/04/2013 6:00:00	21.8	22.0	21.9	21.8	21.9	21.8	21.6	21.6	21.6	
08/04/2013 9:00:00	22.2	21.9	22.0	21.7	21.8	22.0	21.9	21.9	22.0	22.8
08/04/2013 12:00:00	23.6	23.2	23.2	22.5	22.8	23.6	23.2	23.0	23.2	(0.8)
08/04/2013 15:00:00	24.3	23.9	23.9	23.4	23.8	23.5	23.9	24.0	23.9	
08/04/2013 18:00:00	24.1	23.8	23.9	23.6	23.8	23.5	23.8	23.9	23.9	
08/04/2013 21:00:00	23.1	23.1	23.1	22.9	23.1	23.1	22.9	23.0	23.1	
08/05/2013 0:00:00	22.8	22.8	22.7	22.5	22.7	22.8	22.6	22.7	22.8	
08/05/2013 3:00:00	22.5	22.6	22.5	22.3	22.5	22.6	22.4	22.5	22.6	
08/05/2013 6:00:00	22.4	22.4	22.4	22.2	22.3	22.4	22.3	22.3	22.4	
08/05/2013 9:00:00	22.6	22.5	22.5	22.2	22.4	22.6	22.4	22.4	22.5	22.7
08/05/2013 12:00:00	23.1	22.9	22.9	22.5	22.7	22.9	22.8	22.8	22.9	(0.3)
08/05/2013 15:00:00	23.5	23.5	23.2	22.8	23.0	23.2	23.2	23.3	23.3	
08/05/2013 18:00:00	23.2	23.1	22.8	22.5	22.7	22.9	22.8	22.9	22.9	
08/05/2013 21:00:00	22.5	22.6	22.4	22.3	22.4	22.5	22.4	22.4	22.5	
08/06/2013 0:00:00	22.3	22.4	22.3	22.1	22.3	22.3	22.2	22.2	22.3	
08/06/2013 3:00:00	22.2	22.4	22.2	22.0	22.2	22.2	22.1	22.1	22.2	
08/06/2013 6:00:00	22.0	22.1	22.0	21.8	22.0	22.0	21.9	21.9	22.0	
08/06/2013 9:00:00	22.1	22.1	22.0	21.8	22.0	22.0	22.0	22.0	22.1	22.6
08/06/2013 12:00:00	22.9	22.6	22.7	22.3	22.5	22.9	22.7	22.7	22.8	(0.6)
08/06/2013 15:00:00	24.0	23.6	23.6	23.2	23.4	23.7	23.6	23.8	23.7	
08/06/2013 18:00:00	23.2	23.2	23.2	22.9	23.1	23.2	23.0	23.1	23.2	
08/06/2013 21:00:00	22.9	23.0	22.8	22.6	22.7	22.8	22.6	22.7	22.8	
08/07/2013 0:00:00	22.5	22.7	22.4	22.2	22.4	22.4	22.3	22.4	22.4	
08/07/2013 3:00:00	22.2	22.4	22.1	22.0	22.1	22.2	22.0	22.1	22.2	
08/07/2013 6:00:00	22.0	22.2	22.0	21.9	22.0	22.0	21.9	22.0	22.0	
08/07/2013 9:00:00	22.7	22.4	22.4	22.0	22.1	22.6	22.6	22.3	22.4	22.8
08/07/2013 12:00:00	24.0	23.4	23.1	22.5	22.8	23.4	23.3	23.0	23.4	(0.6)
08/07/2013 15:00:00	24.4	23.7	23.7	22.9	23.2	23.5	23.7	24.0	23.9	
08/07/2013 18:00:00	24.0	23.5	23.4	22.9	23.2	23.5	23.3	23.7	23.7	
08/07/2013 21:00:00	22.9	22.9	22.9	22.7	22.8	22.9	22.8	22.8	22.9	
08/08/2013 0:00:00	22.6	22.6	22.6	22.5	22.6	22.7	22.6	22.6	22.7	
08/08/2013 3:00:00	22.6	22.6	22.6	22.4	22.6	22.6	22.5	22.5	22.6	
08/08/2013 6:00:00	22.5	22.4	22.3	22.1	22.3	22.4	22.3	22.4	22.5	
08/08/2013 9:00:00	22.8	22.6	22.5	22.1	22.4	22.6	22.6	22.6	22.7	23.0
08/08/2013 12:00:00	23.6	23.5	23.4	22.9	23.3	23.5	23.5	23.3	23.4	(0.6)
08/08/2013 15:00:00	24.1	24.0	23.9	23.5	23.7	23.9	23.8	24.0	24.0	
08/08/2013 18:00:00	24.2	23.9	23.8	23.4	23.7	23.9	23.7	24.3	24.0	
08/08/2013 21:00:00	23.1	23.1	23.1	22.9	23.0	23.1	23.0	23.0	23.1	

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 11, 16 & 17)
Data Source: File Folder: 11
Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 11)
Location: I:\AEH-13-PSEUDO-05\HOBO Data

Test Article: MBI 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13 to 15, 2013

Exposure Period and Post-exposure Holding Period Water Temperature
Week 3

	Data Logger Number									
Date	1	2	3	4	5	6	7	8	9	Mean
Time (GMT-05:00)	Temperature (°C)									(SD)
08/09/2013 0:00:00	22.7	22.7	22.7	22.5	22.7	22.7	22.6	22.6	22.7	
08/09/2013 3:00:00	22.5	22.5	22.5	22.4	22.5	22.6	22.4	22.5	22.5	
08/09/2013 6:00:00	22.3	22.3	22.3	22.1	22.3	22.3	22.2	22.2	22.3	
08/09/2013 9:00:00	22.9	22.7	22.8	22.4	22.5	23.0	22.8	22.7	22.7	22.7
08/09/2013 12:00:00	23.3	23.5	23.1	22.7	23.2	23.4	23.0	23.1	23.1	(0.3)
08/09/2013 15:00:00	23.4	23.5	23.2	22.8	23.5	23.2	23.1	23.3	23.3	
08/09/2013 18:00:00	22.8	22.8	22.7	22.5	22.7	22.8	22.6	22.7	22.8	
08/09/2013 21:00:00	22.5	22.5	22.5	22.4	22.4	22.5	22.4	22.4	22.5	
08/10/2013 0:00:00	22.3	22.3	22.3	22.2	22.3	22.3	22.2	22.2	22.3	
08/10/2013 3:00:00	22.1	22.2	22.2	22.0	22.1	22.2	22.0	22.1	22.1	
08/10/2013 6:00:00	22.1	22.1	22.1	22.0	22.0	22.1	22.0	22.0	22.1	
08/10/2013 9:00:00	22.9	22.5	22.6	22.2	22.2	22.8	22.6	22.5	22.6	23.0
08/10/2013 12:00:00	23.2	23.0	23.0	22.6	23.0	23.2	22.9	23.0	23.1	(0.9)
08/10/2013 15:00:00	24.4	24.3	24.2	23.7	24.6	24.1	24.0	24.4	24.3	
08/10/2013 18:00:00	24.4	24.3	24.3	24.0	24.2	24.3	24.2	24.3	24.4	
08/10/2013 21:00:00	23.9	23.9	23.8	23.6	23.7	23.8	23.7	23.8	23.8	
08/11/2013 0:00:00	23.4	23.4	23.4	23.2	23.3	23.4	23.2	23.3	23.3	
08/11/2013 3:00:00	23.0	23.0	23.0	22.8	22.9	23.0	22.9	22.9	23.0	
08/11/2013 6:00:00	22.6	22.6	22.6	22.4	22.6	22.7	22.5	22.5	22.6	
08/11/2013 9:00:00	23.2	22.9	23.1	22.6	22.7	23.2	23.0	22.9	22.9	23.4
08/11/2013 12:00:00	24.3	23.9	23.8	23.2	23.9	24.0	23.7	23.6	23.8	(0.5)
08/11/2013 15:00:00	24.4	24.4	24.2	23.7	24.5	24.1	24.1	24.4	24.3	
08/11/2013 18:00:00	24.2	24.0	23.9	23.5	23.7	23.9	23.8	24.0	24.1	
08/11/2013 21:00:00	23.6	23.7	23.5	23.3	23.4	23.4	23.4	23.5	23.6	
08/12/2013 0:00:00	23.1	23.2	23.1	23.0	23.1	23.2	23.0	23.1	23.2	
08/12/2013 3:00:00	22.8	22.9	22.8	22.7	22.8	22.9	22.8	22.8	22.9	
08/12/2013 6:00:00	22.6	22.6	22.6	22.5	22.6	22.7	22.5	22.6	22.6	
08/12/2013 9:00:00	23.2	23.1	23.2	22.7	22.9	23.3	23.3	23.1	23.1	23.3
08/12/2013 12:00:00	24.0	23.9	23.8	23.3	23.9	24.0	23.8	23.7	23.8	(0.5)
08/12/2013 15:00:00	24.3	24.2	24.1	23.6	24.5	24.1	24.2	24.4	24.3	
08/12/2013 18:00:00	24.1	23.9	23.9	23.5	23.7	23.8	23.8	24.0	23.9	
08/12/2013 21:00:00	23.4	23.3	23.3	23.1	23.3	23.3	23.2	23.2	23.3	
08/13/2013 0:00:00	.	.	.	22.9	23.0	23.0	22.9	22.9	23.0	
08/13/2013 3:00:00	.	.	.	22.6	22.7	22.8	22.6	22.7	22.8	
08/13/2013 6:00:00	.	.	.	22.4	22.5	22.5	22.4	22.4	22.5	
08/13/2013 9:00:00	.	.	.	22.6	22.6	23.2	23.0	22.9	22.9	23.3
08/13/2013 12:00:00	.	.	.	23.4	24.1	24.2	23.8	23.8	23.8	(0.6)
08/13/2013 15:00:00	.	.	.	23.6	24.4	23.8	24.0	24.4	24.4	
08/13/2013 18:00:00	.	.	.	23.7	24.0	24.2	24.0	24.5	24.2	
08/13/2013 21:00:00	.	.	.	23.6	23.7	23.8	23.7	23.8	23.8	
08/14/2013 0:00:00	.	.	.	.	23.3	.	.	23.3	23.4	
08/14/2013 3:00:00	.	.	.	.	22.8	.	.	22.7	22.8	
08/14/2013 6:00:00	.	.	.	.	22.5	.	.	22.5	22.6	
08/14/2013 9:00:00	.	.	.	.	22.6	.	.	22.9	22.9	23.5
08/14/2013 12:00:00	.	.	.	.	24.0	.	.	23.8	23.8	(0.7)
08/14/2013 15:00:00	.	.	.	.	24.7	.	.	24.5	24.4	
08/14/2013 18:00:00	.	.	.	.	24.3	.	.	24.6	24.4	
08/14/2013 21:00:00	.	.	.	.	23.7	.	.	23.7	23.8	

File Folder 11
Item Number 4
Page 4 of 4

Sample Description: 3 mL acidified exposure water (0.45 µm filtrated)
Study #: AEH-13-PSEUDO-05

Upper Midwest Environmental Sciences Center
attn: James Luoma
2630 Fanta Reed Road
La Crosse, WI54603

Water Quality Laboratory
Upper Midwest Environmental Sciences Center
USGS
2630 Fanta Reed Road
La Crosse, WI 54603

Analyzing Date:08/8/2013
Report Date: 08/13/2013

REPORT OF ANALYSIS

Sample Code	Results (Total Ammonia Nitrogen, mg NH_3-N/L)
E1T8HC100	0.141
E2T8HC50	0.12
E3T8HC0	0.077
E4T8HC50	0.103
E5T8HC50	0.074
E6T8HC100	0.145
E7T8HC0	0.03
E8T8HC0	0.026
E9T8HC100	0.113

USGS
Water Quality Laboratory
Laboratory Director

[redacted]

Xiaoli Yuan

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Appendix 7. Spectrophotometric Summary and SAS Output, Program, and Log

Item number	Item description	Number of pages	Report page number
1	Spectrophotometric Data – Data Summary	2	182
2	SAS output for Standard Curve Linear Regression and sample concentrations	11	184
3	SAS program for Standard Curve Linear Regression and sample concentrations	2	195
4	SAS log for Standard Curve Linear Regression and sample concentrations	4	197

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (page 14)	Created.....	5-Sep-2013	KLW
Data Source: File Folder: 12	Revised.....	19-Feb-2014	KLW
Forms: "Spectrophotometric Data" datasheet	Reviewed...	19Feb2014	
	Certified.....	2/19/14	
File Name: See filenames as stated below.			

Spectrophotometric Data

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:

1) A linear regression curve was created using the absorbances (660 nm) from known concentrations (25, 50, 100 and 200 mg/L) of MBI-40 SDP.
2) Standard checks were performed at 4 hour by comparing the 25, 50, 100 and 200 mg/L dilutions used to create the linear regression to the linear regression.
3) Data codes used within SAS
   - enc = Test Enclosure ID (1 through 9)
   - thero = theoretical concentration (25, 50, 100 and 200 mg/L)
   - time = sample time (1, 4 and 8 h post-dosing initiation)
   - abs = measured absorbance of sample
   - conc = concentration (mg/L)

4) Information that is not relevant to a sample (i.e., test enclosure ID for standards) or that will be calculated by SAS (i.e., predicted concentration for standard checks and samples) is denoted by a "." for input into SAS.

Data Analysis Methods:

1) A linear regression equation was fit using the SAS ProcReg procedure using the absorbances (660 nm) of 3 replicate dilutions of 25, 50, 100 and 200 mg/L MBI-401 SDP
2) Standard checks and treatment sample concentrations were predicted from the regression analysis

Data Analysis:

1) The following mean treatment concentrations were determined in SAS:
   - 3a) Mean concentration for each individual test enclosure over the entire exposure period
   - 3b) Mean concentration for each treatment group at each sampling time

File Names:

Spectrophotometric Data for SAS Input
I:\AEH-13-PSEUDO-05\Data Summaries\[Ammonia Data.xlsx]Ammonia Coversheet

SAS Program/Code
I:\AEH-13-PSEUDO-05\SAS and sas output\spec analysis program

SAS Log
I:\AEH-13-PSEUDO-05\SAS and sas output\spec analysis log

SAS Output
I:\AEH-13-MBICRADA-01\SAS\spec analysis output

Data Anomalies and Deviations:
NONE

File Folder: 12

Item Number 1
Page 1 of 2

enc	thero	time	abs	conc
.	25	0	0.030	25
.	50	0	0.063	50
.	100	0	0.121	100
.	200	0	0.241	200
.	25	0	0.030	25
.	50	0	0.060	50
.	100	0	0.122	100
.	200	0	0.241	200
.	25	0	0.031	25
.	50	0	0.062	50
.	100	0	0.121	100
.	200	0	0.240	200
.	25	4	0.025	.
.	50	4	0.061	.
.	100	4	0.120	.
.	200	4	0.239	.
3	0	1	0.000	.
7	0	1	0.001	.
8	0	1	0.000	.
2	50	1	0.045	.
4	50	1	0.049	.
5	50	1	0.048	.
1	100	1	0.093	.
6	100	1	0.095	.
9	100	1	0.092	.
3	0	4	0.000	.
7	0	4	-0.001	.
8	0	4	0.001	.
2	50	4	0.040	.
4	50	4	0.046	.
5	50	4	0.045	.
1	100	4	0.095	.
6	100	4	0.090	.
9	100	4	0.090	.
3	0	8	-0.003	.
7	0	8	-0.001	.
8	0	8	-0.001	.
2	50	8	0.038	.
4	50	8	0.040	.
5	50	8	0.040	.
1	100	8	0.084	.
6	100	8	0.087	.
9	100	8	0.085	.

AEH-13-PSEUDO-05

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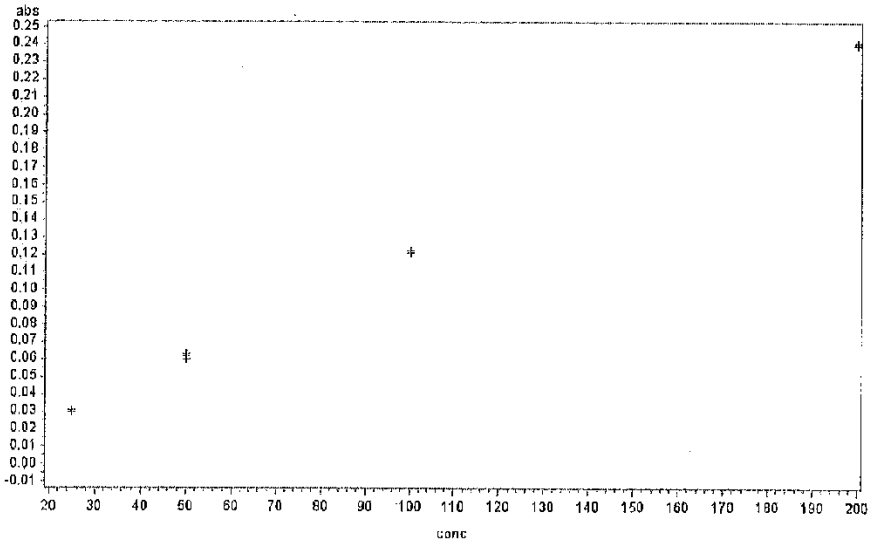
Item No. 1
Pg 2 of 2

Standard Curve Linear Regression and sample concentrations

Lake Darling Spectrophotometer Data Analysis

Study # AEH-13-PSEUDO-05

SAS v. 9.3 Analysis completion date: 06MAR2014 Analysis prepared by: XLW AEH-13-PSEUDO-05



Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14 KW

FF # 12
Item No. 3
Pg 1 of 11

Standard Curve Linear Regression and sample concentrations
Lake Darling Spectrophotometer Data Analysis
Study # AEH-13-PSEUDO-05
SAS v. 9.3 Analysis completion date: 06MAR2014 Analysis prepared by: KLW

AEH-13-PSEUDO-05

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc

Number of Observations Read	43
Number of Observations Used	12
Number of Observations with Missing Values	31

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	53899	53899	77866.2	<.0001
Error	10	6.92205	0.69220		
Corrected Total	11	53906			

Root MSE	0.83199	R-Square	0.9999
Dependent Mean	93.75000	Adj R-Sq	0.9999
Coeff Var	0.88745		

Parameter Estimates

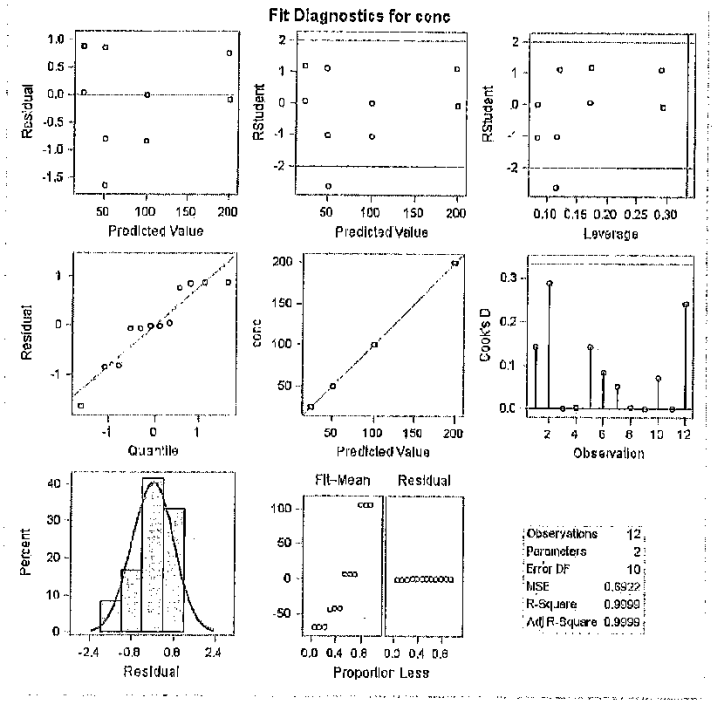
Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	-0.89434	0.41560	-2.15	0.0569
abs	abs	1	833.87086	2.98830	279.05	<.0001

Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

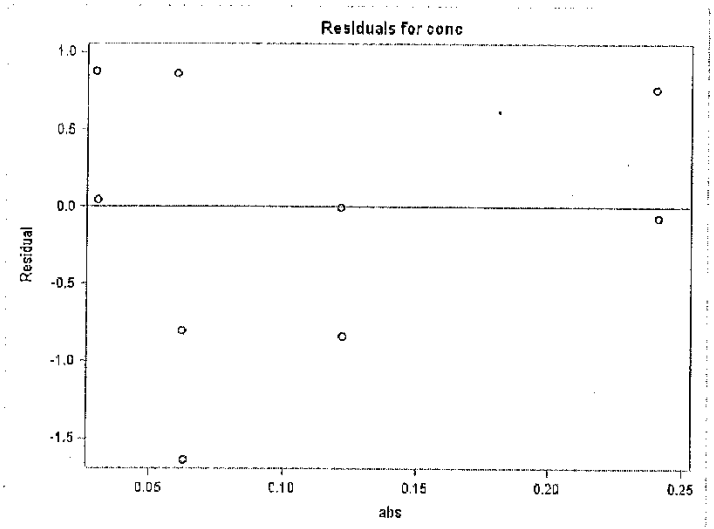
Standard Curve Linear Regression and sample concentrations
Lake Darling Spectrophotometer Data Analysis
Study # AEH-13-PSEUDO-05
SAS v. 9.3 Analysis completion date: 06MAR2014 Analysis prepared by: KLW

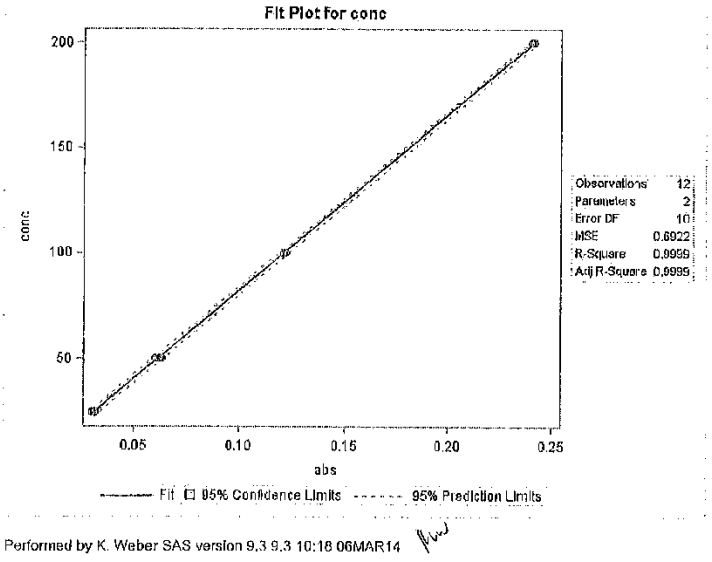
AEH-13-PSEUDO-05

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc



AEH-13-PSEUDO-05





Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

Standard Curve Linear Regression and sample concentrations
Lake Darling Spectrophotometer Data Analysis
Study # AEH-13-PSEUDO-05
SAS v. 9.3 Analysis completion date: 05MAR2014 Analysis prepared by: KLW

AEH-13-PSEUDO-05

Obs	enc	thero	time	abs	conc	predicted_ppm
1	.	25	0	0.030	25	24.122
2	.	50	0	0.063	50	51.640
3	.	100	0	0.121	100	100.004
4	.	200	0	0.241	200	200.069
5	.	25	0	0.030	25	24.122
6	.	50	0	0.060	50	49.138
7	.	100	0	0.122	100	100.838
8	.	200	0	0.241	200	200.069
9	.	25	0	0.031	25	24.956
10	.	50	0	0.062	50	50.806
11	.	100	0	0.121	100	100.004
12	.	200	0	0.240	200	199.235
13	1	100	1	0.093	.	76.656
14	2	50	1	0.045	.	36.630
15	3	0	1	0.000	.	-0.894
16	4	50	1	0.049	.	39.965
17	5	50	1	0.048	.	39.131
18	6	100	1	0.095	.	78.323
19	7	0	1	0.001	.	-0.060
20	8	0	1	0.000	.	-0.894
21	9	100	1	0.092	.	75.822
22	.	25	4	0.025	.	19.952
23	.	50	4	0.061	.	49.972
24	.	100	4	0.120	.	99.170
25	.	200	4	0.239	.	198.401
26	1	100	4	0.095	.	78.323
27	2	50	4	0.040	.	32.460
28	3	0	4	0.000	.	-0.894
29	4	50	4	0.046	.	37.464
30	5	50	4	0.045	.	36.630
31	6	100	4	0.090	.	74.154
32	7	0	4	-0.001	.	-1.728
33	8	0	4	0.001	.	-0.060
34	9	100	4	0.090	.	74.154
35	1	100	8	0.084	.	69.151
36	2	50	8	0.036	.	30.793
37	3	0	8	-0.003	.	-3.396

38	4	50	8	0.040	.	32.460
39	5	50	8	0.040	.	32.460
40	6	100	8	0.087	.	71.652
41	7	0	8	-0.001	.	-1.728
42	8	0	8	-0.001	.	-1.728
43	9	100	8	0.085	.	69.985

Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

\EH-13-PSEUDO-05

klw 3/7/2014

Mean treatment concentration by enclosure for all exposure sampling times
Individual enclosure means

The MEANS Procedure

AEH-13-PSEUDO-05

enc=1 thero=100

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
74.7099	4.8860	62.5724	86.8475

enc=2 thero=50

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
33.2944	3.0086	25.8256	40.7631

enc=3 thero=0

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-1.7282	1.4443	-5.3161	1.8596

enc=4 thero=50

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
36.6298	3.8213	27.1373	46.1224

enc=5 thero=50

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
36.0739	3.3700	27.7023	44.4456

enc=6 thero=100

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
74.7099	3.3700	66.3383	83.0816

enc=7 thero=0

Analysis Variable : predicted_ppm Predicted

Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-1.1723	0.9629	-3.5642	1.2196

I-13-PSEUDO-05

enc=8 thero=0

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-0.8943	0.8339	-2.9658	1.1771

enc=9 thero=100

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
73.3202	3.0066	65.8514	80.7889

Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

6/11/2014

Mean treatment concentration by treatment group at each exposure sampling time

The MEANS Procedure

AEH-13-PSEUDO-05

thero=0 time=1

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-0.6164	0.4814	-1.8123	0.5796

thero=0 time=4

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-0.8843	0.8339	-2.9658	1.1771

thero=0 time=8

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-2.2641	0.9629	-4.6760	0.1078

thero=50 time=1

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
38.5755	1.7358	34.2635	42.8876

thero=50 time=4

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
35.5180	2.6805	28.8592	42.1768

thero=50 time=8

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
31.9046	0.9629	29.5127	34.2965

thero=100 time=1

Analysis Variable : predicted_ppm Predicted Value of conc

TH-13-PSEUDO-05

Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
76.9336	1.2738	73.7694	80.0978

thero=100 time=4

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
75.5438	2.4072	69.5641	81.5236

thero=100 time=8

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
70.2626	1.2738	67.0984	73.4268

Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

Mean treatment concentration by treatment group over all exposure sampling times

The MEANS Procedure

thero=0

AEH-13-PSEUDO-05

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
-1.2650	1.0307	-2.0572	-0.4727

thero=50

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
35.3327	3.3384	32.7666	37.8988

thero=100

Analysis Variable : predicted_ppm Predicted Value of conc			
Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
74.2467	3.3986	71.6343	76.8591

Performed by K. Weber SAS version 9.3 9.3 10:18 06MAR14

FF # 12
Item No. 3
Pg 11 of 11

```
ods html close; /* close previous */;
ods html; /* open new */;
ods graphics on;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version 9.3' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 'Standard Curve Linear Regression and sample concentrations';
title2 'Lake Darling Spectrophotometer Data Analysis';
title3 h=1 'Study # AEH-13-PSEUDO-05';
title4 h=1 'SAS v. 9.3  Analysis completion date:  06MAR2014  Analysis prepare

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  KLW      Page 1 of 2
* Analysis completion date:  06MAR2014
*****************************************************************************/

data Zeq; set pseudo05.Spec_data;
run;
proc sort;
by enc time ; run;

run;
proc gplot data= zeq;
plot abs * conc;
run;
proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
proc sort;
by time enc;
proc print data=output_out;
run;
data zeq2; set output_out;
if enc = "." then delete;
run;
proc sort data = zeq2;
by enc;
run;
/*****************************************************************************
* This procedure produces the mean concentrations for each treatment replicate
* i.e. It gives the mean concentration of each treatment enclosure over the er
*****************************************************************************/
```

FF # 12
Item No. 4
Pg 1 of 3

```
title1 "Mean treatment concentration by enclosure for all exposure sampling ti
title2 "Individual enclosure means";
proc means data = zeq2 mean std lclm uclm fw=8;
by enc thero;
var predicted_ppm;
run;
proc sort;
by thero time;
/*****************************************************************************
* This procedure produces the mean concentrations for each treatment group at
* i.e. It gives the mean concentration of the three control, 50ppm, & 100ppm t
* at each sample time over the entire exposure
*****************************************************************************
title1 "Mean treatment concentration by treatment group at each exposure sampl
proc means data = zeq2 mean std lclm uclm fw=8;
by thero time;
var predicted_ppm;
run;
/*****************************************************************************
* This procedure produces the mean concentrations for each treatment group ove
* i.e. It gives the mean concentration of the three control, 50ppm, & 100ppm t
* over the entire exposure
*****************************************************************************
title1 "Mean treatment concentration by treatment group over all exposure samp
proc means data = zeq2 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;
quit;
run;
```

FF # 12
Item No. 4
Pg 2 of 2

```
4    DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
5
6    FOOTNOTE1 'Performed by K. Weber SAS version 9.3' &SYSVER &SYSTIME
6  ! &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.
7
8    options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
9
10   title1 'Standard Curve Linear Regression and sample concentrations';
11   title2 'Lake Darling Spectrophotometer Data Analysis';
12   title3 h=1 'Study # AEH-13-PSEUDO-05';
13   title4 h=1 'SAS v. 9.3  Analysis completion date:  06MAR2014  Analysis pr
14
15   /*************************************************************************
16   * SAS ver 9.3       Analysis prepared by:  KLW (W)     Page 1 of 4
17   * Analysis completion date:  06MAR2014
18   *************************************************************************
19
20   data Zeq; set pseudo05.Spec_data;
21   run;

NOTE: There were 43 observations read from the data set PSEUDO05.SPEC_DATA.
NOTE: The data set WORK.ZEQ has 43 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.02 seconds
      cpu time            0.03 seconds


22   proc sort;
23   by enc time ; run;

NOTE: There were 43 observations read from the data set WORK.ZEQ.
NOTE: The data set WORK.ZEQ has 43 observations and 5 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


24
25   run;
26   proc gplot data= zeq;
27   plot abs * conc;
28   run;

NOTE: 31 observation(s) contained a MISSING value for the abs * conc request.
```

FF # 12
Item No. 5
Pg 1 of 4

KLW 6 MAR 2014 Pg 2 of 4

```
NOTE: 5 records written to C:\Users\klweber\gplot.png.

NOTE: There were 43 observations read from the data set WORK.ZEQ.
NOTE: PROCEDURE GPLOT used (Total process time):
      real time           0.68 seconds
      cpu time            0.48 seconds


29   proc reg data = zeq;
30   model conc = abs /edf;
31   output out=output_out p=predicted_ppm;
32   run;


NOTE: The data set WORK.OUTPUT_OUT has 43 observations and 6 variables.
NOTE: PROCEDURE REG used (Total process time):
      real time           5.07 seconds
      cpu time            0.54 seconds


33   proc sort;
34   by time enc;

NOTE: There were 43 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.OUTPUT_OUT has 43 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


35   proc print data=output_out;
36   run;

NOTE: There were 43 observations read from the data set WORK.OUTPUT_OUT.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.09 seconds
      cpu time            0.03 seconds


37   data zeq2; set output_out;
38   if enc = "." then delete;
39   run;

NOTE: There were 43 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ2 has 27 observations and 6 variables.
NOTE: DATA statement used (Total process time):
```

```
      real time           0.01 seconds
      cpu time            0.01 seconds


40    proc sort data = zeq2;
41    by enc;
42    run;

NOTE: There were 27 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 27 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


43    /*************************************************************************
43 !  ************************
44    * This procedure produces the mean concentrations for each treatment repl
44 !  exposure sampling times     *
45    * i.e. It gives the mean concentration of each treatment enclosure over t
45 !  period                 *
46    *************************************************************************
46 !  ************************/
47    title1 "Mean treatment concentration by enclosure for all exposure sampli
48    title2 "Individual enclosure means";
49    proc means data = zeq2 mean std lclm uclm fw=8;
50    by enc thero;
51    var predicted_ppm;
52    run;

NOTE: There were 27 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.09 seconds
      cpu time            0.03 seconds


53    proc sort;
54    by thero time;
55    /*************************************************************************
55 !  ***********
56    * This procedure produces the mean concentrations for each treatment grou
56 !  time      *
57    * i.e. It gives the mean concentration of the three control, 50ppm, & 100
57 !  replicates     *
58    * at each sample time over the entire exposure
58 !           *
```

```
59   ************************************************************************
59 ! **********/
60   title1 "Mean treatment concentration by treatment group at each exposure

NOTE: There were 27 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 27 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.02 seconds
      cpu time            0.03 seconds


61   proc means data = zeq2 mean std lclm uclm fw=8;
62   by thero time;
63   var opredicted_ppm;
64   run;

NOTE: There were 27 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.13 seconds
      cpu time            0.04 seconds


65   /************************************************************************
65 ! ***********
66   * This procedure produces the mean concentrations for each treatment grou
66 !  times    *
67   * i.e. It gives the mean concentration of the three control, 50ppm, & 100
67 ! replicates     *
68   * over the entire exposure
68 !           *
69   ************************************************************************
69 ! **********/
70   title1 'Mean treatment concentration by treatment group over all exposure
71   proc means data = zeq2 mean std lclm uclm fw=8;
72   by thero;
73   var predicted_ppm;
74   run;

NOTE: There were 27 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.04 seconds


75   quit;
76   run;
```

FF # 12
Item No. 5
Pg 4 of 4

Appendix 8. Zebra Mussel Density Association Summary Data Analysis

Item number	Item description	Number of pages	Report page number
1	Zebra Mussel Density Associations – Data Summary	15	202

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (pages 9, 10, 15 to 18 & 21 to 22)	Created.....	12-Sep-13	KLW
Data Source Forms: "Initial Assessment of Native and Zebra Mussels" (File Folder 13a)	Revised.....	26-Mar-14	KLW
"Mussel Initial Assessment" (File Folder 13b)	Reviewed...	[illegible]	[illegible]
"Mussel Weight and Length Assessment" (File Folder 13c)	Certified...	3/26/14	SP
"Mussel Survival Assessment" (File Folder 13d)			
		Coversheet	
File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Density Associations.xlsx]ZM Density Coversheet		Page 1 of 2	

Zebra Mussel Density Associations

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A (SDP)); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Explanation for Pre-exposure Zebra Mussel Densities: (Data can be found in FF 13A)
Data that is not calculated (obtained from "Initial Assessment of Native and Zebra Mussels" datasheets)
- Treatment Level (mg/L) = Targeted exposure concentration for the test enclosure (Targeted concentrations were: control (0), 50 or 100 mg/L)
- Test Enclosure ID = Test Enclosure ID; numbered 1 through 9
- Tag ID = Hallprint shellfish tag glued to the native mussel
- Weight with ZM (g) = Weight of unionid with adhering zebra mussels
- Weight without ZM (g) = Weight of unionid after zebra mussel removal

Calculated Data (obtained from calculations in Excel)
- Weight of ZM (g) = Weight with ZM (g) - Weight without ZM (g)
- % Body Mass of ZM on Unionids = (Weight of ZM (g))/(Weight with ZM (g))*100
- Number of ZM/gram of ZM = Number of ZM/Weight of Zebra Mussels (g)
- Mean Number of ZM/gram of ZM = Average number of ZM/gram of Zm by test enclosure
- Grand mean number of Zm/gram = Mean of the average number of ZM/gram of ZM by exposure group

Explanation for Post-exposure Zebra Mussel Densities:
Data that is not calculated (obtained from datasheets)
- Tag ID = Hallprint shellfish tag glued to the native mussel
- **Pre-exposure (Data on "Mussel Initial Assessment" datasheet in FF 13B)**
  - Unionid Wt with ZM(g) = Initial weight of unionid with zebra mussels prior to distribution
- **Post-exposure (Data on " Mussel Weight and Length Assessment" datasheet in FF 13C)**
  - Unionid Wt with ZM (g) = Weight of unionid with zebra mussels post-exposure measured during assessment
  - Unionid Wt without ZM (g) = Weight of unionid after zebra mussel removal post-exposure measured during assessment
- **Post-exposure (Data on "Mussel Survival Assessment" datasheet in FF 13C)**
  - # ZM Adhering (Live) = Number of adhering live zebra mussels post-exposure
  - # Adhering ZM (Dead) = Number of adhering dead zebra mussels post-exposure

Calculated Data (obtained from analysis in Excel)
- **Pre-exposure**
  - Mean # ZM/gram = Average Number of ZM/gram of Zm by test enclosure from pre-exposure zebra mussel counts
  - Estimated Wt of ZM (g) = Pre-exposure Measured Weight with ZM (g) - Post-exposure Measured Weight without ZM (g)
  - Estimated # of Adhering ZM = (Mean number of ZM/gram of ZM from pre-exposure) x (Estimated Weight of ZM (g))
  - Mean Estimated # of Adhering ZM = Average of Estimated # of Adhering ZM prior to exposure
- **Post-exposure**
  - Mean # Live ZM/Unionid = Average # ZM Adhering (Live) post-exposure
  - ZM Burden (% Unionid B.W.) = (Estimated Weight of ZM (g)/Unionid Wt without ZM (g))*100
    - B.W. = Body Weight
  - Mean ZM Burden = Average of ZM Burden (% Unionid B.W.)
- **Burden and Reduction Summary**
  - ZM Reduction = (Initial Number) - (Final Number)
  - % Change = [(ZM Reduction)/(Initial Number)]*100
- **SDP Reduction Coefficient and Efficiency**
  - SPD Applied (g) = Amount of MBI-401 SDP applied to each test enclosure
  - Test Enclosure Area (m^2) = Area treated with MB -401 SDP (= 2.25 m^2)
  - % Change = [(ZM Reduction)/(Initial Number)]*100
  - Reduction Coefficient = (%Change/(SDP applied (g)/Test Enclosure Area (m^2))

Data Codes for SAS Input:
- enc = test enclosure ID (1 through 9)
- treat = treatment group (0, 50, and 100 mg/L)
- time = sample time
  - 1 = Pre-exposure density association
  - 2 = Post-exposure density association
- density = number of zebra mussels per unionid for each test enclosure

File Folder 13c.
Item Number 1
Page 1 of 15

Study Number: AEH-13-PSEUDO-05	Coversheet (cont.)
Electronic Lab Notebook (pages 9, 10, 15 to 18 & 21 to 22)	Page 2 of 2
Data Source Forms: "Initial Assessment of Native and Zebra Mussels" (File Folder 13a)	
"Mussel Initial Assessment" (File Folder 13b)	
"Mussel Weight and Length Assessment" (File Folder 13c)	
"Mussel Survival Assessment" (File Folder 13c)	
File Name: I:\AEH-13-PSEUDO-05\Data Summaries\[Zebra Mussel Density Associations.xlsx]Enclosure 1	

Zebra Mussel Density Associations

Test Article: MBI-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TM669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

The following unionid mussels were mortalities from the assessment (August 13 to 15, 2013). These data will not be used within the post-exposure density association analysis:

Test Enclosure 1 (100 mg/L):	Test Enclosure 6 (100 mg/L):	Test Enclosure 8 (0 mg/L):
C312	C213	C340
C359	C251	C387
Test Enclosure 2 (50 mg/L):	C280	Test Enclosure 9 (100 mg/L):
C436	C307	C202
Test Enclosure 3 (0 mg/L):	C396	C226
C193	Test Enclosure 7 (0 mg/L):	C258
Test Enclosure 4 (50 mg/L):	C248	C430
C271	C289	C411
C334	C299	C416
Test Enclosure 5 (50 mg/L):	C301	
C253		

The following mussels were not recovered during the assessment (August 13 to 15, 2013).

Test Enclosure 6 (100 mg/L):	Test Enclosure 9 (100 mg/L):
C356 - Included in pre-exposure zebra mussel density analysis	C323 - Removed from zebra mussel density analysis

The following mussels were used to estimate the pre-exposure density of zebra mussels adhering to unionids. These data were analyzed separately (Pre-exposure Zebra Mussel Density Associations).

Test Enclosure 1 (100 mg/L):	Test Enclosure 4 (50 mg/L):	Test Enclosure 7 (0 mg/L):
C208	C225	C192
C263	C239	C261
C350	C282	C361
C358	C333	C394
C377	C348	C417
Test Enclosure 2 (50 mg/L):	Test Enclosure 5 (50 mg/L):	Test Enclosure 8 (0 mg/L):
C310	C238	C189
C332	C240	C191
C375	C256	C209
C410	C259	C220
C419	C343	C337
Test Enclosure 3 (0 mg/L):	Test Enclosure 6 (100 mg/L):	Test Enclosure 9 (100 mg/L):
C197	C200	C295
C254	C250	C305
C382	C356	C367
C427	C402	C411
C434	C426	C416

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 10 & 21 to 22)
Data Source: Forms: "Initial Assessment of Native and Zebra Mussels" (File Folder 13a)

Test Article: MBI 401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates August 13-15, 2013

Zebra Mussel Density Associations
Pre-exposure Unionid Mussels

Treatment Level (mg/L)	Test Enclosure ID	Tag ID	Weight w/ZM (g)	Weight w/o ZM (g)	Number of Zebra Mussels (Live)	Weight of ZM (g)	% Body Mass of ZM on Unionids	Number of ZM/gram of ZM	Mean Number of ZM/gram of ZM (SD)	Grand Mean Number of ZM/gram of ZM (SD)
		C197	87.49	29.51	201	57.98	196.48	3.47		
		C254	129.20	64.98	270	64.22	98.83	4.20	4.11	
	3	C382	95.43	41.40	164	54.03	130.51	3.04	(0.87)	
		C427	60.56	30.47	168	30.09	98.75	5.58		
		C434	121.23	75.00	196	46.23	61.64	4.24		
		C192	72.08	30.89	118	41.19	133.34	2.86		
		C261	66.57	30.12	123	36.45	121.02	3.37	3.67	3.82
Control	7	C361	67.80	24.88	152	42.92	172.51	3.54	(0.73)	(0.20)
		C394	53.13	26.77	133	26.36	98.47	5.05		
		C417	91.02	48.99	149	42.03	85.79	3.55		
		C189	81.34	41.04	146	40.30	98.20	3.62		
		C191	85.90	35.24	164	50.66	143.76	3.24	3.69	
	8	C209	92.00	41.12	181	50.88	123.74	3.56	(0.55)	
		C220	82.76	24.20	193	58.56	241.98	3.30		
		C337	91.54	46.67	213	44.87	96.14	4.75		
		C310	60.53	23.64	114	36.89	156.05	3.09		
		C332	109.88	57.36	181	52.52	91.56	3.45	3.55	
	2	C375	74.17	42.09	94	32.08	76.22	2.93	(0.87)	
		C410	60.95	28.90	97	32.05	110.90	3.03		
		C419	111.41	56.12	291	55.29	98.52	5.26		
		C225	56.62	27.72	108	28.90	104.26	3.74		
		C239	76.10	22.10	257	54.00	244.34	4.76	4.08	3.78
50	4	C282	64.49	35.00	88	29.49	84.26	2.98	(0.65)	(0.20)
		C333	48.70	17.33	126	31.37	181.02	4.02		
		C348	116.97	61.39	260	55.58	90.54	4.68		
		C238	85.28	34.40	229	50.88	147.91	4.50		
		C240	67.22	28.16	113	39.06	138.71	2.89	3.77	
	5	C256	87.84	53.99	156	33.85	62.70	4.61	(0.68)	
		C259	70.93	38.85	114	32.08	82.57	3.55		
		C343	63.45	39.03	80	24.42	62.57	3.28		
		C208	88.81	45.45	172	43.36	95.40	3.97		
		C263	98.82	49.74	202	49.08	98.67	4.12	4.29	
	1	C350	70.50	34.33	110	36.17	105.36	3.04	(0.95)	
		C358	68.17	25.09	188	43.08	171.70	4.36		
		C377	52.12	19.35	196	32.77	169.35	5.98		
		C200	85.77	50.64	156	35.13	69.37	4.44		
		C250	71.05	42.35	124	28.70	67.77	4.32	4.04	4.16
100	6	C356	59.64	25.73	148	33.91	131.79	4.36	(0.42)	(0.10)
		C402	111.07	40.37	262	70.70	175.13	3.71		
		C426	70.01	28.66	140	41.35	144.28	3.39		
		C295	67.10	39.75	114	27.35	68.81	4.17		
		C305	56.29	34.93	90	21.36	61.15	4.21	4.13	
	9	C367	123.87	60.76	249	63.11	103.87	3.95	(0.61)	
		C411	111.65	48.10	204	63.55	132.12	3.21		
		C416	53.28	24.17	149	29.11	120.44	5.12		

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 1 (100 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C195	99.10	82.5	43.7	135	73	4.29	55.40	238			126.77	
C207	152.37	114.7	83.0	43	116	4.29	69.37	298			83.58	
C221	70.80	59.0	29.6	113	62	4.29	41.20	177			139.19	
C228	84.23	62.6	35.5	52	84	4.29	48.73	209			137.27	
C233	69.44	57.4	28.6	78	52	4.29	40.84	175			142.80	
C237	46.65	38.5	31.3	10	5	4.29	15.35	66			49.04	
C245	79.59	74.1	30.7	177	29	4.29	48.89	210			159.25	
C266	61.63	55.3	24.4	107	50	4.29	37.23	160	195	85	152.58	140.11
C292	78.89	69.2	49.4	55	38	4.29	29.49	127	(68)	(60)	59.70	(74.06)
C302	63.76	53.4	38.2	28	22	4.29	25.56	110			66.91	
C309	96.29	86.8	20.5	248	42	4.29	75.79	325			369.71	
C314	76.95	45.9	25.8	71	23	4.29	51.15	220			198.26	
C329	66.91	51.5	41.4	13	44	4.29	25.51	110			61.62	
C345	103.34	92.9	36.5	188	33	4.29	66.84	287			183.12	
C360	72.28	59.3	29.4	66	51	4.29	42.88	184			145.85	
C363	63.30	50.7	31.7	32	46	4.29	31.60	136			99.68	
C379	112.68	94.4	54.5	81	78	4.29	58.18	250			106.75	
C380	52.97	53.5	24.5	100	13	4.29	28.47	122			116.20	
C414	79.42	49.3	21.2	61	89	4.29	58.22	250			274.62	
C424	98.39	74.6	42.9	45	105	4.29	55.49	238			129.35	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 2 (50 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C187	96.06	93.4	54.7	168	53	3.55	41.36	147			75.61	
C188	94.04	72.3	43.4	73	74	3.55	50.64	180			116.68	
C203	88.88	78.6	37.8	105	70	3.55	51.08	181			135.13	
C217	111.45	89.1	49.0	82	93	3.55	62.45	222			127.45	
C219	72.60	54.4	38.9	45	53	3.55	33.70	120			86.63	
C232	39.87	36.9	19.8	52	22	3.55	20.07	71			101.36	
C235	62.11	54.1	27.8	80	42	3.55	34.31	122			123.42	
C242	46.83	39.2	18.4	69	41	3.55	28.43	101	147	79	154.51	117.77
C249	65.70	59.9	39.1	52	37	3.55	26.60	94	(51)	(36)	68.03	(32.63)
C276	59.30	52.9	32.6	62	25	3.55	26.70	95			81.90	
C278	65.99	58.6	36.6	63	30	3.55	29.39	104			80.30	
C293	81.12	76.4	36.8	126	55	3.55	44.32	157			120.43	
C298	48.57	38.5	24.9	28	28	3.55	23.67	84			95.06	
C304	119.17	101.1	65.4	67	52	3.55	53.77	191			82.22	
C317	131.35	84.3	48.9	55	80	3.55	82.45	293			168.61	
C321	77.36	69.3	32.4	96	65	3.55	44.96	160			138.77	
C331	56.08	40.4	23.2	30	54	3.55	32.83	117			141.51	
C342	92.78	73.1	40.5	76	64	3.55	52.28	186			129.09	
C395	61.05	48.1	20.4	57	41	3.55	40.65	144			199.26	
C406	75.53	70.4	32.4	138	50	3.55	43.13	153			133.12	
C408	85.63	82.7	40.0	141	54	3.55	45.63	162			114.08	

[a] Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 3 (Control)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C194	87.65	95.1	39.3	163	1	4.11	48.35	199			123.03	
C204	103.85	108.1	45.9	274	2	4.11	57.95	238			126.25	
C210	63.58	68.5	29.9	123	2	4.11	33.68	138			112.64	
C215	95.94	105.1	27.7	261	2	4.11	68.24	280			246.35	
C218	73.57	79.2	28.1	268	2	4.11	45.47	187			161.81	
C231	96.84	100.8	35.4	345	1	4.11	61.44	252			173.56	
C243	94.87	97.7	41.9	204	1	4.11	52.97	217			126.42	
C246	81.69	89.4	31.5	196	2	4.11	50.19	206	182	182	159.33	130.10
C311	85.39	86.8	46.2	119	2	4.11	39.19	161	(46)	(68)	84.83	(39.91)
C325	83.91	90.2	45.4	137	0	4.11	38.51	158			84.82	
C326	92.93	98.3	51.6	125	0	4.11	41.33	170			80.10	
C354	85.06	89.0	45.6	153	0	4.11	39.46	162			86.54	
C364	58.93	61.4	25.4	130	1	4.11	33.53	138			132.01	
C373	73.01	79.6	29.8	215	1	4.11	43.21	177			145.00	
C374	76.37	78.8	27.7	234	1	4.11	48.67	200			175.70	
C384	102.02	104.1	48.8	246	1	4.11	53.22	219			109.06	
C390	40.71	46.8	19.4	91	1	4.11	21.31	87			109.85	
C393	92.07	77.9	38.8	203	1	4.11	53.27	219			137.29	
C405	51.34	53.9	19.2	113	0	4.11	32.14	132			167.40	
C418	76.77	78.5	41.7	112	0	4.11	35.07	144			84.10	
C437	60.54	58.2	29.4	111	0	4.11	31.14	128			105.92	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 4 (50 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C223	77.07	65.2	36.4	89	55	4.03	40.67	164			111.73	
C255	56.70	36.7	21.6	44	37	4.03	35.10	142			162.50	
C270	80.92	51.5	34.7	31	55	4.03	46.72	189			136.61	
C279	99.42	83.6	56.3	63	56	4.03	43.12	174			76.59	
C283	110.89	92.5	41.6	137	111	4.03	69.29	280			166.56	
C285	80.13	67.9	24.9	89	58	4.03	55.23	223			221.81	
C286	36.01	26.9	12.6	33	22	4.03	23.41	94			185.79	
C296	76.58	64.1	28.2	95	65	4.03	48.48	196	167	64	171.91	140.27
C303	112.85	86.7	53.2	69	82	4.03	59.65	241	(51)	(33)	112.12	(38.67)
C339	95.46	76.6	51.1	68	79	4.03	44.36	179			86.81	
C351	56.47	44.4	23.5	42	37	4.03	32.97	133			140.30	
C353	82.43	69.1	28.2	148	75	4.03	54.23	219			192.30	
C388	51.82	35.9	26.1	38	39	4.03	25.72	104			98.54	
C389	69.06	55.0	32.8	49	47	4.03	36.26	146			110.55	
C398	39.97	33.7	15.7	45	41	4.03	24.27	98			154.59	
C403	73.94	57.9	33.8	85	72	4.03	40.14	162			118.76	
C404	73.70	46.5	28.1	40	34	4.03	45.60	184			162.28	
C412	33.23	26.7	17.5	25	20	4.03	15.73	63			89.89	
C421	69.41	41.7	25.1	49	37	4.03	44.31	179			176.53	
C429	77.70	55.8	33.9	36	49	4.03	43.80	177			129.20	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid ({"Column G"/"Column C"}*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 5 (50 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C190	51.36	49.2	29.2	66	8	3.77	22.16	83			75.89	
C214	123.13	102.0	56.0	196	85	3.77	67.13	253			119.88	
C236	82.88	72.0	52.9	44	30	3.77	29.98	113			56.67	
C244	54.96	49.6	23.3	67	25	3.77	31.66	119			135.88	
C265	133.83	108.0	61.0	148	131	3.77	72.83	274			119.39	
C284	103.37	85.3	49.4	79	72	3.77	53.97	203			109.25	
C290	68.65	65.5	44.7	51	19	3.77	23.95	90			53.58	
C294	78.53	66.2	34.4	112	59	3.77	44.13	166	166	79	128.28	119.57
C300	139.04	120.0	72.5	84	83	3.77	66.54	251	(56)	(37)	91.78	(56.60)
C320	91.34	72.4	40.4	51	67	3.77	50.94	192			126.09	
C330	72.55	60.8	30.0	58	63	3.77	42.55	160			141.83	
C335	50.31	40.2	12.6	62	73	3.77	37.71	142			299.29	
C352	78.65	73.6	40.1	68	54	3.77	38.55	145			96.13	
C365	98.76	89.1	62.9	50	33	3.77	35.86	135			57.01	
C368	78.75	68.0	49.6	55	22	3.77	29.15	110			58.77	
C392	59.05	54.2	34.2	46	17	3.77	24.85	94			72.66	
C397	90.93	74.3	34.4	105	71	3.77	56.53	213			164.33	
C407	75.09	63.4	28.1	75	47	3.77	46.99	177			167.22	
C425	82.92	61.7	31.0	64	71	3.77	51.92	196			167.48	
C431	96.41	78.8	33.5	120	100	3.77	62.91	237			187.79	
C433	72.55	62.2	39.9	59	66	3.77	32.65	123			81.83	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid {["Column G"/"Column C"]*100}.

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 6 (100 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C205	53.68	45.6	26.6	54	27	4.04	37.08	150			139.40	
C252	91.97	58.9	40.7	8	126	4.04	51.27	207			125.97	
C277	122.06	81.6	44.4	65	110	4.04	77.66	314			174.91	
C281	72.19	60.5	42.6	37	82	4.04	29.59	120			69.46	
C297	100.71	78.7	36.5	91	92	4.04	64.21	260			175.92	
C308	48.66	40.9	36.1	7	7	4.04	12.56	51			34.79	
C313	76.47	60.3	36.4	42	81	4.04	40.07	162			110.08	
C315	80.80	58.4	25.0	100	41	4.04	55.80	226	174	42	223.20	123.96
C316	64.65	49.9	31.9	19	34	4.04	32.75	132	(61)	(26)	102.66	(42.65)
C322	68.57	49.0	31.8	33	43	4.04	36.77	149			115.63	
C336	46.49	37.9	18.2	53	50	4.04	28.29	114			155.44	
C362	83.25	54.9	39.1	22	68	4.04	44.15	179			112.92	
C372	98.65	73.2	50.5	36	90	4.04	48.15	195			95.35	
C378	92.51	73.5	38.2	70	109	4.04	54.31	220			142.17	
C383	77.74	48.8	37.6	14	34	4.04	40.14	162			106.76	
C385	90.98	67.7	38.9	31	55	4.04	52.08	211			133.88	
C423	60.41	50.3	32.0	30	59	4.04	28.41	115			88.78	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 7 (Control)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C212	94.16	97.3	36.8	182	2	3.67	57.36	211			155.87	
C234	69.20	67.0	26.5	144	2	3.67	42.70	157			161.13	
C247	62.94	65.6	29.4	109	0	3.67	33.54	123			114.08	
C257	69.07	71.8	32.0	132	2	3.67	37.07	136			115.84	
C260	56.79	59.4	21.5	180	2	3.67	35.29	130			164.14	
C268	84.13	88.1	40.7	181	0	3.67	43.43	160			106.71	
C272	78.30	78.3	36.7	184	1	3.67	41.60	153			113.35	
C273	62.99	66.4	20.3	155	2	3.67	42.69	157	155	169	210.30	134.41
C275	64.78	67.3	33.5	114	1	3.67	31.28	115	(22)	(32)	93.37	(31.47)
C306	87.58	91.0	44.7	143	2	3.67	42.88	158			95.93	
C319	65.15	65.0	23.8	206	2	3.67	41.35	152			173.74	
C347	67.92	73.2	31.1	161	0	3.67	36.82	135			118.39	
C349	84.18	87.7	36.5	195	1	3.67	47.68	175			130.63	
C355	94.03	99.0	44.3	151	1	3.67	49.73	183			112.26	
C357	71.43	77.2	30.1	205	0	3.67	41.33	152			137.31	
C370	68.73	72.1	26.8	202	5	3.67	41.93	154			156.46	
C401	91.70	94.3	46.3	165	2	3.67	45.40	167			98.06	
C413	77.52	82.0	29.6	228	3	3.67	47.92	176			161.89	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 8 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 8 (Control)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C186	49.88	51.0	31.4	105	0	3.69	18.48	68			58.85	
C196	107.40	94.9	48.1	273	2	3.69	59.30	219			123.28	
C198	46.92	46.9	22.3	103	0	3.69	24.62	91			110.40	
C199	88.30	91.0	44.5	253	3	3.69	43.80	162			98.43	
C211	79.44	82.1	37.0	136	2	3.69	42.44	157			114.70	
C216	46.21	48.5	20.9	107	0	3.69	25.31	93			121.10	
C222	92.44	90.8	45.7	187	1	3.69	46.74	173			102.28	
C230	64.03	64.5	24.9	193	3	3.69	39.13	144	157	185	157.15	125.57
C241	76.37	79.6	44.9	135	2	3.69	31.47	116	(51)	(58)	70.09	(46.72)
C264	86.88	91.8	27.4	273	0	3.69	59.48	220			217.08	
C269	89.99	91.3	47.1	186	1	3.69	42.89	158			91.06	
C274	73.45	71.9	33.8	133	2	3.69	39.65	146			117.31	
C324	71.59	76.9	35.1	186	2	3.69	36.49	135			103.96	
C366	75.55	78.5	48.0	108	3	3.69	27.55	102			57.40	
C369	57.85	60.5	17.4	193	2	3.69	40.45	149			232.47	
C391	85.21	80.3	30.4	192	7	3.69	54.81	202			180.30	
C399	97.93	103.8	45.5	215	4	3.69	52.43	194			115.23	
C415	63.89	65.1	26.8	178	1	3.69	37.09	137			138.40	
C428	80.34	83.1	27.9	281	0	3.69	52.44	194			187.96	
C435	145.23	146.7	67.9	254	3	3.69	77.33	286			113.89	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)
"Mussel Weight and Length Assessment" (File Folder 13c)
"Mussel Survival Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4569-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Density Associations
Post-exposure - Test Enclosure 9 (100 mg/L)

	Collected Data					Calculated Values						
	exposure	Post-exposure				Pre-exposure				Post-exposure		
	A	B	C	D	E	F	G	H	I	J	K	L
Unionid Tag ID	Unionid Wt with ZM (g)	Unionid Wt with ZM (g)	Unionid Wt without ZM (g)	# ZM Adhering (Live)	# ZM Adhering (Dead)	Mean # ZM/gram[a]	Estimated Wt of ZM (g)[b]	Estimated # of Adhering ZM[c]	Mean Estimated # of Adhering ZM (SD)	Mean # of Live ZM/Unionid (SD)	ZM Burden[d] (% Unionid Body Weight)	Mean ZM Burden (SD)
C206	83.11	68.4	43.1	48	75	4.13	40.01	165			92.83	
C227	78.97	63.4	32.0	115	48	4.13	46.97	194			146.78	
C229	116.33	79.5	55.3	38	70	4.13	61.03	252			110.36	
C262	106.54	85.2	46.3	92	79	4.13	60.24	249			130.11	
C287	105.78	80.5	56.7	25	93	4.13	49.08	203			86.56	
C327	72.37	62.3	42.6	68	26	4.13	29.77	123			69.88	
C328	58.20	36.6	25.5	7	37	4.13	32.70	135			128.24	
C338	65.20	61.4	25.0	148	61	4.13	40.20	166	172	51	160.80	117.33
C344	41.34	38.7	25.5	32	15	4.13	15.84	65	(64)	(38)	62.12	(37.53)
C346	51.11	40.9	28.5	27	28	4.13	22.61	93			79.33	
C376	93.22	66.2	43.6	19	132	4.13	49.62	205			113.81	
C381	38.81	37.4	22.5	50	6	4.13	15.31	67			72.49	
C386	41.10	29.3	16.1	21	44	4.13	25.00	103			155.28	
C409	88.67	60.9	29.9	85	82	4.13	58.77	243			196.56	
C420	84.11	52.4	35.2	35	42	4.13	48.91	202			138.95	
C422	86.18	60.5	44.9	21	52	4.13	41.28	171			91.94	
C432	112.26	65.6	43.4	28	100	4.13	68.86	284			158.66	

[a] - Value obtained from "Zebra Mussel Density Association - Pre-exposure Unionid Mussels" data summary page (i.e., page 3 of this data summary).

[b] - Value obtained by subtracting "Column C" from "Column A"

[c] - Value obtained by multiplying "Column F" by "Column G"

[d] - ZM Burden = Compares weight of adhering zebra mussels to weight of unionid mussel. Expressed as percent body weight of unionid (["Column G"/"Column C"]*100).

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Zebra Mussel Burden and Reduction on Unionid Mussels

			Zebra Mussels/Unionid			
Treatment Level (mg/L)	Enclosure ID	ZM Burden (% Unionid Body Weight)	Initial Number Adhering	Final Number Adhering	Number Adhering Reduction	% Change
0	3	130.10	182	182	0	0
	7	134.41	155	169	-14	-9
	8	125.57	157	185	-28	-18
	Mean	130.03	165	179	-14	-9
	(SD)	(4.42)	(15)	(9)	(14)	(9)
50	2	117.77	147	79	68	46
	4	140.27	167	64	103	62
	5	119.57	166	79	87	52
	Mean	125.87	160	74	86	53
	(SD)	(12.50)	(11)	(9)	(18)	(8)
100	1	140.11	195	85	110	56
	6	123.96	174	42	132	76
	9	117.33	172	51	121	70
	Mean	127.14	180	59	121	68
	(SD)	(11.72)	(13)	(23)	(11)	(10)

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 15 to 16 & 18)
Data Source: Forms: "Mussel Initial Assessment" (File Folder 13b)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

SDP Reduction Coefficient

Treatment Level (mg/L)	Enclosure ID	SDP Applied (g)	Test Enclosure Area (m^2)	% Change	Reduction Coefficient
0	3	N/A	2.25	0	N/A
	7	N/A	2.25	-9	N/A
	8	N/A	2.25	-18	N/A
	Mean	N/A	2.25	-9	N/A
	(SD)	N/A	0.00	(9)	N/A
50	2	262.09	2.25	46	0.397
	4	277.53	2.25	62	0.500
	5	277.59	2.25	52	0.425
	Mean	272.40	2.25	53	0.441
	(SD)	(8.93)	0.00	(8)	(0.043)
100	1	544.73	2.25	56	0.233
	6	544.61	2.25	76	0.313
	9	563.27	2.25	70	0.281
	Mean	550.87	2.25	68	0.276
	(SD)	(10.74)	0.00	(10)	(0.033)

enc	treat	time	density
1	100	1	195
1	100	2	85
2	50	1	147
2	50	2	79
3	0	1	182
3	0	2	182
4	50	1	167
4	50	2	64
5	50	1	166
5	50	2	79
6	100	1	174
6	100	2	42
7	0	1	155
7	0	2	169
8	0	1	157
8	0	2	185
9	100	1	172
9	100	2	51

Appendix 9. Unionid Mussel Survival Assessment Summary

Item number	Item description	Number of pages	Report page number
1	Unionid Mussel Survival – Data Summary	5	218

Study Number: AEH-13-PSEUDO-05	Action	Date	Initials
Electronic Lab Notebook (pages 9, 10, 15 to 17 & 21)	Created.....	20-Feb-14	KLW
Data Source: Forms: "Mussel Species Identification" (File Folder 7b)	Revised.....	6-Mar-14	KLW
"Initial Assessment of Native and Zebra Mussels" (File Folder 13a)	Reviewed...	6 MAR 14	
"Mussel Weight and Length Assessment" (File Folder 13c)	Certified..	3/6/14	

File Name: See filenames as stated below.

Unionid Mussel Survival

Test Article: M8I-401 SDP (Pseudomonas fluorescens Pf-CL 145A [SDP]); Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Data Explanation:

1) Unionid mussel survival status was determined 18 to 20 days post-exposure (August 13-15, 2013).
2) Data codes used within SAS

- cnc = Test Enclosure ID (1 through 9)
- treat = treatment level (0, 50 and 100)
  - 0 = control (0 mg/L)
  - 50 = 50 mg/L
  - 100 = 100 mg/L
- group = zebra mussel assessment time
  - 1 = pre-exposure (i.e., 45 unionid mussels used to determine zebra mussel density associations)
  - 2 = post-exposure
- tag = Hallprint shellfish tag glued to the native mussel
- species = unionid species
  - FAM = Fatmucket (*Lampsilis siliquoidea*)
  - FPS = Fragile Papershell (*Leptodea fragilis*)
  - UNK = Unknown
- status = status of unionid mussel during post-exposure assessment
  - L = Live
  - D = Dead
  - U = Unrecovered

Data Anomalies and Deviations:

NONE

File Folder 13c
Item Number 2
Page 1 of 5

Study Number: AEH-13-PSEUDO-05

Electronic Lab Notebook (pages 9, 10, 15 to 17 & 21)

Data Source: Forms: "Mussel Species Identification" (File Folder 7b)

"Initial Assessment of Native and Zebra Mussels" (File Folder 13a)

"Mussel Weight and Length Assessment" (File Folder 13c)

Test Article: MBI-401 SDP

Lot #: TR4669-4-3

Exposure Date: July 26, 2013

Assessment Dates: August 13-15, 2013

Unionid Mussel Survival
Control Test Enclosures

Alive/ Dead	Enclosure ID	Group	Species	N	Tag ID							
Alive	3	1	FAM	5	C197	C254	C382	C427	C434			
		2	FAM	21	C194	C204	C210	C215	C218	C231	C243	C246
					C311	C325	C326	C354	C364	C373	C374	C384
					C390	C393	C405	C418	C437			
	7	1	FAM	4	C192	C261	C361	C417				
			FPS	1	C394							
		2	FAM	18	C212	C234	C247	C257	C260	C268	C272	C273
					C347	C349	C355	C357	C370	C401	C413	C275
					C306	C319						
	8	1	FAM	5	C189	C191	C209	C220	C337			
		2	FAM	20	C186	C196	C198	C216	C222	C230	C241	C264
					C269	C274	C324	C199	C211	C391	C399	C415
					C428	C435	C369	C366				
Dead	3	2	FAM	1	C193							
	7	2	FAM	4	C248	C289	C299	C301				
	8	2	FAM	2	C340	C387						

Study Number: AEH-13-PSEUDO-05
Electronic Lab Notebook (pages 9, 10, 15 to 17 & 21)
Data Source: Forms: "Mussel Species Identification" (File Folder 7b)
"Initial Assessment of Native and Zebra Mussels" (File Folder 13a)
"Mussel Weight and Length Assessment" (File Folder 13c)

Test Article: MBI-401 SDP
Lot #: TR4669-4-3
Exposure Date: July 26, 2013
Assessment Dates: August 13-15, 2013

Unionid Mussel Survival
50 mg/L Test Enclosures

Alive/ Dead	Enclosure ID	Group	Species	N	Tag ID							
Alive	2	1	FAM	5	C310	C332	C375	C410	C419			
		2	FPS	2	C331	C342						
			FAM	19	C187	C188	C203	C217	C219	C232	C235	C242
					C249	C276	C278	C293	C298	C304	C317	C321
					C395	C406	C408					
	4	1	FAM	5	C225	C239	C282	C333	C348			
		2	FPS	1	C398							
			FAM	19	C223	C255	C270	C279	C283	C285	C286	C296
					C303	C339	C351	C353	C388	C389	C403	C404
					C412	C421	C429					
	5	1	FAM	5	C238	C240	C256	C259	C343			
		2	FPS	1	C335							
			FAM	20	C190	C214	C236	C244	C265	C284	C290	C294
					C300	C320	C330	C352	C365	C368	C392	C397
					C407	C425	C431	C433				
Dead	2	2	FAM	1	C436							
	4	2	FAM	2	C271	C334						
	5	2	FAM	1	C253							

Study Number: AEH-13-PSEUDO-05

Electronic Lab Notebook (pages 9, 10, 15 to 17 & 21)

Data Source: Forms: "Mussel Species Identification" (File Folder 7b)

"Initial Assessment of Native and Zebra Mussels" (File Folder 13a)

"Mussel Weight and Length Assessment" (File Folder 13c)

Test Article: MBI-401 SDP

Lot #: TR4669-4-3

Exposure Date: July 26, 2013

Assessment Dates: August 13-15, 2013

Unionid Mussel Survival
100 mg/L Test Enclosures

Alive/ Dead	Enclosure ID	Group	Species	N	Tag ID							
Alive	1	1	FAM	5	C208	C263	C350	C358	C377			
		2	FPS	2	C207	C363						
			FAM	18	C195	C221	C228	C233	C237	C245	C266	C292
					C302	C309	C314	C329	C345	C360	C379	C380
					C414	C424						
	6	1	FAM	4	C200	C250	C402	C426				
		2	FPS	2	C336	C362						
			FAM	15	C205	C252	C277	C281	C297	C308	C313	C315
					C316	C322	C372	C378	C383	C385	C423	
	9	1	FAM	3	C295	C305	C367					
		2	FPS	2	C344	C386						
			FAM	15	C206	C227	C229	C262	C287	C327	C328	C338
					C346	C376	C381	C409	C420	C422	C432	
Dead	1	2	FAM	2	C312	C359						
	6	2	FPS	1	C307							
			FAM	4	C213	C251	C280	C396				
	9	1	FAM	2	C411	C416						
		2	FAM	4	C202	C226	C258	C430				
Not Recovered	6	1	Unknown	1	C356							
	9	2	Unknown	1	C323							

enc	treat	group	species	tot	dead	unrec	tdead
1	100	1	FAM	5	0	0	0
1	100	2	FAM	20	2	0	2
1	100	1	FPS	0	0	0	0
1	100	2	FPS	2	0	0	0
2	50	1	FAM	5	0	0	0
2	50	2	FAM	20	1	0	1
2	50	1	FPS	0	0	0	0
2	50	2	FPS	2	0	0	0
3	0	1	FAM	5	0	0	0
3	0	2	FAM	22	1	0	1
3	0	1	FPS	0	0	0	0
3	0	2	FPS	0	0	0	0
4	50	1	FAM	5	0	0	0
4	50	2	FAM	21	2	0	2
4	50	1	FPS	0	0	0	0
4	50	2	FPS	1	0	0	0
5	50	1	FAM	5	0	0	0
5	50	2	FAM	21	1	0	1
5	50	1	FPS	0	0	0	0
5	50	2	FPS	1	0	0	0
6	100	1	FAM	5	0	1	1
6	100	2	FAM	19	4	0	4
6	100	1	FPS	0	0	0	0
6	100	2	FPS	3	1	0	1
7	0	1	FAM	4	0	0	0
7	0	2	FAM	22	4	0	4
7	0	1	FPS	1	0	0	0
7	0	2	FPS	0	0	0	0
8	0	1	FAM	5	0	0	0
8	0	2	FAM	22	2	0	2
8	0	1	FPS	0	0	0	0
8	0	2	FPS	0	0	0	0
9	100	1	FAM	5	2	0	2
9	100	2	FAM	20	4	1	5
9	100	1	FPS	0	0	0	0
9	100	2	FPS	2	0	0	0

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Item No. 2
Pg 5 of 5

Appendix 10. Statistical Analysis, Including SAS Programs, Outputs, and Logs for Unionid Mussel Survival, Zebra Mussel Colonization Density Associations, and Zebra Mussel Test Animal Lengths

Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP
for controlling zebra mussels within field enclosures
Unionid Mussel Survival
all data

AEH-13-PSEUDO-05

Obs	enc	treat	group	species	tot	dead	unrep	tdead	potsurv	potmort	treata
1	2	50	1	FAM	5	0	0	0	100.000	0.0000	A
2	4	50	1	FAM	5	0	0	0	100.000	0.0000	A
3	5	50	1	FAM	5	0	0	0	100.000	0.0000	A
4	1	100	1	FAM	5	0	0	0	100.000	0.0000	B
5	6	100	1	FAM	5	0	1	1	80.000	20.0000	B
6	9	100	1	FAM	5	2	0	2	60.000	40.0000	B
7	3	0	1	FAM	5	0	0	0	100.000	0.0000	C
8	7	0	1	FAM	4	0	0	0	100.000	0.0000	C
9	8	0	1	FAM	5	0	0	0	100.000	0.0000	C
10	2	50	2	FAM	20	1	0	1	95.000	5.0000	A
11	4	50	2	FAM	21	2	0	2	90.476	9.5238	A
12	5	50	2	FAM	21	1	0	1	95.238	4.7619	A
13	1	100	2	FAM	20	2	0	2	90.000	10.0000	B
14	6	100	2	FAM	19	4	0	4	78.947	21.0526	B
15	9	100	2	FAM	20	4	1	5	75.000	25.0000	B
16	3	0	2	FAM	22	1	0	1	95.455	4.5455	C
17	7	0	2	FAM	22	4	0	4	81.818	18.1818	C
18	8	0	2	FAM	22	2	0	2	90.909	9.0909	C
19	7	0	1	FPS	1	0	0	0	100.000	0.0000	C
20	2	50	2	FPS	2	0	0	0	100.000	0.0000	A
21	4	50	2	FPS	1	0	0	0	100.000	0.0000	A
22	5	50	2	FPS	1	0	0	0	100.000	0.0000	A
23	1	100	2	FPS	2	0	0	0	100.000	0.0000	B
24	6	100	2	FPS	3	1	0	1	66.667	33.3333	B
25	9	100	2	FPS	2	0	0	0	100.000	0.0000	B

Performed by K. Weber SAS version 9.3 13:31 18MAR14

File Folder: 16

Item Number: 1

AEH-13-PSEUDO-05

Mean Survival by Species (sorted by treatment level and group)

The MEANS Procedure

species=FAM

treata	group	N Obs	Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
A	1	3	potsurv	100.0	0	.	.
			potmort	0	0	.	.
	2	3	potsurv	93.5714	2.8832	86.9060	100.2
			potmort	6.4286	2.8832	-0.2369	13.0940
B	1	3	potsurv	80.0000	20.0000	30.3172	129.7
			potmort	20.0000	20.0000	-29.6828	69.6828
	2	3	potsurv	81.3158	7.7754	62.0006	100.6
			potmort	18.6842	7.7754	-0.6310	37.9994
C	1	3	potsurv	100.0	0	.	.
			potmort	0	0	.	.
	2	3	potsurv	89.3939	6.9433	72.1458	106.6
			potmort	10.6061	6.9433	-6.8420	27.8542

species=FPS

treata	group	N Obs	Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
A	2	3	potsurv	100.0	0	.	.
			potmort	0	0	.	.
B	2	3	potsurv	88.8889	19.2450	41.0816	136.7
			potmort	11.1111	19.2450	-36.6961	58.9184
C	1	1	potsurv	100.0	.	.	.
			potmort	0	.	.	.

Performed by K. Weber SAS version 9.3 13:31 18MAR14

Mean Survival by Species (sorted by treatment level)

The MEANS Procedure

species=FAM

treata	N Obs	Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
A	6	potsurv	96.7857	3.9087	92.8838	100.8
		potmort	3.2143	3.9087	-0.6876	7.3162
B	6	potsurv	80.6579	13.5905	66.3955	94.9203
		potmort	19.3421	13.5905	5.0797	33.6045
C	6	potsurv	94.6970	7.2822	87.0548	102.3
		potmort	5.3030	7.2822	-2.3392	12.9452

species=FPS

treata	N Obs	Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
A	3	potsurv	100.0	0	.	.
		potmort	0	0	.	.
B	3	potsurv	88.8889	19.2450	41.0816	136.7
		potmort	11.1111	19.2450	-36.6861	58.9184
C	1	potsurv	100.0	.	.	.
		potmort	0	.	.	.

Performed by K. Weber SAS version 9.3 13:31 18MAR14

Mean Survival by Treatment Level and Group (combined species)

The MEANS Procedure

treata=A group=1

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	100.0	0	.	.
pctmort	0	0	.	.

treata=A group=2

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	96.7857	3.9087	92.8838	100.9
pctmort	3.2143	3.9087	-0.8876	7.3162

treata=B group=1

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	80.0000	20.0000	30.3172	129.7
pctmort	20.0000	20.0000	-29.6828	69.6828

treata=B group=2

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	85.1023	13.7672	70.6545	99.5502
pctmort	14.8977	13.7672	0.4498	29.3455

treata=C group=1

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	100.0	0	.	.
pctmort	0	0	.	.

treata=C group=2

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
pctsurv	89.3939	6.9433	72.1458	106.6
pctmort	10.6061	6.9433	-6.6420	27.8542

Performed by K. Weber SAS version 9.3 13:31 18MAR14

Mean Survival by Treatment (combined species and group)

The MEANS Procedure

treata=A

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
petsurv	97.8571	3.4830	95.1793	100.5
petmort	2.1429	3.4830	-0.5344	4.8202

treata=B

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
petsurv	83.4016	14.9990	71.8723	94.9308
petmort	16.5984	14.9990	5.0692	28.1277

treata=C

Variable	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
petsurv	95.4545	6.9433	80.0331	101.9
petmort	4.5455	6.9433	-1.8760	10.9669

Performed by K. Weber SAS version 9.3 13:31 18MAR14

AEH-13-PSEUDO-05

Proc Glimmix Analysis of Survival and Interactions (Group 1 and 2 combined)

FW 1/31/2014

Native Unionid Survival

The GLIMMIX Procedure

Model Information	
Data Set	WORK.MUSSEL
Response Variable (Events)	dead
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
treata	3	A B C

Number of Observations Read	25
Number of Observations Used	25
Number of Events	28
Number of Trials	243

Dimensions	
Covariance Parameters	1
Columns in X	3
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	25

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	3
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	21.752424490	.	0.407133
1	0	3	21.585841936	0.16658258	0.026042
2	0	3	21.585219359	0.00062258	0.000175
3	0	3	21.585219336	0.00000002	8.693E-9

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	43.17
AIC (smaller is better)	49.17
AICC (smaller is better)	50.31
BIC (smaller is better)	52.83
CAIC (smaller is better)	55.83
HQIC (smaller is better)	50.18
Pearson Chi-Square	11.98
Pearson Chi-Square / DF	0.54

Parameter Estimates

Effect	treata	Estimate	Standard Error	DF	t Value	Pr > |t|
treata	A	-2.9575	0.3784	22	-7.82	<.0001
treata	B	-1.4815	0.2110	22	-7.02	<.0001
treata	C	-2.3582	0.2917	22	-8.08	<.0001
Residu		0.5443				

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treata	3	22	58.58	<.0001

Odds Ratio Estimates

treata	_treata	Estimate	DF	95% Confidence Limits	
A	C	0.546	22	0.204	1.479
B	C	2.403	22	1.139	5.070

treata Least Squares Means

treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
A	-2.9575	0.3784	22	-7.82	<.0001	0.05	-3.7422	-2.1729	0.04638	0.01776	0.02316	0.1022
B	-1.4815	0.2110	22	-7.02	<.0001	0.05	-1.9193	-1.0439	0.1852	0.03184	0.1279	0.2604
C	-2.3582	0.2917	22	-8.08	<.0001	0.05	-2.9632	-1.7531	0.08642	0.02303	0.04912	0.1477

Differences of treata Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

treata	_treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio	Adj Lower Odds Ratio	Adj Upper Odds Ratio
A	B	-1.4759	0.4332	22	-3.41	0.0025	0.0069	0.05	-2.3744	-0.5775	-2.5642	-0.3876	0.229	0.093	0.561	0.077	0.679
A	C	-0.5994	0.4778	22	-1.26	0.2228	0.4360	0.05	-1.5902	0.3915	-1.7986	0.6000	0.549	0.204	1.479	0.165	1.824
B	C	0.8766	0.3601	22	2.43	0.0235	0.0557	0.05	0.1298	1.6233	-0.02797	1.7811	2.403	1.139	5.070	0.972	5.938

Performed by K. Weber SAS version 9.3 13:31 18MAR14

Proc Glimmix Analysis of Survival - Group 1 (animals cleaned prior to exposure to determine initial ZM colonization)

SUMMARY

Native Unionid Survival

The GLIMMIX Procedure

Model Information	
Data Set	WORK.MUSSEL2
Response Variable (Events)	ldead
Response Variable (Trials)	lot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
treata	3	A B C

Number of Observations Read	10
Number of Observations Used	10
Number of Events	3
Number of Trials	45

Dimensions	
Covariance Parameters	1
Columns in X	3
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	10

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	3
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	4.627142553	.	0.348373
1	0	3	3.9537411209	0.67340143	0.1264
2	0	3	3.7252303424	0.22851078	0.046274
3	0	3	3.6421395879	0.08309077	0.016993
4	0	3	3.6116993893	0.03044118	0.006247
5	0	3	3.6005156839	0.01118173	0.002298
6	0	3	3.5964054261	0.00411124	0.000845
7	0	3	3.5948932957	0.00151213	0.000311
8	0	3	3.5943378559	0.00055624	0.000114
9	0	3	3.5941324324	0.00020482	0.000042
10	0	3	3.5940571563	0.00007528	0.000015
11	0	3	3.5940294639	0.00002769	5.695E-6

Convergence criterion (ABSGCONV=0.00001) satisfied.

Fit Statistics

-2 Log Likelihood	7.19
AIC (smaller is better)	13.19
AICC (smaller is better)	17.19
BIC (smaller is better)	14.10
CAIC (smaller is better)	17.10
HQIC (smaller is better)	12.19
Pearson Chi-Square	2.50
Pearson Chi-Square / DF	0.36

Parameter Estimates

Effect	treata	Estimate	Standard Error	DF	t Value	Pr > |t|
treata	A	-14.6365	221.28	7	-0.07	0.9495
treata	B	-1.3883	0.3858	7	-3.59	0.0088
treata	C	-14.3463	201.20	7	-0.07	0.9452
Residu		0.3571	.	.	.	.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treata	3	7	4.31	0.0510

Odds Ratio Estimates

treata	_treata	Estimate	DF	95% Confidence Limits	
A	C	0.827	7	<0.001	>999.999
B	C	>999.999	7	<0.001	>999.999

treata Least Squares Means

treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
A	-14.6365	221.28	7	-0.07	0.9495	0.05	-537.78	508.70	4.863E-7	0.000108	2E-235	1.0000
B	-1.3883	0.3858	7	-3.59	0.0088	0.05	-2.2985	-0.4741	0.2000	0.06172	0.09125	0.3836
C	-14.3463	201.20	7	-0.07	0.9452	0.05	-490.11	461.42	5.681E-7	0.000113	1E-214	1.0000

Differences of treata Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

treata	_treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio	Adj Lower Odds Ratio	Adj Upper Odds Ratio
A	B	-13.1502	221.28	7	-0.06	0.9543	0.9981	0.05	-536.39	510.09	-564.82	538.52	<0.001	<0.001	>999.999	<0.001	>999.999
A	C	-0.1902	299.05	7	-0.00	0.9995	1.0000	0.05	-707.59	707.01	-880.98	880.60	0.827	<0.001	>999.999	<0.001	>999.999
B	C	12.9600	201.20	7	0.06	0.9504	0.9977	0.05	-452.81	488.73	-579.59	605.51	>999.999	<0.001	>999.999	<0.001	>999.999

Performed by K. Weber SAS version 9.3 13:31 18MAR14

Proc Glimmix Analysis of Survival - Group 2 (animals with adhering ZM)

Native Unionid Survival

The GLIMMIX Procedure

Model Information	
Data Set	WORK.MUSSEL3
Response Variable (Events)	idead
Response Variable (Trials)	tot
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
treata	3	A B C

Number of Observations Read	15
Number of Observations Used	15
Number of Events	23
Number of Trials	198

Dimensions	
Covariance Parameters	1
Columns in X	3
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	15

Optimization Information	
Optimization Technique	Newton-Raphson
Parameters in Optimization	3
Lower Boundaries	0
Upper Boundaries	0
Fixed Effects	Not Profiled

Iteration History

Iteration	Restarts	Evaluations	Objective Function	Change	Max Gradient
0	0	4	15.716610776	.	0.425279
1	0	3	15.628622663	0.08198812	0.015563
2	0	3	15.628477872	0.00014498	0.000043
3	0	3	15.628477871	0.00000000	4.09E-13

Convergence criterion (GCONV=1E-8) satisfied.

Fit Statistics	
-2 Log Likelihood	31.26
AIC (smaller is better)	37.26
AICC (smaller is better)	39.44
BIC (smaller is better)	39.38
CAIC (smaller is better)	42.38
HQIC (smaller is better)	37.23
Pearson Chi-Square	6.02
Pearson Chi-Square / DF	0.50

AEH-13-PSEUDO-05

Parameter Estimates

Effect	treata	Estimate	Standard Error	DF	t Value	Pr > |t|
treata	A	-2.7406	0.3654	12	-7.50	<.0001
treata	B	-1.5041	0.2261	12	-6.65	<.0001
treata	C	-2.1316	0.2832	12	-7.53	<.0001
Residu		0.5016				

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treata	3	12	52.39	<.0001

Odds Ratio Estimates

treata	_treata	Estimate	DF	95% Confidence Limits	
A	C	0.544	12	0.189	1.489
B	C	1.673	12	0.850	4.125

treata Least Squares Means

treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
A	-2.7406	0.3654	12	-7.50	<.0001	0.05	-3.537	-1.9446	0.06061	0.02061	0.02828	0.1251
B	-1.5041	0.2261	12	-6.65	<.0001	0.05	-1.9966	-1.0116	0.1818	0.03363	0.1186	0.2667
C	-2.1316	0.2832	12	-7.53	<.0001	0.05	-2.7486	-1.5146	0.1061	0.02685	0.06016	0.1803

Differences of treata Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

treata	_treata	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Upper Confidence Limit for Odds Ratio	Adj Lower Odds Ratio	Adj Upper Odds Ratio
A	B	-1.2365	0.4297	12	-2.88	0.0139	0.0345	0.05	-2.1750	-0.3005	-2.3832	-0.09036	0.290	0.114	0.740	0.092	0.914
A	C	-0.6092	0.4623	12	-1.32	0.2122	0.4126	0.05	-1.6165	0.3981	-1.8426	0.6242	0.544	0.199	1.489	0.158	1.867
B	C	0.6275	0.3624	12	1.73	0.1069	0.2338	0.05	-0.1820	1.4170	-0.3391	1.5942	1.873	0.850	4.125	0.712	4.926

Performed by K. Weber SAS version 9.3 13:31 18MAR14

File Folder: 16

Item Number: 1

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
title2 h=2 'for controlling zebra mussels within field enclosures';
title3 h=2 'Unionid Mussel Survival';
title4 h=1.5'Study Number AEH-13-PSEUDO-05';
title5 h=1 'SAS v. 9.3  Analysis completion date:  18MAR14  Analysis prepared

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by: KLW      Page 1 of 3
* Analysis completion date:  18MAR14
*****************************************************************************/

/*****************************************************************************
* Variable Names:
* enc = enclosure ID (1 through 9)
* treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 mg/L
* group = zebra mussel asssessment time
*         1 = pre-exposure (i.e., zebra mussels from 45 unionid mussels used
*         2 = post-exposure
* species = unionid mussel species
*         FAM = Fatmucket (Lampsilis siliquoidea)
*         FPS = Fragil papershell (Leptodea fragilis)
*         UNK = Unknown (i.e., mussel unrecovered)
* tot = total number of unionid mussels
* dead = number of dead unionid mussels at post-exposure assessment
* unrec = number of unrecovered unionid mussels from the post-exposure assessm
* tdead = number of dead unionids + number of unrecovered unionid mussels
*****************************************************************************/

data mussel; set Pseudo05.Unionid_survival;
if tot = 0 then delete;
pctsurv = (tot-tdead)/tot*100;
pctmort = tdead/tot*100;
if treat = 0 then treata = 'C';
if treat = 50 then treata = 'A ';
if treat = 100 then treata = 'B';
run;

proc sort data=mussel; by species group treata; run;
proc print data=mussel; title4 h=1 'all data'; run;
```

FF # 16
Item No. 2
Pg 1 of 3

```
Title1 h=2 'Mean Survival by Species (sorted by treatment level and group)';
proc means data = mussel mean std lclm uclm fw=8;
by species;
class treata group;
var pctsurv pctmort;
run;

Title1 h=2 'Mean Survival by Species (sorted by treatment level)';
proc means data = mussel mean std lclm uclm fw=8;
by species;
class treata;
var pctsurv pctmort;
run;

proc sort data=mussel; by treata group; run;
Title1 h=2 'Mean Survival by Treatment Level and Group (combined species)';
proc means data = mussel mean std lclm uclm fw=8;
by treata group;
var pctsurv pctmort;
run;

Title1 h=2 'Mean Survival by Treatment (combined species and group)';
proc means data = mussel mean std lclm uclm fw=8;
by treata;
var pctsurv pctmort;
run;

Title1 h=2 'Proc Glimmix Analysis of Survival and Interactions (Group 1 and 2
proc glimmix data = mussel;
title4 'Native Unionid Survival';
class treata;
model tdead/tot  = treata / d = bin link = logit noint s or;
  lsmeans treata/pdiff cl ilink or adjust=tukey;
  random _residual_;
  run;

data mussel2; set mussel;
if group = 2 then delete;

Title1 h=2 'Proc Glimmix Analysis of Survival - Group 1 (animals cleaned prior
proc glimmix data = mussel2;
title4 'Native Unionid Survival';
class treata;
model tdead/tot  = treata / d = bin link = logit noint s or;
  lsmeans treata/pdiff cl ilink or adjust=tukey;
  random _residual_;
```

```
run;

data mussel3; set mussel;
if group = 1 then delete;

Title1 h=2 'Proc Glimmix Analysis of Survival - Group 2 (animals with adherinç
proc glimmix data = mussel3;
title4 'Native Unionid Survival';
class treata;
model tdead/tot  = treata / d = bin link = logit noint s or;
  lsmeans treata/pdiff cl ilink or adjust=tukey;
  random _residual_;
  run;
  quit;
```

FF # 16
Item No. 2
Pg 3 of 3

```
1    DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
2
3    FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE
3  ! ;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.
4
5    options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
6
7    title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
8    title2 h=2 'for controlling zebra mussels within field enclosures';
9    title3 h=2 'Unionid Mussel Survival';
10   title4 h=1.5'Study Number AEH-13-PSEUDO-05';
11   title5 h=1 'SAS v. 9.3  Analysis completion date:  18MAR14  Analysis prep
12
13   /*************************************************************************
14   * SAS ver 9.3       Analysis prepared by:  KLW       Page 1 of 6
15   * Analysis completion date:  18MAR14
16   *************************************************************************
17
18   /*************************************************************************
18 ! *************
19   * Variable Names:
19 !              *
20   * enc = enclosure ID (1 through 9)
20 !              *
21   * treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 m
21 !              *
22   * group = zebra mussel asssessment time
22 !              *
23   *          1 = pre-exposure (i.e., zebra mussels from 45 unionid mussels
23 ! density)     *
24   *          2 = post-exposure
24 !              *
25   * species = unionid mussel species
25 !              *
26   *          FAM = Fatmucket (Lampsilis siliquoidea)
26 !              *
27   *          FPS = Fragil papershell (Leptodea fragilis)
27 !              *
28   *          UNK = Unknown (i.e., mussel unrecovered)
28 !              *
29   * tot = total number of unionid mussels
29 !              *
30   * dead = number of dead unionid mussels at post-exposure assessment
30 !              *
```

FF # 16
Item No. 5
Pg 1 of 6

```
31   * unrec = number of unrecovered unionid mussels from the post-exposure as
31 |            *
32   * tdead = number of dead unionids + number of unrecovered unionid mussels
32 |             *
33   ************************************************************************
33 | ************/
34
35   data mussel; set Pseudo05.Unionid_survival;
36   if tot = 0 then delete;
37   pctsurv = (tot-tdead)/tot*100;
38   pctmort = tdead/tot*100;
39   if treat = 0 then treata = 'C';
40   if treat = 50 then treata = 'A';
41   if treat = 100 then treata = 'B';
42   run;

NOTE: There were 36 observations read from the data set PSEUDO05.UNIONID_SURVI
NOTE: The data set WORK.MUSSEL has 25 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.04 seconds
      cpu time            0.01 seconds


43
44   proc sort data=mussel; by species group treata; run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL has 25 observations and 11 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


45   proc print data=mussel; title4 h=1 'all data'; run;
NOTE: Writing HTML Body file: sashtml.htm
NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           3.93 seconds
      cpu time            0.28 seconds


46
47   Title1 h=2 'Mean Survival by Species (sorted by treatment level and group
48   proc means data = mussel mean std lclm uclm fw=8;
49   by species;
50   class treata group;
```

```
51   var pctsurv pctmort;
52   run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.12 seconds
      cpu time            0.07 seconds


53
54   Title1 h=2 'Mean Survival by Species (sorted by treatment level)';
55   proc means data = mussel mean std lclm uclm fw=8;
56   by species;
57   class treata;
58   var pctsurv pctmort;
59   run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.06 seconds


60
61   proc sort data=mussel; by treata group; run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL has 25 observations and 11 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


62   Title1 h=2 'Mean Survival by Treatment Level and Group (combined species)
63   proc means data = mussel mean std lclm uclm fw=8;
64   by treata group;
65   var pctsurv pctmort;
66   run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.06 seconds


67
```

```
68   Title1 h=2 'Mean Survival by Treatment (combined species and group)';
69   proc means data = mussel mean std lclm uclm fw=8;
70   by treata;
71   var pctsurv pctmort;
72   run;

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.09 seconds
      cpu time            0.04 seconds


73
74   Title1 h=2 'Proc Glimmix Analysis of Survival and Interactions (Group 1 a
75   proc glimmix data = mussel;
76   title4 'Native Unionid Survival';
77   class treata;
78   model tdead/tot  = treata / d = bin link = logit noint s or;
79     lsmeans treata/pdiff cl ilink or adjust=tukey;
80     random _residual_;
81     run;



NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.29 seconds
      cpu time            0.20 seconds


82
83   data mussel2; set mussel;
84   if group = 2 then delete;
85
86   Title1 h=2 'Proc Glimmix Analysis of Survival - Group 1 (animals cleaned
86 ! to determine initial ZM colonization)';

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL2 has 10 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

```
87   proc glimmix data = mussel2;
88   title4 'Native Unionid Survival';
89   class treata;
90   model tdead/tot  = treata / d = bin link = logit noint s or;
91     lsmeans treata/pdiff cl ilink or adjust=tukey;
92     random _residual_;
93   run;



NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.26 seconds
      cpu time            0.15 seconds


94
95   data mussel3; set mussel;
96   if group = 1 then delete;
97
98   Title1 h=2 'Proc Glimmix Analysis of Survival - Group 2 (animals with adr

NOTE: There were 25 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL3 has 15 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


99   proc glimmix data = mussel3;
100  title4 'Native Unionid Survival';
101  class treata;
102  model tdead/tot  = treata / d = bin link = logit noint s or;
103    lsmeans treata/pdiff cl ilink or adjust=tukey;
104    random _residual_;
105    run;



NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.29 seconds
```

```
      cpu time                0.20 seconds

106   quit;
```

FF # 16
Item No. 3
Pg 6 of 6

AEH-13-PSEUDO-05

Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP
for controlling zebra mussels within field enclosures
Zebra Mussel Colonization Changes
SAS v. 9.3 Analysis completion date: 25MAR14 Analysis prepared by: KLW

Obs	enc	treat	time	density
1	3	0	1	182
2	3	0	2	182
3	7	0	1	155
4	7	0	2	169
5	8	0	1	157
6	8	0	2	185
7	2	50	1	147
8	2	50	2	79
9	4	50	1	167
10	4	50	2	64
11	5	50	1	166
12	5	50	2	79
13	1	100	1	195
14	1	100	2	85
15	6	100	1	174
16	6	100	2	42
17	9	100	1	172
18	9	100	2	51

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File Folder: 16

Item Number: 4

AEH-13-PSEUDO-05

KW 25MAR14

ZM Density:
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: density (density)
treat = 0
time = 1

Moments			
N	3	Sum Weights	3
Mean	164.666667	Sum Observations	494
Std Deviation	15.0443788	Variance	226.333333
Skewness	1.69767732	Kurtosis	
Uncorrected SS	81798	Corrected SS	452.666667
Coeff Variation	9.13626243	Std Error Mean	8.68587615

Basic Statistical Measures			
Location		Variability	
Mean	164.6667	Std Deviation	15.04438
Median	157.0000	Variance	226.33333
Mode	.	Range	27.00000
		Interquartile Range	27.00000

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	18.95798	Pr > |t|	0.0028
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.805228	Pr < W	0.1270
Kolmogorov-Smirnov	D	0.361502	Pr > D	0.1217
Cramer-von Mises	W-Sq	0.076265	Pr > W-Sq	0.1619
Anderson-Darling	A-Sq	0.418086	Pr > A-Sq	0.1028

Quantiles (Definition 5)

AEH-13-PSEUDO-05

Quantile	Estimate
100% Max	182
99%	182
95%	182
90%	182
75% Q3	182
50% Median	157
25% Q1	155
10%	155
5%	155
1%	155
0% Min	155

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
155	2	155	2
157	3	157	3
182	1	182	1

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ZM Density:
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: density (density)
treat = 50
time = 1

Moments			
N	3	Sum Weights	3
Mean	160	Sum Observations	480
Std Deviation	11.2694277	Variance	127
Skewness	-1.7167204	Kurtosis	.
Uncorrected SS	77054	Corrected SS	254
Coeff Variation	7.04339229	Std Error Mean	6.5064071

Basic Statistical Measures			
Location		Variability	
Mean	160.0000	Std Deviation	11.26943
Median	166.0000	Variance	127.00000
Mode	.	Range	20.00000
		Interquartile Range	20.00000

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	24.59114	Pr > |t|	0.0016
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.787402	Pr < W	0.0848
Kolmogorov-Smirnov	D	0.369447	Pr > D	0.1017
Cramer-von Mises	W-Sq	0.080806	Pr > W-Sq	0.1374
Anderson-Darling	A-Sq	0.440311	Pr > A-Sq	0.0878

Quantiles (Definition 5)

Quantile	Estimate
100% Max	167
99%	167
95%	167
90%	167
75% Q3	167
50% Median	166
25% Q1	147
10%	147
5%	147
1%	147
0% Min	147

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
147	4	147	4
166	6	166	6
167	5	167	5

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ZM Density:
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: density (density)
treat = 100
time = 1

Moments			
N	3	Sum Weights	3
Mean	180.333333	Sum Observations	541
Std Deviation	12.7410099	Variance	162.333333
Skewness	1.6841605	Kurtosis	.
Uncorrected SS	97885	Corrected SS	324.666667
Coeff Variation	7.06525503	Std Error Mean	7.3560255

Basic Statistical Measures			
Location		Variability	
Mean	180.3333	Std Deviation	12.74101
Median	174.0000	Variance	162.33333
Mode	.	Range	23.00000
		Interquartile Range	23.00000

Tests for Location: Mu0=0				
Test		Statistic	p Value	
Student's t	t	24.51505	Pr > |t|	0.0017
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.814682	Pr < W	0.1501
Kolmogorov-Smirnov	D	0.357101	Pr > D	0.1327
Cramer-von Mises	W-Sq	0.07387	Pr > W-Sq	0.1783
Anderson-Darling	A-Sq	0.406399	Pr > A-Sq	0.1153

Quantiles (Definition 5)	

AEH-13-PSEUDO-05

Quantile	Estimate
100% Max	195
99%	195
95%	195
90%	195
75% Q3	195
50% Median	174
25% Q1	172
10%	172
5%	172
1%	172
0% Min	172

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
172	9	172	9
174	8	174	8
195	7	195	7

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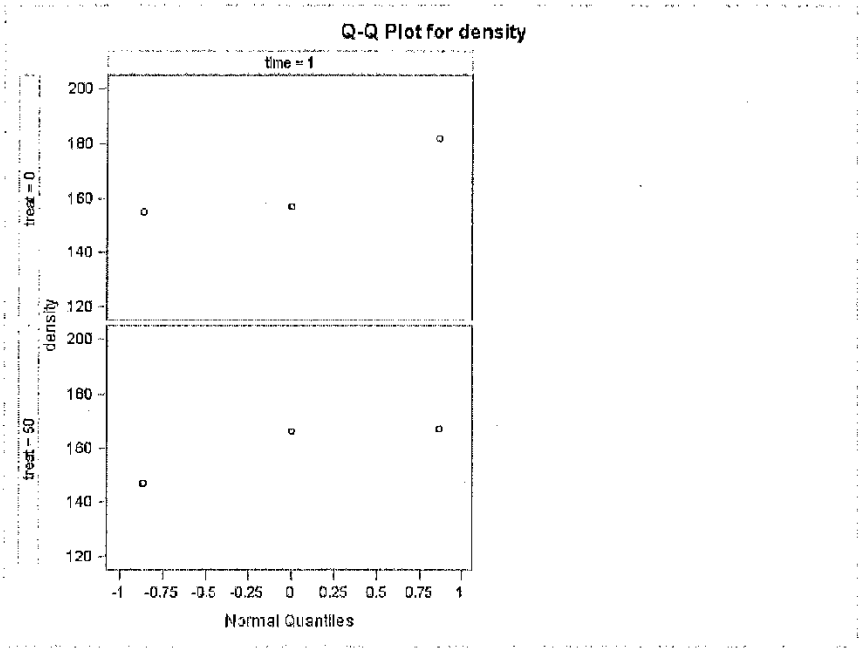
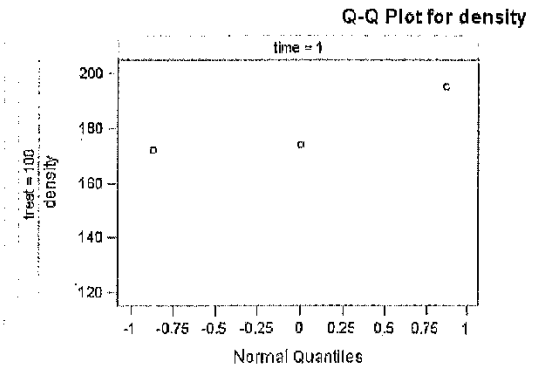
ZM Density:
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure





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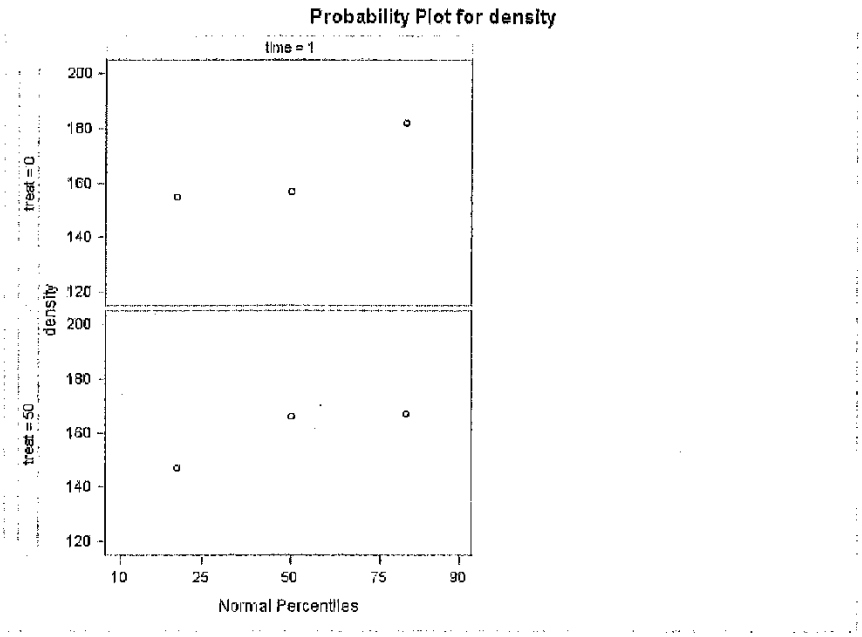
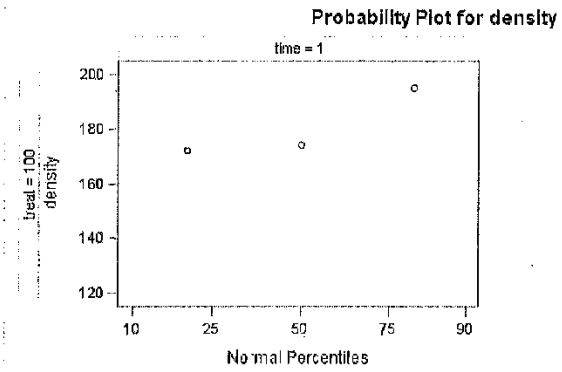
ZM Density:
Expressed as #ZM per unionId; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure



AEH-13-PSEUDO-05



Performed by K. Weber SAS version 9.3 08:43 25MAR14

Density: Bartletts test of homogeneity of variance
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Class Level Information		
Class	Levels	Values
time	2	1 2

Number of Observations Read	18
Number of Observations Used	18

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Density: Bartletts test of homogeneity of variance
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

KW 3/7/14

The GLM Procedure

Dependent Variable: density density

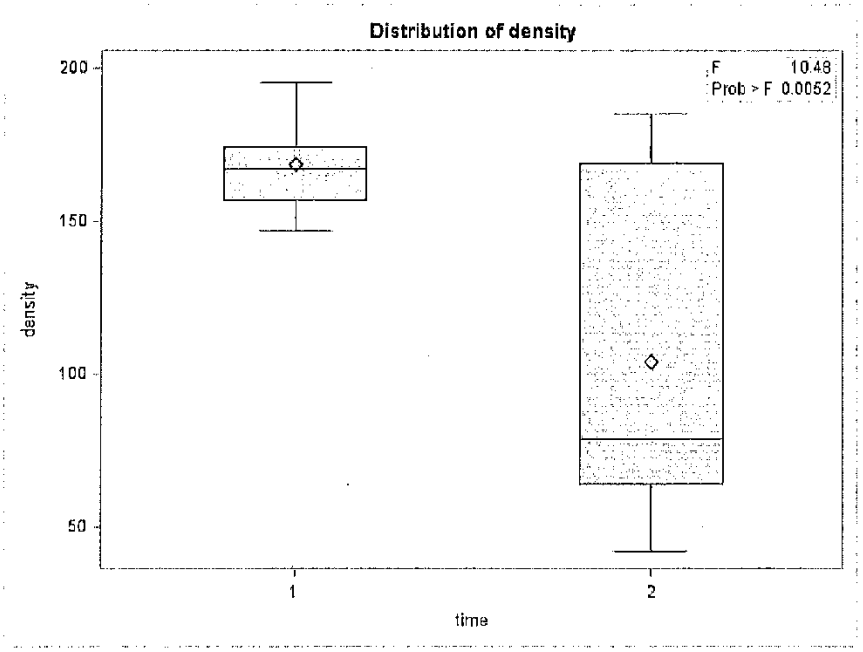
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	18624.50000	18624.50000	10.48	0.0052
Error	16	28446.00000	1777.87500		
Corrected Total	17	47070.50000			

R-Square	Coeff Var	Root MSE	density Mean
0.395672	30.96562	42.16486	136.1667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
time	1	18624.50000	18624.50000	10.48	0.0052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	18624.50000	18624.50000	10.48	0.0052

AEH-13-PSEUDO-05



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Density: Bartletts test of homogeneity of variance
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Bartlett's Test for Homogeneity of density Variance			
Source	DF	Chi-Square	Pr > ChiSq
time	1	11.1896	0.0008

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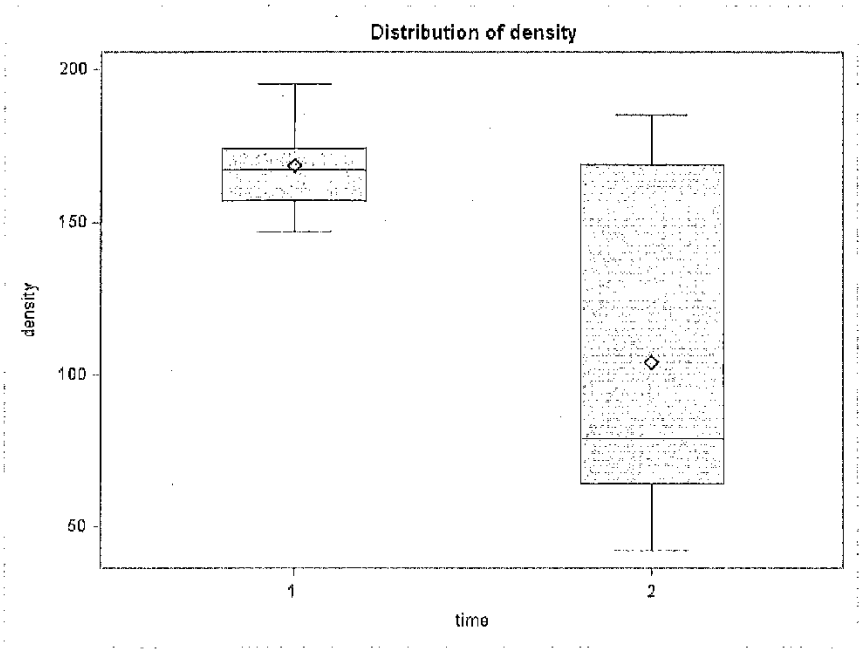
Density: Bartletts test of homogeneity of variance
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure



Level of time	N	density Mean	density Std Dev
1	9	168.333333	14.6287388
2	9	104.000000	57.8078714

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Zebra mussel densities pre- and post-exposure comparison
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Class Level Information		
Class	Levels	Values
enc	9	1 2 3 4 5 6 7 8 9
treat	3	0 50 100
time	2	1 2

Number of Observations Read	18
Number of Observations Used	18

Performed by K. Weber SAS version 9.3 08:43 25MAR14

Zebra mussel densities pre- and post-exposure comparison
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Dependent Variable: density density

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	378460.3333	63076.7222	321.46	<.0001
Error	12	2354.6667	196.2222		
Uncorrected Total	18	380815.0000			

R-Square	Coeff Var	Root MSE	density Mean
0.949976	10.28734	14.00793	136.1667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	3	345110.8333	115036.9444	586.26	<.0001
time	1	18624.5000	18624.5000	94.92	<.0001
treat*time	2	14725.0000	7362.5000	37.52	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	2	11366.33333	5683.16667	28.96	<.0001
time	1	18624.50000	18624.50000	94.92	<.0001
treat*time	2	14725.00000	7362.50000	37.52	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
treat 0	178.6666667	B	8.08748462	22.09	<.0001
treat 50	74.0000000	B	8.08748462	9.15	<.0001
treat 100	59.3333333	B	8.08748462	7.34	<.0001
time 1	121.0000000	B	11.43743043	10.58	<.0001
time 2	0.0000000	B	.	.	.
treat*time 0 1	-135.0000000	B	16.17496923	-8.35	<.0001
treat*time 0 2	0.0000000	B	.	.	.
treat*time 50 1	-35.0000000	B	16.17496923	-2.16	0.0514
treat*time 50 2	0.0000000	B	.	.	.
treat*time 100 1	0.0000000	B	.	.	.
treat*time 100 2	0.0000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Performed by K. Weber SAS version 9.3 08:43 25MAR14

Zebra mussel densities pre- and post-exposure comparison
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Observation	Observed	Predicted	Residual	95% Confidence Limits for Mean Predicted Value	
1	182.0000000	164.6666667	17.3333333	147.0455514	182.2877819
2	182.0000000	178.6666667	3.3333333	161.0455514	196.2877819
3	155.0000000	164.6666667	-9.6666667	147.0455514	182.2877819
4	169.0000000	178.6666667	-9.6666667	161.0455514	196.2877819
5	157.0000000	164.6666667	-7.6666667	147.0455514	182.2877819
6	185.0000000	178.6666667	6.3333333	161.0455514	196.2877819
7	147.0000000	160.0000000	-13.0000000	142.3788848	177.6211152
8	79.0000000	74.0000000	5.0000000	56.3788848	91.6211152
9	167.0000000	160.0000000	7.0000000	142.3788848	177.6211152
10	64.0000000	74.0000000	-10.0000000	56.3788848	91.6211152
11	166.0000000	160.0000000	6.0000000	142.3788848	177.6211152
12	79.0000000	74.0000000	5.0000000	56.3788848	91.6211152
13	195.0000000	180.3333333	14.6666667	162.7122181	197.9544486
14	85.0000000	59.3333333	25.6666667	41.7122181	76.9544486
15	174.0000000	180.3333333	-6.3333333	162.7122181	197.9544486
16	42.0000000	59.3333333	-17.3333333	41.7122181	76.9544486
17	172.0000000	180.3333333	-8.3333333	162.7122181	197.9544486
18	51.0000000	59.3333333	-8.3333333	41.7122181	76.9544486

Sum of Residuals	-0.000000
Sum of Squared Residuals	2354.666667
Sum of Squared Residuals - Error SS	-0.000000
PRESS Statistic	5298.000000
First Order Autocorrelation	0.230653
Durbin-Watson D	1.381606

Performed by K. Weber SAS version 9.3 08:43 25MAR14

Zebra mussel densities pre- and post-exposure comparison
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	time	density LSMEAN	LSMEAN Number
0	1	164.666667	1
0	2	178.666667	2
50	1	160.000000	3
50	2	74.000000	4
100	1	180.333333	5
100	2	59.333333	6

Least Squares Means for effect treat*time
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: density

i/j	1	2	3	4	5	6
1		0.8175	0.9982	<.0001	0.7432	<.0001
2	0.8175		0.5950	<.0001	1.0000	<.0001
3	0.9982	0.5950		<.0001	0.5124	<.0001
4	<.0001	<.0001	<.0001		<.0001	0.7889
5	0.7432	1.0000	0.5124	<.0001		<.0001
6	<.0001	<.0001	<.0001	0.7889	<.0001	

treat	time	density LSMEAN	95% Confidence Limits	
0	1	164.666667	147.045551	182.287782
0	2	178.666667	161.045551	196.287782
50	1	160.000000	142.378885	177.621115
50	2	74.000000	56.378885	91.621115
100	1	180.333333	162.712218	197.954449
100	2	59.333333	41.712218	76.954449

Least Squares Means for Effect treat*time

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-14.000000	-52.417204	24.417204

1	3	4.666667	-33.750537	43.083870
1	4	90.666667	52.249463	129.083870
1	5	-15.666667	-54.083870	22.750537
1	6	105.333333	66.916130	143.750537
2	3	18.666667	-19.750537	57.083870
2	4	104.666667	66.249463	143.083870
2	5	-1.666667	-40.083870	36.750537
2	6	119.333333	80.916130	157.750537
3	4	86.000000	47.582796	124.417204
3	5	-20.333333	-58.750537	18.083870
3	6	100.666667	62.249463	139.083870
4	5	-106.333333	-144.750537	-67.916130
4	6	14.666667	-23.750537	53.083870
5	6	121.000000	82.582796	159.417204

Performed by K. Weber SAS version 9.3 08:43 25MAR14

Mean zebra mussel densities pre- and post-exposure by treatment level
Expressed as #ZM per unionid; pre- and post-exposure
Mean Density on all unionids/test enclosure/sampling event
Study Number AEH-13-PSEUDO-05

The MEANS Procedure

treat=0

Analysis Variable : density density					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	164.7	15.0444	127.3	202.0
2	3	176.7	8.5049	157.5	199.8

treat=50

Analysis Variable : density density					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	160.0	11.2694	132.0	188.0
2	3	74.0000	8.6603	52.4867	95.5133

treat=100

Analysis Variable : density density					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	180.3	12.7410	148.7	212.0
2	3	59.3333	22.6789	2.9958	115.7

Performed by K. Weber SAS version 9.3 08:43 25MAR14

File Folder: 16

Item Number: 4

```
ods html close; /* close previous */;
ods html; /* open new */;

DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
title2 h=2 'for controlling zebra mussels within field enclosures';
title3 h=2 'Zebra Mussel Colonization Changes';
title4 h=2 'SAS v. 9.3 Analysis completion date:  25MAR14  Analysis prepared b

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  KLW kw     Page 1 of 2
* Analysis completion date:  25MAR2014
*****************************************************************************/

/*****************************************************************************
* Variable Names:
* enc = test enclosure ID (1 through 9)
* treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 mg/L
* time = sample time; 1 = pre-exposure; 2 = post-exposure
* denisty= density of live zebra mussels on the unionid (# ZM/unionid)
*****************************************************************************/

data mussel; set Pseudo05.Density_mean_num;
run;
proc sort;
by treat enc; run;
proc print; run;

data mussel2; set mussel;
if time = 2 then delete;
proc univariate data=mussel2 normal notabcontents;
title1 h=2 'ZM Density: ';
title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';

class treat time;
var density;
probplot density;
qqplot density;
run;
```

FF # 16
Item No. 3
Pg 1 of 2

```
/*************************************************************************
*  This analysis determines the variability among treatments             *
*************************************************************************/
ods graphics on;
proc glm data= mussel;
title1 h=2  Density: Bartletts test of homogeneity of variance';
title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
title3 h=2 'Mean Dersity on all unionids/test enclosure/sampling event';
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
class time;
model density=time;
means time / HOVTEST=BARTLETT; run;

ods graphics off;
/*************************************************************************
*  This analysis compares the test enclosure densities by treatment and time
*************************************************************************/
title1 h=2 'Zebra mussel densities pre- and post-exposure comparison";
title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
proc glm data = mussel;
class enc treat time;
model density = treat|time / noint solution cli clm;
  lsmeans treat*time / pdiff=all cl;
  run;
  quit;

/*************************************************************************
* This analysis determines the densities by treatment level pre- and post-expo
*************************************************************************/
title1 h=2 'Mean zebra mussel densities pre- and post-exposure by treatment le
title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
proc means data = mussel mean std lclm uclm fw=8;
by treat;
class time;
var density;
run;
```

FF # 16
Item No. 5
Pg 2 of 2

```
3
4    DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
5
6    FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE
6  ! ;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.
7
8    options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
9
10   title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
11   title2 h=2 'for controlling zebra mussels within field enclosures';
12   title3 h=2 'Zebra Mussel Colonization Changes';
13   title4 h=2 'SAS v. 9.3 Analysis completion date:  25MAR14  Analysis prepa
14
15   /*************************************************************************
16   * SAS ver 9.3       Analysis prepared by:  KLW        Page 1 of 4
17   * Analysis completion date:  25MAR2014       KW
18   *************************************************************************
19
20   /*************************************************************************
20 ! *************
21   * Variable Names:
21 !             *
22   * enc = test enclosure ID (1 through 9)
22 !             *
23   * treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 m
23 !             *
24   * time = sample time; 1 = pre-exposure; 2 = post-exposure
24 !             *
25   * denisty= density of live zebra mussels on the unionid (# ZM/unionid)
25 !             *
26   *************************************************************************
26 ! ************/
27
28   data mussel; set Pseudo05.Density_mean_num;
29   run;

NOTE: There were 18 observations read from the data set PSEUDO05.DENSITY_MEAN_
NOTE: The data set WORK.MUSSEL has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


30   proc sort;
```

FF # 16
Item No. 6
Pg 1 of 4

```
31   by treat enc; run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


32   proc print; run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.08 seconds
      cpu time            0.03 seconds


33
34   data mussel2; set mussel;
35   if time = 2 then delete;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds


36   proc univariate data=mussel2 normal notabcontents;
37   title1 h=2 'ZM Density: ';
38   title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure ;
39   title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
40   title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
41
42   class treat time;
43   var density;
44   probplot density;
45   qqplot density;
46   run;

NOTE: PROCEDURE UNIVARIATE used (Total process time):
      real time           4.25 seconds
      cpu time            0.46 seconds

47
```

```
48
49
50   /*************************************************************************
51   *  This analysis determines the variability among treatments
52   *************************************************************************
53   ods graphics on;
54   proc glm data= mussel;
55   title1 h=2 'Density: Bartletts test of homogeneity of variance';
56   title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure ';
57   title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
58   title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
59   class time;
60   model density=time;
61   means time / HOVTEST=BARTLETT; run;

62
63   ods graphics off;
64   /*************************************************************************
65   *  This analysis compares the test enclosure densities by treatment and t
66   *************************************************************************
67   title1 h=2 "Zebra mussel densities pre- and post-exposure comparison";
68   title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
69   title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
70   title4 h=1.5 'Study Number AEH-13-PSEUDO-05';

NOTE: PROCEDURE GLM used (Total process time):
      real time           0.85 seconds
      cpu time            0.26 seconds


71   proc glm data = mussel;
72   class enc treat time;
73   model density = treat|time / noint solution cli clm;
74     lsmeans treat*time / pdiff=all cl;
75     run;

NOTE: Due to the presence of CLASS variables, an intercept is implicitly fitte
      been corrected for the mean.
76     quit;

NOTE: PROCEDURE GLM used (Total process time):
      real time           0.33 seconds
      cpu time            0.17 seconds
```



```
77
```

```
78   /*************************************************************************
79   * This analysis determines the densities by treatment level pre- and post
80   *************************************************************************
81   title1 h=2 'Mean zebra mussel densities pre- and post-exposure by treatme
82   title2 h=2 'Expressed as #ZM per unionid; pre- and post-exposure';
83   title3 h=2 'Mean Density on all unionids/test enclosure/sampling event';
84   title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
85   proc means data = mussel mean std lclm uclm fw=8;
86   by treat;
87   class time;
88   var density;
89   run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.09 seconds
      cpu time            0.04 seconds
```

FF # 16
Item No. 6
Pg 4 of 4

AEH-13-PSEUDO-05

Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling zebra mussels within field enclosures
Zebra Mussel Length Analysis
Study Number AEH-13-PSEUDO-05
SAS v. 9.3 Analysis completion date: 18MAR14 Analysis prepared by: KLW

Obs	enc	treat	time	length
1	3	0	1	12.0407
2	3	0	2	12.5773
3	7	0	1	11.9452
4	7	0	2	12.4593
5	8	0	1	12.1144
6	8	0	2	8.6073
7	2	50	1	12.6307
8	2	50	2	11.6271
9	4	50	1	11.6470
10	4	50	2	11.3896
11	5	50	1	11.8969
12	5	50	2	12.2949
13	1	100	1	11.3217
14	1	100	2	12.3281
15	6	100	1	11.5253
16	6	100	2	11.4374
17	9	100	1	10.8151
18	9	100	2	12.0955

Performed by K. Weber SAS version 9.3 14:27 18MAR14 KW

File Folder: 16

Item Number: 7

Length of Live Zebra Mussels in Each Treatment
Means of Lengths measured from samples collected from all unionid mussels/test enclosure
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: length (length)
treat = 0
time = 1

Moments			
N	3	Sum Weights	3
Mean	12.0334094	Sum Observations	36.1002281
Std Deviation	0.08483153	Variance	0.00719639
Skewness	-0.3819681	Kurtosis	
Uncorrected SS	434.423216	Corrected SS	0.01439278
Coeff Variation	0.70496672	Std Error Mean	0.04897751

Basic Statistical Measures			
Location		Variability	
Mean	12.03341	Std Deviation	0.08483
Median	12.04066	Variance	0.00720
Mode	.	Range	0.16920
		Interquartile Range	0.16920

Tests for Location: Mu0=0				
Test		Statistic	p Value	
Student's t	t	245.6926	Pr > |t|	<.0001
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.994516	Pr < W	0.8584
Kolmogorov-Smirnov	D	0.200737	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.029255	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.195608	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

AEH-13-PSEUDO-05

Quantile	Estimate
100% Max	12.1144
99%	12.1144
95%	12.1144
90%	12.1144
75% Q3	12.1144
50% Median	12.0407
25% Q1	11.9452
10%	11.9452
5%	11.9452
1%	11.9452
0% Min	11.9452

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
11.9452	2	11.9452	2
12.0407	1	12.0407	1
12.1144	3	12.1144	3

Performed by K. Weber SAS version 9.3 14:27 18MAR14

AEH-13-PSEUDO-05

KLW 13MAR2014

Length of Live Zebra Mussels in Each Treatment

Means of Lengths measured from samples collected from all unionid mussels/test enclosure

Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: length (length)
treat = 50
time = 1

Moments			
N	3	Sum Weights	3
Mean	12.0581995	Sum Observations	36.1745986
Std Deviation	0.51129389	Variance	0.26142144
Skewness	1.27819581	Kurtosis	.
Uncorrected SS	436.723371	Corrected SS	0.52284288
Coeff Variation	4.24021753	Std Error Mean	0.29519566

Basic Statistical Measures			
Location		Variability	
Mean	12.05820	Std Deviation	0.51129
Median	11.89692	Variance	0.26142
Mode	.	Range	0.98370
		Interquartile Range	0.98370

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	40.84816	Pr > |t|	0.0006
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.92538	Pr < W	0.4716
Kolmogorov-Smirnov	D	0.290448	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.046274	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.27423	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

AEH-13-PSEUDO-05

Quantile	Estimate
100% Max	12.6307
99%	12.6307
95%	12.6307
90%	12.6307
75% Q3	12.6307
50% Median	11.8969
25% Q1	11.6470
10%	11.6470
5%	11.6470
1%	11.6470
0% Min	11.6470

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
11.6470	5	11.6470	5
11.8969	6	11.8969	6
12.6307	4	12.6307	4

Performed by K. Weber SAS version 9.3 14:27 18MAR14

KW 16MAR2014

Length of Live Zebra Mussels in Each Treatment

Means of Lengths measured from samples collected from all unionid mussels/test enclosure

Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure
Variable: length (length)
treat = 100
time = 1

Moments			
N	3	Sum Weights	3
Mean	11.2207028	Sum Observations	33.6621083
Std Deviation	0.36567833	Variance	0.13372064
Skewness	-1.1482579	Kurtosis	.
Uncorrected SS	377.979953	Corrected SS	0.26744128
Coeff Variation	3.25896103	Std Error Mean	0.21112448

Basic Statistical Measures			
Location		Variability	
Mean	11.22070	Std Deviation	0.36568
Median	11.32172	Variance	0.13372
Mode	.	Range	0.71012
		Interquartile Range	0.71012

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	53.14733	Pr > |t|	0.0004
Sign	M	1.5	Pr >= |M|	0.2500
Signed Rank	S	3	Pr >= |S|	0.2500

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.942764	Pr < W	0.5386
Kolmogorov-Smirnov	D	0.275489	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.041989	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.254197	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

AEH-13-PSEUDO-05

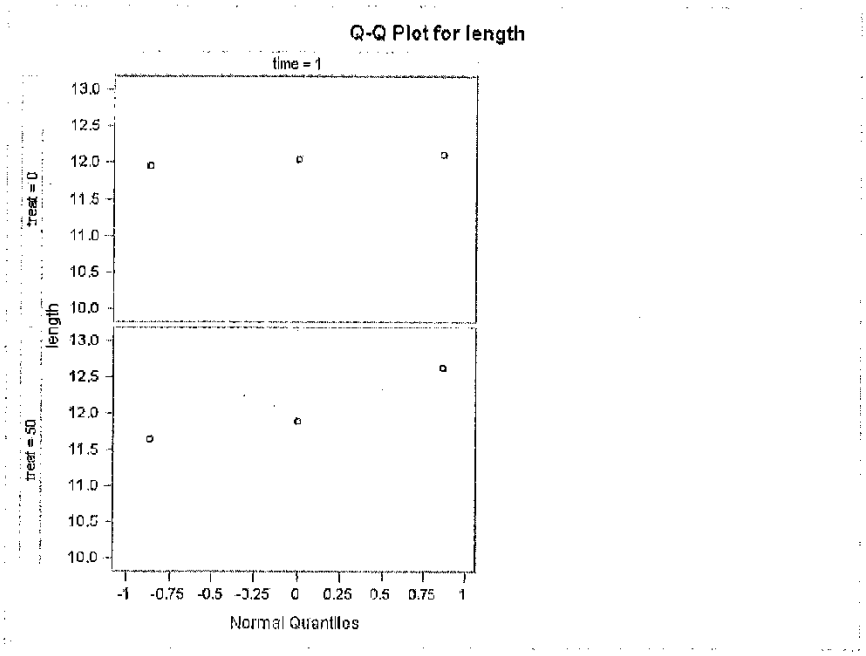
Quantile	Estimate
100% Max	11.5253
99%	11.5253
95%	11.5253
90%	11.5253
75% Q3	11.5253
50% Median	11.3217
25% Q1	10.8151
10%	10.8151
5%	10.8151
1%	10.8151
0% Min	10.8151

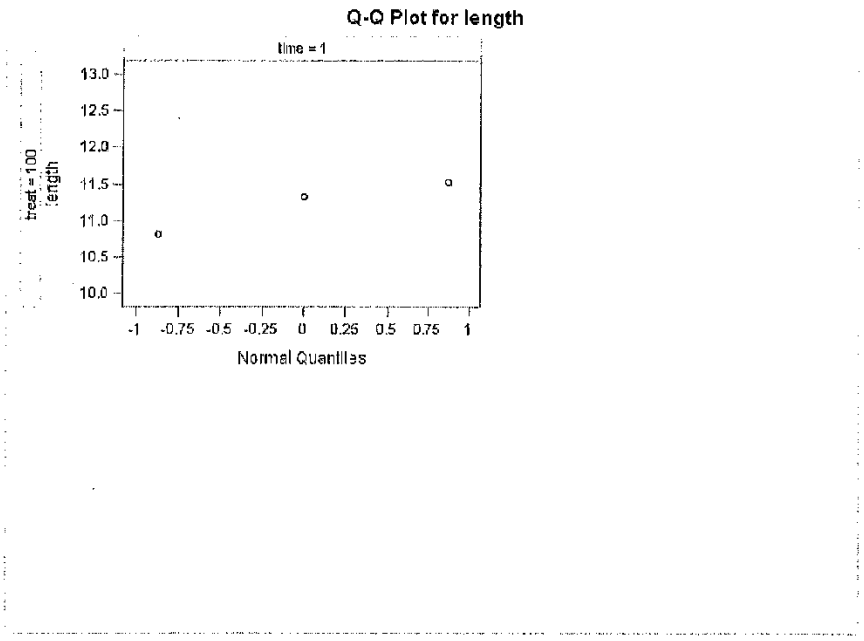
Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
10.8151	9	10.8151	9
11.3217	7	11.3217	7
11.5253	8	11.5253	8

Performed by K. Weber SAS version 9.3 14:27 18MAR14

Length of Live Zebra Mussels in Each Treatment
Means of Lengths measured from samples collected from all unionid mussels/test enclosure
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure

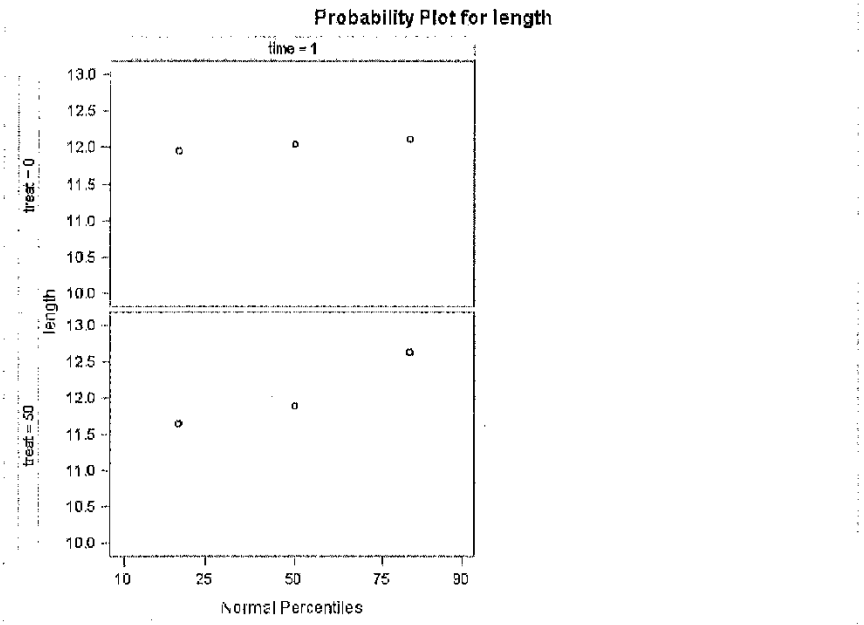




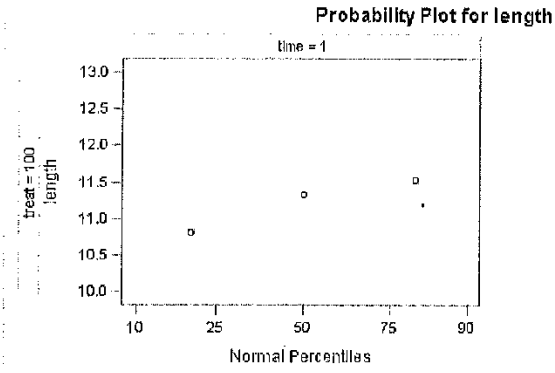
Performed by K. Weber SAS version 9.3 14:27 18MAR14

Length of Live Zebra Mussels in Each Treatment
Means of Lengths measured from samples collected from all unionid mussels/test enclosure
Study Number AEH-13-PSEUDO-05

The UNIVARIATE Procedure



AEH-13-PSEUDO-05



Performed by K. Weber SAS version 9.3 14:27 18MAR14

ZM Length:Bartletts test of homogeneity of variance
Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Class Level Information		
Class	Levels	Values
time	2	1 2

Number of Observations Read	18
Number of Observations Used	18

Performed by K. Weber SAS version 9.3 14:27 18MAR14

AEH-13-PSEUDO-05

ZM Length:Bartletts test of homogeneity of variance
Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Dependent Variable: length length

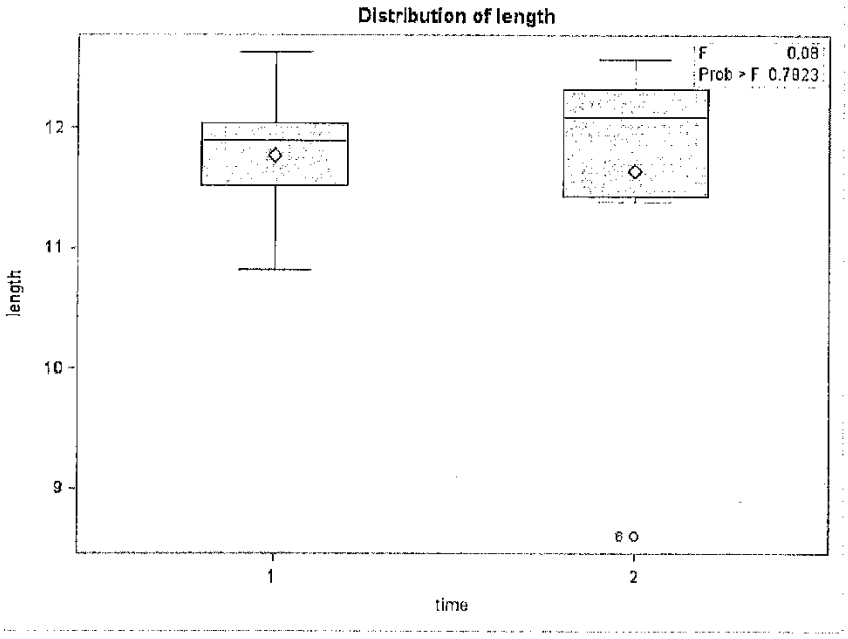
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.06973195	0.06973195	0.08	0.7823
Error	16	14.12736600	0.88296037		
Corrected Total	17	14.19709794			

R-Square	Coeff Var	Root MSE	length Mean
0.004912	8.025429	0.939660	11.70853

Source	DF	Type I SS	Mean Square	F Value	Pr > F
time	1	0.06973195	0.06973195	0.08	0.7823

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.06973195	0.06973195	0.08	0.7823

AEH-13-PSEUDO-05



Performed by K. Weber SAS version 9.3 14:27 18MAR14

ZM Length:Bartletts test of homogeneity of variance
Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

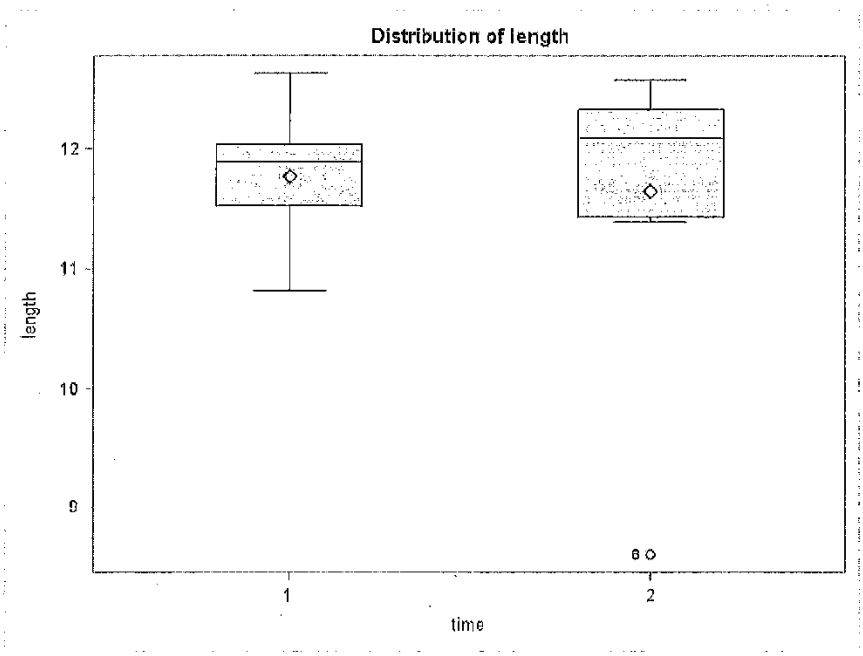
The GLM Procedure

Bartlett's Test for Homogeneity of length Variance			
Source	DF	Chi-Square	Pr > ChiSq
time	1	4.9312	0.0264

Performed by K. Weber SAS version 9.3 14:27 18MAR14

ZM Length:Bartletts test of homogeneity of variance
Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure



Level of time	N	length Mean	length Std Dev
1	9	11.7707706	0.52047867
2	9	11.6462877	1.22271121

Performed by K. Weber SAS version 9.3 14:27 18MAR14

AEH-13-PSEUDO-05

Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Class Level Information		
Class	Levels	Values
enc	9	1 2 3 4 5 6 7 8 9
treat	3	0 50 100
time	2	1 2

Number of Observations Read	18
Number of Observations Used	18

Performed by K. Weber SAS version 9.3 14:27 18MAR14

AEH-13-PSEUDO-05

3/18/2014

Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Dependent Variable: length length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2469.934665	411.655778	415.95	<.0001
Error	12	11.876204	0.989684		
Uncorrected Total	18	2481.810870			

R-Square	Coeff Var	Root MSE	length Mean
0.163477	8.496614	0.994828	11.70853

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	3	2467.999186	822.666395	831.24	<.0001
time	1	0.069732	0.069732	0.07	0.7952
treat*time	2	1.865748	0.932874	0.94	0.4167

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	2	0.38541418	0.19270709	0.19	0.8256
time	1	0.06973195	0.06973195	0.07	0.7952
treat*time	2	1.86574762	0.93287381	0.94	0.4167

Parameter	Estimate		Standard Error	t Value	Pr > |t|
treat 0	11.21466092	B	0.57436448	19.53	<.0001
treat 50	11.77054328	B	0.57436448	20.49	<.0001
treat 100	11.95365881	B	0.57436448	20.81	<.0001
time 1	-0.73295605	B	0.81227404	-0.90	0.3846
time 2	0.00000000	B	.	.	.
treat*time 0 1	1.55170450	B	1.14872897	1.35	0.2017
treat*time 0 2	0.00000000	B	.	.	.
treat*time 50 1	1.02061229	B	1.14872897	0.89	0.3917
treat*time 50 2	0.00000000	B	.	.	.
treat*time 100 1	0.00000000	B	.	.	.
treat*time 100 2	0.00000000	B	.	.	.

AEH-13-PSEUDO-05

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Performed by K. Weber SAS version 9.3 14:27 18MAR14

AEH-13-PSEUDO-05

Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure

Observation	Observed	Predicted	Residual	95% Confidence Limits for Mean Predicted Value	
1	12.04066306	12.03340937	0.00725369	10.78197666	13.28484208
2	12.57734200	11.21466092	1.36268108	9.96322821	12.46609363
3	11.94518391	12.03340937	-0.08822547	10.78197666	13.28484208
4	12.45929985	11.21466092	1.24463893	9.96322821	12.46609363
5	12.11438115	12.03340937	0.08097178	10.78197666	13.28484208
6	8.60734090	11.21466092	-2.60732002	9.96322821	12.46609363
7	12.63068496	12.05819953	0.57248543	10.80676682	13.30963224
8	11.62714409	11.77054328	-0.14339919	10.51911057	13.02197599
9	11.64698975	12.05819953	-0.41120977	10.80676682	13.30963224
10	11.38962384	11.77054328	-0.38091944	10.51911057	13.02197599
11	11.89692387	12.05819953	-0.16127566	10.80676682	13.30963224
12	12.29486191	11.77054328	0.52431863	10.51911057	13.02197599
13	11.32172148	11.22070276	0.10101872	9.96927006	12.47213547
14	12.32808047	11.95365881	0.37442166	10.70222610	13.20509152
15	11.52525263	11.22070276	0.30454987	9.96927006	12.47213547
16	11.43741649	11.95365881	-0.51624232	10.70222610	13.20509152
17	10.81513418	11.22070276	-0.40556859	9.96927006	12.47213547
18	12.09547947	11.95365881	0.14182066	10.70222610	13.20509152

Sum of Residuals	0.00000000
Sum of Squared Residuals	11.87620420
Sum of Squared Residuals - Error SS	-0.00000000
PRESS Statistic	26.72145945
First Order Autocorrelation	-0.12742312
Durbin-Watson D	2.25314825

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Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	time	length LSMEAN	LSMEAN Number
0	1	12.0334094	1
0	2	11.2146609	2
50	1	12.0581995	3
50	2	11.7705433	4
100	1	11.2207028	5
100	2	11.9536588	6

Least Squares Means for effect treat*time
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: length

i/j	1	2	3	4	5	6
1		0.9066	1.0000	0.9994	0.9091	1.0000
2	0.9066		0.8958	0.9804	1.0000	0.9366
3	1.0000	0.8958		0.9991	0.8985	1.0000
4	0.9994	0.9804	0.9991		0.9813	0.9999
5	0.9091	1.0000	0.8985	0.9813		0.9386
6	1.0000	0.9366	1.0000	0.9999	0.9386	

treat	time	length LSMEAN	95% Confidence Limits	
0	1	12.033409	10.781977	13.284842
0	2	11.214661	9.963228	12.466094
50	1	12.058200	10.806767	13.309632
50	2	11.770543	10.519111	13.021976
100	1	11.220703	9.969270	12.472135
100	2	11.953659	10.702226	13.205092

Least Squares Means for Effect treat*time

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	0.818748	-1.909600	3.547097

1	3	-0.024790	-2.753139	2.703558
1	4	0.262866	-2.465483	2.991215
1	5	0.812707	-1.916642	3.541055
1	6	0.079751	-2.648598	2.808099
2	3	-0.843539	-3.571887	1.884810
2	4	-0.555882	-3.284231	2.172466
2	5	-0.006042	-2.734390	2.722307
2	6	-0.738998	-3.467346	1.989351
3	4	0.287656	-2.440692	3.016005
3	5	0.837497	-1.890852	3.565845
3	6	0.104541	-2.623808	2.832889
4	5	0.549841	-2.178508	3.278189
4	6	-0.183116	-2.911464	2.545233
5	6	-0.732956	-3.461305	1.995393

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Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure
Grand Means of Lengths measured from samples collected from all unionid mussels/test enclosure/sample time
Study Number AEH-13-PSEUDO-05

The MEANS Procedure

treat=0

Analysis Variable : length length					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	12.0334	0.0848	11.8227	12.2441
2	3	11.2147	2.2588	5.6035	16.8258

treat=50

Analysis Variable : length length					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	12.0582	0.5113	10.7881	13.3283
2	3	11.7705	0.4693	10.6046	12.9365

treat=100

Analysis Variable : length length					
time	N Obs	Mean	Std Dev	Lower 95% CL for Mean	Upper 95% CL for Mean
1	3	11.2207	0.3657	10.3123	12.1291
2	3	11.9537	0.4620	10.8061	13.1012

Performed by K. Weber SAS version 9.3 14:27 18MAR14

File Folder: 16

Item Number: 7

```
ods html close; /* close previous */;
ods html; /* open new */;

DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
title2 h=2 'for controlling zebra mussels within field enclosures';
title3 h=2 'Zebra Mussel Length Analysis';
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
title5 h=1 'SAS v. 9.3  Analysis completion date:  18MAR14  Analysis prepared

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  KLW      Page 1 of 2
* Analysis completion date:  18MAR14
*****************************************************************************/

/*****************************************************************************
* Variable Names:
* enc = enclosure ID (1 through 9)
* treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 mg/L
* time = sample time; 1 = pre-exposure; 2 = post-exposure
* length= mean length of live zebra mussels per enclosure (grand mean from all
*              collecteded from all unionid mussels in each enclosure per sam
*****************************************************************************/

data mussel; set Pseudo05.ZM_lengths;
run;
proc sort;
by treat enc; run;
proc print; run;

data mussel2; set mussel;
if time = 2 then delete;
proc univariate data=mussel2 normal notabcontents;
title1 'Length of Live Zebra Mussels in Each Treatment';
title2 'Means of Lengths measured from samples collected from all unionid muss
title3 h=1.5 'Study Number AEH-13-PSEUDO-05';

class treat time;
var length;
probplot length;
qqplot length;
```

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```
run;


/*************************************************************************
*  This analysis determines the variability among treatments             *
*************************************************************************/
ods graphics on;
proc glm data= mussel;
title1 'ZM Length:Bartletts test of homogeneity of variance';
title2 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure'
title3 'Means of Lengths measured from samples collected from all unionid muss
title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
class time;
model length = time;
means time / HOVTEST=BARTLETT; run;

ods graphics off;


/*************************************************************************
*  This analysis compares the zebra mussel lenghts among treatments pre- and p
*************************************************************************/
title1 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure'
title2 'Means of Lengths measured from samples collected from all unionid muss
title3 h=1.5 'Study Number AEH-13-PSEUDO-05';
proc glm data = mussel;
class enc treat time;
model length = treat|time / noint solution cli clm;
  lsmeans treat*time / pdiff=all cl;
  run;
  quit;


/*************************************************************************
*  This analysis determines the grand mean zebra mussel lengths among treatmer
*************************************************************************/
title1 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-exposure'
title2 'Grand Means of Lengths measured from samples collected from all unioni
title3 h=1.5 'Study Number AEH-13-PSEUDO-05';
proc means data = mussel mean std lclm uclm fw=8;
by treat;
class time;
var length;
run;
```

FF # 16
Item No. 8
Pg 2 of 2

```
3
4    DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPU";
5
6    FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE
6  ! ;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.
7
8    options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
9
10   title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP';
11   title2 h=2 'for controlling zebra mussels within field enclosures';
12   title3 h=2 'Zebra Mussel Length Analysis';
13   title4 h=1.6 'Study Number AEH-13-PSEUDO-05';
14   title5 h=1 'SAS v. 9.3  Analysis completion date:  18MAR14  Analysis prep
15
16   /*************************************************************************
17   * SAS ver 9.3       Analysis prepared by:  KLW        Page 1 of 4
18   * Analysis completion date:  18MAR14
19   *************************************************************************
20
21   /*************************************************************************
21 ! *************
22   * Variable Names:
22 !              *
23   * enc = enclosure ID (1 through 9)
23 !              *
24   * treat= treatment level; 0 = Control [0 mg/L], 50 = 50 mg/L, 100 = 100 m
24 !              *
25   * time = sample time; 1 = pre-exposure; 2 = post-exposure
25 !         /    *
26   * length= mean length of live zebra mussels per enclosure (grand mean fro
26 ! mussels          *
27   *               collecteded from all unionid mussels in each enclosure pe
27 !              *
28   *************************************************************************
28 ! ************/
29
30   data mussel; set Pseudo05.ZM_lengths;
31   run;

NOTE: There were 18 observations read from the data set PSEUDO05.ZM_LENGTHS.
NOTE: The data set WORK.MUSSEL has 18 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.01 seconds
```

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Item No. 4
Pg 1 of 4

```
32   proc sort;
33   by treat enc; run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL has 18 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


34   proc print; run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.07 seconds
      cpu time            0.01 seconds


35
36   data mussel2; set mussel;
37   if time = 2 then delete;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


38   proc univariate data=mussel2 normal notabcontents;
39   title1 'Length of Live Zebra Mussels in Each Treatment';
40   title2 'Means of Lengths measured from samples collected from all unionic
40 ! enclosure';
41   title3 h=1.5 'Study Number AEH-13-PSEUDO-05';
42
43   class treat time;
44   var length;
45   probplot length;
46   qqplot length;
47   run;

NOTE: PROCEDURE UNIVARIATE used (Total process time):
      real time           4.43 seconds
      cpu time            0.51 seconds
```

```
48
49
50
51   /*************************************************************************
52   *  This analysis determines the variability among treatments
53   *************************************************************************
54   ods graphics on;
55   proc glm data= mussel;
56   title1 'ZM Length:Bartletts test of homogeneity of variance';
57   title2 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-expo
58   title3 'Means of Lengths measured from samples collected from all unionic
58 ! enclosure/sample time';
59   title4 h=1.5 'Study Number AEH-13-PSEUDO-05';
60   class time;
61   model length = time;
62   means time / HOVTEST=BARTLETT; run;

63
64   ods graphics off;
65
66
67
68   /*************************************************************************
68 ! **
69   *  This analysis compares the zebra mussel lenghts among treatments pre-
69 !  *
70   *************************************************************************
70 ! */
71   title1 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-expo
72   title2 'Means of Lengths measured from samples collected from all unionic
72 ! enclosure/sample time';
73   title3 h=1.5 'Study Number AEH-13-PSEUDO-05';

NOTE: PROCEDURE GLM used (Total process time):
      real time           0.84 seconds
      cpu time            0.21 seconds


74   proc glm data = mussel;
75   class enc treat time;
76   model length = treat|time / noint solution cli clm;
77     lsmeans treat*time / pdiff-all cl;
78     run;
```

```
NOTE: Due to the presence of CLASS variables, an intercept is implicitly fitte
      been corrected for the mean.
79     quit;

NOTE: PROCEDURE GLM used (Total process time):
      real time           0.34 seconds
      cpu time            0.23 seconds


80
81
82   /*************************************************************************
82 ! **************
83   *  This analysis determines the grand mean zebra mussel lengths among tre
83 ! post-exposure   *
84   *************************************************************************
84 ! **************/
85   title1 'Length of Live Zebra Mussels in Each Treatment Pre- and Post-expo
86   title2 'Grand Means of Lengths measured from samples collected from all t
86 !  enclosure/sample time';
87   title3 h=1.5 'Study Number AEH-13-PSEUDO-05';
88   proc means data = mussel mean std lclm uclm fw=8;
89   by treat;
90   class time;
91   var length;
92   run;

NOTE: There were 18 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.01 seconds
```

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