# APPENDIX A

# DATABASE OF SEDIMENT CHEMISTRY DATA

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RECEIVED
JAN 21 1997
MPCA
Water Quality Div.

January 16, 1997

Judy L.Crane, Ph. D.
Minnesota Pollution Control Agency
Water Quality Division
520 Lafayette Rd. N.
St. Paul, MN 55155-4194

SUBJECT: Electronic data for 1993 Mudpuppy sampling - Duluth/Superior Harbor

Dear Ms. Crane: Judy

Please find enclosed a diskette with data from the 1993 Mudpuppy project. The data has been formatted in MS Excel according to the GLNPO data reporting format, provided to you last August. All the files, with the exception of one, adhere to this format. The one exception is the station file (dsstatn.xls), which follows the Station Reporting Standard, a hard copy of which I have enclosed.

The Mudpuppy data contains three types of files. The station file (dsstatn.xls) contains station descriptions and location information. The field file (dsfield.xls) contains detailed sample information, and all the remaining files contain analytical results. Each result file represents a different analytical method (e.g., dspcb.xls and dspcbimm.xls contain PCB and PCB/immunoassay data, respectively).

Files containing the lists of allowable codes for the Station Reporting Standard are contained on a second diskette. Each file contains codes for a single column within the Station Reporting Standard.

A list containing short descriptions of file contents is enclosed. If you have any questions or comments, please call me at (312) 353-3565.

Sincerely,

Brian Stage

Brian Stage

Enclosures

cc: Callie Bolattino (letter only)

| File Name | Description |
|---|---|

## 93 Mudpuppy files

**Note: 'ds' prefix stands for Duluth/Superior**

| File Name | Description |
|---|---|
| dsdiox&f.xls | dioxin & furan |
| dsfield.xls | field file |
| dsmetals.xls | metals other than As and Hg |
| dsmetas.xls | arsenic (As) |
| dsmethg.xls | mercury (Hg) |
| dsmetxrf.xls | metals by x-ray fluorescence |
| dsnh3.xls | ammonia |
| dspahall.xls | PAH's |
| dspahflr.xls | PAH, by fluorescence |
| dspcb.xls | PCB's |
| dspcbimm.xls | PCB's by immunoassay |
| dspest.xls | pesticides |
| dsstatn.xls | station file |
| dstoc.xls | TOC |

## Station Reporting Standard files
**(for use with dsstatn.xls)**

| File Name | Description |
|---|---|
| alp_type.xls | absolute location point type |
| country.xls | country |
| county.xls | FIPS county |
| datum_h.xls | geopositioning horizontal datum |
| datum_v.xls | geopositioning vertical datum |
| dist_shr.xls | distance to shore |
| huc.xls | FIPS hydrologic unit code |
| native.xls | native american lands |
| poll_rel.xls | pollutant spatial relation |
| poll.src.xls | pollutant source |
| reln.shr.xls | relation to shore |
| stn_shap.xls | station shape |
| stn_typ.xls | station type |

# *STATIC* Reporting Standards

# Station/Location Reporting Standard

This reporting standard includes two spreadsheet templates for entering station and location information. When entering data, you first should enter all data into the station spreadsheet template. Then, you will enter the data in the absolute location point template. You also need to link the data in the two spreadsheets by using the first column of both spreadsheets (*i.e.*, station GLNPO code).

**Most importantly to submit data using this reporting standard, you should read through the following directions carefully *before entering any data* into either spreadsheet template.**

## Template Layout

The template includes all the information about the data model that you need to know to enter data. For example, the column headings denote the table and column names, the cardinality among the data in the template, and additional information that may be useful. The *presentation* of the column headings also is intended to provide you with useful information. For example, CAPITALIZATION denotes mandatory. Underlined entries specify whether you need to include a valid reference table code. These concepts are described in more detail below. The following descriptions also can be used as reference material until you become familiar with the general template layout.

### Column Headings

Each template has several column headings. Each row of the column heading has a different purpose as described below.

1st row—***Logical Data Unit*** = describes the group of columns that fall between the pair of dark black lines.

2nd row—***Cardinality Explanation*** = describes how many rows should be included for the logical data unit (*i.e.*, the columns that fall between the pair of dark black lines).

3rd row—***Entity Type/Table Name*** = references the entity type/table name where the data will be stored in the target database.

The reference tables that are included in the station spreadsheet template include:

(1) Absolute Location Point Type

(2) Map or Photo

(3) Geopositioning Map or Photo Scale

(4) Geopositioning Horizontal Method

(5) Geopositioning Horizontal Datum

(6) Geopositioning Vertical Method

(7) Geopositioning Vertical Datum

(8) FIPS County

(9) USDA District (to be determined)

(10) FIPS HUC

(11) Native American Land

(12) EPA RF1 River Reach

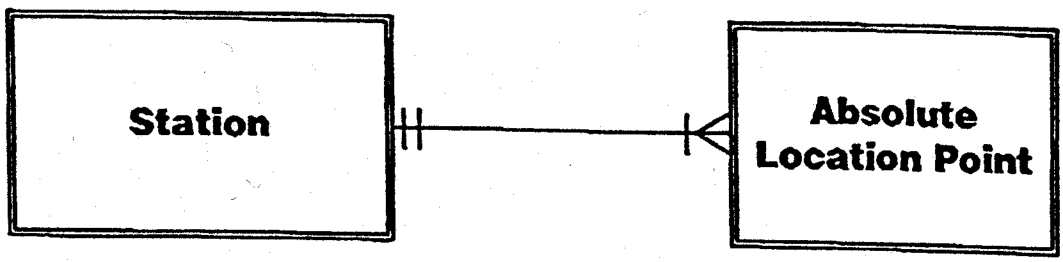
(13) Unit of Measure

Reference tables with the valid code for data entry are attached to these instructions. *Do not enter codes that do not exist in the attached tables.* You also should not add entries and new codes to the attached reference tables. (If you absolutely need a code that is not listed, contact the project manager. He will research your request and provide an answer, usually within a few days.)

## Linking Stations to Absolute Location Points

Although stations and absolute locations points are reported on separate spreadsheets, the data in both spreadsheets are related. In other words, a row in the station spreadsheet is related to a row(s) in the absolute location point spreadsheet. Therefore, when you use this reporting standard, you need to make a link between the two spreadsheets so that the data can be related in the database.

The logical connection between the rows in the two spreadsheets are as follows:




A *STATION* must have one or more *ABSOLUTE LOCATION POINTs*.

An *ABSOLUTE LOCATION POINT* must have one and only one *STATION*.

For each logical unit, there is a pre-defined cardinality between station and the logical unit. In other words, each station could have many entries in a logical unit such as Station Pollutant Source information. For example, a station may be polluted by more than one type of pollutant source (*e.g.*, urban runoff, industrial discharge).

These cardinalities are described in the second row of the template. When there is ONE Entry per Station, the user should enter only one row of data for any given station. When this row states MANY Entries per Station, the user may enter one or more rows of data.

The following table provides a high-level example of how the template should be used. To simplify the explanation, this example does not include all the template columns.

**Figure 3: Simplified Station Template**

*(In comparison to the real template, some columns and rows of column headings have been deleted in this simplified version.)*

| Primary Station Info. | | Station Description | | Distance to Shore | | Pollutant Source | |
|---|---|---|---|---|---|---|---|
| GLNPO CODE | Establishment Date | TYPE | SHAPE | Distance to Shore | DIST TO SHORE TYPE | Pollutant Source Type | Pollutant Spatial Relation |
| 1 | 081596 | RVR | LN | 5 | LEFT | ID | CROSS |
| 1 | | | | 10 | RIGHT | | |
| 2 | 081596 | RVR | PT | | | ORU | IN |
| 2 | | | | | | ID | IN |
| 2 | | | | | | CSO | IN |

To include data in the template, the user should begin in the left-most column (*i.e.*, station GLNPO code) and continue to the right. According to the entries in the simplified version of the template, there are two stations being reported. The stations are uniquely defined by GLNPO as 1 and 2. These two stations were established on 8/15/96, and they are both RVR (*i.e.*, river) stations. More specifically, station 1 is a LN (*i.e.*, line shaped station) that is 5 meters to the LEFT shore and 10 meters to the RIGHT shore. Station 2 is a PT (*i.e.*, point station) where the user decided not to measure the distance to shore. (This omission is acceptable because Distance to Shore is not mandatory based on the template convention that non-capitalized table names are optional). Finally, both stations are being polluted by an ID (industrial discharge). In addition, station 2 is effected by a CSO (combined sewer overflow) and an ORU (overland runoff, urban). For station 1, the station is located cross-stream from an industrial discharge. For station 2, the station is located in-stream (in the pollutant stream) of all pollutant sources listed.

In addition to the entries that should be included in specific cells, this example also shows how the cardinality rules work. In this case, a station can have only one set of station descriptions. Therefore, the description information is listed on the same row as the original station information. At the same time, the station can have many distances to shore and pollutant sources. When the user gets to the first logical unit that allows *many* entries per station (*i.e.*, distance to shore), the user can enter as many rows as necessary. The first row of entries must be in the same row as the related primary station information. After entering all rows for the current logical unit (*e.g.*, distance to shore), the user should move to the next logical unit

Like the station template, the spreadsheets are divided into logical units of data entry as denoted by the thick, solid black lines. For example in figure 6 above, the logical unit is standard location information. Figure 5 includes the logical units called latitude/longitude and geopositioning explanation. In this template, the cardinality among these logical units is one entry for every absolute location point.

To enter data in the template, the user should begin in the left-most column (*i.e.*, station GLNPO code) and continue to the right. On every row, you must not only enter a station GLNPO code in the first column, but the code must match a GLNPO code that was provided in the station template. If this GLNPO code does not correspond to an entry in the station template, there is no way to relate the absolute location information to a station.

# APPENDIX B

# SEDIMENT TOXICITY TEST REPORTS FOR *HYALELLA AZTECA* AND *CHIRONOMUS TENTANS*

# ACUTE TOXICITY TESTS
# WITH
# *HYALELLA AZTECA* AND *CHIRONOMUS TENTANS*
# ON SEDIMENTS FROM THE DULUTH/SUPERIOR HARBOR:
# 1993 Sampling Results - Batches # 1 and 2

Conducted by

Minnesota Pollution Control Agency
Monitoring and Assessment Section
520 Lafayette Road
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February 1997

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## INTRODUCTION

As part of the 1993 survey of sediment quality in the Duluth/Superior Harbor, sediment toxicity tests were conducted to assess acute (survival) and chronic (growth) toxicity to benthic invertebrates. Acute effects were measured in separate 10-day toxicity tests to *Hyalella azteca* (*H. azteca*) and *Chironomus tentans* (*C. tentans*). Growth was measured at the end of the *C. tentans* test to assess chronic effects. Survival and growth endpoints were compared to organisms similarly exposed to a reference control sediment collected from West Bearskin Lake (Cook County, MN).

A total of 40 sediment samples were collected for toxicity testing. This report presents the results of nine of these sediment samples run in two separate batches with separate controls.

## SAMPLE COLLECTION AND HANDLING

Between September 13-23, 1993, Minnesota Pollution Control Agency (MPCA) staff collected the nine sediments referred to in this report. The samples were collected from the harbor using a Ponar sampler and were taken to the University of Minnesota-Duluth Chemical Toxicology Research Laboratory. The samples were stored at 4°C until they were transported to the MPCA Toxicology Laboratory in St. Paul, MN on October 4, 1993.

## METHODS

Nine sediment samples and two control sediment samples were subjected to the 10-day sediment toxicity tests using the modified procedures described in ASTM (1993). However, the specific test system used for these assays is not indicated in the methods. The test organisms (*H. azteca* and *C. tentans*) were exposed to sediment samples for ten days in a portable, mini-flow system described in Benoit et al. (1993). The test apparatus consists of 300 mL, glass-beaker test chambers held in a glass box supplied with water from an acrylic plastic headbox. The beakers have two, 1.5 cm holes covered with stainless steel mesh, to allow for water exchange, while containing the test organisms. The headbox has a pipette tip drain calibrated to deliver water at an average rate of 32.5 mL/min. The glass box is fitted with a self-starting siphon to provide exchange of overlying water.

The *H. azteca* used for this test were 1 to 3 mm long, and the *C. tentans* were approximately 14 days old. These organisms were supplied by Environmental Consulting and Testing in Superior, WI. On the day of the Batch #1 test set up, MPCA personnel picked up the organisms from the supplier and transported them to the MPCA Toxicology Laboratory. An insufficient number of *H. azteca* were received to set up the toxicity tests. Thus, another batch of *H. azteca* was received from the supplier the next day via Federal Express.

On October 4, 1993, four samples (DSH 08, DSH 12, DSH 21, and DSH 40) and the control sediment were separately homogenized by hand, and 100 mL of each sediment was placed in a test beaker (Batch #1). On October 5, 1993, five more samples (DSH 16, DSH 18, DSH 19, DSH 23, and DSH 29) and another control sediment were homogenized and placed in beakers

(Batch #2). Aerated, artesian well water was added to the beakers, and the sediments were allowed to settle for approximately two hours before the organisms were added. The sediment samples for DSH 18 and DSH 19 had accidentally frozen during storage. These sediment samples were thawed in a water bath the morning of October 5 before homogenizing them.

Each sediment test was set up with three replicates of *H. azteca* and three replicates of *C. tentans.* Ten organisms were placed in each of six beakers in a random fashion. The organisms were exposed to 16 hours of light and eight hours of darkness for the duration of the ten-day test. Each day, two liters of aerated water from the artesian well at Stroh Brewery in St. Paul were exchanged in each test chamber. On weekdays, this was done in two equal aliquots. On weekends, the two liters were passed through the chambers all at once. Water quality measurements (i.e., pH, temperature, and dissolved oxygen) of the overlying water were taken in one beaker of each of the triplicate sets of each of the sediments. The results, along with daily observations involving the physical appearance of the sediments and organisms, were recorded in a laboratory notebook.

The test was terminated on October 14, 1993 for Batch #1 and on October 15, 1993 for Batch #2. The sediments were sieved through 40 mesh screens, and the sieved material was sorted for organisms. The organisms found were counted, and the number of alive and dead organisms were recorded. Organisms not found were recorded as missing and presumed dead. The *C. tentans* that survived were placed in aluminum weighing dishes, dried at approximately 90°C for at least four hours, desiccated to room temperature, and weighed.

Growth (weight) of the *C. tentans* and survival of both organisms were used as the endpoints for these tests. The resulting survival data were analyzed using TOXSTAT (Gulley and WEST, Inc., 1994), a statistical software package obtained from the University of Wyoming; however, due to a quality assurance problem, the growth data were not analyzed.

A 96-hour, reference toxicant test with *H. azteca* in sodium chloride (NaCl) was run in conjunction with these toxicity tests to determine the acceptability of the *H. azteca* used. Four concentrations of NaCl solution (i.e., 5, 2.5, 1.25, and 0.625 g/L) and a control (aerated, artesian well water) were used in this test. Three replicates of five organisms each were set up per concentration.

## RESULTS

Water Quality

Measurements of pH, dissolved oxygen concentration, and temperature in the overlying water of the test beakers were made daily. These measurements are summarized below and in Tables 1, 2, and 3, respectively, for both batches of tests.

*Batch # 1 Water Chemistry*

In Batch #1, the range of pH values in the beakers containing *H. azteca* was 7.2 to 7.7 (Table 1). The water in the *C. tentans* beakers had a pH range of 7.0 to 7.5 (Table 1). The pH fluctuations during these tests were acceptable since it did not vary more than 50% within each treatment (U.S. EPA, 1994).

The dissolved oxygen concentration ranged from 3.8 to 7.6 mg/L in the *H. azteca* beakers and from 1.6 to 7.2 mg/L in the *C. tentans* beakers (Table 2). It should be noted that on days 2, 3, 5, 6, and 9, the dissolved oxygen concentration in the DSH 40 sediment beaker containing *C. tentans* was less than 40% saturated, which is out of the acceptable test range for dissolved oxygen.

The temperature of the overlying water in each glass box was measured and ranged from 20.0°C to 22.5°C (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

*Batch # 2 Water Chemistry*

In Batch #2, the range of pH values in the beakers containing *H. azteca* was 6.9 to 7.7 (Table 1). The water in the *C. tentans* beakers had a pH range of 6.8 to 7.7 (Table 1). These pH ranges were acceptable for these tests.

The dissolved oxygen concentration ranged from 4.4 to 6.9 mg/L in the *H. azteca* beakers and from 3.2 to 6.7 mg/L in the *C. tentans* beakers (Table 2). It should be noted that on day 5, the dissolved oxygen concentration in the DSH 19 sediment beaker containing *C. tentans* was less than 40% saturated. On day 9, sample DSH 29 and Control #2 also had low dissolved oxygen concentrations in the *C. tentans* tests.

The range of temperature values in the beakers was measured and ranged from 20.0°C to 22.5°C (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

Test Endpoints

The mean percent survival of the test organisms is summarized below and in Table 4. The sediments for DSH 18 and DSH 19 had frozen during sample storage. Changes in the sample matrix that may have taken place during the freezing and thawing of these sediments could not be determined. Thus, it is not known whether similar survival data would have resulted from using unfrozen sediments for these toxicity tests.

The mean percent survival of *H. azteca* in Control #1 was 13% with a range of 0% to 30%. For Control #2, the mean percent survival was 33% with a range of 10% to 50%. Survival for both of these controls was less than 80% and, therefore, unacceptable. Thus, both test batches for *H. azteca* failed.

For the control sediment containing *C. tentans*, percent survival ranged from 90% to 100% with a mean of 93% for Control #1 and a range of 80% to 100% with a mean of 90% for Control #2. Mean percent survival of *C. tentans* in Batch #1 in the test sediments ranged from 83% in the DSH 40 sample to 100% in the DSH 08 sample. Mean percent survival of *C. tentans* in Batch #2 ranged from 77% in the DSH 19 sample to 97% in the DSH 23 sample.

Although the dried *C. tentans* were weighed, the balance on which they were weighed was not calibrated with standard weights; therefore, the data are suspect since the internal calibration of the balance may have drifted with time.

Data Analysis

Survival data for both batches of test sediments containing *C. tentans*, except DSH 08 (100% survival) and DSH 21 (90% survival), were transformed using an arc sine-square root transformation before being analyzed statistically using Dunnett's test. A one-tailed test was used to test the alternative hypothesis that sample survival was less than control survival. Thus, it was not necessary to include the sample survival data which exceeded the control survival in the Dunnett's test (e.g., survival data for DSH 08). For DSH 21, survival (90%) was within the variability of 30-50% necessary to see any significant difference between the control and any given sediment (T. Norberg-King, U.S. EPA, Duluth, MN, personal communication). Thus, it is reasonable to assume that the effect that DSH 21 had on the test organisms was not significantly less than that of the control.

For both batches of test, none of the test sediment survivals were statistically less than the control at $p=0.05$ (Appendix A). For test batch #2, all of the survival results were included in the Dunnett's test even though the survival in DSH 23 and DSH 29 exceeded the control survival. This was because the statistical analysis had been run prior to implementing a policy at the MPCA Toxicology Laboratory to exclude results exceeding the control survival.

Reference Toxicant Test with *Hyalella azteca* in Sodium Chloride Solution

The pH of the overlying water in the reference toxicant test ranged from 7.1 to 8.0. The dissolved oxygen ranged from 7.4 to 8.4 mg/L and the temperature was 21°C on the first day of the test (temperature was not measured during the remainder of the test). Mean percent survival of the organisms in the control was less than 90% (i.e., 40%) which was unacceptable. Thus, the health of the test organisms was suspect, and the test failed.

## SUMMARY

Survival of *H. azteca* in the control sediments was unacceptable (i.e., less than 80%), and the reference toxicant test with *H. azteca* failed. Therefore, no conclusions can be drawn about the effect that the sediments had on *H. azteca*.

Control survival was acceptable in both batches of *C. tentans* tests, and the survival of organisms in the test sediments was not statistically less than the control sediments.

## REFERENCES


ASTM. 1993. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. E1383-93. In *Annual Book of ASTM Standards, Vol. 11.04*. American Society for Testing and Materials, Philadelphia, PA. pp. 1173-1199.

Benoit, D.A., G. Phipps, and G.T. Ankley. 1993. A sediment testing intermittent renewal system for the automated renewal of overlying water in toxicity tests with contaminated sediments. Water Research 27:1403-1412.

Gulley, D.D. and WEST, Inc. 1994. TOXSTAT 3.4. WEST, Inc., Cheyenne, WY.

U.S. EPA. 1994. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN. EPA/600/R-94/024.

## TABLE 1. Daily Overlying Water pH Measurements

**Batch # 1**

| Day | Control 1 *C. tentans* | Control 1 *H. azteca* | DSH 08 *C. tentans* | DSH 08 *H. azteca* | DSH 12 *C. tentans* | DSH 12 *H. azteca* | DSH 21 *C. tentans* | DSH 21 *H. azteca* | DSH 40 *C. tentans* | DSH 40 *H. azteca* |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 7.1 | 7.2 | 7.5 | 7.4 | 7.3 | 7.3 | 7.5 | 7.5 | 7.2 | 7.2 |
| 1 | 7.2 | 7.3 | 7.4 | 7.5 | 7.2 | 7.2 | 7.4 | 7.5 | 7.3 | 7.2 |
| 2 | 7.1 | 7.2 | 7.4 | 7.4 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.2 |
| 3 | 7.3 | 7.4 | 7.5 | 7.6 | 7.3 | 7.3 | 7.5 | 7.5 | 7.2 | 7.4 |
| 4 | 7.3 | 7.3 | 7.4 | 7.5 | 7.3 | 7.4 | 7.4 | 7.5 | 7.3 | 7.3 |
| 5 | 7.3 | 7.3 | 7.5 | 7.5 | 7.3 | 7.3 | 7.3 | 7.3 | 7.4 | 7.4 |
| 6 | 7.2 | 7.2 | 7.3 | 7.3 | 7.4 | 7.4 | 7.3 | 7.2 | 7.3 | 7.4 |
| 7 | 7.2 | 7.4 | 7.5 | 7.7 | 7.4 | 7.4 | 7.5 | 7.5 | 7.2 | 7.4 |
| 8 | 7.2 | 7.4 | 7.5 | 7.7 | 7.3 | 7.4 | 7.4 | 7.7 | 7.3 | 7.3 |
| 9 | 7.0 | 7.5 | 7.3 | 7.5 | 7.3 | 7.3 | 7.3 | 7.6 | 7.1 | 7.2 |
| Mean | 7.2 | 7.3 | 7.4 | 7.5 | 7.3 | 7.3 | 7.4 | 7.5 | 7.3 | 7.3 |
| Range | 7.0-7.3 | 7.2 - 7.5 | 7.3 - 7.5 | 7.3 - 7.7 | 7.2 - 7.4 | 7.2 - 7.4 | 7.3 - 7.5 | 7.2 - 7.7 | 7.1 - 7.4 | 7.2 - 7.4 |

**Batch # 2**

| Day | Control 2 *C. tentans* | Control 2 *H. azteca* | DSH 16 *C. tentans* | DSH 16 *H. azteca* | DSH 18 *C. tentans* | DSH 18 *H. azteca* | DSH 19 *C. tentans* | DSH 19 *H. azteca* | DSH 23 *C. tentans* | DSH 23 *H. azteca* | DSH 29 *C. tentans* | DSH 29 *H. azteca* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 7.3 | 7.3 | 7.4 | 7.4 | 7.2 | 7.1 | 7.3 | 7.2 | 7.4 | 7.4 | 6.8 | 7.0 |
| 1 | 7.0 | 6.9 | 7.2 | 7.2 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.1 | 7.2 |
| 2 | 7.4 | 7.6 | 7.5 | 7.7 | 7.4 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.3 | 7.3 |
| 3 | 7.5 | 7.6 | 7.5 | 7.7 | 7.4 | 7.5 | 7.5 | 7.6 | 7.5 | 7.6 | 7.3 | 7.3 |
| 4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.5 | 7.4 | 7.4 | 7.5 | 7.5 | 7.4 | 7.4 |
| 5 | 7.4 | 7.4 | 7.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.4 | 7.5 | 7.5 | 7.5 | 7.4 |
| 6 | 7.4 | 7.6 | 7.4 | 7.7 | 7.5 | 7.6 | 7.5 | 7.6 | 7.6 | 7.6 | 7.3 | 7.4 |
| 7 | 7.5 | 7.6 | 7.7 | 7.7 | 7.5 | 7.5 | 7.4 | 7.6 | 7.5 | 7.5 | 7.3 | 7.4 |
| 8 | 7.2 | 7.4 | 7.4 | 7.6 | 7.2 | 7.3 | 7.2 | 7.5 | 7.2 | 7.3 | 7.1 | 7.4 |
| 9 | 7.2 | 7.3 | 7.3 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.1 | 7.2 |
| Mean | 7.3 | 7.4 | 7.4 | 7.5 | 7.4 | 7.4 | 7.4 | 7.5 | 7.4 | 7.5 | 7.2 | 7.3 |
| Range | 7.0 - 7.5 | 6.9 - 7.6 | 7.2 - 7.7 | 7.2 - 7.7 | 7.2 - 7.5 | 7.1 - 7.6 | 7.2 - 7.5 | 7.2 - 7.6 | 7.2 - 7.6 | 7.3 - 7.6 | 6.8 - 7.5 | 7.0 - 7.4 |

## TABLE 2. Daily Overlying Water Dissolved Oxygen Concentrations (mg/L)

**Batch # 1**

| | Control 1 | | DSH 08 | | DSH 12 | | DSH 21 | | DSH 40 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 6.9 | 6.8 | 7.2 | 7.0 | 6.7 | 6.7 | 7.2 | 7.3 | 6.6 | 6.2 |
| 1 | 5.9 | 6.7 | 5.3 | 6.3 | 6.0 | 5.7 | 6.4 | 7.0 | 4.6 | 5.1 |
| 2 | 5.0 | 6.3 | 5.5 | 6.5 | 4.3 | 5.7 | 5.8 | 6.4 | 3.3 | 3.8 |
| 3 | 6.1 | 6.4 | 5.5 | 6.5 | 4.4 | 5.7 | 5.5 | 5.9 | 3.2 | 5.3 |
| 4 | 5.3 | 6.8 | 5.2 | 6.4 | 5.1 | 6.1 | 5.8 | 6.8 | 4.3 | 4.6 |
| 5 | 4.2 | 6.1 | 5.1 | 6.2 | 4.5 | 5.2 | 4.1 | 5.9 | 1.7 | 5.0 |
| 6 | 4.0 | 5.8 | 4.9 | 6.0 | 4.2 | 5.1 | 4.0 | 6.0 | 1.6 | 4.8 |
| 7 | 5.7 | 6.7 | 6.0 | 7.5 | 5.7 | 6.0 | 6.5 | 7.0 | 3.6 | 5.3 |
| 8 | 5.7 | 6.6 | 6.4 | 7.1 | 5.5 | 5.8 | 6.1 | 7.6 | 4.2 | 4.6 |
| 9 | 4.4 | 6.5 | 4.7 | 6.5 | 4.1 | 5.1 | 4.8 | 6.8 | 3.0 | 3.8 |
| Mean | 5.3 | 6.5 | 5.6 | 6.6 | 5.1 | 5.7 | 5.6 | 6.7 | 3.6 | 4.9 |
| Range | 4.0-6.9 | 5.8-6.8 | 4.7-7.2 | 6.0-7.5 | 4.1-6.7 | 5.1-6.7 | 4.0-7.2 | 5.9-7.6 | 1.6-6.6 | 3.8-6.2 |

**Batch # 2**

| | Control 2 | | DSH 16 | | DSH 18 | | DSH 19 | | DSH 23 | | DSH 29 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 6.7 | 5.8 | 6.6 | 6.9 | 5.0 | 5.2 | 4.7 | 4.4 | 5.7 | 5.4 | 6.7 | 6.9 |
| 1 | 5.2 | 6.0 | 5.3 | 5.8 | 5.3 | 5.7 | 4.2 | 5.0 | 5.4 | 5.9 | 5.0 | 5.4 |
| 2 | 5.3 | 6.4 | 4.9 | 6.0 | 5.2 | 5.8 | 5.1 | 5.4 | 5.5 | 5.9 | 5.0 | 5.4 |
| 3 | 5.8 | 6.9 | 5.2 | 6.5 | 4.9 | 6.4 | 6.0 | 6.4 | 5.9 | 6.6 | 4.8 | 6.1 |
| 4 | 5.5 | 6.4 | 5.1 | 6.3 | 4.5 | 6.3 | 3.5 | 6.1 | 4.4 | 5.8 | 4.5 | 6.0 |
| 5 | 5.3 | 6.2 | 5.0 | 5.8 | 4.2 | 6.0 | 3.2 | 5.7 | 4.0 | 5.3 | 4.3 | 6.1 |
| 6 | 5.5 | 6.7 | 4.5 | 6.6 | 6.0 | 6.9 | 5.3 | 6.8 | 6.4 | 6.7 | 4.7 | 6.3 |
| 7 | 5.6 | 6.7 | 6.2 | 6.7 | 6.5 | 6.0 | 5.0 | 6.6 | 5.8 | 6.3 | 5.1 | 5.9 |
| 8 | 3.7 | 6.2 | 4.5 | 6.1 | 4.3 | 5.8 | 3.9 | 6.0 | 4.1 | 5.4 | 3.8 | 6.0 |
| 9 | 3.4 | 5.5 | 4.1 | 5.8 | 4.2 | 5.5 | 3.5 | 5.6 | 4.3 | 5.6 | 3.4 | 5.4 |
| Mean | 5.2 | 6.3 | 5.1 | 6.3 | 5.0 | 6.0 | 4.4 | 5.8 | 5.2 | 5.9 | 4.7 | 6.0 |
| Range | 3.4-6.7 | 5.5-6.9 | 4.1-6.6 | 5.8-6.9 | 4.2-6.5 | 5.2-6.9 | 3.2-6.0 | 4.4-6.8 | 4.0-6.4 | 5.3-6.7 | 3.4-6.7 | 5.4-6.9 |

## TABLE 3. Daily Overlying Water Temperatures (Degrees Celsius)

Batch # 1

| | Control 1 | | DSH 08 | | DSH 12 | | DSH 21 | | DSH 40 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 1 | 21.5 | 21.5 | 21.5 | 21.5 | 20.5 | 21.0 | 21.5 | 21.5 | 21.0 | 21.0 |
| 2 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 3 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 |
| 4 | 20.5 | 20.5 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| 5 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 6 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 7 | 22.0 | 22.0 | 21.5 | 21.5 | 21.5 | 21.5 | 22.0 | 22.0 | 21.5 | 21.5 |
| 8 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| 9 | 20.5 | 20.5 | 20.5 | * | * | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| Mean | 21.6 | 21.6 | 21.5 | 21.6 | 21.4 | 21.4 | 21.5 | 21.5 | 21.4 | 21.4 |
| Range | 20.5-22.5 | 20.5-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 |

* Temperature was not recorded.

Batch # 2

| | Control 2 | | DSH 16 | | DSH 18 | | DSH 19 | | DSH 23 | | DSH 29 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 22.0 | 22.0 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.0 | 21.0 | 21.5 | 21.5 |
| 1 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 2 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 |
| 3 | 20.0 | 20.0 | 20.0 | 20.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.0 | 20.0 | 20.0 | 20.0 |
| 4 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 5 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 6 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 21.5 | 22.0 |
| 7 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 8 | 20.5 | 20.5 | 20.5 | 20.5 | 21.0 | 21.0 | 20.5 | 20.5 | 21.0 | 21.0 | 20.5 | 20.5 |
| 9 | 21.0 | 21.0 | 21.0 | 21.0 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.0 | 21.0 |
| Mean | 21.5 | 21.5 | 21.5 | 21.5 | 21.6 | 21.6 | 21.6 | 21.6 | 21.5 | 21.5 | 21.4 | 21.5 |
| Range | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 | 20.0-22.5 |

**TABLE 4. Mean Percent Survival of *Hyalella azteca* and *Chironomus tentans***

| | **Mean Percent Survival** | |
|---|---|---|
| | *Hyalella azteca* [1] | *Chironomus tentans* |
| Batch # 1 | | |
| CONTROL #1 | 13% | 93% |
| DSH 08 | 33% | 100% |
| DSH 12 | 27% | 90% |
| DSH 21 | 23% | 90% |
| DSH 40 | 27% | 83% |
| Batch # 2 | | |
| CONTROL #2 | 33% | 90% |
| DSH 16 | 60% | 83% |
| DSH 18 | 50% | 90% |
| DSH 19 | 40% | 77% |
| DSH 23 | 30% | 97% |
| DSH 29 | 37% | 93% |

[1] Controls were unacceptable (< 80% survival). Thus, the *Hyalella azteca* tests failed for both batches of samples.

# APPENDIX A

# TOXSTAT Analysis

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
4
3
3
3
3
CONTROL
1.00000000
0.90000000
0.90000000
DSH 12
0.80000000
1.00000000
0.90000000
DSH 40
0.80000000
0.70000000
1.00000000

TITLE: 93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
FILE: 93mpr2CA.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 3

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | CONTROL | 1 | 1.0000 | 1.4120 |
| 1 | CONTROL | 2 | 0.9000 | 1.2490 |
| 1 | CONTROL | 3 | 0.9000 | 1.2490 |
| 2 | DSH 12 | 1 | 0.8000 | 1.1071 |
| 2 | DSH 12 | 2 | 1.0000 | 1.4120 |
| 2 | DSH 12 | 3 | 0.9000 | 1.2490 |
| 3 | DSH 40 | 1 | 0.8000 | 1.1071 |
| 3 | DSH 40 | 2 | 0.7000 | 0.9912 |
| 3 | DSH 40 | 3 | 1.0000 | 1.4120 |

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | 1.249 | 1.412 | 1.303 |
| 2 | DSH 12 | 3 | 1.107 | 1.412 | 1.256 |
| 3 | DSH 40 | 3 | 0.991 | 1.412 | 1.170 |

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | CONTROL | 0.009 | 0.094 | 0.054 | 7.22 |
| 2 | DSH 12 | 0.023 | 0.153 | 0.088 | 12.15 |
| 3 | DSH 40 | 0.047 | 0.217 | 0.126 | 18.58 |

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 2 | 0.027 | 0.014 | 0.517 |
| Within (Error) | 6 | 0.159 | 0.026 | |
| Total | 8 | 0.186 | | |

Critical F value = 5.14 (0.05,2,6)
Since F < Critical F FAIL TO REJECT Ho: All equal

```
93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT              Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality
----------------------------------------------------------------------

D =    0.159

W =    0.934

Critical W (P = 0.05) (n =  9) = 0.829
Critical W (P = 0.01) (n =  9) = 0.764
----------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.
```

```
93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT               Transform: ARC SINE(SQUARE ROOT(Y))

----------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    1.05

----------------------------------------------------------------------
Table Chi-square value =    9.21  (alpha = 0.01, df =   2)
Table Chi-square value =    5.99  (alpha = 0.05, df =   2)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | CONTROL | 1.303 | 0.933 | | |
| 2 | DSH 12 | 1.256 | 0.900 | 0.356 | |
| 3 | DSH 40 | 1.170 | 0.833 | 1.003 | |

Dunnett table value = 2.34 (1 Tailed Value, P=0.05, df=6,2)

93 MUDPUPPY RUN #2A CHIRONOMIDS 10/4/93
File: 93mpr2CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | | | |
| 2 | DSH 12 | 3 | 0.229 | 24.5 | 0.033 |
| 3 | DSH 40 | 3 | 0.229 | 24.5 | 0.100 |

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
6
3
3
3
3
3
3
CONTROL
0.8
1.0
0.9
DSH 16
0.8
0.8
0.9
DSH 18
1.0
0.8
0.9
DSH 19
0.6
0.8
0.9
DSH 23
0.9
1.0
1.0
DSH 29
0.9
1.0
0.9

TITLE: 93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | CONTROL | 1 | 0.8000 | 1.1071 |
| 1 | CONTROL | 2 | 1.0000 | 1.4120 |
| 1 | CONTROL | 3 | 0.9000 | 1.2490 |
| 2 | DSH 16 | 1 | 0.8000 | 1.1071 |
| 2 | DSH 16 | 2 | 0.8000 | 1.1071 |
| 2 | DSH 16 | 3 | 0.9000 | 1.2490 |
| 3 | DSH 18 | 1 | 1.0000 | 1.4120 |
| 3 | DSH 18 | 2 | 0.8000 | 1.1071 |
| 3 | DSH 18 | 3 | 0.9000 | 1.2490 |
| 4 | DSH 19 | 1 | 0.6000 | 0.8861 |
| 4 | DSH 19 | 2 | 0.8000 | 1.1071 |
| 4 | DSH 19 | 3 | 0.9000 | 1.2490 |
| 5 | DSH 23 | 1 | 0.9000 | 1.2490 |
| 5 | DSH 23 | 2 | 1.0000 | 1.4120 |
| 5 | DSH 23 | 3 | 1.0000 | 1.4120 |
| 6 | DSH 29 | 1 | 0.9000 | 1.2490 |
| 6 | DSH 29 | 2 | 1.0000 | 1.4120 |
| 6 | DSH 29 | 3 | 0.9000 | 1.2490 |

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | 1.107 | 1.412 | 1.256 |
| 2 | DSH 16 | 3 | 1.107 | 1.249 | 1.154 |
| 3 | DSH 18 | 3 | 1.107 | 1.412 | 1.256 |
| 4 | DSH 19 | 3 | 0.886 | 1.249 | 1.081 |
| 5 | DSH 23 | 3 | 1.249 | 1.412 | 1.358 |
| 6 | DSH 29 | 3 | 1.249 | 1.412 | 1.303 |

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | CONTROL | 0.023 | 0.153 | 0.088 | 12.15 |
| 2 | DSH 16 | 0.007 | 0.082 | 0.047 | 7.10 |
| 3 | DSH 18 | 0.023 | 0.153 | 0.088 | 12.15 |
| 4 | DSH 19 | 0.033 | 0.183 | 0.106 | 16.92 |
| 5 | DSH 23 | 0.009 | 0.094 | 0.054 | 6.93 |
| 6 | DSH 29 | 0.009 | 0.094 | 0.054 | 7.22 |

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 0.153 | 0.031 | 1.755 |
| Within (Error) | 12 | 0.209 | 0.017 | |
| Total | 17 | 0.362 | | |

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho: All equal

```
93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
----------------------------------------------------------------------

D =    0.209

W =    0.958

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
----------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.
```

```
93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

----------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    1.79

----------------------------------------------------------------------
Table Chi-square value =   15.09  (alpha = 0.01, df =   5)
Table Chi-square value =   11.07  (alpha = 0.05, df =   5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | CONTROL | 1.256 | 0.900 | | |
| 2 | DSH 16 | 1.154 | 0.833 | 0.943 | |
| 3 | DSH 18 | 1.256 | 0.900 | 0.000 | |
| 4 | DSH 19 | 1.081 | 0.767 | 1.628 | |
| 5 | DSH 23 | 1.358 | 0.967 | -0.943 | |
| 6 | DSH 29 | 1.303 | 0.933 | -0.439 | |

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

93 MUDPUPPY RUN #2B CHIRONOMIDS 10/5/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR2CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | | | |
| 2 | DSH 16 | 3 | 0.208 | 23.1 | 0.067 |
| 3 | DSH 18 | 3 | 0.208 | 23.1 | 0.000 |
| 4 | DSH 19 | 3 | 0.208 | 23.1 | 0.133 |
| 5 | DSH 23 | 3 | 0.208 | 23.1 | -0.067 |
| 6 | DSH 29 | 3 | 0.208 | 23.1 | -0.033 |

# ACUTE TOXICITY TESTS
# WITH
# *HYALELLA AZTECA* AND *CHIRONOMUS TENTANS*
# ON SEDIMENTS FROM THE DULUTH/SUPERIOR HARBOR:
## 1993 Sampling Results - Batches # 3 and 4

Conducted by

Minnesota Pollution Control Agency
Monitoring and Assessment Section
520 Lafayette Road
St. Paul, Minnesota  55155-4194

February 1997

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LIST OF TABLES

## INTRODUCTION

As part of the 1993 survey of sediment quality in the Duluth/Superior Harbor, sediment toxicity tests were conducted to assess acute (survival) and chronic (growth) toxicity to benthic invertebrates. Acute effects were measured in separate 10-day toxicity tests to *Hyalella azteca* (*H. azteca*) and *Chironomus tentans* (*C. tentans*). Growth was measured at the end of the *C tentans* test to assess chronic effects. Survival and growth endpoints were compared to organisms similarly exposed to a reference control sediment collected from West Bearskin Lake (Cook County, MN).

A total of 40 sediment samples were collected for toxicity testing. This report presents the results of thirteen of these sediment samples run in two separate batches with separate controls.

## SAMPLE COLLECTION AND HANDLING

During September 14-23, 1993, Minnesota Pollution Control Agency (MPCA) staff collected the thirteen sediments referred to in this report. The samples were collected from the harbor using a Ponar sampler and were taken to the University of Minnesota-Duluth Chemical Toxicology Research Laboratory. The samples were stored at 4°C until they were transported to the MPCA Toxicology Laboratory in St. Paul, MN.

## METHODS

Thirteen sediment samples and two control sediment samples were subjected to the 10-day sediment toxicity tests using the modified procedures described in ASTM (1993). However, the specific test system used for these assays is not indicated in the methods. The test organisms (*H. azteca* and *C. tentans*) were exposed to sediment samples for ten days in a portable, mini-flow system described in Benoit et al. (1993). The test apparatus consists of 300 mL, glass-beaker test chambers held in a glass box supplied with water from an acrylic plastic headbox. The beakers have two, 1.5 cm holes covered with stainless steel mesh, to allow for water exchange, while containing the test organisms. The headbox has a pipette tip drain calibrated to deliver water at an average rate of 32.5 mL/min. The glass box is fitted with a self-starting siphon to provide exchange of overlying water.

The *H. azteca* used for this test were 1 to 3 mm long, and the *C. tentans* were approximately 14 days old. These organisms were supplied by Environmental Consulting and Testing, Superior, WI and were shipped to St. Paul the night before the test was set up. The organisms arrived at 10 p.m. and were stored at the St. Paul bus depot until 9 a.m. the next morning. The organisms were then transported to the MPCA Toxicology Laboratory. The majority of the organisms were then placed in glass vessels and transferred to the test beakers by 1:30 p.m. The remaining organisms were aerated in these vessels until they were placed in the test beakers the following day.

On October 18, 1993, eight samples (DSH 01, DSH 02, DSH 06, DSH 07, DSH 14, DSH 22, DSH 26, and DSH 30) and the control sediment were separately homogenized by hand, and 100 mL of each sediment was placed in a test beaker (Batch #3). On October 19, 1993, five more samples (DSH 03, DSH 04, DSH 13, DSH 17, and DSH 24) and another control sediment were homogenized and placed in beakers (Batch #4). Each sediment test was set up with three replicates of *H. azteca* and three replicates of *C. tentans*. Aerated, artesian well water was added to the beakers, and the sediments were allowed to settle for approximately two hours before the organisms were added. For each toxicity test, ten organisms were placed in each beaker in a random fashion.

The organisms were exposed to 16 hours of light and eight hours of darkness for the duration of the ten-day test. Each day, two liters of aerated water from the artesian well at Stroh Brewery in St. Paul, MN were exchanged in each test chamber. On weekdays, 1-L was exchanged in the morning and 1-L in the afternoon. On weekends, the two liters were passed through the chambers all at once. Water quality measurements (i.e., pH, temperature, and dissolved oxygen) of the overlying water were taken in one beaker of each of the triplicate sets of each of the sediments. The results, along with daily observations involving the physical appearance of the sediments and organisms, were recorded in a laboratory notebook. This notebook is retained on file at the MPCA.

The test was terminated on October 28, 1993 for Batch #3 and on October 29, 1993 for Batch #4. The sediments were sieved through 40 mesh screens, and the sieved material was sorted for organisms. The organisms found were counted, and the number of alive and dead organisms were recorded. Organisms not found were recorded as missing and presumed dead. The *C. tentans* that survived were placed in aluminum weighing dishes, dried at approximately 90°C for at least four hours, desiccated to room temperature, and weighed.

Growth (weight) of the *C. tentans* and survival of both organisms were used as the endpoints for these tests. The resulting survival data were analyzed using TOXSTAT (Gulley and WEST, Inc., 1994), a statistical software package obtained from the University of Wyoming; however, due to a quality assurance problem, the growth data were not analyzed.

A 96-hour, reference toxicant test with *H. azteca* in sodium chloride (NaCl) was run in conjunction with these toxicity tests to determine the acceptability of the *H. azteca* used. Four concentrations of NaCl solution (i.e., 5, 2.5, 1.25, and 0.625 g/L) and a control (aerated, artesian well water) were used in this test. Three replicates of five organisms each were set up per concentration.

## RESULTS

Water Quality

Measurements of pH, dissolved oxygen, and temperature in the overlying water of the test beakers were made daily. These measurements are summarized below and in Tables 1, 2, and 3, respectively, for both batches of tests.

*Batch # 3 Water Chemistry*

In Batch #3, the range of pH values in the beakers containing *H. azteca* was 6.0 to 7.9 (Table 1). The water in the *C. tentans* beakers had a pH range of 6.8 to 7.7 (Table 1). The pH fluctuation during this test was acceptable since it did not vary more than 50% within each treatment (U.S. EPA, 1994).

The dissolved oxygen concentration ranged from 4.3 to 7.8 mg/L in the *H. azteca* beakers and from 3.3 to 8.1 mg/L in the *C. tentans* beakers (Table 2).

The temperature of the overlying water in each glass box was measured and ranged from 19.5°C to 22.0°C (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

*Batch # 4 Water Chemistry*

In Batch #4, the range of pH values in the beakers containing *H. azteca* was 7.2 to 8.0 (Table 1). The water in the *C. tentans* beakers had a pH range of 7.0 to 8.0 (Table 1). These pH ranges are acceptable for this test.

The dissolved oxygen concentration ranged from 3.6 to 6.9 mg/L in the *H. azteca* beakers and from 3.4 to 7.0 mg/L in the *C. tentans* beakers (Table 2).

The temperature of the overlying water in each glass box was measured and ranged from 20.5°C to 22.5°C (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

Test Endpoints

The mean percent survival of test organisms is summarized below and in Table 4.

*Batch #3 Survival Data*

The mean percent survival of *H. azteca* in Control #3 was 73% with a range of 70% to 80%. For this test, the mean percent survival must be at least 80% in the controls for the test to pass. For the control sediment containing *C. tentans,* percent survival ranged from 80% to 100% with a mean of 90%. Survival for these controls was greater than 70% and, therefore, acceptable.

Mean percent survival of *H. azteca* in the test sediments of Batch #3 ranged from 53% in the DSH 30 sample to 87% in the DSH 14 sample. Mean percent survival of *C. tentans* in Batch #3 test sediments ranged from 43% in the DSH 14 sample to 100% in the DSH 01 sample.

*Batch #4 Survival Data*

For Control #4 containing *H. azteca*, the mean percent survival was 73% with a range of 60% to 90%. The control survival for this test was unacceptable (<80% survival). Therefore, all of the *H. azteca* tests for Batch #4 failed. Survival in the control sediment containing *C. tentans* ranged from 80% to 100% with a mean of 90%; this was acceptable, and the test passed.

Mean percent survival of *H. azteca* in Batch #4 ranged from 60% in the DSH 24 sample to 80% in the DSH 17 sample. Mean percent survival of *C. tentans* in Batch #4 ranged from 0% in the DSH 24 sample to 93% in the DSH 13 sample.

*C. tentans Growth Data*

Although the dried *C. tentans* were weighed, the balance on which they were weighed was not calibrated with standard weights; therefore, the data are suspect since the internal calibration of the balance may have drifted with time.

Data Analysis

Survival data for both batches of test sediments containing *C. tentans,* except DSH 01, 03, and 24, were transformed using an arc sine-square root transformation before being analyzed statistically using Dunnett's test. The aforementioned data were eliminated from the analysis because there was zero variance between replicates. Although nonparametric statistics can be used to analyze zero variance data, a minimum of four replicates per sediment is needed. Only three replicates per sediment were run in this toxicity test.

A one-tailed test was used to test the alternative hypothesis that sample survival was significantly less than control survival. Thus, it was not necessary to include the sample survival data which exceeded the control survival in the Dunnett's test [e.g., survival data for DSH 01 (100%) and DSH 03 (90%)]. Since it is assumed that variability of 30-50% is necessary to see any significant difference between the control and any given sediment (T. Norberg-King, U.S. EPA, Duluth, MN, personal communication), and since DSH 24 had 0% survival, it is reasonable to assume that survival in DSH 24 was significantly less than the control. The only other sample survival that was significantly less than the control was site DSH 14. Results of the statistical analysis of the data are included in Appendix A.

Reference Toxicant Test with *Hyalella azteca* in Sodium Chloride Solution

The pH of the overlying water in the reference toxicant test ranged from 7.1 to 8.2. The dissolved oxygen ranged from 7.8 to 8.7 mg/L, and the temperature ranged between 19.5°C and 22.0°C. Mean percent survival of the organisms in the control was less than 90% (i.e., 67%) which was unacceptable. Thus, the health of the test organisms was suspect, and the test failed.

## SUMMARY

Survival of *H. azteca* in both of the control sediments was unacceptable (i.e., less than 80% survival), and the reference toxicant test failed. Therefore, no conclusions can be drawn about the effect that the sediments had on *H. azteca.*

Control survival was acceptable in both batches of samples containing *C. tentans*. The mean percent survival of *C. tentans* in the DSH 14 and DSH 24 samples was significantly less than their respective test controls. Survival of *C. tentans* in all other samples analyzed was not significantly different from the respective test controls

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# TABLE 1. Daily Overlying Water pH Measurements

## Batch #3

| | Control 3 | | DSH 01 | | DSH 02 | | DSH 06 | | DSH 07 | | DSH 14 | | DSH 22 | | DSH 26 | | DSH 30 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.2 | 6.0 | 6.9 | 6.8 | 7.3 | 7.3 | 7.2 | 7.2 | 6.9 | 6.8 | 6.9 | 6.9 | 7.3 | 7.2 | 6.8 | 6.9 | 7.0 | 6.9 |
| 1 | 7.0 | 7.1 | 7.2 | 7.3 | 7.4 | 7.6 | 7.5 | 7.5 | 7.3 | 7.4 | 7.4 | 7.4 | 7.6 | 7.6 | 7.3 | 7.5 | 7.4 | 7.6 |
| 2 | 7.2 | 7.4 | 7.3 | 7.4 | 7.5 | 7.5 | 7.5 | 7.5 | 7.3 | 7.4 | 7.5 | 7.6 | 7.5 | 7.5 | 7.6 | 7.7 | 7.4 | 7.4 |
| 3 | 6.9 | 7.2 | 7.2 | 7.3 | 7.4 | 7.5 | 7.4 | 7.5 | 7.3 | 7.4 | 7.4 | 7.6 | 7.4 | 7.6 | 7.3 | 7.4 | 7.3 | 7.4 |
| 4 | 7.0 | 7.3 | 7.3 | 7.4 | 7.3 | 7.4 | 7.3 | 7.5 | 7.2 | 7.4 | 7.4 | 7.6 | 7.4 | 7.5 | 7.3 | 7.5 | 7.3 | 7.5 |
| 5 | 7.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| 6 | 7.4 | 7.5 | 7.3 | 7.3 | 7.4 | 7.5 | 7.4 | 7.7 | 7.3 | 7.4 | 7.5 | 7.6 | 7.5 | 7.6 | 7.5 | 7.5 | 7.3 | 7.6 |
| 7 | 7.4 | 7.5 | 7.5 | 7.6 | 7.4 | 7.6 | 7.2 | 7.5 | 7.5 | 7.6 | 7.4 | 7.7 | 7.4 | 7.6 | 7.5 | 7.6 | 7.4 | 7.6 |
| 8 | 7.2 | 7.3 | 7.3 | 7.4 | 7.3 | 7.5 | 7.4 | 7.5 | 7.3 | 7.5 | 7.3 | 7.4 | 7.4 | 7.6 | 7.4 | 7.5 | 7.3 | 7.4 |
| 9 | 7.7 | 7.7 | 7.2 | 7.4 | 7.4 | 7.6 | 7.2 | 7.5 | 7.5 | 7.7 | 7.6 | 7.9 | 7.5 | 7.6 | 7.2 | 7.3 | 7.4 | 7.6 |
| Mean | 7.2 | 7.2 | 7.3 | 7.3 | 7.4 | 7.5 | 7.4 | 7.5 | 7.3 | 7.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.3 | 7.4 | 7.3 | 7.5 |
| Range | 6.9-7.7 | 6.0-7.7 | 6.9-7.5 | 6.8-7.6 | 7.3-7.5 | 7.3-7.6 | 7.2-7.5 | 7.2-7.7 | 6.9-7.5 | 6.8-7.7 | 6.9-7.6 | 6.9-7.9 | 7.3-7.6 | 7.2-7.6 | 6.8-7.6 | 6.9-7.7 | 7.0-7.5 | 6.9-7.6 |

## Batch #4

| | Control 4 | | DSH 03 | | DSH 04 | | DSH 13 | | DSH 17 | | DSH 24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.4 | 7.3 | 7.3 | 7.2 | 7.5 | 7.4 | 7.5 | 7.3 | 7.7 | 7.6 | 7.8 | 7.8 |
| 1 | 7.0 | 7.4 | 7.3 | 7.3 | 7.5 | 7.6 | 7.5 | 7.5 | 7.5 | 7.6 | 7.7 | 7.8 |
| 2 | 7.3 | 7.5 | 7.3 | 7.3 | 7.5 | 7.5 | 7.5 | 7.6 | 7.5 | 7.7 | 7.8 | 7.8 |
| 3 | 7.3 | 7.4 | 7.3 | 7.3 | 7.4 | 7.5 | 7.5 | 7.6 | 7.6 | 7.6 | 7.7 | 7.7 |
| 4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.5 | 7.4 | 7.4 | 7.5 | 7.5 |
| 5 | 7.1 | 7.4 | 7.3 | 7.4 | 7.3 | 7.5 | 7.5 | 7.6 | 7.4 | 7.6 | 7.6 | 7.6 |
| 6 | 7.4 | 7.5 | 7.4 | 7.6 | 7.5 | 7.7 | 7.5 | 7.7 | 7.5 | 7.7 | 7.6 | 7.7 |
| 7 | 7.4 | 7.4 | 7.3 | 7.5 | 7.3 | 7.5 | 7.5 | 7.6 | 7.5 | 7.6 | 7.6 | 7.7 |
| 8 | 7.7 | 7.9 | 7.7 | 7.9 | 7.9 | 8.0 | 7.9 | 8.0 | 7.9 | 8.0 | 8.0 | 8.0 |
| 9 | 7.2 | 7.3 | 7.3 | 7.4 | 7.8 | 7.9 | 7.8 | 7.9 | 7.9 | 7.9 | 7.7 | 7.8 |
| Mean | 7.3 | 7.5 | 7.4 | 7.4 | 7.5 | 7.6 | 7.6 | 7.6 | 7.6 | 7.7 | 7.7 | 7.7 |
| Range | 7.0-7.7 | 7.3-7.9 | 7.3-7.7 | 7.2-7.9 | 7.3-7.9 | 7.4-7-8.0 | 7.5-7.9 | 7.3-8.0 | 7.4-7.9 | 7.4-8.0 | 7.5-8.0 | 7.5-8.0 |

## TABLE 2. Daily Overlying Water Dissolved Oxygen Concentrations (mg/L)

### Batch #3

| Day | Control 3 | | DSH 01 | | DSH 02 | | DSH 06 | | DSH 07 | | DSH 14 | | DSH 22 | | DSH 26 | | DSH 30 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.5 | 6.8 | 6.3 | 6.6 | 6.9 | 6.8 | 6.9 | 7.0 | 6.7 | 6.1 | 4.1 | 4.3 | 7.0 | 6.9 | 8.1 | 7.7 | 6.2 | 7.8 |
| 1 | 6.3 | 6.7 | 6.0 | 6.5 | 6.2 | 6.9 | 6.6 | 6.6 | 6.1 | 6.5 | 5.4 | 5.8 | 6.6 | 6.2 | 6.0 | 7.0 | 6.2 | 7.0 |
| 2 | 5.4 | 5.0 | 5.3 | 6.2 | 5.2 | 6.3 | 6.1 | 6.7 | 5.1 | 5.7 | 5.3 | 6.1 | 5.8 | 6.4 | 6.4 | 6.9 | 5.8 | 6.3 |
| 3 | 5.3 | 6.8 | 4.3 | 5.9 | 5.2 | 6.2 | 5.4 | 6.6 | 4.1 | 5.5 | 5.0 | 6.2 | 5.4 | 6.7 | 4.9 | 6.4 | 4.6 | 6.5 |
| 4 | 4.7 | 6.7 | 4.8 | 6.0 | 4.7 | 6.2 | 4.9 | 6.4 | 4.4 | 5.9 | 4.7 | 6.2 | 4.8 | 6.5 | 4.7 | 6.9 | 4.6 | 6.4 |
| 5 | 4.5 | 6.0 | 4.1 | 5.6 | 4.5 | 5.9 | 4.5 | 5.8 | 3.8 | 5.1 | 4.2 | 5.8 | 4.3 | 5.0 | 4.2 | 6.0 | 4.0 | 5.0 |
| 6 | 5.0 | 6.0 | 4.9 | 5.4 | 3.7 | 5.7 | 4.2 | 6.7 | 3.5 | 4.4 | 5.4 | 5.2 | 5.0 | 6.0 | 5.4 | 6.0 | 4.4 | 5.9 |
| 7 | 5.2 | 6.2 | 4.1 | 6.3 | 4.0 | 6.8 | 4.7 | 6.2 | 4.7 | 6.3 | 5.0 | 6.1 | 4.2 | 6.6 | 3.9 | 6.2 | 4.5 | 6.3 |
| 8 | 5.5 | 6.0 | 4.5 | 6.0 | 3.9 | 5.6 | 4.1 | 5.9 | 4.0 | 6.3 | 3.5 | 5.2 | 4.0 | 6.1 | 5.3 | 5.9 | 4.4 | 5.7 |
| 9 | 5.4 | 6.2 | 3.3 | 6.2 | 5.0 | 6.0 | 4.0 | 6.5 | 4.5 | 6.4 | 4.0 | 5.9 | 5.3 | 6.7 | 5.5 | 6.2 | 4.0 | 6.4 |
| Mean | 5.5 | 6.2 | 4.8 | 6.1 | 4.9 | 6.2 | 5.1 | 6.4 | 4.7 | 5.8 | 4.7 | 5.7 | 5.2 | 6.3 | 5.4 | 6.5 | 4.9 | 6.3 |
| Range | 4.5-7.5 | 5.0-6.8 | 3.3-6.3 | 5.4-6.6 | 3.7-6.9 | 5.6-6.9 | 4.0-6.9 | 5.8-7.0 | 3.5-6.7 | 4.4-6.5 | 3.5-5.4 | 4.3-6.2 | 4.0-7.0 | 5.0-6.9 | 3.9-8.1 | 5.9-7.7 | 4.0-6.2 | 5.0-7.8 |

### Batch #4

| Day | Control 4 | | DSH 03 | | DSH 04 | | DSH 13 | | DSH 17 | | DSH 24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 6.8 | 6.5 | 6.6 | 5.7 | 6.7 | 6.3 | 6.7 | 6.0 | 7.0 | 6.6 | 6.4 | 6.3 |
| 1 | 6.3 | 6.6 | 5.8 | 5.9 | 6.0 | 6.4 | 6.2 | 6.3 | 6.0 | 6.7 | 5.7 | 6.2 |
| 2 | 5.6 | 6.9 | 4.9 | 5.4 | 5.7 | 6.2 | 6.3 | 6.3 | 6.0 | 6.4 | 5.4 | 6.1 |
| 3 | 5.2 | 6.6 | 5.1 | 5.6 | 5.3 | 6.3 | 6.1 | 6.7 | 6.1 | 6.7 | 5.1 | 5.7 |
| 4 | 4.3 | 5.9 | 3.7 | 5.5 | 4.0 | 5.3 | 5.0 | 5.2 | 4.8 | 5.1 | 4.8 | 3.6 |
| 5 | 4.8 | 6.3 | 4.9 | 5.8 | 3.7 | 6.0 | 6.0 | 5.0 | 4.4 | 5.8 | 4.3 | 4.3 |
| 6 | 4.7 | 5.9 | 4.6 | 6.4 | 4.2 | 6.5 | 4.9 | 5.6 | 4.3 | 6.0 | 3.4 | 5.0 |
| 7 | 4.5 | 5.7 | 4.8 | 6.2 | 3.8 | 5.9 | 5.1 | 6.7 | 5.1 | 6.2 | 4.3 | 5.1 |
| 8 | 4.4 | 6.4 | 3.5 | 6.0 | 4.5 | 6.8 | 4.5 | 6.9 | 5.5 | 6.0 | 4.5 | 4.5 |
| 9 | 4.7 | 6.6 | 3.6 | 5.5 | 4.8 | 6.5 | 5.5 | 5.9 | 5.8 | 6.1 | 3.6 | 4.3 |
| Mean | 5.1 | 6.3 | 4.8 | 5.8 | 4.9 | 6.2 | 5.6 | 6.1 | 5.5 | 6.2 | 4.8 | 5.1 |
| Range | 4.3-6.8 | 5.7-6.9 | 3.5-6.6 | 5.4-6.4 | 3.7-6.7 | 5.3-6.8 | 4.5-6.7 | 5.0-6.9 | 4.3-7.0 | 5.1-6.7 | 3.4-6.4 | 3.6-6.3 |

**TABLE 3. Daily Overlying Water Temperatures (Degrees Celsius)**

## Batch #3

| Day | Control 3 *C. tentans* | Control 3 *H. azteca* | DSH 01 *C. tentans* | DSH 01 *H. azteca* | DSH 02 *C. tentans* | DSH 02 *H. azteca* | DSH 06 *C. tentans* | DSH 06 *H. azteca* | DSH 07 *C. tentans* | DSH 07 *H. azteca* | DSH 14 *C. tentans* | DSH 14 *H. azteca* | DSH 22 *C. tentans* | DSH 22 *H. azteca* | DSH 26 *C. tentans* | DSH 26 *H. azteca* | DSH 30 *C. tentans* | DSH 30 *H. azteca* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 19.5 | 19.5 | 19.5 | 19.5 | 21.0 | 21.0 | 21.0 | 21.0 | 20.0 | 20.0 | 20.0 | 20.0 | 21.0 | 21.0 | 19.5 | 19.5 | 20.0 | 20.0 |
| 1 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.0 | 21.0 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 |
| 2 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| 3 | 20.5 | 20.5 | 20.0 | 20.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.0 | 20.0 | 20.0 | 20.0 | 20.5 | 20.5 | 20.0 | 20.0 | 20.0 | 20.0 |
| 4 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | NA | 20.5 | NA | 20.5 | 20.5 | 20.5 | NA | 20.5 | NA | 20.5 | 20.5 | 20.5 | 20.5 |
| 5 | 22.0 | 22.0 | 22.0 | NA | 22.0 | NA | 22.0 | 22.0 | NA | 22.0 | NA | 22.0 | 22.0 | NA | 22.0 | NA | NA | 22.0 |
| 6 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 7 | 21.5 | 21.5 | 21.5 | 21.5 | 22.0 | 22.0 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 |
| 8 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 9 | 21.0 | 21.0 | 20.5 | 20.5 | 21.0 | 21.0 | 21.0 | 21.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.0 | 20.0 |
| Mean | 21.0 | 20.9 | 20.9 | 20.8 | 21.2 | 21.2 | 21.2 | 21.1 | 20.9 | 20.7 | 20.9 | 20.8 | 21.1 | 21.1 | 20.9 | 20.8 | 20.9 | 20.8 |
| Range | 19.5-22.0 | 19.5-22.0 | 19.5-22.0 | 19.5-22.0 | 20.5-22.0 | 20.5-22.0 | 20.5-22.0 | 20.5-22.0 | 20.0-22.0 | 20.0-22.0 | 20.0-22.0 | 20.0-22.0 | 20.5-22.0 | 20.5-22.0 | 19.5-22.0 | 19.5-22.0 | 20.0-22.0 | 20.0-22.0 |

## Batch #4

| Day | Control 4 *C. tentans* | Control 4 *H. azteca* | DSH 03 *C. tentans* | DSH 03 *H. azteca* | DSH 04 *C. tentans* | DSH 04 *H. azteca* | DSH 13 *C. tentans* | DSH 13 *H. azteca* | DSH 17 *C. tentans* | DSH 17 *H. azteca* | DSH 24 *C. tentans* | DSH 24 *H. azteca* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 |
| 1 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 2 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| 3 | 20.5 | NA | 20.5 | NA | 20.5 | NA | 20.5 | NA | 20.5 | NA | 20.5 | NA |
| 4 | NA | 22.0 | NA | 22.0 | 22.0 | NA | 22.0 | NA | NA | 22.0 | NA | 22.0 |
| 5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.0 | 22.0 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 |
| 6 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 7 | 21.5 | 21.5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.5 | 21.5 | 21.0 | 21.0 |
| 8 | 21.0 | 21.0 | 21.0 | 21.0 | 21.5 | 21.5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 9 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| Mean | 21.4 | 21.4 | 21.3 | 21.3 | 21.3 | 21.4 | 21.3 | 21.3 | 21.4 | 21.4 | 21.3 | 21.3 |
| Range | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.0 | 20.5-22.0 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 | 20.5-22.5 |

NA = Not applicable, no measurement taken.

**TABLE 4. Mean Percent Survival of *Hyalella azteca* and *Chironomus tentans***

| | Mean Percent Survival | | |
|---|---|---|---|
| | *Hyalella azteca*[1] | *Chironomus tentans* | |
| Batch # 3 | | | |
| CONTROL #3 | 73% | 90% | |
| DSH 01 | 63% | 100% | |
| DSH 02 | 70% | 93% | |
| DSH 06 | 57% | 97% | |
| DSH 07 | 63% | 87% | |
| DSH 14 | 87% | 43% | * |
| DSH 22 | 77% | 80% | |
| DSH 26 | 60% | 83% | |
| DSH 30 | 53% | 97% | |
| Batch # 4 | | | |
| CONTROL #2 | 73% | 90% | |
| DSH 03 | 77% | 90% | |
| DSH 04 | 63% | 87% | |
| DSH 13 | 70% | 93% | |
| DSH 17 | 80% | 90% | |
| DSH 24 | 60% | 0% | * |

[1] Controls were unacceptable (<80% survival). Thus, the *Hyalella azteca* tests failed for both batches of samples.

* Significantly different from the control, p = 0.05.

# APPENDIX A

# TOXSTAT Analysis

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
8
3
3
3
3
3
3
3
3
CONTROL
1.0
0.8
0.9
DSH 30
0.9
1.00000000
1.00000000
DSH 02
1.00000000
0.90000000
0.90000000
DSH 06
0.90000000
1.00000000
1.00000000
DSH 07
0.80000000
0.90000000
0.90000000
DSH 14
0.60000000
0.30000000
0.40000000
DSH 22
0.90000000
0.80000000
0.70000000
DSH 26
0.80000000
0.90000000
0.80000000

TITLE: 93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 8

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | CONTROL | 1 | 1.0000 | 1.4120 |
| 1 | CONTROL | 2 | 0.8000 | 1.1071 |
| 1 | CONTROL | 3 | 0.9000 | 1.2490 |
| 2 | DSH 30 | 1 | 0.9000 | 1.2490 |
| 2 | DSH 30 | 2 | 1.0000 | 1.4120 |
| 2 | DSH 30 | 3 | 1.0000 | 1.4120 |
| 3 | DSH 02 | 1 | 1.0000 | 1.4120 |
| 3 | DSH 02 | 2 | 0.9000 | 1.2490 |
| 3 | DSH 02 | 3 | 0.9000 | 1.2490 |
| 4 | DSH 06 | 1 | 0.9000 | 1.2490 |
| 4 | DSH 06 | 2 | 1.0000 | 1.4120 |
| 4 | DSH 06 | 3 | 1.0000 | 1.4120 |
| 5 | DSH 07 | 1 | 0.8000 | 1.1071 |
| 5 | DSH 07 | 2 | 0.9000 | 1.2490 |
| 5 | DSH 07 | 3 | 0.9000 | 1.2490 |
| 6 | DSH 14 | 1 | 0.6000 | 0.8861 |
| 6 | DSH 14 | 2 | 0.3000 | 0.5796 |
| 6 | DSH 14 | 3 | 0.4000 | 0.6847 |
| 7 | DSH 22 | 1 | 0.9000 | 1.2490 |
| 7 | DSH 22 | 2 | 0.8000 | 1.1071 |
| 7 | DSH 22 | 3 | 0.7000 | 0.9912 |
| 8 | DSH 26 | 1 | 0.8000 | 1.1071 |
| 8 | DSH 26 | 2 | 0.9000 | 1.2490 |
| 8 | DSH 26 | 3 | 0.8000 | 1.1071 |

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | 1.107 | 1.412 | 1.256 |
| 2 | DSH 30 | 3 | 1.249 | 1.412 | 1.358 |
| 3 | DSH 02 | 3 | 1.249 | 1.412 | 1.303 |
| 4 | DSH 06 | 3 | 1.249 | 1.412 | 1.358 |
| 5 | DSH 07 | 3 | 1.107 | 1.249 | 1.202 |
| 6 | DSH 14 | 3 | 0.580 | 0.886 | 0.717 |
| 7 | DSH 22 | 3 | 0.991 | 1.249 | 1.116 |
| 8 | DSH 26 | 3 | 1.107 | 1.249 | 1.154 |

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | CONTROL | 0.023 | 0.153 | 0.088 | 12.15 |
| 2 | DSH 30 | 0.009 | 0.094 | 0.054 | 6.93 |
| 3 | DSH 02 | 0.009 | 0.094 | 0.054 | 7.22 |
| 4 | DSH 06 | 0.009 | 0.094 | 0.054 | 6.93 |
| 5 | DSH 07 | 0.007 | 0.082 | 0.047 | 6.82 |
| 6 | DSH 14 | 0.024 | 0.156 | 0.090 | 21.72 |
| 7 | DSH 22 | 0.017 | 0.129 | 0.075 | 11.58 |
| 8 | DSH 26 | 0.007 | 0.082 | 0.047 | 7.10 |

```
93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
------------------------------------------------------------------------------

D =    0.208

W =    0.952

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.
```

```
93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

------------------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    1.74

------------------------------------------------------------------------------
Table Chi-square value =   18.48  (alpha = 0.01, df =   7)
Table Chi-square value =   14.07  (alpha = 0.05, df =   7)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 7 | 0.912 | 0.130 | 10.000 |
| Within (Error) | 16 | 0.208 | 0.013 | |
| Total | 23 | 1.120 | | |

Critical F value = 2.66 (0.05,7,16)
Since F > Critical F REJECT Ho: All equal

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | CONTROL | 1.256 | 0.900 | | |
| 2 | DSH 30 | 1.358 | 0.967 | -1.091 | |
| 3 | DSH 02 | 1.303 | 0.933 | -0.508 | |
| 4 | DSH 06 | 1.358 | 0.967 | -1.091 | |
| 5 | DSH 07 | 1.202 | 0.867 | 0.583 | |
| 6 | DSH 14 | 0.717 | 0.433 | 5.787 | * |
| 7 | DSH 22 | 1.116 | 0.800 | 1.506 | |
| 8 | DSH 26 | 1.154 | 0.833 | 1.091 | |

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

93 MUDPUPPY RUN #3A CHIRONOMIDS 10/18/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | | | |
| 2 | DSH 30 | 3 | 0.180 | 20.0 | -0.067 |
| 3 | DSH 02 | 3 | 0.180 | 20.0 | -0.033 |
| 4 | DSH 06 | 3 | 0.180 | 20.0 | -0.067 |
| 5 | DSH 07 | 3 | 0.180 | 20.0 | 0.033 |
| 6 | DSH 14 | 3 | 0.180 | 20.0 | 0.467 |
| 7 | DSH 22 | 3 | 0.180 | 20.0 | 0.100 |
| 8 | DSH 26 | 3 | 0.180 | 20.0 | 0.067 |

```
93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
4
3
3
3
3
CONTROL
0.80000000
0.90000000
1.00000000
DSH 17
1.0
0.9
0.8
DSH 04
0.90000000
0.80000000
0.90000000
DSH 13
1.00000000
0.90000000
0.90000000
```

TITLE: 93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 4

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | CONTROL | 1 | 0.8000 | 1.1071 |
| 1 | CONTROL | 2 | 0.9000 | 1.2490 |
| 1 | CONTROL | 3 | 1.0000 | 1.4120 |
| 2 | DSH 17 | 1 | 1.0000 | 1.4120 |
| 2 | DSH 17 | 2 | 0.9000 | 1.2490 |
| 2 | DSH 17 | 3 | 0.8000 | 1.1071 |
| 3 | DSH 04 | 1 | 0.9000 | 1.2490 |
| 3 | DSH 04 | 2 | 0.8000 | 1.1071 |
| 3 | DSH 04 | 3 | 0.9000 | 1.2490 |
| 4 | DSH 13 | 1 | 1.0000 | 1.4120 |
| 4 | DSH 13 | 2 | 0.9000 | 1.2490 |
| 4 | DSH 13 | 3 | 0.9000 | 1.2490 |

93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | 1.107 | 1.412 | 1.256 |
| 2 | DSH 17 | 3 | 1.107 | 1.412 | 1.256 |
| 3 | DSH 04 | 3 | 1.107 | 1.249 | 1.202 |
| 4 | DSH 13 | 3 | 1.249 | 1.412 | 1.303 |

93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | CONTROL | 0.023 | 0.153 | 0.088 | 12.15 |
| 2 | DSH 17 | 0.023 | 0.153 | 0.088 | 12.15 |
| 3 | DSH 04 | 0.007 | 0.082 | 0.047 | 6.82 |
| 4 | DSH 13 | 0.009 | 0.094 | 0.054 | 7.22 |

93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 3 | 0.016 | 0.005 | 0.333 |
| Within (Error) | 8 | 0.124 | 0.016 | |
| Total | 11 | 0.140 | | |

Critical F value = 4.07 (0.05,3,8)
Since F < Critical F FAIL TO REJECT Ho: All equal

```
93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
-----------------------------------------------------------------------------

D =    0.124

W =    0.939

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805
-----------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

-----------------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    0.98

-----------------------------------------------------------------------------
Table Chi-square value =   11.34  (alpha = 0.01, df =   3)
Table Chi-square value =    7.81  (alpha = 0.05, df =   3)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

**93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment**

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | CONTROL | 1.256 | 0.900 | | |
| 2 | DSH 17 | 1.256 | 0.900 | 0.000 | |
| 3 | DSH 04 | 1.202 | 0.867 | 0.534 | |
| 4 | DSH 13 | 1.303 | 0.933 | -0.465 | |

**Dunnett table value = 2.42 (1 Tailed Value, P=0.05, df=8,3)**

**93 MUDPUPPY RUN #3B CHIRONOMIDS 10/19/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR3CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment**

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | | | |
| 2 | DSH 17 | 3 | 0.187 | 20.8 | 0.000 |
| 3 | DSH 04 | 3 | 0.187 | 20.8 | 0.033 |
| 4 | DSH 13 | 3 | 0.187 | 20.8 | -0.033 |

# ACUTE TOXICITY TESTS
# WITH
# *HYALELLA AZTECA* AND *CHIRONOMUS TENTANS*
# ON SEDIMENTS FROM THE DULUTH/SUPERIOR HARBOR:
1993 Sampling Results - Batches # 5 and 6

Conducted by

Minnesota Pollution Control Agency
Monitoring and Assessment Section
520 Lafayette Road
St. Paul, Minnesota  55155-4194

February 1997

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## INTRODUCTION

As part of the 1993 survey of sediment quality in the Duluth/Superior Harbor, sediment toxicity tests were conducted to assess acute (survival) and chronic (growth) toxicity to benthic invertebrates. Acute effects were measured in separate 10-day toxicity tests to *Hyalella azteca* (*H. azteca*) and *Chironomus tentans* (*C. tentans*). Growth was measured at the end of the *C. tentans* test to assess chronic effects. Survival and growth endpoints were compared to organisms similarly exposed to a reference control sediment collected from West Bearskin Lake (Cook County, MN).

A total of 40 sediment samples were collected for toxicity testing. This report presents the results of twelve of these sediment samples run in two separate batches with separate controls.

## SAMPLE COLLECTION AND HANDLING

During September 22-27, 1993, Minnesota Pollution Control Agency (MPCA) staff collected the twelve sediments referred to in this report. The samples were collected from the harbor using a Ponar sampler and were taken to the University of Minnesota-Duluth Chemical Toxicology Research Laboratory. The samples were stored at 4°C until they were transported to the MPCA Toxicology Laboratory in St. Paul, MN.

## METHODS

Twelve sediment samples and two control sediment samples were subjected to the 10-day sediment toxicity tests using the modified procedures described in ASTM (1993). However, the specific test system used for these assays is not indicated in the methods. The test organisms (*H. azteca* and *C. tentans*) were exposed to sediment samples for ten days in a portable, mini-flow system described in Benoit et al. (1993). The test apparatus consists of 300 mL, glass-beaker test chambers held in a glass box supplied with water from an acrylic plastic headbox. The beakers have two, 1.5 cm holes covered with stainless steel mesh, to allow for water exchange, while containing the test organisms. The headbox has a pipette tip drain calibrated to deliver water at an average rate of 32.5 mL/min. The glass box is fitted with a self-starting siphon to provide exchange of overlying water.

The *H. azteca* used for this test were 1 to 3 mm long, and the *C. tentans* were approximately 14 days old. These organisms were supplied by Environmental Consulting and Testing, Superior, WI on the day of the test.

On November 1, 1993, eight samples (DSH 05, DSH 09, DSH 10, DSH 11, DSH 25, DSH 27, DSH 31, and DSH 32) and the control sediment were separately homogenized by hand, and 100 mL of each sediment was placed in a test beaker (Batch #5). On November 2, 1993, four more samples (DSH 15, DSH 28, DSH 34, and DSH 35) and another control sediment were homogenized and placed in beakers (Batch #6). Each sediment test was set up with three replicates of *H. azteca* and three replicates of *C. tentans.* Aerated, artesian well water was added

to the beakers, and the sediments were allowed to settle for approximately two hours before the organisms were added. For each toxicity test, ten organisms were placed in each beaker in a random fashion.

The organisms were exposed to 16 hours of light and eight hours of darkness for the duration of the ten-day test. Each day, two liters of aerated water from the artesian well at Stroh Brewery in St. Paul, MN were exchanged in each test chamber. On weekdays, 1-L was exchanged in the morning and 1-L in the afternoon. On weekends, the two liters were passed through the chambers all at once. Water quality measurements (i.e., pH, temperature, and dissolved oxygen) of the overlying water were taken in one beaker of each of the triplicate sets of each of the sediments. The results, along with daily observations involving the physical appearance of the sediments and organisms, were recorded in a laboratory notebook. This notebook is retained on file at the MPCA.

The test was terminated on November 11, 1993 for Batch #5 and on November 12, 1993 for Batch #6. The sediments were sieved through 40 mesh screens, and the sieved material was sorted for organisms. The organisms found were counted, and the number of alive and dead organisms were recorded. Organisms not found were recorded as missing and presumed dead. The *C. tentans* that survived were placed in aluminum weighing dishes, dried at approximately 90°C for at least four hours, desiccated to room temperature, and weighed.

Growth (weight) of the *C. tentans* and survival of both organisms were used as the endpoints for these tests. The resulting survival data were analyzed using TOXSTAT (Gulley and WEST, Inc., 1994), a statistical software package obtained from the University of Wyoming; however, due to a quality assurance problem, the growth data were not analyzed.

A 96-hour, reference toxicant test with *H. azteca* in sodium chloride (NaCl) was run in conjunction with these toxicity tests to determine the acceptability of the *H. azteca* used. Four concentrations of NaCl solution (i.e., 5, 2.5, 1.25, and 0.625 g/L) and a control (aerated, artesian well water) were used in this test. Three replicates of five organisms each were set up per concentration.

## RESULTS

### <u>Water Quality</u>

Measurements of pH, dissolved oxygen, and temperature in the overlying water of the test beakers were made daily. These measurements are summarized below and in Tables 1, 2, and 3, respectively, for both batches of tests.

#### *Batch # 5 Water Chemistry*

In Batch #5, the range of pH values in the beakers containing *H. azteca* was 7.0 to 8.6 (Table 1). The water in the *C. tentans* beakers had a pH range of 6.8 to 8.6 (Table 1). The pH fluctuation

during these tests was acceptable since it did not vary more than 50% within each treatment (U.S. EPA, 1994).

The dissolved oxygen concentration ranged from 5.5 to 7.4 mg/L in the *H. azteca* beakers and from 2.3 to 7.3 mg/L in the *C. tentans* beakers (Table 2). The recommended dissolved oxygen concentration for these tests is greater than 40% saturation. The dissolved oxygen dipped below 40% saturation on day 6 in most of the *C. tentans* beakers (i.e., the control, DSH 9, 10, 11, 25, 27, 31, and 32) and in the control on days 8 and 9. Feeding of the organisms was suspended on these days. The chambers were not aerated.

The range of temperature values in the *H. azteca* beakers was 19.0°C to 21.0°C, whereas the range was 18.9°C to 21.0°C in the *C. tentans* beakers (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

*Batch #6 Water Chemistry*

In Batch #6, the range of pH values in the beakers containing *H. azteca* was 7.8 to 8.4 (Table 1). The water in the *C. tentans* beakers had a pH range of 7.5 to 8.3 (Table 1). These pH ranges are acceptable for these tests.

The dissolved oxygen concentration ranged from 5.0 to 7.9 mg/L in the *H. azteca* beakers and from 2.2 to 8.0 mg/L in the *C. tentans* beakers (Table 2). The dissolved oxygen in some of the *C. tentans* chambers dropped below 40% saturation. Levels were lower than acceptable on day 5 in chambers holding sediments DSH 15, 28, and 35. On days 7, 8, and 9, levels were too low in DSH 35. Dissolved oxygen levels were unacceptable in the control on days 8 and 9. Feeding of the organisms was suspended on these days. The chambers were not aerated.

The range of temperature values in the *H. azteca* beakers was 18.9°C to 21.0°C, whereas the range was 18.9°C to 21.0°C in the *C. tentans* beakers (Table 3). The recommended temperature range for this test is 23 ± 1°C (U.S. EPA, 1994).

<u>Test Endpoints</u>

The mean percent survival of test organisms is summarized below and in Table 4.

*Batch #5 Survival Data*

The mean percent survival of *H. azteca* in Control #5 was 97% with a range of 90% to 100%. For the control sediment containing *C. tentans*, percent survival ranged from 70% to 80% with a mean of 77%. Survival for these controls was acceptable, and both tests passed.

Mean percent survival of *H. azteca* in the test sediments of Batch #5 ranged from 83% in the DSH 09 sample to 100% in the DSH 27 sample. Mean percent survival of *C. tentans* in Batch #5 test sediments ranged from 73% in the DSH 31 sample to 97% in the DSH 25 sample.

*Batch #6 Survival Data*

For Control #6 containing *H. azteca*, the mean percent survival was 87% with a range of 80% to 90%. For the control sediment containing *C. tentans*, the range was 90% to 100% with a mean of 97%. Both of these survival measurements were acceptable.

Mean percent survival of *H. azteca* in Batch #6 ranged from 77% in the DSH 34 and DSH 35 samples to 97% in the DSH 28 sample. Mean percent survival of *C. tentans* in Batch #6 ranged from 47% in the DSH 34 sample to 93% in the DSH 35 sample.

*C. tentans Growth Data*

Although the dried *C. tentans* were weighed, the balance on which they were weighed was not calibrated with standard weights; therefore, the data are suspect since the internal calibration of the balance may have drifted with time and no conclusions regarding chronic toxicity (growth) can be made.

Data Analysis

Survival data for all of the sediments tested, except DSH 05 containing *C. tentans* and DSH 15, 25 and 27 containing *H. azteca*, were transformed using an arc sine-square root transformation before being analyzed statistically using Dunnett's test. The aforementioned samples were eliminated from the analysis because there was zero variance between replicates. Although nonparmetric statistics can be used to analyze zero variance data, a minimum of four replicates per sediment is needed. Only three replicates per sediment were run in these toxicity tests. Since it is assumed that variability of 30-50% is necessary to see any significant difference between the control and any given sediment, and since survival of the organisms in the sediments in question was equal to or greater than 90%, it is reasonable to assume that the effect these sediments had on the organisms tested was not significantly less than that of their respective controls (T. Norberg-King, USEPA, Duluth, MN, personal communication).

The mean percent survival of *C. tentans* in the DSH 34 sample was significantly less than the control as determined by a 1-tailed Dunnett's test, p=0.05. The survival results of all other organisms in all other samples run in these tests were not significantly less than their respective controls. Results of the statistical analysis of the data are included in Appendix A.

Reference Toxicant Test with *Hyalella azteca* in Sodium Chloride Solution

The pH of the overlying water in the reference toxicant test ranged from 7.8 to 8.5. The dissolved oxygen ranged from 7.8 to 8.5 mg/L, and the temperature ranged between 19.0°C and 20.0°C. Mean percent survival of the organisms in the control was less than 90% (i.e., 73%) which was unacceptable. Thus, the reference toxicant test failed. The cause of this failure could not be determined. Since the control survivals in Batch #5 and Batch #6 were acceptable, the organisms appeared to be healthy.

## SUMMARY

Survival of *H. azteca* in the control sediments was acceptable (greater than 80%), however, the reference toxicant test failed, leaving the health of the organisms suspect and, therefore, no conclusion can be drawn about the effect that the sediments had on *H. azteca*.

Control survival was acceptable in both batches of samples containing *C. tentans*. The mean percent survival of *C. tentans* in the DSH 34 sample was significantly less than the control (p=0.05). Survival of *C. tentans* in all other samples analyzed were not significantly different than the control.

## REFERENCES


ASTM. 1993. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. E1383-93. In *Annual Book of ASTM Standards*, *Vol. 11.04*. American Society for Testing and Materials, Philadelphia, PA. pp. 1173-1199.

Benoit, D.A., G. Phipps, and G.T. Ankley. 1993. A sediment testing intermittent renewal system for the automated renewal of overlying water in toxicity tests with contaminated sediments. Water Research 27:1403-1412.

Gulley, D.D. and WEST, Inc. 1994. TOXSTAT 3.4. WEST, Inc., Cheyenne, WY.

U.S. EPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN. EPA/600/R-94/024.

**TABLE 1. Daily Overlying Water pH Measurements**

**Batch #5**

| | Control 5 | | DSH 05 | | DSH 09 | | DSH 10 | | DSH 11 | | DSH 25 | | DSH 27 | | DSH 31 | | DSH 32 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.8 | 7.9 | 7.9 | 7.9 | 7.8 | 7.9 | 7.8 | 7.8 | 7.8 | 7.8 | 7.4 | 7.4 | 7.8 | 7.8 | 7.8 | 7.9 | 6.9 | 7.0 |
| 1 | 7.6 | 7.9 | 7.9 | 7.9 | 8.0 | 8.0 | 7.6 | 7.6 | 7.2 | 7.4 | 7.8 | 7.8 | 7.9 | 7.8 | 7.7 | 7.8 | 7.8 | 7.8 |
| 2 | 7.8 | 7.9 | 7.9 | 8.1 | 7.9 | 8.3 | 7.7 | 7.9 | 7.6 | 7.8 | 7.6 | 8.0 | 8.0 | 8.1 | 7.8 | 7.9 | 7.8 | 8.0 |
| 3 | 7.7 | 7.9 | 7.9 | 8.1 | 7.9 | 8.2 | 7.6 | 7.9 | 7.6 | 8.0 | 7.7 | 8.1 | 7.9 | 7.9 | 7.8 | 7.9 | 7.6 | 7.9 |
| 4 | 7.7 | 8.0 | 8.2 | 8.3 | 7.8 | 8.2 | 7.8 | 8.0 | 7.6 | 8.0 | 7.7 | 8.0 | 8.6 | 8.6 | 8.0 | 8.1 | 7.8 | 8.1 |
| 5 | 7.7 | 8.0 | 7.8 | 8.0 | 7.8 | 8.1 | 7.8 | 7.9 | 6.8 | 7.2 | 7.7 | 7.9 | 7.8 | 8.0 | 7.7 | 7.9 | 7.8 | 7.9 |
| 6 | 7.4 | 7.8 | 7.7 | 8.1 | 7.7 | 8.1 | 7.5 | 7.8 | 7.6 | 7.9 | 7.6 | 7.7 | 7.7 | 7.7 | 7.6 | 7.8 | 7.6 | 7.8 |
| 7 | 7.6 | 8.1 | 8.0 | 8.3 | 8.1 | 8.2 | 7.8 | 8.0 | 7.9 | 8.1 | 7.8 | 8.0 | 8.0 | 8.3 | 7.9 | 8.0 | 7.7 | 8.1 |
| 8 | 7.6 | 7.9 | 7.9 | 8.2 | 8.1 | 8.2 | 7.8 | 8.0 | 7.7 | 8.0 | 7.8 | 7.9 | 7.9 | 8.2 | 7.9 | 8.0 | 7.8 | 8.0 |
| 9 | 7.6 | 8.0 | 7.8 | 8.1 | 8.0 | 8.2 | 7.7 | 7.9 | 7.7 | 7.9 | 7.8 | 7.9 | 8.0 | 8.2 | 7.8 | 7.9 | 7.8 | 8.0 |
| Mean | 7.7 | 7.9 | 7.9 | 8.1 | 7.9 | 8.1 | 7.7 | 7.9 | 7.6 | 7.8 | 7.7 | 7.9 | 8.0 | 8.1 | 7.8 | 7.9 | 7.7 | 7.9 |
| Range | 7.4-7.8 | 7.8-8.1 | 7.7-8.2 | 7.9-8.3 | 7.7-8.1 | 7.9-8.3 | 7.5-7.8 | 7.6-8.0 | 6.8-7.9 | 7.2-8.1 | 7.4-7.8 | 7.4-8.1 | 7.7-8.6 | 7.7-8.6 | 7.6-8.0 | 7.8-8.1 | 6.9-7.8 | 7.0-8.1 |

**Batch #6**

| | Control 6 | | DSH 15 | | DSH 28 | | DSH 34 | | DSH 35 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.5 | 7.8 | 8.3 | 8.3 | 8.2 | 8.1 | 8.0 | 8.0 | 8.0 | 8.1 |
| 1 | 7.8 | 8.0 | 8.2 | 8.3 | 7.6 | 7.8 | 7.7 | 7.8 | 7.8 | 8.0 |
| 2 | 7.8 | 8.0 | 8.2 | 8.3 | 7.7 | 8.0 | 7.8 | 7.9 | 7.8 | 8.0 |
| 3 | 7.8 | 8.1 | 8.2 | 8.4 | 7.9 | 8.2 | 8.1 | 8.0 | 7.8 | 8.1 |
| 4 | 7.7 | 7.9 | 7.8 | 8.0 | 7.8 | 7.8 | 7.7 | 7.8 | 7.8 | 7.8 |
| 5 | 7.5 | 7.8 | 7.9 | 8.2 | 7.5 | 7.9 | 7.8 | 7.8 | 7.6 | 7.8 |
| 6 | 7.8 | 8.1 | 8.1 | 8.4 | 7.8 | 8.0 | 7.9 | 8.0 | 7.9 | 8.0 |
| 7 | 7.7 | 8.1 | 8.0 | 8.2 | 7.7 | 8.0 | 7.8 | 8.0 | 7.8 | 7.9 |
| 8 | 7.6 | 8.0 | 7.9 | 8.2 | 7.6 | 8.0 | 7.7 | 7.9 | 7.7 | 8.1 |
| 9 | 7.8 | 8.1 | 8.0 | 8.2 | 7.8 | 8.1 | 7.7 | 7.9 | 7.8 | 8.0 |
| Mean | 7.7 | 8.0 | 8.1 | 8.3 | 7.8 | 8.0 | 7.8 | 7.9 | 7.8 | 8.0 |
| Range | 7.5-7.8 | 7.8-8.1 | 7.8-8.3 | 8.0-8.4 | 7.5-8.2 | 7.8-8.2 | 7.7-8.1 | 7.8-8.0 | 7.6-8.0 | 7.8-8.1 |

**TABLE 2. Daily Overlying Water Dissolved Oxygen Concentrations (mg/L)**

**Batch #5**

| | Control 5 | | DSH 05 | | DSH 09 | | DSH 10 | | DSH 11 | | DSH 25 | | DSH 27 | | DSH 31 | | DSH 32 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.0 | 6.5 | 7.3 | 7.1 | 7.3 | 6.9 | 7.0 | 6.3 | 6.9 | 6.5 | 6.9 | 6.8 | 7.3 | 6.7 | 6.7 | 6.3 | 6.7 | 6.2 |
| 1 | 6.1 | 6.8 | 6.6 | 6.9 | 6.6 | 6.8 | 6.2 | 6.1 | 6.0 | 6.5 | 6.3 | 6.8 | 6.9 | 6.6 | 6.3 | 6.3 | 6.4 | 6.5 |
| 2 | 5.2 | 6.3 | 5.4 | 6.4 | 4.3 | 6.7 | 5.3 | 6.2 | 4.6 | 6.1 | 4.0 | 6.0 | 5.7 | 6.1 | 5.7 | 6.2 | 4.6 | 6.9 |
| 3 | 4.3 | 6.9 | 4.6 | 6.7 | 4.9 | 6.9 | 4.5 | 6.6 | 4.5 | 6.6 | 4.0 | 6.5 | 5.4 | 6.4 | 4.3 | 6.1 | 4.2 | 6.1 |
| 4 | 4.6 | 6.8 | 4.7 | 6.4 | 4.4 | 7.0 | 4.5 | 6.8 | 4.3 | 6.8 | 4.9 | 6.3 | 5.8 | 6.6 | 4.8 | 6.4 | 5.2 | 6.4 |
| 5 | 4.5 | 6.9 | 4.6 | 6.7 | 3.9 | 7.0 | 5.2 | 6.7 | 4.3 | 6.5 | 3.6 | 6.0 | 4.9 | 6.9 | 4.3 | 6.4 | 4.5 | 6.3 |
| 6 | 2.9 | 6.3 | 3.5 | 6.8 | 2.4 | 6.6 | 2.8 | 5.8 | 2.9 | 6.5 | 2.3 | 5.5 | 3.1 | 6.6 | 2.6 | 5.9 | 2.9 | 5.9 |
| 7 | 3.6 | 6.8 | 3.4 | 7.1 | 3.4 | 7.1 | 3.4 | 6.4 | 3.4 | 7.0 | 4.4 | 6.4 | 4.3 | 6.7 | 4.7 | 6.6 | 4.0 | 6.7 |
| 8 | 3.1 | 6.4 | 4.4 | 7.4 | 3.4 | 7.0 | 4.0 | 6.5 | 4.0 | 7.0 | 3.6 | 6.4 | 4.5 | 7.0 | 4.5 | 6.1 | 4.6 | 6.8 |
| 9 | 2.9 | 6.3 | 4.0 | 7.3 | 5.7 | 7.0 | 3.4 | 6.5 | 4.5 | 6.9 | 3.9 | 6.5 | 4.0 | 7.0 | 3.6 | 5.9 | 4.8 | 6.6 |
| Mean | 4.4 | 6.6 | 4.9 | 6.9 | 4.6 | 6.9 | 4.6 | 6.4 | 4.5 | 6.6 | 4.4 | 6.3 | 5.2 | 6.7 | 4.8 | 6.2 | 4.8 | 6.4 |
| Range | 2.9-7.0 | 6.3-6.9 | 3.4-7.3 | 6.4-7.4 | 2.4-7.3 | 6.6-7.1 | 2.8-7.0 | 5.8-6.8 | 2.9-6.9 | 6.1-7.0 | 2.3-6.9 | 5.5-6.8 | 3.1-7.3 | 6.1-7.0 | 2.6-6.7 | 5.9-6.6 | 2.9-6.7 | 5.9-6.9 |

**Batch #6**

| | Control 6 | | DSH 15 | | DSH 28 | | DSH 34 | | DSH 35 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.4 | 7.4 | 8.0 | 7.9 | 7.8 | 7.6 | 7.6 | 7.5 | 7.5 | 7.6 |
| 1 | 5.6 | 6.3 | 5.8 | 6.3 | 5.6 | 6.6 | 5.5 | 5.9 | 5.3 | 6.1 |
| 2 | 5.5 | 6.7 | 6.0 | 6.8 | 5.0 | 6.6 | 5.8 | 6.4 | 5.2 | 6.4 |
| 3 | 5.2 | 6.7 | 6.0 | 7.1 | 4.7 | 6.9 | 5.7 | 6.4 | 4.3 | 6.3 |
| 4 | 4.8 | 6.6 | 4.6 | 6.7 | 5.2 | 6.5 | 4.9 | 6.4 | 4.1 | 6.6 |
| 5 | 4.0 | 6.4 | 2.8 | 6.6 | 3.3 | 6.2 | 4.8 | 5.9 | 2.2 | 5.4 |
| 6 | 4.5 | 6.6 | 4.4 | 7.2 | 4.6 | 7.0 | 5.3 | 6.4 | 3.6 | 6.7 |
| 7 | 5.1 | 6.8 | 4.8 | 7.0 | 4.5 | 6.9 | 5.7 | 6.1 | 3.2 | 7.1 |
| 8 | 3.2 | 6.4 | 6.0 | 6.8 | 3.6 | 6.8 | 3.8 | 5.0 | 3.0 | 6.4 |
| 9 | 3.2 | 6.2 | 4.6 | 6.5 | 3.6 | 6.5 | 4.3 | 5.6 | 2.6 | 6.0 |
| Mean | 4.9 | 6.6 | 5.3 | 6.9 | 4.8 | 6.8 | 5.3 | 6.2 | 4.1 | 6.5 |
| Range | 3.2-7.4 | 6.2-7.4 | 2.8-8.0 | 6.3-7.9 | 3.3-7.8 | 6.2-7.6 | 3.8-7.6 | 5.0-7.5 | 2.2-7.5 | 5.4-7.6 |

## Table 3. Daily Overlying Water Temperatures (Degrees Celsius)

### Batch #5

| | Control 5 | | DSH 05 | | DSH 09 | | DSH 10 | | DSH 11 | | DSH 25 | | DSH 27 | | DSH 31 | | DSH 32 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| 1 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 | 21.0 | 21.0 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| 2 | 21.0 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 21.0 | 21.0 | 21.0 | 21.0 | 20.5 | 20.5 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 |
| 3 | 19.9 | 19.9 | 19.8 | 19.8 | 20.1 | 20.1 | 19.9 | 19.9 | 20.1 | 20.1 | 20.1 | 20.1 | 19.9 | 19.9 | 19.6 | 19.9 | 20.4 | 20.4 |
| 4 | 19.2 | 19.2 | 19.4 | 19.4 | 19.5 | 19.6 | 19.3 | 19.2 | 19.8 | 19.2 | 19.5 | 19.5 | 19.4 | 19.4 | 19.5 | 19.5 | 19.5 | 19.5 |
| 5 | 20.5 | 20.5 | 20.5 | 20.5 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 | 21.0 | 21.0 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| 6 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 7 | 19.3 | 19.3 | 19.3 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.7 | 19.7 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.3 | 19.4 |
| 8 | 19.0 | 19.0 | 19.0 | 19.0 | 19.1 | 19.3 | 19.1 | 19.1 | 19.3 | 19.3 | 18.9 | 19.0 | 19.1 | 19.1 | 19.0 | 19.1 | 19.1 | 19.0 |
| 9 | 19.2 | 19.2 | 19.3 | 19.3 | 19.3 | 19.6 | 19.3 | 19.3 | 19.5 | 19.5 | 19.2 | 19.4 | 19.1 | 19.3 | 19.3 | 19.4 | 19.2 | 19.4 |
| Mean | 20.0 | 20.0 | 20.0 | 20.0 | 20.1 | 20.1 | 20.1 | 20.1 | 20.2 | 20.1 | 20.0 | 20.1 | 20.0 | 20.0 | 19.9 | 20.0 | 20.0 | 20.0 |
| Range | 19.0-21.0 | 19.0-21.0 | 19.0-21.0 | 19.0-21.0 | 19.1-21.0 | 19.3-21.0 | 19.1-21.0 | 19.1-21.0 | 19.3-21.0 | 19.2-21.0 | 18.9-21.0 | 19.0-21.0 | 19.1-21.0 | 19.1-21.0 | 19.0-21.0 | 19.1-21.0 | 19.1-21.0 | 19.0-21.0 |

### Batch #6

| | Control 6 | | DSH 15 | | DSH 28 | | DSH 34 | | DSH 35 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 20.5 | 20.5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 1 | 20.5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 2 | 19.5 | 20.2 | 20.5 | 20.5 | 20.5 | 20.5 | 20.2 | 20.6 | 20.0 | 20.3 |
| 3 | 19.6 | 19.6 | 19.8 | 19.9 | 20.0 | 20.0 | 19.8 | 19.7 | 19.6 | 19.8 |
| 4 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 5 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| 6 | 19.4 | 19.7 | 19.6 | 20.1 | 20.0 | 20.1 | 19.7 | 19.8 | 19.6 | 19.6 |
| 7 | 18.9 | 19.3 | 19.5 | 19.6 | 19.6 | 19.7 | 19.5 | 19.5 | 19.4 | 19.4 |
| 8 | 19.5 | 19.7 | 19.6 | 19.6 | 19.8 | 20.0 | 19.6 | 19.8 | 19.6 | 19.8 |
| 9 | 20.0 | 20.0 | 19.7 | 20.0 | 20.1 | 20.3 | 20.0 | 20.0 | 20.0 | 19.9 |
| Mean | 20.0 | 20.2 | 20.3 | 20.4 | 20.4 | 20.5 | 20.2 | 20.3 | 20.2 | 20.3 |
| Range | 18.9-21.0 | 19.3-21.0 | 19.5-21.0 | 19.6-21.0 | 19.6-21.0 | 19.7-21.0 | 19.5-21.0 | 19.5-21.0 | 19.4-21.0 | 19.4-21.0 |

**TABLE 4. Mean Percent Survival of *Hyalella azteca* and *Chironomus tentans***

| | Mean Percent Survival | |
|---|---|---|
| | *Hyalella azteca* | *Chironomus tentans* |
| Batch # 5 | | |
| CONTROL #5 | 97% | 77% |
| DSH 05 | 87% | 90% |
| DSH 09 | 83% | 87% |
| DSH 10 | 93% | 90% |
| DSH 11 | 93% | 87% |
| DSH 25 | 90% | 97% |
| DSH 27 | 100% | 83% |
| DSH 31 | 90% | 73% |
| DSH 32 | 93% | 77% |
| Batch # 6 | | |
| CONTROL #6 | 87% | 97% |
| DSH 15 | 90% | 83% |
| DSH 28 | 97% | 93% |
| DSH 34 | 77% | 47% * |
| DSH 35 | 77% | 93% |

* Significantly different from the control, p=0.05.

# APPENDIX A

## TOXSTAT Analysis

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
8
3
3
3
3
3
3
3
3
CONTROL
0.8
0.7
0.8
DSH 09
0.7
0.9
1.0
DSH 10
0.9
1.0
0.8
DSH 11
0.8
1.0
0.8
DSH 25
0.9
1.0
1.0
DSH 27
0.9
0.7
0.9
DSH 31
0.8
0.6
0.8
DSH 32
0.7
0.8
0.8

TITLE: 93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
FILE: 93MPR4CA.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 8

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | CONTROL | 1 | 0.8000 | 1.1071 |
| 1 | CONTROL | 2 | 0.7000 | 0.9912 |
| 1 | CONTROL | 3 | 0.8000 | 1.1071 |
| 2 | DSH 09 | 1 | 0.7000 | 0.9912 |
| 2 | DSH 09 | 2 | 0.9000 | 1.2490 |
| 2 | DSH 09 | 3 | 1.0000 | 1.4120 |
| 3 | DSH 10 | 1 | 0.9000 | 1.2490 |
| 3 | DSH 10 | 2 | 1.0000 | 1.4120 |
| 3 | DSH 10 | 3 | 0.8000 | 1.1071 |
| 4 | DSH 11 | 1 | 0.8000 | 1.1071 |
| 4 | DSH 11 | 2 | 1.0000 | 1.4120 |
| 4 | DSH 11 | 3 | 0.8000 | 1.1071 |
| 5 | DSH 25 | 1 | 0.9000 | 1.2490 |
| 5 | DSH 25 | 2 | 1.0000 | 1.4120 |
| 5 | DSH 25 | 3 | 1.0000 | 1.4120 |
| 6 | DSH 27 | 1 | 0.9000 | 1.2490 |
| 6 | DSH 27 | 2 | 0.7000 | 0.9912 |
| 6 | DSH 27 | 3 | 0.9000 | 1.2490 |
| 7 | DSH 31 | 1 | 0.8000 | 1.1071 |
| 7 | DSH 31 | 2 | 0.6000 | 0.8861 |
| 7 | DSH 31 | 3 | 0.8000 | 1.1071 |
| 8 | DSH 32 | 1 | 0.7000 | 0.9912 |
| 8 | DSH 32 | 2 | 0.8000 | 1.1071 |
| 8 | DSH 32 | 3 | 0.8000 | 1.1071 |

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | 0.991 | 1.107 | 1.068 |
| 2 | DSH 09 | 3 | 0.991 | 1.412 | 1.217 |
| 3 | DSH 10 | 3 | 1.107 | 1.412 | 1.256 |
| 4 | DSH 11 | 3 | 1.107 | 1.412 | 1.209 |
| 5 | DSH 25 | 3 | 1.249 | 1.412 | 1.358 |
| 6 | DSH 27 | 3 | 0.991 | 1.249 | 1.163 |
| 7 | DSH 31 | 3 | 0.886 | 1.107 | 1.033 |
| 8 | DSH 32 | 3 | 0.991 | 1.107 | 1.068 |

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | CONTROL | 0.004 | 0.067 | 0.039 | 6.27 |
| 2 | DSH 09 | 0.045 | 0.212 | 0.123 | 17.43 |
| 3 | DSH 10 | 0.023 | 0.153 | 0.088 | 12.15 |
| 4 | DSH 11 | 0.031 | 0.176 | 0.102 | 14.56 |
| 5 | DSH 25 | 0.009 | 0.094 | 0.054 | 6.93 |
| 6 | DSH 27 | 0.022 | 0.149 | 0.086 | 12.80 |
| 7 | DSH 31 | 0.016 | 0.128 | 0.074 | 12.35 |
| 8 | DSH 32 | 0.004 | 0.067 | 0.039 | 6.27 |

```
93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: 93MPR4CA.DAT       Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality
--------------------------------------------------------------------

D =    0.311

W =    0.950

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
--------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: 93MPR4CA.DAT       Transform: ARC SINE(SQUARE ROOT(Y))

--------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    3.84

---------------------------------------------------------------------
Table Chi-square value =   18.48  (alpha = 0.01, df =   7)
Table Chi-square value =   14.07  (alpha = 0.05, df =   7)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: 93MPR4CA.DAT        Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 7 | 0.257 | 0.037 | 1.888 |
| Within (Error) | 16 | 0.311 | 0.019 | |
| Total | 23 | 0.568 | | |

Critical F value = 2.66 (0.05,7,16)
Since F < Critical F FAIL TO REJECT Ho: All equal

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: 93MPR4CA.DAT        Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2        Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | CONTROL | 1.068 | 0.767 | | |
| 2 | DSH 09 | 1.217 | 0.867 | -1.308 | |
| 3 | DSH 10 | 1.256 | 0.900 | -1.648 | |
| 4 | DSH 11 | 1.209 | 0.867 | -1.232 | |
| 5 | DSH 25 | 1.358 | 0.967 | -2.540 | |
| 6 | DSH 27 | 1.163 | 0.833 | -0.831 | |
| 7 | DSH 31 | 1.033 | 0.733 | 0.308 | |
| 8 | DSH 32 | 1.068 | 0.767 | 0.000 | |

Dunnett table value = 2.56     (1 Tailed Value, P=0.05, df=16,7)

93 MUDPUPPY RUN 34 CHIRONOMIDS 11/01/93
File: 93MPR4CA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | CONTROL | 3 | | | |
| 2 | DSH 09 | 3 | 0.277 | 36.1 | -0.100 |
| 3 | DSH 10 | 3 | 0.277 | 36.1 | -0.133 |
| 4 | DSH 11 | 3 | 0.277 | 36.1 | -0.100 |
| 5 | DSH 25 | 3 | 0.277 | 36.1 | -0.200 |
| 6 | DSH 27 | 3 | 0.277 | 36.1 | -0.067 |
| 7 | DSH 31 | 3 | 0.277 | 36.1 | 0.033 |
| 8 | DSH 32 | 3 | 0.277 | 36.1 | 0.000 |

93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
5
3
3
3
3
3
control
1.0
0.9
1.0
dsh 15
0.8
1.0
0.7
dsh 28
1.0
0.9
0.9
dsh 34
0.4
0.5
0.5
dsh 35
0.9
0.9
1.0

```
93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
-------------------------------------------------------------------

D =    0.154

W =    0.942

Critical W (P = 0.05) (n = 15) = 0.881
Critical W (P = 0.01) (n = 15) = 0.835
--------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

--------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    3.45

--------------------------------------------------------------------
Table Chi-square value =   13.28  (alpha = 0.01, df =   4)
Table Chi-square value =    9.49  (alpha = 0.05, df =   4)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

TITLE: 93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 5

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | control | 1 | 1.0000 | 1.4120 |
| 1 | control | 2 | 0.9000 | 1.2490 |
| 1 | control | 3 | 1.0000 | 1.4120 |
| 2 | dsh 15 | 1 | 0.8000 | 1.1071 |
| 2 | dsh 15 | 2 | 1.0000 | 1.4120 |
| 2 | dsh 15 | 3 | 0.7000 | 0.9912 |
| 3 | dsh 28 | 1 | 1.0000 | 1.4120 |
| 3 | dsh 28 | 2 | 0.9000 | 1.2490 |
| 3 | dsh 28 | 3 | 0.9000 | 1.2490 |
| 4 | dsh 34 | 1 | 0.4000 | 0.6847 |
| 4 | dsh 34 | 2 | 0.5000 | 0.7854 |
| 4 | dsh 34 | 3 | 0.5000 | 0.7854 |
| 5 | dsh 35 | 1 | 0.9000 | 1.2490 |
| 5 | dsh 35 | 2 | 0.9000 | 1.2490 |
| 5 | dsh 35 | 3 | 1.0000 | 1.4120 |

93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | control | 3 | 1.249 | 1.412 | 1.358 |
| 2 | dsh 15 | 3 | 0.991 | 1.412 | 1.170 |
| 3 | dsh 28 | 3 | 1.249 | 1.412 | 1.303 |
| 4 | dsh 34 | 3 | 0.685 | 0.785 | 0.752 |
| 5 | dsh 35 | 3 | 1.249 | 1.412 | 1.303 |

**93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2**

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | control | 0.009 | 0.094 | 0.054 | 6.93 |
| 2 | dsh 15 | 0.047 | 0.217 | 0.126 | 18.58 |
| 3 | dsh 28 | 0.009 | 0.094 | 0.054 | 6.93 |
| 4 | dsh 34 | 0.003 | 0.058 | 0.034 | 7.73 |
| 5 | dsh 35 | 0.009 | 0.094 | 0.054 | 7.22 |

**93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**ANOVA TABLE**

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 4 | 0.784 | 0.196 | 12.701 |
| Within (Error) | 10 | 0.154 | 0.015 | |
| Total | 14 | 0.939 | | |

**Critical F value = 3.48 (0.05,4,10)**
**Since F > Critical F REJECT Ho: All equal**

93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.358 | 0.967 | | |
| 2 | dsh 15 | 1.170 | 0.833 | 1.849 | |
| 3 | dsh 28 | 1.303 | 0.933 | 0.000 | |
| 4 | dsh 34 | 0.752 | 0.467 | 5.972 | * |
| 5 | dsh 35 | 1.303 | 0.933 | 0.535 | |

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

93 MUDPUPPY RUN #4B CHIRONOMIDS 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4CB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | control | 3 | | | |
| 2 | dsh 15 | 3 | 0.155 | 16.1 | 0.133 |
| 3 | dsh 28 | 3 | 0.155 | 16.1 | 0.000 |
| 4 | dsh 34 | 3 | 0.155 | 16.1 | 0.500 |
| 5 | dsh 35 | 3 | 0.155 | 16.1 | 0.033 |

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
7
3
3
3
3
3
3
3
control
1.0
0.9
1.0
DSH 05
1.0
0.8
0.8
DSH 09
0.9
0.9
0.7
DSH 10
1.0
0.9
0.9
DSH 11
0.9
0.9
1.0
DSH 31
0.8
0.9
1.0
DSH 32
0.8
1.0
1.0

TITLE: 93 MUDPUPPY RUN #4A HYALELLA 11/01/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | control | 1 | 1.0000 | 1.4120 |
| 1 | control | 2 | 0.9000 | 1.2490 |
| 1 | control | 3 | 1.0000 | 1.4120 |
| 2 | DSH 05 | 1 | 1.0000 | 1.4120 |
| 2 | DSH 05 | 2 | 0.8000 | 1.1071 |
| 2 | DSH 05 | 3 | 0.8000 | 1.1071 |
| 3 | DSH 09 | 1 | 0.9000 | 1.2490 |
| 3 | DSH 09 | 2 | 0.9000 | 1.2490 |
| 3 | DSH 09 | 3 | 0.7000 | 0.9912 |
| 4 | DSH 10 | 1 | 1.0000 | 1.4120 |
| 4 | DSH 10 | 2 | 0.9000 | 1.2490 |
| 4 | DSH 10 | 3 | 0.9000 | 1.2490 |
| 5 | DSH 11 | 1 | 0.9000 | 1.2490 |
| 5 | DSH 11 | 2 | 0.9000 | 1.2490 |
| 5 | DSH 11 | 3 | 1.0000 | 1.4120 |
| 6 | DSH 31 | 1 | 0.8000 | 1.1071 |
| 6 | DSH 31 | 2 | 0.9000 | 1.2490 |
| 6 | DSH 31 | 3 | 1.0000 | 1.4120 |
| 7 | DSH 32 | 1 | 0.8000 | 1.1071 |
| 7 | DSH 32 | 2 | 1.0000 | 1.4120 |
| 7 | DSH 32 | 3 | 1.0000 | 1.4120 |

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | control | 3 | 1.249 | 1.412 | 1.358 |
| 2 | DSH 05 | 3 | 1.107 | 1.412 | 1.209 |
| 3 | DSH 09 | 3 | 0.991 | 1.249 | 1.163 |
| 4 | DSH 10 | 3 | 1.249 | 1.412 | 1.303 |
| 5 | DSH 11 | 3 | 1.249 | 1.412 | 1.303 |
| 6 | DSH 31 | 3 | 1.107 | 1.412 | 1.256 |
| 7 | DSH 32 | 3 | 1.107 | 1.412 | 1.310 |

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | control | 0.009 | 0.094 | 0.054 | 6.93 |
| 2 | DSH 05 | 0.031 | 0.176 | 0.102 | 14.56 |
| 3 | DSH 09 | 0.022 | 0.149 | 0.086 | 12.80 |
| 4 | DSH 10 | 0.009 | 0.094 | 0.054 | 7.22 |
| 5 | DSH 11 | 0.009 | 0.094 | 0.054 | 7.22 |
| 6 | DSH 31 | 0.023 | 0.153 | 0.088 | 12.15 |
| 7 | DSH 32 | 0.031 | 0.176 | 0.102 | 13.43 |

```
93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
----------------------------------------------------------------------

D =    0.268

W =    0.950

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
---------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.
```


```
93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))
```


```
----------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    1.69

---------------------------------------------------------------------
Table Chi-square value =   16.81  (alpha = 0.01, df =   6)
Table Chi-square value =   12.59  (alpha = 0.05, df =   6)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 6 | 0.081 | 0.013 | 0.703 |
| Within (Error) | 14 | 0.268 | 0.019 | |
| Total | 20 | 0.349 | | |

Critical F value = 2.85 (0.05,6,14)
Since F < Critical F FAIL TO REJECT Ho: All equal

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.358 | 0.967 | | |
| 2 | DSH 05 | 1.209 | 0.867 | 1.318 | |
| 3 | DSH 09 | 1.163 | 0.833 | 1.723 | |
| 4 | DSH 10 | 1.303 | 0.933 | 0.481 | |
| 5 | DSH 11 | 1.303 | 0.933 | 0.481 | |
| 6 | DSH 31 | 1.256 | 0.900 | 0.900 | |
| 7 | DSH 32 | 1.310 | 0.933 | 0.419 | |

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

93 MUDPUPPY RUN #4A HYALELLA 11/01/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HA.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | control | 3 | | | |
| 2 | DSH 05 | 3 | 0.184 | 19.1 | 0.100 |
| 3 | DSH 09 | 3 | 0.184 | 19.1 | 0.133 |
| 4 | DSH 10 | 3 | 0.184 | 19.1 | 0.033 |
| 5 | DSH 11 | 3 | 0.184 | 19.1 | 0.033 |
| 6 | DSH 31 | 3 | 0.184 | 19.1 | 0.067 |
| 7 | DSH 32 | 3 | 0.184 | 19.1 | 0.033 |

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
4
3
3
3
3
control
0.9
0.9
0.8
DSH 28
0.9
1.0
1.0
DSH 34
0.9
0.7
0.7
DSH 35
0.7
0.8
0.8

TITLE: 93 MUDPUPPY RUN #4B HYALELLA 11/02/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 4

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | control | 1 | 0.9000 | 1.2490 |
| 1 | control | 2 | 0.9000 | 1.2490 |
| 1 | control | 3 | 0.8000 | 1.1071 |
| 2 | DSH 28 | 1 | 0.9000 | 1.2490 |
| 2 | DSH 28 | 2 | 1.0000 | 1.4120 |
| 2 | DSH 28 | 3 | 1.0000 | 1.4120 |
| 3 | DSH 34 | 1 | 0.9000 | 1.2490 |
| 3 | DSH 34 | 2 | 0.7000 | 0.9912 |
| 3 | DSH 34 | 3 | 0.7000 | 0.9912 |
| 4 | DSH 35 | 1 | 0.7000 | 0.9912 |
| 4 | DSH 35 | 2 | 0.8000 | 1.1071 |
| 4 | DSH 35 | 3 | 0.8000 | 1.1071 |

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | control | 3 | 1.107 | 1.249 | 1.202 |
| 2 | DSH 28 | 3 | 1.249 | 1.412 | 1.358 |
| 3 | DSH 34 | 3 | 0.991 | 1.249 | 1.077 |
| 4 | DSH 35 | 3 | 0.991 | 1.107 | 1.068 |

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | control | 0.007 | 0.082 | 0.047 | 6.82 |
| 2 | DSH 28 | 0.009 | 0.094 | 0.054 | 6.93 |
| 3 | DSH 34 | 0.022 | 0.149 | 0.086 | 13.82 |
| 4 | DSH 35 | 0.004 | 0.067 | 0.039 | 6.27 |

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 3 | 0.165 | 0.055 | 5.212 |
| Within (Error) | 8 | 0.084 | 0.011 | |
| Total | 11 | 0.249 | | |

Critical F value = 4.07 (0.05,3,8)
Since F > Critical F REJECT Ho: All equal

```
93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
----------------------------------------------------------------------

D =    0.084

W =    0.855

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805
----------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

----------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    1.23

----------------------------------------------------------------------
Table Chi-square value =   11.34  (alpha = 0.01, df =   3)
Table Chi-square value =    7.81  (alpha = 0.05, df =   3)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT        Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2        Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.202 | 0.867 | | |
| 2 | DSH 28 | 1.358 | 0.967 | -1.859 | |
| 3 | DSH 34 | 1.077 | 0.767 | 1.486 | |
| 4 | DSH 35 | 1.068 | 0.767 | 1.589 | |

Dunnett table value = 2.42     (1 Tailed Value, P=0.05, df=8,3)

93 MUDPUPPY RUN #4B HYALELLA 11/02/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR4HB.DAT        Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2        Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | control | 3 | | | |
| 2 | DSH 28 | 3 | 0.163 | 18.8 | -0.100 |
| 3 | DSH 34 | 3 | 0.163 | 18.8 | 0.100 |
| 4 | DSH 35 | 3 | 0.163 | 18.8 | 0.100 |

# ACUTE TOXICITY TESTS
# WITH
# *HYALELLA AZTECA* AND *CHIRONOMUS TENTANS*
# ON SEDIMENTS FROM THE DULUTH/SUPERIOR HARBOR:
1993 Sampling Results - Batch # 7

Conducted by

Minnesota Pollution Control Agency
Monitoring and Assessment Section
520 Lafayette Road
St. Paul, Minnesota  55155-4194

February 1997

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## INTRODUCTION

As part of the 1993 survey of sediment quality in the Duluth/Superior Harbor, sediment toxicity tests were conducted to assess acute (survival) and chronic (growth) toxicity to benthic invertebrates. Acute effects were measured in separate 10-day toxicity tests to *Hyalella azteca* (*H. azteca*) and *Chironomus tentans* (*C. tentans*). Growth was measured at the end of the *C. tentans* test to assess chronic effects. Survival and growth endpoints were compared to organisms similarly exposed to a reference control sediment collected from West Bearskin Lake (Cook County, MN).

A total of 40 sediment samples were collected for toxicity testing. This report presents the results of six of these sediment samples.

## SAMPLE COLLECTION AND HANDLING

During September 27-28, 1993, Minnesota Pollution Control Agency (MPCA) staff collected the six sediments referred to in this report. The samples were collected from the harbor using a Ponar sampler and were taken to the University of Minnesota-Duluth Chemical Toxicology Research Laboratory. The samples were stored at 4°C until they were transported to the MPCA Toxicology Laboratory in St. Paul, MN.

## METHODS

Six sediment samples and a control sediment sample were subjected to the 10-day sediment toxicity tests using the modified procedures described in ASTM (1993). However, the specific test system used for these assays is not indicated in the methods. The test organisms (*H. azteca* and *C. tentans*) were exposed to sediment samples in a portable, mini-flow system described in Benoit et al. (1993). The test apparatus consists of 300 mL, glass-beaker test chambers held in a glass box supplied with water from an acrylic plastic headbox. The beakers have two, 1.5 cm holes covered with stainless steel mesh, to allow for water exchange, while containing the test organisms. The headbox has a pipette tip drain calibrated to deliver water at an average rate of 32.5 mL/min. The glass box is fitted with a self-starting siphon to provide exchange of overlying water.

The *H. azteca* used for this test were 1 to 3 mm long, and the *C. tentans* were approximately 14 days old. These organisms were supplied by Environmental Consulting and Testing, Superior, WI on the day of the test.

On November 12, 1993, six samples (DSH 20, DSH 33, DSH 36, DSH 37, DSH 38, and DSH 39) and the control sediment were separately homogenized by hand, and 100 mL of each sediment was placed in a test beaker (Batch #7). Each sediment test was set up with three replicates of *H. azteca* and three replicates of *C. tentans*. Aerated, artesian well water was added to the beakers, and the sediments were allowed to settle for approximately two hours before the organisms were added. For each toxicity test, ten organisms were placed in each beaker in a random fashion.

The organisms were exposed to 16 hours of light and eight hours of darkness for the duration of the ten-day test. Each day, two liters of aerated water from the artesian well at Stroh Brewery in St. Paul, MN were exchanged in each test chamber. On weekdays, 1-L was exchanged in the morning and 1-L in the afternoon. On weekends, the two liters were passed through the chambers all at once. Water quality measurements (i.e., pH, temperature, and dissolved oxygen) of the overlying water were taken in one beaker of each of the triplicate sets of each of the sediments. The results, along with daily observations involving the physical appearance of the sediments and organisms, were recorded in a laboratory notebook. This notebook is retained on file at the MPCA.

The test was terminated on November 22, 1993. The sediments were sieved through 40 mesh screens, and the sieved material was sorted for organisms. The organisms found were counted, and the number of alive and dead organisms was recorded. Organisms not found were recorded as missing and presumed dead. The *C. tentans* that survived were placed in aluminum weighing dishes, dried at approximately 90°C for at least four hours, desiccated to room temperature, and weighed.

Growth (weight) of the *C. tentans* and survival of both organisms were used as the endpoints for these tests. The resulting survival data were analyzed using TOXSTAT (Gulley and WEST, Inc., 1994), a statistical software package obtained from the University of Wyoming; however, due to a quality assurance problem, the growth data were not analyzed.

A 96-hour, reference toxicant test with *H. azteca* in sodium chloride (NaCl) was run in conjunction with these toxicity tests to determine the acceptability of the *H. azteca* used. Four concentrations of NaCl solution (i.e., 5, 2.5, 1.25, and 0.625 g/L) and a control (aerated, artesian well water) were used in this test. Three replicates of five organisms each were set up per concentration.

**RESULTS**

Water Chemistry

Measurements of pH, dissolved oxygen, and temperature in the overlying water of the test beakers were made daily. These measurements are summarized below and in Tables 1, 2, and 3, respectively.

The range of pH values in the beakers containing *H. azteca* was 7.5 to 8.2 (Table 1). The water in the *C. tentans* beakers had a pH range of 7.3 to 8.1 (Table 1). The pH fluctuation during these tests was acceptable since it did not vary more than 50% within each treatment (U.S. EPA, 1994).

The dissolved oxygen concentration ranged from 5.6 to 7.3 mg/L in the *H. azteca* beakers and from 4.1 to 7.2 mg/L in the *C. tentans* beakers (Table 2). The recommended dissolved oxygen

concentration for these tests is greater than 40% saturation; therefore, these dissolved oxygen ranges were acceptable.

The range of temperature values in the beakers containing the *H. azteca* was 19.1°C to 22.0°C (Table 3). For the *C. tentans* test, the water temperature ranged from 18.9°C to 22°C (Table 3). The recommended temperature for this test is 23 ± 1°C (U.S. EPA, 1994).

<u>Test Endpoints</u>

The mean percent survival of *H. azteca* in the control was 37% which was unacceptable (Table 4). At least 80% survival in the control is necessary for the test to pass. Since the control survival of *H. azteca* in the 4-day reference toxicant test was acceptable at 93%, this would indicate that the culture was healthy. The reason for the poor control survival in the toxicity test could not be determined. For *C. tentans,* the mean percent survival in the control was 87% which was acceptable.

Mean percent survival of *H. azteca* in the test sediments ranged from 57% in the DSH 38 sample to 77% in the DSH 33 sample. Mean percent survival of *C. tentans* in the test sediments ranged from 53% in the DSH 33 and DSH 37 samples to 80% in the DSH 38 sample.

Although the dried *C. tentans* were weighed, the balance on which they were weighed was not calibrated with standard weights; therefore, the data are suspect since the internal calibration of the balance may have drifted with time.

<u>Data Analysis</u>

All *C. tentans* survival data were transformed using an arc sine-square root transformation before being analyzed statistically using Dunnett's test. The mean percent survival of *C. tentans* in all the samples was not significantly different from the control as determined by a 1-tailed Dunnett's test, p=0.05. Results of the statistical analyses of the data are included in Appendix A.

<u>Reference Toxicant Test with *Hyalella azteca* in Sodium Chloride Solution</u>

The pH of the overlying water in the reference toxicant test ranged from 8.2 to 8.7. The dissolved oxygen ranged from 7.5 to 8.6 mg/L, and the temperature ranged between 18.0°C and 22.0°C. The mean percent survival of the control was 93% which met quality assurance requirements (i.e., ≥ 90% control survival). The $LC_{50}$ value for this test was 3.2 g/L NaC1 as determined by the Trimmed Spearman-Karber method. A control chart will be developed for this test once five data points are obtained.

**SUMMARY**

Survival of *H. azteca* in the control sediment was unacceptable (less than 80%). Therefore, no conclusions can be drawn about the effect that the sediments had on *H. azteca*. The reference toxicant test for *H. azteca* was acceptable, and a $LC_{50}$ value of 3.2 g/L NaCl was determined for this test.

Control survival was acceptable in the control containing *C. tentans*. The mean percent survival of *C. tentans* in the sediment samples was not significantly different from the control.

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**TABLE 1. Daily Overlying Water pH Measurements**

| Day | Control #7 | | DSH 20 | | DSH 33 | | DSH 36 | |
|---|---|---|---|---|---|---|---|---|
| | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.7 | 7.7 | 7.7 | 7.8 | 7.9 | 7.8 | 7.9 | 7.8 |
| 1 | 7.6 | 7.8 | 7.5 | 7.6 | 7.5 | 7.6 | 7.4 | 7.5 |
| 2 | 7.3 | 7.5 | 7.6 | 7.6 | 7.6 | 7.7 | 7.5 | 7.6 |
| 3 | 7.8 | 7.9 | 7.7 | 7.7 | 7.7 | 7.8 | 7.7 | 7.7 |
| 4 | 8.1 | 8.1 | 7.9 | 7.8 | 7.8 | 7.8 | 7.8 | 7.9 |
| 5 | 7.8 | 8.0 | 7.8 | 7.9 | 7.9 | 7.9 | 7.8 | 7.8 |
| 6 | 7.9 | 8.0 | 7.8 | 7.9 | 7.8 | 7.9 | 7.7 | 7.8 |
| 7 | 7.8 | 7.9 | 8.0 | 8.0 | 7.8 | 7.9 | 7.7 | 7.8 |
| 8 | 7.7 | 8.0 | 8.0 | 8.0 | 7.8 | 8.0 | 7.7 | 7.9 |
| 9 | 7.8 | 7.9 | 8.0 | 8.0 | 7.9 | 8.0 | 7.8 | 7.9 |
| Mean | 7.8 | 7.9 | 7.8 | 7.8 | 7.8 | 7.8 | 7.7 | 7.8 |
| Range | 7.3-8.1 | 7.5-8.1 | 7.5-8.0 | 7.6-8.0 | 7.5-7.9 | 7.6-8.0 | 7.4-7.9 | 7.5-7.9 |

| Day | DSH 37 | | DSH 38 | | DSH 39 | |
|---|---|---|---|---|---|---|
| | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 7.8 | 7.7 | 7.8 | 7.9 | 8.0 | 7.7 |
| 1 | 7.5 | 7.5 | 7.6 | 7.7 | 8.1 | 8.0 |
| 2 | 7.4 | 7.5 | 7.6 | 7.7 | 7.7 | 7.7 |
| 3 | 7.7 | 7.7 | 7.7 | 7.8 | 7.8 | 8.0 |
| 4 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.9 |
| 5 | 7.8 | 7.8 | 7.8 | 7.9 | 7.9 | 8.1 |
| 6 | 7.8 | 7.8 | 7.9 | 8.0 | 7.9 | 8.2 |
| 7 | 7.8 | 7.8 | 7.8 | 7.9 | 7.7 | 8.0 |
| 8 | 7.7 | 7.8 | 7.8 | 8.0 | 7.8 | 8.2 |
| 9 | 7.8 | 7.9 | 8.0 | 8.0 | 8.0 | 8.1 |
| Mean | 7.7 | 7.7 | 7.8 | 7.9 | 7.9 | 8.0 |
| Range | 7.4-7.8 | 7.5-7.9 | 7.6-8.0 | 7.7-8.0 | 7.7-8.1 | 7.7-8.2 |

**TABLE 2. Daily Overlying Water Dissolved Oxygen Concentrations (mg/L)**

| Day | Control #7 *C. tentans* | Control #7 *H. azteca* | DSH 20 *C. tentans* | DSH 20 *H. azteca* | DSH 33 *C. tentans* | DSH 33 *H. azteca* | DSH 36 *C. tentans* | DSH 36 *H. azteca* |
|---|---|---|---|---|---|---|---|---|
| 0 | 7.0 | 7.2 | 7.2 | 7.2 | 7.1 | 7.2 | 7.2 | 6.9 |
| 1 | 6.3 | 6.5 | 5.8 | 6.0 | 5.7 | 5.8 | 5.8 | 5.8 |
| 2 | 5.5 | 6.6 | 5.8 | 6.3 | 5.6 | 6.3 | 5.2 | 6.0 |
| 3 | 4.5 | 6.4 | 5.5 | 6.0 | 4.4 | 6.0 | 4.4 | 5.8 |
| 4 | 5.9 | 6.8 | 6.2 | 6.5 | 5.5 | 6.5 | 5.5 | 6.3 |
| 5 | 5.3 | 6.7 | 5.8 | 6.6 | 5.3 | 6.3 | 5.1 | 6.2 |
| 6 | 5.0 | 6.6 | 5.9 | 7.3 | 5.2 | 6.9 | 4.6 | 6.7 |
| 7 | 5.6 | 6.3 | 6.0 | 7.0 | 5.3 | 6.7 | 4.6 | 6.6 |
| 8 | 5.4 | 6.3 | 5.8 | 6.5 | 5.3 | 6.9 | 4.6 | 6.4 |
| 9 | 4.6 | 6.2 | 5.8 | 6.3 | 4.9 | 6.6 | 4.4 | 5.6 |
| Mean | 5.5 | 6.6 | 6.0 | 6.6 | 5.4 | 6.5 | 5.1 | 6.2 |
| Range | 4.5-7.0 | 6.2-7.2 | 5.5-7.2 | 6.0-7.3 | 4.4-7.1 | 5.8-7.2 | 4.4-7.2 | 5.6-6.9 |

| Day | DSH 37 *C. tentans* | DSH 37 *H. azteca* | DSH 38 *C. tentans* | DSH 38 *H. azteca* | DSH 39 *C. tentans* | DSH 39 *H. azteca* |
|---|---|---|---|---|---|---|
| 0 | 6.7 | 6.0 | 7.0 | 7.3 | 7.2 | 6.6 |
| 1 | 5.9 | 6.0 | 6.0 | 6.0 | 6.6 | 6.4 |
| 2 | 5.5 | 6.2 | 5.8 | 6.3 | 6.2 | 6.8 |
| 3 | 4.7 | 5.6 | 4.7 | 6.0 | 5.1 | 6.6 |
| 4 | 5.6 | 6.2 | 5.7 | 6.4 | 4.9 | 6.6 |
| 5 | 5.7 | 6.1 | 5.4 | 6.7 | 5.9 | 6.9 |
| 6 | 6.0 | 6.1 | 6.0 | 6.9 | 6.1 | 7.0 |
| 7 | 6.0 | 6.2 | 6.5 | 6.7 | 5.3 | 7.0 |
| 8 | 4.1 | 6.0 | 6.3 | 6.2 | 4.7 | 6.8 |
| 9 | 4.7 | 6.3 | 6.0 | 6.0 | 4.8 | 6.9 |
| Mean | 5.5 | 6.1 | 5.9 | 6.5 | 5.7 | 6.8 |
| Range | 4.1-6.7 | 5.6-6.3 | 4.7-7.0 | 6.0-7.3 | 4.7-7.2 | 6.4-7.0 |

**TABLE 3. Daily Overlying Water Temperatures (Degrees Celsius)**

| | Control #7 | | DSH 20 | | DSH 33 | | DSH 36 | |
|---|---|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 19.6 | 19.5 | 19.2 | 19.2 | 19.1 | 19.1 | 19.0 | 19.1 |
| 1 | 21.4 | 21.3 | 20.5 | 20.7 | 20.7 | 20.6 | 20.8 | 20.9 |
| 2 | 21.7 | 21.6 | 21.3 | 21.3 | 21.1 | 21.2 | 21.3 | 21.4 |
| 3 | 20.5 | 20.5 | 19.7 | 19.6 | 19.6 | 19.5 | 19.5 | 19.8 |
| 4 | 20.0 | 19.8 | 19.5 | 19.5 | 19.5 | 19.5 | 19.6 | 19.6 |
| 5 | 20.1 | 20.0 | 19.6 | 19.6 | 19.6 | 19.6 | 19.5 | 19.5 |
| 6 | 20.2 | 20.1 | 19.6 | 19.6 | 19.6 | 19.6 | 19.6 | 19.6 |
| 7 | 19.8 | 19.8 | 19.5 | 19.6 | 19.6 | 19.6 | 19.6 | 19.6 |
| 8 | 21.2 | 21.1 | 20.8 | 20.7 | 20.6 | 20.6 | 20.9 | 20.9 |
| 9 | 21.5 | 21.5 | 21.4 | 21.4 | 21.3 | 21.2 | 21.4 | 21.4 |
| Mean | 20.6 | 20.5 | 20.1 | 20.1 | 20.1 | 20.1 | 20.1 | 20.2 |
| Range | 19.6-21.7 | 19.5-21.6 | 19.2-21.4 | 19.2-21.4 | 19.1-21.3 | 19.1-21.2 | 19.0-21.4 | 19.1-21.4 |

| | DSH 37 | | DSH 38 | | DSH 39 | |
|---|---|---|---|---|---|---|
| Day | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* | *C. tentans* | *H. azteca* |
| 0 | 19.3 | 19.3 | 19.2 | 19.3 | 18.9 | 19.1 |
| 1 | 21.0 | 21.0 | 21.0 | 21.0 | 22.0 | 22.0 |
| 2 | 21.2 | 21.3 | 21.3 | 21.3 | 21.2 | 22.0 |
| 3 | 20.5 | 20.5 | 19.7 | 19.7 | 19.6 | 19.7 |
| 4 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.7 |
| 5 | 19.7 | 19.7 | 19.5 | 19.5 | 19.2 | 19.1 |
| 6 | 19.9 | 19.9 | 19.7 | 19.7 | 19.2 | 19.6 |
| 7 | 19.8 | 19.8 | 19.7 | 19.7 | 19.7 | 19.8 |
| 8 | 21.0 | 21.0 | 20.9 | 20.9 | 20.9 | 21.0 |
| 9 | 21.5 | 21.5 | 21.5 | 21.5 | 21.3 | 21.3 |
| Mean | 20.3 | 20.4 | 20.2 | 20.2 | 20.2 | 20.3 |
| Range | 19.3-21.5 | 19.3-21.5 | 19.2-21.5 | 19.3-21.5 | 18.9-22.0 | 19.1-22.0 |

**TABLE 4. Mean Percent Survival of *Hyalella azteca* and *Chironomus tentans***

| Batch #7 | Mean Percent Survival | |
|---|---|---|
| **Sample** | ***Hyalella azteca*[1]** | ***Chironomus tentans*** |
| CONTROL #7 | 37% | 87% |
| DSH 20 | 70% | 60% |
| DSH 33 | 77% | 53% |
| DSH 36 | 63% | 73% |
| DSH 37 | 60% | 53% |
| DSH 38 | 57% | 80% |
| DSH 39 | 63% | 70% |

[1] Control survival was unacceptable (<80% survival). Therefore, the test failed.

# APPENDIX A

## TOXSTAT Analysis

93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
7
3
3
3
3
3
3
3
control
0.9
0.7
1.0
dsh 37
0.5
0.6
0.5
dsh 36
0.7
0.7
0.8
dsh 33
0.8
0.3
0.5
dsh 38
0.7
0.8
0.9
dsh 20
0.3
0.9
0.6
dsh 39
0.8
0.7
0.6

TITLE: 93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
FILE: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

| GRP | IDENTIFICATION | REP | VALUE | TRANS VALUE |
|---|---|---|---|---|
| 1 | control | 1 | 0.9000 | 1.2490 |
| 1 | control | 2 | 0.7000 | 0.9912 |
| 1 | control | 3 | 1.0000 | 1.4120 |
| 2 | dsh 37 | 1 | 0.5000 | 0.7854 |
| 2 | dsh 37 | 2 | 0.6000 | 0.8861 |
| 2 | dsh 37 | 3 | 0.5000 | 0.7854 |
| 3 | dsh 36 | 1 | 0.7000 | 0.9912 |
| 3 | dsh 36 | 2 | 0.7000 | 0.9912 |
| 3 | dsh 36 | 3 | 0.8000 | 1.1071 |
| 4 | dsh 33 | 1 | 0.8000 | 1.1071 |
| 4 | dsh 33 | 2 | 0.3000 | 0.5796 |
| 4 | dsh 33 | 3 | 0.5000 | 0.7854 |
| 5 | dsh 38 | 1 | 0.7000 | 0.9912 |
| 5 | dsh 38 | 2 | 0.8000 | 1.1071 |
| 5 | dsh 38 | 3 | 0.9000 | 1.2490 |
| 6 | dsh 20 | 1 | 0.3000 | 0.5796 |
| 6 | dsh 20 | 2 | 0.9000 | 1.2490 |
| 6 | dsh 20 | 3 | 0.6000 | 0.8861 |
| 7 | dsh 39 | 1 | 0.8000 | 1.1071 |
| 7 | dsh 39 | 2 | 0.7000 | 0.9912 |
| 7 | dsh 39 | 3 | 0.6000 | 0.8861 |

93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

| GRP | IDENTIFICATION | N | MIN | MAX | MEAN |
|---|---|---|---|---|---|
| 1 | control | 3 | 0.991 | 1.412 | 1.217 |
| 2 | dsh 37 | 3 | 0.785 | 0.886 | 0.819 |
| 3 | dsh 36 | 3 | 0.991 | 1.107 | 1.030 |
| 4 | dsh 33 | 3 | 0.580 | 1.107 | 0.824 |
| 5 | dsh 38 | 3 | 0.991 | 1.249 | 1.116 |
| 6 | dsh 20 | 3 | 0.580 | 1.249 | 0.905 |
| 7 | dsh 39 | 3 | 0.886 | 1.107 | 0.995 |

**93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2**

| GRP | IDENTIFICATION | VARIANCE | SD | SEM | C.V. % |
|---|---|---|---|---|---|
| 1 | control | 0.045 | 0.212 | 0.123 | 17.43 |
| 2 | dsh 37 | 0.003 | 0.058 | 0.034 | 7.10 |
| 3 | dsh 36 | 0.004 | 0.067 | 0.039 | 6.50 |
| 4 | dsh 33 | 0.071 | 0.266 | 0.154 | 32.26 |
| 5 | dsh 38 | 0.017 | 0.129 | 0.075 | 11.58 |
| 6 | dsh 20 | 0.112 | 0.335 | 0.193 | 37.03 |
| 7 | dsh 39 | 0.012 | 0.111 | 0.064 | 11.12 |

**93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93**
**File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT Transform: ARC SINE(SQUARE ROOT(Y))**

**ANOVA TABLE**

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 6 | 0.399 | 0.067 | 1.759 |
| Within (Error) | 14 | 0.530 | 0.038 | |
| Total | 20 | 0.929 | | |

**Critical F value = 2.85 (0.05,6,14)**
**Since F < Critical F FAIL TO REJECT Ho: All equal**

```
93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

Shapiro - Wilk's test for normality
------------------------------------------------------------------------------

D =    0.530

W =    0.968

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.
```

```
93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT       Transform: ARC SINE(SQUARE
ROOT(Y))

------------------------------------------------------------------------------
Bartlett's test for homogeneity of variance
Calculated B1 statistic =    7.74

------------------------------------------------------------------------------
Table Chi-square value =   16.81  (alpha = 0.01, df =   6)
Table Chi-square value =   12.59  (alpha = 0.05, df =   6)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.
```

93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.217 | 0.867 | | |
| 2 | dsh 37 | 0.819 | 0.533 | 2.509 | |
| 3 | dsh 36 | 1.030 | 0.733 | 1.181 | |
| 4 | dsh 33 | 0.824 | 0.533 | 2.477 | |
| 5 | dsh 38 | 1.116 | 0.800 | 0.640 | |
| 6 | dsh 20 | 0.905 | 0.600 | 1.968 | |
| 7 | dsh 39 | 0.995 | 0.700 | 1.402 | |

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

93 MUDPUPPY RUN #5 CHIRONOMIDS 11/12/93
File: S:\MA\CHUBBAR\TSD\93MUD\93MPR5C.DAT Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | control | 3 | | | |
| 2 | dsh 37 | 3 | 0.350 | 40.4 | 0.333 |
| 3 | dsh 36 | 3 | 0.350 | 40.4 | 0.133 |
| 4 | dsh 33 | 3 | 0.350 | 40.4 | 0.333 |
| 5 | dsh 38 | 3 | 0.350 | 40.4 | 0.067 |
| 6 | dsh 20 | 3 | 0.350 | 40.4 | 0.267 |
| 7 | dsh 39 | 3 | 0.350 | 40.4 | 0.167 |