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Recent Intra- and Interlaboratory Studies Related to the Development and Standardization of Environment Canada's Biological Test Methods for Measuring Sediment Toxicity Using Freshwater Amphipods (*Hyalella azteca)* or Midge Larvae (*Chironomus riparius*)

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1.0 INTRODUCTION

1.1 Background

The freshwater amphipod, *Hyalella azteca*, and midge larvae, *Chironomus riparius* and *C. tentans* have been used in the assessment of sediment toxicity for the past twenty years ,Wentsel *et al.*, 1977; Cairns *et al.*, 1984; Nebeker *et al.*, 1984; Kosalwat and Knight, 1987; Pascoe *et al.*, 1989; Burton, 1991; Burton et al., 1992). Despite their frequent use, it has only been in the last few years that standardized methods have been published (Borgmann and Munawar, 1989; Ingersoll and Nelson, 1990; ASTM, 1995; USEPA, 1994). Since 1993, Environment Canada (EC) has been developing guidance documents with standardized methods for conducting solid-phase sediment toxicity tests with the above species. As support for the information contained in these two documents, studies involving the use and evaluation of two testing options: a static system, where the overlying water is not replaced throughout the exposure period; and a static-renewal system which involves the automated renewal of the overlying water at 12 - h intervals, have been conducted. In addition, comparisons of different food types and feeding rates on the survival and growth of each species in a range of sediment types have been evaluated. Throughout these tests, Environment Canada has tried, wherever possible, to harmonize its test methods with those of the USEPA (1994).

Prior to finalizing and publishing Environment Canada's biological test methods for undertaking "growth-and-survival" tests of (freshwater) sediment toxicity using *H. azteca* or midge larvae, it was considered necessary to complete the standardization of these two-option test methods and to validate them by interlaboratory comparisons. Reported here are the findings of a number of intralaboratory (within NWRI) and interlaboratory investigations undertaken in this regard.

1.2 Objectives

USEPA (1994) utilizes an automated static-renewal system in its sediment toxicity tests with *H. azteca* or *C. tentans* whilst Environment Canada (1995a,b) recommends both the static and the static-renewal systems. The first objective of this study was to compare the performance of the static vs. the static-renewal option. For the initial comparison, survival and growth of *C. riparius* and *H. azteca* were examined in a variety of clean, contaminated and artificially-formulated

and *H. azteca* were examined in a variety of clean, contaminated and artificially-formulated sediments. These sediments varied in total organic carbon (TOC) content as well as particle size distribution and thus allowed a determination of the effects of sediment characteristics of uncontaminated sediments on test end points. As part of these investigations, it was also necessary to establish a type of food and feeding rate that would be appropriate for this range of sediment types, as well as for the use of a formulated sediment to be used in spiked, whole-sediment dose-response tests.

The second objective of this study was to evaluate different food types and feeding rates for the static and static-renewal test options using *H. azteca* or *C. riparius*. USEPA (1994) recommends daily feeding, whereas EC (1995a,b) recommend a feeding rate of 3X weekly for *H. azteca* and four times over 10 days for *C. riparius* in order to reduce the labour requirements for the test.

A third objective was to ascertain a suitable criterion for minimum acceptable growth of control animals in clean sediment, according to each test option and species. At present, toxicity test methods recommended by USEPA (1994) for *H. azteca* are designed primarily as a test of survival with the end point which measures an increase in growth as dry weight recommended only as a test option. Their toxicity test method for *C. tentans* is designed as a test of both survival and growth. USEPA (1994) initiates its 10-d survival test for *H. azteca* with 7- to 14-day old juveniles. There is no minimally-acceptable level of growth designated at the end of the exposure period for this species in clean sediment. In contrast, EC (1995a) recommends that the test with *H. azteca* begins with 2 - to 9-day old juveniles and terminates after 14 days. The end points measured at the end of this exposure period are both survival and growth (mg dry wt/individual) which necessitates the establishment of a minimum acceptable level of growth in control animals.

A minimum-acceptable level of growth of 0.6 mg dry wt/individual has been established for the midge larvae, *C. tentans*, after 10 days in control sediment(s) (Ankley *et al.*, 1993; USEPA, 1994); no such level has been established for *C. riparius*. Therefore, an additional objective of the present study was to provide a minimum acceptable level of growth for *C. riparius* in clean sediment for the EC biological test method.

A fourth objective for the study was to evaluate the reproducibility of each of the two test options, as determined by a series of interlaboratory studies using Environment Canada's standardized test

methods for *H. azteca* or *C. riparius* (EC, 1995a,b). In these interlaboratory round-robins (or 'ring' tests), a number of volunteer government and private laboratories conducted tests with both semi-artificial formulated sediments spiked with copper in a dose-response test, as well as several contaminated sediments collected from sites in the field. Food type and feeding rates used by each participating laboratory were identical and standardized earlier as part of the investigations reported here. The interlaboratory round-robins were designed to evaluate variability within and between laboratories using coefficients of variation (CVs) and the consistency statistics *h* and *k* (ASTM ,1992; Burton *et al.*, 1996). The consistency statistic *h* describes between-laboratory variation while *k* measures within-laboratory consistency.

2.0 METHODS

2.1 Culture Techniques

2.1.1 *Chironomus riparius*

Source of C. riparius

Egg masses of *C. riparius* were acquired from Dr. C. Ingersoll, USGS, Columbia, Missouri, in 1991, and the culture of this species has been maintained at the National Water Research Institute (NWRI) for the past six years. Laboratories participating in Phase I of the interlaboratory studies (see Section 2.3.4) were supplied with *C. riparius* by NWRI. In Phase II (see Section 2.3.4), one laboratory obtained *C. riparius* from an independent supplier.

Culture of C. riparius

Detailed culturing methods are outlined in Day *et al.* (1993), Reynoldson *et al.* (1994), Hamr *et al.* (1994), NWRI Standard Operating Procedures (unpublished) and EC (1995b). In brief, organisms were cultured in 10 L aquaria with 2 cm of silica sand substrate and 8 L of culture water using an environmental chamber set at 23 ± 1°C and 16L:8D light regime. Fitted plexiglass additions were constructed and placed over the aquaria to contain emerging adults. Cultures were initiated by the addition of three egg masses to each aquarium. Food in the form of moistened Nutrafin^{R} flakes was added *ad libitum*. Emergence of males occurred at approximately 15-20

days, followed by females at 18-23 days (Day *et al.*, 1993). Once mating and egg deposition occurred, egg masses were removed on a daily basis and kept in a 150 mL beaker with 100 mL culture water until the first instar emerged from the gelatinous egg masses. Upon hatching, the organisms were used to initiate another culture, or, were used for testing purposes.

Culture water

Carbon filtered, dechlorinated (vigorous aeration), City of Burlington, (Lake Ontario) tap water was employed in culturing the test organisms and in the various tests. The characteristics of this water source included: pH 7.7 - 8.5; conductivity 273 - 347 μS/cm; hardness 120 - 140 mg/L; alkalinity 76 - 102 mg/L. The water was filtered through a charcoal filter and aerated for four to five days prior to use. Testing of the water for hardness, nutrients, and major ions was performed on a monthly basis by the National Laboratory for Environmental Testing (NLET) at NWRI, Burlington.

2.1.2 *Hyalella azteca*

Source of H. azteca

The initial culture of *H. azteca* was acquired from Dr. U. Borgmann, Department of Fisheries and Oceans, Burlington, Ontario, and has been maintained for approximately six years at NWRI, Burlington. Organisms of *H. azteca* were provided from this laboratory to one other laboratory participating in the interlaboratory studies for the establishment of their cultures. The remaining laboratories used their own cultures of *H. azteca* which originated from independent suppliers.

Culture of H. azteca

Detailed culturing methods are outlined in Borgmann *et al.* (1989), Hamr *et al.* (1994) and EC (1995a). A maintenance culture of a mixture of adult and juvenile amphipods was kept in large aquaria as a source of mating pairs for the production of known-aged young for each test. In order to provide known-aged young for a test, 20 to 30 adult *Hyalella* (preferably mating pairs) were maintained in 2 L wide mouth jars with 1 L culture water. Approximately 25-40 jars were used in order to provide enough juveniles for any particular test. Each jar was fed 5 mg of Nutrafin^{R} fish

flakes 3 times per week on non-consecutive days. Young were separated from the adults on a weekly basis (Tuesdays), and kept aside in 1 L culture water for two days prior to use in tests. From 300 - 1000 young were produced on a weekly basis following these procedures.

Culture water

The culture water and its range of characteristics were the same as that used to culture *C. riparius* (see Section 2.1.1).

2.2 Intralaboratory Standardization and Comparison of Static and Static-Renewal Test Options

A series of intralaboratory tests were conducted at NWRI in order to standardize each of the static and static-renewal options for performing sediment-toxicity tests using *H. azteca* or midge larvae, which are described in Environment Canada's draft test-method documents for these organisms (EC, 1995a,b). These studies dealt with three of the four stated objectives for the present testing program (see Section 1.1). Tests with midge larvae were restricted to *C. riparius*.

For each series of tests using *H. azteca* or *C. riparius*, both the static system and the static-renewal system employed 300 mL beakers with 100 mL sediment and 175 mL overlying water per beaker. Beakers within each static test were aerated continuously (gently) throughout the test, by means of oil-free aeration pump(s) (Optima), and with the use of 5 3/4 mm Pateur pipets for air delivery. Overlying water lost due to evaporation in the static beakers was replenished with distilled water if necessary. In general, the amount of distilled water added during a test was ≤ 25 mL. The static-renewal mode used a modified Zumwalt *et al.* (1994) system for all tests. The static-renewal option involved the twice daily (automatically at 12- h intervals by means of timers; Mastercraft No. 52-8851-2) renewal of 175 mL of overlying culture water.

Water employed in all tests was that described in Section 2.1.1. Specific measurements performed on the overlying water in all tests using both static and static-renewal systems were according to EC (1995a,b). In brief, dissolved oxygen was measured ≥3X per week in at least one replicate from each treatment, using a YSI meter (model No. 58). Total ammonia (as un-ionized ammonia) was measured at the start and end of each test using an ammonia electrode (Orion No. 95-12).

Total hardness and total alkalinity were measured at the start and end of each test using titration kits (Can. Sci. No.'s k-4520 and r-9815 respectively). Conductivity and pH were measured at the start and end of each test using a conductivity/TDS meter (Orion, No.124), and a pH electrode, respectively.

2.2.1 10-day tests using *C. riparius*

Tests were conducted according to conditions and procedures described in EC (1995a, b). Four replicates per treatment were used in each study. A summary of the static vs. static-renewal comparisons as well as the feeding regimes for these experiments and the interlaboratory test, (see Section 2.3) performed with *C. riparius* is presented in Table 1.

Test sediment

Three uncontaminated field-collected sediments were used in these trials chosen from previous studies outlined in Reynoldson and Day (1994) and Reynoldson *et al.* (1995) which demonstrated good survival and growth of *C. riparius* in other samples of sediments collected from these same three locales. Sediments chosen covered a range of total organic carbon content (TOC) from low to high, as follows:

Sediment #1: Long Point Marsh, Lake Erie, (TOC = 8.8%) (high)
Sediment #2: Reference Site #108, Lake Erie, (TOC = 1.9%) (moderate)
Sediment #3: Off Wasaga Beach (WB), Georgian Bay, (TOC = 0.6%) (low)

All three sediments had demonstrated an acceptability criterion of ≥70% survival set by USEPA (1994), ASTM (1995) and EC (1995b) for this or related (*i.e., C. tentans*) species. The physical and chemical characteristics of each of these three sediments are given in Table 2.

Diet

Test organisms were fed Nutrafin^{R} fish flakes (crushed <500 μm) at the four feeding rates listed below. Food was prepared by adding 1-2 g of Nutrafin^{R} to 100 mL distilled water and placing on a magnetic stir plate. Approximately 1 mL of the slurry was pipetted into each of several pre-weighed aluminum pans and dried for 1 h at 60°C. In order to achieve the desired amount of food

(mg) per test beaker, the amount of slurry (mL) added was adjusted based on the mean dry weight of 1 mL of the prepared slurry.

Diet #1: 4 mg fed daily (total: 40 mg)
Diet #2: 6 mg fed daily (total: 60 mg)
Diet #3: 10 mg fed 4 times throughout the test (10 mg/4X; total: 40 mg; fed on non-consecutive days)
Diet #4: 15 mg fed 4 times throughout the test (15 mg/4X; total: 60 mg; fed on non-consecutive days)

2.2.2 14-day tests using *H. azteca*

Previous experiments have shown that exposure of the freshwater amphipod *H. azteca* to contaminated sediment(s) for 14 days is a suitable time for effects on survival and growth to be detected (Hamr *et al.*, 1994;Day and Reynoldson, 1995; Day *et al.*, 1995b; Kubitz *et al.*, 1995). Based on these results, the growth-and-survival tests in this study were conducted for 14 days duration using the procedures described in EC (1995a). Four or five replicates per treatment were used in each feeding trial. A summary of the test conditions for the static vs. static-renewal comparisons as well as the feeding regimes for these experiments and the interlaboratory tests (see Section 2.3) performed with *H. azteca* is presented in Table 3.

Test sediment

As with *C. riparius*, three field-collected sediments with TOC ranging from 0.7 to 8.1% (see below) were used in the experiments with *H. azteca*. These sediments were chosen on the basis of earlier studies showing that they provided good survival and growth of *H. azteca* (Reynoldson *et al.*, 1995; Reynoldson and Day, 1994) well as achieving a criterion specified for acceptable control survival of ≥ 80% (USEPA, 1994; ASTM, 1995: EC, 1995a) for this species. The physical and chemical characteristics of these three sediments are given in Table 4.

Sediment #1: Long Point, Lake Erie, (TOC = 8.1%) (high)
Sediment #2: Reference Site #1213, Georgian Bay, (TOC = 2.1%) (moderate)
Sediment #3: Reference Site #100, Lake Huron, (TOC =0.1%) (low)

Diet

Test organisms were fed either of two types of food: Nutrafin^R fish food flakes (crushed <500 μm) or Yeast-Cerophyll-Trout Chow (YCT), at the rates listed below. The YCT was made according to the recipe given by USEPA (1994), found in Appendix G of EC (1995a). The dry solid content was checked by drying the food at 60°C for 1 h, and was subsequently adjusted with distilled water to achieve a desired 1.7 - 1.9 mg/mL of dry solids (EC, 1995a). Nutrafin^R was prepared as described in Section 2.2.1 to achieve 4 mg per feeding.

Diet #1: 1.5 mL YCT fed daily (total added 21 mL or ≈ 38 mg)

Diet #2: 3.5 mL YCT fed 3X/week (total added 21 mL ≈ 38 mg; fed on non-consecutive days)

Diet #3: 4 mg Nutrafin^R flakes fed 3X/week (total added 24 mg; fed on non-consecutive days)

2.3 Interlaboratory Studies with *C. riparius* or *H. azteca*

Two phases of interlaboratory experiments were performed with *C. riparius* or *H. azteca*. For each species, Phase I consisted of the use of a semi-artificial formulated sediment spiked with a range of copper concentrations. Test concentrations were based on the results of preliminary studies conducted at NWRI in 1995 which measured the survival and growth of each species in these and other concentrations of copper. In addition, Suedel *et al.* (1996a) have shown that formulated sediments can be used successfully with copper in spiked-sediment experiments. A second phase of experiments (Phase II) for *C. riparius* consisted of repeating the test with copper in a dose-response series of concentrations with the incorporation of a sample of uncontaminated (clean, control) sediment collected from the field and a sediment collected from a field site thought to contaminated with toxicant(s) which reduce the growth of midge larvae.

Phase II tests with *H. azteca* included a sample of uncontaminated (clean, control) sediment collected from the field, as well as three field sediments thought to be contaminated with toxicant(s) which reduce the growth of juvenile *H. azteca*.

2.3.1 General

Laboratories which participated in the interlaboratory studies were those which had experience in performing sediment-toxicity tests, some familiarity with the test organisms, and could volunteer their time and expertise. Each participating laboratory simultaneously exposed their test organisms to similar test sediments spiked and/or supplied by the organizer (NWRI) in order to investigate interlaboratory variability and precision. Standard Operating Procedures (SOP), based on methods outlined in Environment Canada's draft biological test methods for *C. riparius* (EC, 1995b) or *H. azteca* (EC, 1995a) as well as procedural improvements determined during the intralaboratory investigations reported here, were prepared for each species by NWRI, and distributed to the participating laboratories. A copy of these SOPs is provided as Appendix A. Each SOP described in detail the procedures that the laboratories were to follow in all aspects of the test (e.g., handling of sediments, handling of test organisms, test methods, water quality parameters, test take-down). In order to reduce certain potential sources of interlaboratory variability, each laboratory was supplied with the appropriate food for the test, as well as the copper stock solution and substrate for the reference-toxicity test. Each sediment was given a particular coding (*i.e.*, A, B, C, etc) known only to NWRI, in an attempt to remove bias from the testing. Criteria used to judge each test as valid, as well as the results of acceptability for statistical analysis, were: for *C. riparius*, ≥70% survival in the control sediment; and for *H. azteca*, ≥80% survival in the control sediment. The criterion used to judge a "water only" reference-toxicity test as valid (and the results of acceptability for statistical analysis) was ≥ 90% survival in the controls.

2.3.2 Phase I - spiking techniques for copper

2.3.2.1 *Semi-artificial formulated sediment*

Sediment used for preparing a range of copper-spiked sediment in a dose-response scenario consisted of a formulated 1:1 (by volume) mixture of clean sediment from Long Point Marsh, Lake Erie, Ontario and a mixture of 3 kg of Allen R clay (kaolin), 3 kg of silica sand #75, and 4 L of culture water (Hamr *et al.*, 1994). This formulation of semi-artificial sediment has been shown in previous experiments to provide reproducible and dose-dependent results (Hamr *et al.*, 1994; NWRI, unpublished data).

A batch of formulated semi-artificial sediment was spiked with an appropriate aliquot of copper chloride ($CuCl_2 \cdot 2H_2O$) to yield a range of nominal concentrations of copper expressed as μg Cu/g dry weight of sediment. Concentrations for spiking were based on the mean dry weight of the formulated sediment mixture, which was determined by drying several subsamples of the (unspiked) mixture at 60°C for 24 hours. Each test concentration was prepared by dispensing a 1-L aliquot of thoroughly homogenized formulated sediment into a 2-L wide mouth glass jar, and spiking it with an appropriate aliquot of copper. The copper-spiked sediment was then mixed on a rotational shaker (175 agitations per minute) for 80 minutes. Since only 1 L of sediment per concentration was spiked at given time, it was necessary to repeat the spiking process three to four times to achieve the volume necessary to supply all participating laboratories. After each shaking process, the contents of the jars spiked at a given concentration were mixed together in a 10 L bucket. The sediment was then distributed into 1-L leak proof acid-rinsed polyethylene containers, and stored at 4 °C. Sediment was shipped by air to participating laboratories the following day, for delivery within 24 hours.

Upon receipt of the containers of spiked sediment, participating laboratories homogenized each concentration of sediment by shaking vigorously. Aliquots of 100 mL of each concentration of sediment were allocated to 300 mL beakers and 175 mL of overlying water was added by slowly pouring along the sides of the test chambers to minimize disturbance of the sediment. All test beakers were covered with loose-fitting petri dishes and placed in a 4°C cold room for two weeks minus 1 day to test initiation (equilibration period). Each participating laboratory used their own water unique to their locality.

2.3.2.2 *Analytical determination of copper*

Analyses for concentrations of copper in the overlying water, pore water (interstitial water), and bulk sediment for the sediment spiked with copper chloride which was provided to each laboratory participating in the round-robins were performed by the National Laboratory for Evaluation and Testing (NLET) located in Burlington, Ontario, at NWRI, using their standard procedures (Environment Canada, 1994). In brief, the method followed for water was the Inductively-Coupled Plasma Optical Emission Spectrometric (ICP-OES) determination and quantification of trace amounts of copper in surface waters (McLaren, 1981) after a manual digestion and o..ent ti.n p..e.u.e an. u..a..ni. n...isa...n (M..... #0.-.001, .n...n..en. Cana.a,

1994). For bulk sediment, the sample was dried, ground, homogenized and digested with a combination of acids (hydrofluoric, hydrochloric, nitric and perchloric) followed by atomic absorption spectroscopy (Method 02-2401, Environment Canada, 1994).

A subsample of bulk sediment from each container of sediment spiked with copper was taken for analysis immediately following spiking and homogenization. Additionally, for each concentration of copper-spiked sediment set-up at NWRI during the round-robin, two extra beakers were provided during the experimental procedure. These two beakers were provided specifically for the quantification of copper in the test beakers after the equilibration period (Day 0 of test initiation) and at the end of each test, respectively (*i.e.*, Day 10 or Day 14, for tests with *C. riparius* or *H. azteca*). The beakers were exposed to the same conditions as the experimental beakers and contained animals. However, the animals in these extra beakers were not included in the overall bioassay results.

Overlying water, pore water, and bulk sediment samples were taken on Day 0 (pre-test; containing no organisms) and Day 10 or Day 14 (post-test; with organisms added) of each round-robin. For these samples, overlying water was poured gently from each beaker and its volume recorded. A 15-20 mL aliquot of the overlying water was then preserved in a scintillation vial by acidifying with concentrated nitric acid to 0.2%. Animals were removed from the bulk sediment in the extra beakers by gently swirling the beakers and capturing the animals with tweezers or a pipette. Once animals were removed, the bulk sediment was placed in 100-mL polycarbonate centrifuge tubes and centrifuged at 10,000 rpm for one hour. The pore water (*i.e.*, interstitial water) which formed the supernatant was subsequently removed from the centrifuge tube and its volume was measured and recorded. A 15-20 mL aliquot of the pore water was then preserved as per overlying water. The centrifuged sediment was weighed, dried at 60° C to constant weight, reweighed, then crushed and placed in scintillation vials for analysis.

2.3.3 Field-collected sediment

Sediment(s) used in all interlaboratory tests (and in the intralaboratory studies described in Section 2.2) were collected by means of a mini-Ponar, or by an Eckman grab, and stored upon arrival in the laboratory at 4° C. All sediments were wet-sieved through a 250 μm mesh screen for removal of indigenous organisms. Each sample was thoroughly homogenized prior to dispensing

into 1-L leak proof acid-rinsed polyethylene containers for shipment to participating laboratories for each round-robin. The sediment used as the negative control in all tests was the sediment collected from Long Point marsh, Lake Erie, Ontario. This sediment has been used as a control sediment for over six years at NWRI, and is known to provide a consistent level of test acceptability for both survival and growth of *C. riparius* or *H. azteca* (Reynoldson *et al.*, 1995; Reynoldson and Day, 1995).

The field-collected sediments used in the round-robin with *C. riparius* were as follows:

Field Sediment #1: Long Point marsh, Lake Erie, (TOC = 7.2%)
Field Sediment #2: Hamilton Harbour, Lake Ontario (TOC =0.8%)

The physical and chemical characteristics of these samples of sediment as well as the semi-artificial formulated sediment used in Phase-I and Phase-II Interlaboratory studies with *C. riparius* are given in Table 5.

The field-collected sediments used in the round-robin with *H. azteca* were as follows:

Sediment A: Toronto Harbour, Lake Ontario (TOC = 1.2%)
Sediment B: Hamilton Harbour, Lake Ontario (TOC = 3.1%)
Sediment C: Long Point marsh, Lake Erie (TOC = 7.2%)
Sediment D: Montreal Harbour, St. Lawrence River (TOC = 3.1%)

Physical and chemical characteristics of these samples and of the semi-artificial formulated sediment used in Phase-I Interlaboratory studies with *H. azteca* are given in Table 6.

2.3.4 Round-robin tests with *C. riparius*

Participating laboratories

Three laboratories participated the sediment toxicity tests with *C. riparius* using both the static and the static-renewal options. A fourth laboratory participated only in tests which employed the static-renewal option.

Diet

Test organisms were fed 15 mg Nutrafin^R flakes (crushed <500 μm), four times over the course of the test, for a total of 60 mg food and feedings were on non-consecutive days. Food was prepared in advance by NWRI and shipped to the participating laboratories. For each test, each laboratory received 250 mL food slurry containing 5 g Nutrafin^R. The food was pre-weighed and adjusted as described in Section 2.2.1 to achieve 15 mg dry weight per aliquot.

2.3.4.1 *10-day tests with field -collected or semi-artificial formulated sediment*

Tests were divided into two phases: Phase I consisted of a copper-spiked dose response test with nominal concentrations (on a sediment dry weight basis) of 0, 100, 250, 500, 1000, and 2000 μg Cu/g. These concentrations were determined from previous range-finding tests (NWRI, unpublished data). Phase II tests incorporated a repeat of the copper-spiked dose response test by each participating laboratory as well as two field-collected sediments described in Section 2.3.3.

2.3.4.2 *96-h "water only" tests with reference toxicant*

A "water-only" 96-hour reference-toxicity test was performed by each laboratory, using copper (as $CuCl_2 \cdot 2H_2O$), and the same batch of organisms that was used in the whole-sediment tests. At each laboratory, test organisms not used in the sediment-toxicity tests (see Section. 2.3.4.1) were placed in an aquarium (with silica sand substrate) for three to five days until second instar was achieved. Animals were fed *ad libitum* during this time with Nutrafin^R fish flakes. Nominal concentrations of copper tested were 0, 100, 250, 500, 1000, and 2000 μg/L Cu. One to three replicates were used by each of the laboratories, depending on the number of animals available and time constraints on personnel in each laboratory.

2.3.5 Round-robin tests with *H. azteca*

Participating laboratories

Four laboratories participated in the round-robin spiked-sediment toxicity tests with *H. azteca* using both the static option and the static-renewal options, while a fifth laboratory participated only in the tests which employed the static-renewal option.

Diet

Test organisms were fed 3.5 mL YCT, six times over the course of the test, for a total of 21 mL and food additions were on non-consecutive days. Food was prepared in advance by NWRI, as described in Section 2.2.2 and a 1-L aliquot was shipped to each participating laboratory for each round-robin test.

2.3.5.1 *14-day tests with field-collected or semi-artificial formulated sediment*

As with *C. riparius*, the interlaboratory tests with *H. azteca* were divided into two phases: Phase I consisted of a copper-spiked dose response test, with laboratory formulated semi-artificial sediment spiked at nominal concentrations of 0, 50, 125, 250, 500, and 1000 μg Cu/g d.w. These concentrations were determined from previous range-finding tests (NWRI, unpublished data).

Phase II experiments involved 14-d growth-and-survival tests using the four field-collected sediments described in Section 2.3.3 and 2 to 9 day-old juveniles of *H. azteca*.

2.3.5.2 *96-h "water only" tests with reference toxicant*

A "water-only" 96-h reference toxicity test was performed by each laboratory using copper (as $CuCl_2 \cdot 2H_2O$), and the same batch of organisms that was used in the sediment-toxicity tests. Nominal concentrations tested at each laboratory were 0, 50, 125, 250, 500, and 1000 μg Cu/L. One to three replicates were used by each of the laboratories, depending on the number of animals available and time constraints on personnel in each laboratory.

2.4 Sediment Characterization

Each sample of sediment employed in the intra- and interlaboratory tests was analyzed for physical and chemical parameters at NLET, Burlington, Ontario. Particle size determination was performed on lyophilized samples, following the procedure outlined by Duncan and LaHaie

(1979). In brief, the sediment was placed in sodium metaphosphate solution, mixed for fifteen minutes, and wet sieved through a 0.063 μm mesh screen. The material remaining on the sieve was dried and recorded as percent sand. The remaining suspension was analyzed using a sedigraph analyzer, with results expressed as percent silt and clay.

Chemical analysis

Sub-samples of each field sediment were analyzed by Seprotech Laboratories, Ottawa, Ontario for chemical analysis. Analysis was conducted on whole sediment for total organic carbon, total nitrogen, total phosphorus, and metals. Metal determination was by acid digestion followed by ICP-AES analysis (Multi-channel Jarrell-ASH AtomComp 1100) (McLaren, 1981).

2.5 Statistical Analysis

The measured end points for survival and growth from all tests using field-collected sediment were tested for normality and homogeneity of variance, and a One-Way Analysis of Variance (ANOVA) was used for statistical comparison. If data passed the tests for normality and homogeneity, then comparison of means to the control sediment were performed using Dunnett's or Bonferroni's test. Students t-test was performed to compare the two systems (static and static-renewal) within the same treatments. The Sigmastat™ (Jandel, California v. 2.1) software package was employed using a significance level of $p \leq 0.05$. An LC50 was determined on mortality data derived from the copper-spiked dose response tests and the "water-only" reference-toxicity tests, using probit analysis or the trimmed Spearman-Karber method if probit analysis was not appropriate (Hamilton *et al.*, 1977). The inhibition concentration estimate (IC_p) was performed at the 25% level on growth in the copper-spiked dose response tests using the linear interpolation method, with the confidence intervals determined using the bootstrap method (Norberg-King, 1994).

For evaluating the precision of the end points from the biological data within and between laboratories, it has been recently recommended (Burton *et al.*, 1996) that the coefficient of variation (CV) not be the only estimate used. Accordingly, the precision of all survival and growth data derived from the interlaboratory studies with *H. azteca* or *C. riparius* was analyzed using the intralaboratory consistency statistic "k", and the interlaboratory consistency statistic "h"

(for a detailed description of these statistics, see ASTM, 1992). For each end point statistic and study, the limits of variability for "h" and "k" were determined using a critical-value *t* for the interlaboratory consistency statistic "h", and the F ratio for the intralaboratory consistency statistic "k" (ASTM, 1992). The critical value for *h* is based on the number of labs participating (*p*) while *k* depends on both the number of labs and the number of repeated test results (*n*) per lab per sample. The consistency statistic *h* is an indicator of how a laboratory's sample average compares with the average of the other laboratories. The *k* statistic is an indicator of how the laboratory's within-laboratory variability on a sample compares with all the laboratories combined. These limits were plotted as horizontal lines on graphs which included "h" or "k" values and their critical limits. Individual values for "h" or "k" exceeding these critical limit values represent unacceptable intra- or interlaboratory variability.

The Litchfield and Wilcoxon formula (Sprague and Fogels, 1977) was used to determine significant differences for pairwise (*i.e.*, static vs. static-renewal) comparisons of LC50 values for survival, and IC25 values for growth, as derived in the copper-spiked dose response tests.

2.6 Minimum Acceptable Dry Weight for Controls

Values representing a minimum acceptable dry weight for control organisms at test end were calculated for *H. azteca* and *C. riparius*. Calculations were based on dry weights measured for the respective species under standardized test conditions (EC, 1995a, 1995b) used here and in earlier studies at NWRI. The minimum dry weights for controls at test end were determined from data derived for control sediment and clean reference sediment at NWRI, and from the dry weights for controls in the interlaboratory tests that achieved the species-specific minimum acceptable survival criteria. Mean dry weights at test end were determined from each sediment type *(i.e.*, Long Point marsh sediment, other clean sediments and semi-artificial formulated sediment). Mean dry weight from each sediment type was then combined to give an overall grand mean and standard deviation. Two standard deviations were subtracted from the grand mean to give the final minimum recommended mean dry weight for individual control organisms at test end, for each species.

3.0 RESULTS

3.1 Intralaboratory Standardization and Comparison of Static and Static-Renewal Test Options

3.1.1 *C. riparius*

The mean percent survival and growth (i.e., average dry weight for individuals at test end) for *C. riparius* in side-by-side 10-d tests using either the static or static-renewal options are illustrated in Figures 1 and 2. Results are presented as averages plus or minus one standard deviation (SD) for percent survival and growth (mg dry weight/individual organism) for each test option when animals were exposed to uncontaminated natural sediments and fed different rations of food, either daily or on non-consecutive days three times weekly.

3.1.1.1 *Survival*

Static

Mean percent survival of *C. riparius* in the static system (white bars) was consistently above the proposed acceptability criterion of 70%, for a valid test using this species (EC, 1995a) and values ranging from 82.5 to 95%. Variability in the results was low with coefficient of variations (CVs) ranging from 5.7 to 16.2%. No significant differences in survival rates were observed among the four rates of feeding in each type of sediment.

Static-renewal

In the concurrent static-renewal experiments (coloured bars), mean percent survival was also above the proposed acceptability criterium of 70% for all types of sediment and food rations with the exception of sediment #2 (moderate TOC) under a food regime of 40 mg Nutrafin^R added four times over 10 days. Mean percent survival in this treatment was only 55.0 ±26.5%. Higher variability was noted for % survival in the static-renewal system vs. the static system with CVs ranging from 0 to 48.1%.

Static vs. static-renewal

With one exception, in each of the four feeding regimes the mean % survival of midge larvae in the static vs. the static-renewal system was not statistically different (Figure 1). Percent survival was significantly lower in the static-renewal system vs. the static system for sediment # 2 given ration #3 ($p \leq 0.05$). For sediments #1 (high TOC) and #3 (low TOC), survival rates were not influenced by test option (i.e., static versus static-renewal) or feeding regime. Using either test option, no influence of sediment organic content on mean survival rates for *C. riparius* was apparent for the range tested (0.6 to 8.8% TOC).

3.1.1.2 *Growth*

Static

For each of the three sediments tested, mean growth was higher for animals fed the greater ration (*i.e.*, 60 mg dry weight, either as 6 mg/day or 15 mg on non-consecutive days over 10 d). Differences in growth due to the level of ration were only significant for sediments #1 and #2. Addition of the food either daily, or four times on non-consecutive days over the 10-d period of exposure did not have a significant effect on growth within a type of sediment provided that the total ration added during the test (40 or 60 mg) was the same (Figure 2). The CVs for growth in the static system were low and ranged from 1.9 to 10.3 % in all sediments.

Growth was affected by the type of sediment to which the animals were exposed. For example, with few exceptions growth was significantly higher in sediment # 3 (Table7).

Static-renewal

Unlike the results for growth in the static system, the level of ration (*i.e.*, 40 mg or 60 mg over test duration) did not affect mean growth in a consistent manner in the static-renewal system (Figure 2). Significantly higher growth in the static-renewal system was only observed at the higher food ration of 15 mg added four times on non-consecutive days over 10 days vs. a daily feeding rate of 4 mg and only in sediment #1 ($p \leq .05$). As in the static system, the type of sediment and its organic carbon content appears to have a greater effect on increase in biomass

than feeding regime with the highest growth exhibited in sediment #3 which had the lowest TOC (Figure 2). Variability in the CVs for the static-renewal system were also slightly higher and ranged from 5.9 to 34% in all sediments.

Static vs static-renewal

In general, higher growth rates were observed in the static system vs. the static-renewal system for all types of sediment. Many of these growth differences were statistically significant (alpha<0.05) and four of five were at the higher feeding rates (Figure 2).

3.1.1.3 *Water Quality*

Ammonia

For two of the three sediments tested, concentrations of total ammonia in the overlying water at test end were consistently non-detectable or low, regardless of food ration or test system used (see Table B-2, Appendix B). Relatively high values (6 - 12 mg/L) were found at test end for sediment #3 (all feeding modes) using the static option. Sediment #3 also exhibited the highest pore water ammonia, measured prior to the start of the test (Table 2). However, this did not appear to affect survival or growth in sediment #3 where both end points were the highest in both the static and the static-renewal systems compared to the other sediments.

Dissolved oxygen

Oxygen levels during the exposure period were lower in the static-renewal system vs. the static system and were independent of sediment type. Some values, on average, dropped below the 40% saturation criterion established by USEPA (1994) in the static-renewal option only; this decline was most pronounced in sediment #3 for the highest feeding regime (Table B-2, Appendix B).

3.1.2 *H. azteca*

Mean percent survival and growth of *H. azteca* in the static and the static-renewal systems for three sediments with low, moderate and high organic carbon content and three feeding regimes are presented in Figures 3 and 4, respectively.

3.1.2.1 *Survival*

Static

For all three types of sediment, mean % survival of *H. azteca* in the static system was well above the acceptability criterion of ≥80% in control sediments set by USEPA (1994) and ASTM (1995); % survival ranged from 88 to 100% (Figure 3). Variability in the results was low, with CVs ranging from 0 to 14.8%. For each of the three sediments tested, no significant differences in % survival for groups or organisms fed different rations (either YCT or Nutrafin^{R}) or feeding regimes (daily or thrice weekly) were found (Figure 3).

Static-renewal

Mean survival of *H. azteca* in the 3 sediments types ranged from 82.5 to 100% in the static-renewal system. CVs ranged from 0 to 20.7%. As with the static system, no significant differences in survival rates for groups fed different rations (YCT or Nutrafin^{R}) or feeding regimes (daily or thrice weekly) were found for the static-renewal tests.

Static vs static- renewal

For each of three sediments and each of the diets provided, pairwise comparisons of same-sediment/same-diet treatments using static versus static-renewal test options showed no statistical differences in % survival (Figure 3).

3.1.2.2 *Growth*

Static

Type of food and feeding regime did not have a statistically significant effect on growth of *H. azteca* in sediments #1 and #3 in the static system; however, growth was higher in animals feeding on YCT (either added daily or thrice weekly) vs. Nutrafin^{R} in these two sediment types (Figure 4).

For sediment #2, growth was much lower independent of food type or amount given, in comparison with the other two sediments (Table 8). The highest growth observed in this sediment was in animals receiving the Nutrafin^R diet vs. YCT but in general even this growth was below that achieved in sediments #1 and #3.

For each of the three types of sediment, the growth of *H. azteca* was not affected significantly by the frequency of the ration given (i.e., daily vs 3X/week).

Static-renewal

For sediments #1 and #3, both daily feeding with YCT and feeding with YCT thrice week elicited statistically significant higher growth in *H. azteca* compared to the Nutrafin^R diet. As in the static option, growth of animals fed YCT in the static-renewal system did not differ significantly when the feeding frequency was either daily or 3X/week. Growth of *H. azteca* was reduced in sediment #2 in comparison to growth of animals held in sediments #1 and #3, regardless of ration type or feeding regime.

Static vs static-renewal

Overall, growth of *H. azteca* was higher in the static system relative to the static-renewal system, with significantly greater growth noted using the static system for each of the three test sediments (Figure 4). In sediment #1, there was significantly higher growth in the static system vs. the static-renewal system and under all 3 feeding regimes. In sediment #2, there was significantly higher growth in the static system vs. the static-renewal system with the Nutrafin^R diet only. In sediment #3, there was significantly better growth in the static system vs. the static-renewal system for each of the two YCT diets but not the Nutrafin^R diet .

3.1.2.3 *Water Quality*

Ammonia

Un-ionized ammonia in the overlying water was≤ 0.1 ppm in both static and static-renewal systems for all types of sediment and with all feeding regimes (Table B-4, Appendix B).

Dissolved oxygen

All dissolved oxygen levels were within acceptable limits for all tests and did not drop below 40% saturation during the experiments (Table B-4, Appendix B).

3.2 Interlaboratory Studies

3.2.1 *Chironomus riparius*

3.2.1.1 *"Water-only" reference toxicant test*

One participating laboratory did not achieve the proposed minimum acceptable control survival of ≥ 90% (EC, 1995b) for a "water-only" reference-toxicity test (Table 9). When the results from this particular laboratory were excluded, the mean 96-h LC50 was 860.6 μg Cu/L, with a range of 493 to 1650 μg Cu/L. The CVs for % control survival and LC50s were 4.6% and 57.2%, respectively. There were statistically significant differences between the LC50s determined within and between laboratories. No trends in LC50s with respect to the hardness of the dilution water were apparent.

3.2.1.2 *Whole-sediment exposures*

Effects on survival in copper-spiked sediment

Not all laboratories were able to participate in both the static and the static-renewal portions of the two round-robins conducted with copper-spiked sediment. Three of the four laboratories using the static system were able to achieve the proposed (EC, 1995b) minimum acceptable criterion for control survival of ≥70% for this species (Table D-1, Appendix D). One laboratory failed to meet this criterion with a control survival of 40% in the first spiked-sediment test. This laboratory was unable to participate in the repeat of the copper-spiking round-robin using the static system in Phase II.

All three laboratories which used the static-renewal system achieved the proposed acceptability criterion for survival in control sediment of ≥70% with the exception of Laboratory C in Phase I

(Table D-2, Appendix D). Mean survival of *C. riparius* in the control sediment was 94.5% in the static systems (results from Lab C not included) and 89.3% in the static-renewal system (results from Lab C, Phase I not included). Mean CVs for percent survival in the control sediment were low, *i.e.*, 5.8% in the static system and 12.8% in the static-renewal system (Table 10).

In general, interlaboratory variability for replicate treatments was greater using the static-renewal system with CVs for individual test concentrations ranging from 9.7 to 143% compared to the static system where CV's ranged from 5.8 to 25.1% (Table 10). Per-treatment interlaboratory variability also increased as the test concentrations increased (esp. at 1000 and/or 2000 μg Cu/g nominal). Appreciable mortalities of midge larvae were evident in some replicates at these concentrations.

There was complete mortality at the highest concentration of copper (2000 μg Cu/g) in the static system whereas some organisms were able to survive (mean % survival of 16.7) in this same concentration when the static-renewal system was operating (Figure 5).

LC50s in static vs. static-renewal systems

Round-robin tests with a range of concentrations of copper-spiked sediment resulted in some statistical differences between laboratories using a particular system (Table 11). For each series of round-robin tests performed using the static system, interlaboratory LC50s did not differ significantly. In contrast, LC50s for static-renewal tests differed significantly between laboratories in both Phase I and Phase II round-robins with copper-spiked sediment (Table 11).

Within a particular laboratory, there were no statistical differences in the reported LC50s using the static vs. the static-renewal system and the same population of test organisms.

Grand mean LC50s for all the participating laboratories were 1110.2 μg Cu/g d.w. using the static system and 1139.3 μg Cu/g d.w. using the static-renewal system. These values do not differ significantly ($p \leq 0.05$) and the CVs are reasonably low, ranging from 10.8 to 27.8% (Table 11). The variability in the static system (grand CV, 10.8%) was lower than that using the static-renewal system (grand CV, 27.8%).

Effects on growth in copper-spiked sediment

The mean dry weight of individual animals exposed to control sediment for 10 days varied appreciably among laboratories and ranged from 0.54 to 1.09 mg in the static system and 0.52 to 1.27 mg in the static-renewal system (Tables D-3 and D-4, Appendix D). No consistent differences in control growth for laboratories or test series (*i.e.*, Phase I or Phase II) using copper-spiked sediment were evident. However, the grand mean CV (all laboratories, both round-robins) for 10-d growth in clean sediment using the static system (17.9%) was appreciably lower than that for tests using the static-renewal system (43.4%) (see Tables D-3 and D-4, Appendix D).

For each laboratory and dose-response series, growth of *C. riparius* was reduced as the concentrations of copper spiked into sediment increased for both the static and the renewal systems (Figure 6). As with % survival, the CVs for growth were higher in the static-renewal system compared to the static system, especially when the toxicity of the sediment (and the concentration of copper) increased (Tables D-3 and D-4, Appendix D).

IC25s in static vs. static-renewal systems

As with the LC50s for survival, between-laboratory IC25s differed statistically. However, intralaboratory IC25s for static versus static-renewal systems were found to differ significantly in only one of four comparisons (Table 11) and the mean IC25s (all laboratories) for growth derived using either system did not differ significantly. As was evident for the mean CVs for % survival, the mean CV for growth using the static system (37.4%) was appreciably lower than that using the static-renewal system (68.4%) (Table 11).

Effects on survival of organisms exposed to natural sediments

Using either the static or static-renewal systems, all laboratories achieved ≥70% survival rates during 10-d exposures to each of the two natural sediments collected from field sites and tested in the laboratory (see Tables D-1 and D-2, Appendix D). Sediment collected from Hamilton Harbour has been shown to contain contaminants such as metals, PAHs and PCBs which can affect the survival and growth of midge larvae (Day *et al.*, 1995a). However, in this study, no detrimental effects were observed.

Effects on growth of organisms exposed to natural sediments

In Phase II, growth of *C. riparius* exposed to sediment collected from Hamilton Harbour, Lake Ontario, under static conditions was slightly but consistently reduced relative to growth in the sediment from Long Point marsh, Lake Erie, for each of the three participating laboratories (Table D-3, Appendix D). This difference, however, was not statistically significant.

In the static-renewal system, no consistent decrease in growth of midge larvae in the Hamilton Harbour sediment was evident for any laboratory and mean values for growth (all laboratories) were similar (Table D-4).

Comparison of h and k consistency statistics

The values of the consistency statistic *h* for interlaboratory survival and growth are shown in Appendix E, Figures E-1 and E-2. For both the Phase-I and Phase-II studies, all *h* values for each laboratory, using static or static-renewal systems, fell within the limits of the statistically-derived critical value.

The values of the consistency statistic *k* for intralaboratory survival and growth are shown in Appendix E, Figures E- 3 and E-4. For Phase-I survival, no laboratories that met the proposed (EC, 1995b) control survival criterion of ≥70% exceeded the critical value for *k* in the static and the static-renewal systems. For Phase-II round robins, only one laboratory exceeded the critical *k* value for survival, and this occurred in only one instance (*i.e.*, control sediment in the static-renewal system, laboratory C) (Figure E-4).

For Phase-I growth, one laboratory that met the proposed control survival criterion of ≥70% exceeded the critical value in the static-renewal system, and one laboratory exceeded the critical value in the static-renewal system for Phase-II .

3.2.1.3 *Chemical analysis of copper*

The concentrations of copper in the overlying water, pore water and bulk sediment for each dose of spiked-sediment provide by NWRI to each of the participating laboratories in Phase I of the interlaboratory experiments are shown in Figure F-1, Appendix F, for both the static and the static-renewal systems. Concentrations of copper in the bulk sediment were determined at -14-d (day of spiking), day 0 of test initiation and day 10 (test termination). Concentrations of copper in the overlying and pore water were only determined on day 0 and day 10.

Concentrations of copper in the bulk sediment closely approximated the desired nominal concentrations in either system and concentrations increased as amount used in spiking increased. Concentrations of copper in the overlying water were low (μg/L) but also increased with increasing dose especially in the static system. Concentrations of copper on the overlying water in the static-renewal system plateaud at the higher concentrations due to the twice-daily flushing of the overlying water in this system. Concentrations in the overlying water in the static system remained fairly constant over the course of the 10-d experiment, indicating that equilibrium of copper between the aqueous and the solid phase was occurring. Concentrations of copper were higher in the pore water than in the overlying water (mg/L vs. μg/L) in both systems and were similar for either static or static-renewal. In addition, concentrations in the pore water declined from day 0 to day 10.

3.2.2 *Hyalella azteca*

3.2.2.1 *Water-only reference toxicity tests*

The test-validity criterion of ≥90% control survival (USEPA, 1994; EC, 1995a) for water-only reference-toxicity tests with *H. azteca* was achieved in only 60% (*i.e.*, 6 of 10) of the acute lethality tests with copper which were performed by five participating laboratories (Table 12). The mean 96-hour LC50 was 175.5 μg Cu/L with a range of 99 to 293 μg Cu/L for tests with acceptable control survival. There were significant differences in the calculated LC50s between laboratories. No trends in LC50s with respect to the hardness of the dilution water were apparent.

3.2.2.2 *Whole-sediment exposures*

Effects on survival in copper-spiked sediment

All laboratories achieved the proposed minimum acceptable criterion of ≥ 80% survival for *H. azteca* in control sediment (EC, 1995a) using either static or static-renewal systems (Figure 7). Mean survival was similar for both the static (97.3%) or the static-renewal systems (93.3%) and variances were low (Table 13). Percent survival of *H. azteca* decreased with increasing nominal concentrations of copper in both the static and the static-renewal systems in a dose-response manner. A significant reduction ($p < 0.05$) in % survival at nominal concentrations of 250 μg Cu/g d.w. sediment in the static system and at 500 μg Cu/g d.w. sediment in the static-renewal system compared to the control sediments was observed (Tables D-5 and D-6, Appendix D). Within each system, a higher degree of variability was observed at the higher concentrations of copper; this variability was slightly less in the static-renewal vs. the static system (Figure 7; Table 13).

LC50s in static vs. static-renewal systems

A statistical comparison of the LC50s derived by each laboratory for the range of concentrations of copper-spiked sediment used in the study showed some significant differences among laboratories using the same test system (static or static-renewal) (Table 14). Additionally, a comparison of intralaboratory findings with either static or static-renewal systems, indicated a significantly lower LC50 in the static system for two of the three side-by-side tests amenable to this comparison. The grand-mean LC50 calculated for survival data from all laboratories using the static mode was 379.5 µg Cu/g d.w.sediment, whereas the grand mean LC50 for the static-renewal mode was 742.5 µg Cu/g d.w.sediment; CVs for these grand means were high (38.2%, static; 52.9%, static-renewal).

Effects on growth in copper-spiked sediment

In each laboratory, the 14-d level of growth achieved by *H. azteca* exposed to a range of concentrations of copper-spiked sediment decreased with increasing concentrations of copper in both the static and the static-renewal systems (Figure 8). Mean dry weight of organisms exposed

to control sediment varied among laboratories and ranged from 0.08 to 0.24 mg in the static system and from 0.11 to 0.21 mg in the static-renewal system (Tables D-7 and D-8, Appendix D).

Interlaboratory variability was high using both systems, with CVs for grand-mean dry weights ranging from 36.8 to 100.5% in the static system and 29.4 to 50.4% in the static-renewal system (Table 13).

IC25s in static vs. static-renewal systems

As with the LC50s for survival, IC25s derived by each laboratory for growth in the range of concentrations of copper-spiked sediment differed significantly from laboratory to laboratory independent of whether the static or the static-renewal system was utilized (Table 14). intralaboratory results for side-by-side comparisons using both systems indicated consistently-lower IC25s using the static system. However, pairwise statistical comparisons of results from each of the four laboratories performing both static and static-renewal tests showed that, in each instance, the IC25s did not differ significantly.

Effects on survival of organisms exposed to natural sediment

All laboratories achieved the proposed minimum acceptable criterion for survival in control sediment of ≥80% in both the static and the renewal systems when natural sediments were tested (Figure 9). Notwithstanding, there were statistically-significant differences ($p < 0.05$) in the % survival of *H. azteca* in the control sediment, Sediment C, vs. a contaminated sediment, Sediment D, for three of four laboratories using the static system and for one of four laboratories using the static-renewal system (Tables D-9 and D-10, Appendix D). For each of these comparisons, survival rates in Sediment D were significantly lower than those in the control sediment (Sediment C). Percent survival in Sediments A and B did not differ significantly from that in the control sediment.

Effects on growth of organisms exposed to natural sediments

For each of the participating laboratories, the mean dry weights of *H. azteca* exposed to control sediment (Sediment C) for 14 days were greater than those for animals held in Sediments A, B, or

D using either the static system or the static-renewal system (Figure 10). In most instances, these differences proved statistically significant (see Tables D-11 and D-12, Appendix D). The grand means for dry weights of *H. azteca* exposed to each of these four sediments in side-by-side tests using static versus static-renewal systems were similar for individual samples, and showed no consistent trend due to test mode (Table 15).

Toxicity ranking for field-collected natural sediment using static or static-renewal systems

A by-sample statistical correlation of sample toxicity, using the survival and growth data derived by each of the participating laboratories and statistics such as the Spearman's or Kendall's rank-correlation coefficients (Zar, 1984), could not be utilized in the present instance since the number of participating laboratories was ≤5. However, within-laboratory ranking of the toxicity of each of the four field sediments based on data for % survival (Table 16) or growth (Table 17) showed good agreement between laboratories. Based on survival data and using a ranking scale of 1 to 4 (where 1 represents the highest survival rate and 4 the lowest survival rate), Sediment C (the "control" sediment) was consistently ranked as 1 or 2 by all laboratories except for F (static-renewal test only), independent of test type (static or static-renewal) (Table 16). Similarly, based on growth data and using a ranking scale of 1 to 4 where 1 represents the greatest mean dry weight at test end and 4 represents the least dry weight), Sediment C was consistently ranked as 1 (i.e., best growth) by each of the six laboratories regardless of test type. Sediment D was consistently ranked as that sediment giving the lowest survival rate (4) or tied with one other sediment in this regard (3.5), by all laboratories (Table 16); this sediment also showed the least growth of amphipods for six of eight intralaboratory rankings (Table 17). Thus, regardless of test system (static or static-renewal) or test end point (survival or growth), this ranking scheme indicated a trend, both within and between laboratories, of lesser/least toxicity for Sediment C (control) and greater/most toxicity for Sediment D.

Comparison of h and k consistency statistics

The values of the consistency statistic *h* for interlaboratory survival and growth are shown in Appendix E, Figures E-5 and E-6. For survival, two laboratories that met the proposed (EC, 1995a) criterion of ≥80% for control survival exceeded the critical value using the static system in Phase-I, and one laboratory exceeded it in Phase-II. For growth, all laboratories were within the

critical values using both systems and either copper-spiked sediment or field-collected sediment.

The values for the consistency statistic *k* for intralaboratory survival and growth are shown in Appendix E, Figures E-7 and E-8. For survival, one laboratory exceeded the critical value in the static system in Phase-I, while none exceeded it in Phase-II. For growth, one laboratory exceeded the critical value in the renewal system in Phase-I, while none exceeded in Phase-II.

3.2.2.3 *Chemical analysis of copper*

Concentrations of copper in the overlying water, pore water and bulk sediment for both the static and the static-renewal systems are shown in Figure F-2, Appendix F. As in the *C. riparius* experiment, concentrations of copper in the bulk sediment closely approximated nominal concentrations in both systems. Concentrations in the overlying water were low and increased with increasing nominal concentrations of spiking in the static system. Concentrations in the overlying water plateaud as concentrations increased in the static-renewal system. Concentrations of copper were therefore consistently higher in the overlying water in the static system vs. the static-renewal system on both Day 0 and Day 14 for all nominal concentrations. Concentrations in the pore water were higher on day 0 vs. day 14 for the static system.

3.3 Minimum Acceptable Dry Weight for Control Animals

3.3.1 *Chironomus riparius*

Table G-1, Appendix G, summarizes the available data for growth measured as mg dry wt./individual midge larvae for organisms exposed to a variety of clean sediments for 10 days. Organisms were fed at the standard rate of 6 mg Nutrafin^{R} daily or 15 mg/4X over 10 days. A grand mean of 0.92 ± 0.10 mg d.w. (CV = 10.4%) was determined for the static system vs. a grand mean of 0.74 ± 0.40 mg d.w. (CV = 23.5%) for the static-renewal system.

3.3.2 *Hyalella azteca*

Table G-2, Appendix G, summarizes the available data for growth of *H. azteca* measured as mg dry wt./individual juvenile for organisms exposed to a variety of clean sediments for 14 days.

Organisms were fed at the standard rate of 1.5 mL YCT daily or 3.5 mL YCT three times weekly on non-consecutive days. A grand mean of 0.22 ± 0.66 mg d.w. (CV = 31.7%) was determined for the static system vs. a grand mean of 0.19 ± 0.04 mg. d.w.(CV = 23.5%) for the static-renewal system.

4.0 DISCUSSION

The use of laboratory toxicity tests with benthic organisms and solid-phase sediments has become an important regulatory tool to assess the potential impacts of sediment-associated contaminants on invertebrates found in aquatic ecosystems. Several species such as the amphipod *H. azteca* and midges *C. tentans* and *C. riparius* are routinely recommended as organisms of choice for tests conducted in Canadian laboratories. Despite their frequent use, there are currently no published guidance documents which outline standardized procedures for private or government laboratories to follow when such tests are required. In addition, there are differences in opinion among the Canadian scientific community regarding the size of test containers, the necessity of the renewal of the overlying water during a test as recommended by USEPA (1994) and the type of food and frequency of feeding required to maintain adequate survival and growth over a 10- to 14-d period of exposure.

This study was designed to provide information and recommendations on the use of a static and\or a static-renewal system and a feeding regime for solid-phase sediment toxicity tests with two species of benthic invertebrates, the amphipod *H. azteca*, and the midge *C. riparius*. The information was to come from two sets of experiments, intralaboratory comparisons conducted at NWRI and interlaboratory "round-robins" where multiple laboratories simultaneously exposed organisms to the same test materials under similar, standardized conditions. The recommended procedures from these initial experiments are to be incorporated into the final drafts of two Canadian guidance documents on biological test methods for solid-phase toxicity tests with *H. azteca* and *C. riparius* (EC, 1995a; EC, 1995b).

All test methods have inherent variability which must be taken into account for practical interpretation of test results. This variability may result from a number of factors such as unavoidable random errors, equipment failure, calibration of equipment, environmental factors, test material differences, source of test animals, source of test water and proficiency of operators

and laboratory personnel (Schlekat *et al.*, 1995; Burton *et al.*, 1996). As part of the validation process for the two draft Canadian guidance documents, interlaboratory testing was conducted to determine the method variances for both protocols and used toxic sediments provided by the host laboratory. The results of the study are discussed below.

4.1 Comparison of Static vs. Static-Renewal Systems in Intralaboratory Tests

As discussed by Ankley *et al.* (1993) and Kubitz and Giesy (1995), static conditions can result in unacceptable overlying water quality in whole-sediment bioassays using sediments with a high oxygen demand even when constant aeration is provided. Conversely, the renewal of the overlying water and daily feeding regime suggested by USEPA (1994) requires more equipment as well as technical expertise and increased labour in comparison to the static mode of operation; in addition, if renewal of the overlying water occurs, contaminants in the sediment may be depleted through the flushing of the interstitial (pore) water, thereby lessening exposure of the test organisms to contaminants. In the current study, a static and a static-renewal system were compared in side-by-side experiments using three non-contaminated ("clean") field-collected sediments and two species of benthic invertebrates for the end points of percent survival and growth measured as dry weight at test completion.

4.1.1 *Chironomus riparius*

Survival

Survival of *C. riparius* was consistently high in a variety of clean sediments regardless of the feeding regime and type of system utilized with one exception *i.e.*, survival was reduced below the acceptability criterion of 70% in the static-renewal system containing sediment collected from Lake Erie (reference site # 108). Variability among replicate beakers for this sediment and feeding regime was large *i.e.*, survival in 2 out of 5 beakers was <50% whereas two other replicates had ≥90% survival. The reason for this variability and lowered survival is not known at this time but may be attributed to observations in our laboratory regarding potential losses of smaller instars through the mesh covering the openings in the beakers receiving twice-daily renewals of overlying water during the early stages of the 10-d exposures. Sediment # 108 also

had a higher percentage of clay (62.6%) compared to the other two sediments (25.9% for sediment # 1 and 0% for sediment # 3) which may have made it difficult for *C. riparius* to burrow into contributing to their susceptibility to flushing.

The addition of two rations of food either daily or every three days over the course of the exposure had no consistent detrimental effect on survival indicating that the animals were receiving an adequate level of food. Neither system (static nor static-renewal) was consistently more precise when CVs for % survival were compared in two of three sediments. However, higher variability was again observed in the static-renewal system for survival of animals exposed to reference sediment # 108. Again, no reason for this higher variability in survival for the static-renewal system is known but it could be attributed to the flushing of organisms during the renewal process or some unknown habitat characteristic which was detrimental to the organisms.

Growth

Growth measured as dry weight of *C. riparius* was also influenced by the type of sediment to which the animals were exposed as well as the amount of food added. The significantly higher growth observed at the higher feeding rates in the static system (*i.e.*, 60 mg dry weight food per test added either as 6 mg daily or 15 mg every 3 days) in two of the three sediments suggests that the higher feeding rate is preferable in order to maximize growth. In the static-renewal system, the higher food ration did not seem to affect the growth of the animals regardless of frequency. The overall lower growth rates observed in the static-renewal system and the lack of correlation with quantity added suggest that there may have been less food available to the animals and this could result from the twice-daily flushing of overlying water.

The type of sediment to which the animals were exposed also had an influence on growth. Sediment characteristics such as particle size distribution and organic carbon content have been shown by Ankley *et al.* (1993) and Suedel *et al.* (1996a, 1996b) to influence the amount of growth of benthic invertebrates regardless of the presence or absence of contaminants or the feeding regime. *C. riparius* is known to inhabit enriched environments and is an indicator of eutrophication of aquatic ecosystems (Postma *et al.*, 1994). The higher growth of this species in sediment #3 which had a high silt content (97.2%) vs. sediment # 1 or # 2 with high sand (72.3%) and clay (62.4%) content, respectively, may reflect the more desirable habitat characteristics of this sediment.

In general, the data for growth in sediments with no renewal were more precise than the data for growth in sediments exposed to the static-renewal system. For example, the CVs for growth in the static system ranged from 3.2 to 10% whereas the Cvs for growth in the static-renewal system ranged from 5.9 to 34%. Again, this variability in growth may be attributed to a lower amount of food in the static-renewal system due to the flushing of the overlying water. The higher precision achieved in the static system would offer an advantage in terms of the discriminatory power of the test when growth is the desired end point (USEPA, 1994).

Water quality

Overlying water quality has been shown to be compromised under static conditions in sediments with high oxygen demand (Ankley *et al.* ,1993; Kubitz and Giesy, 1995). Ammonia in unionized form may be a potential toxicant to benthic invertebrates under these conditions and should be measured regularly during whole-sediment toxicity tests to avoid misinterpretation of the cause of any toxicity observed in solid-phase bioassays (Borgmann ,1994; Ankley et al., 1995).

Ammonia In the present study, slightly higher ammonia concentrations were recorded in the static system (range <0.01 to 12 mg/L) vs. the static-renewal system (range 0.18 to 1.8 mg/L) especially in sediment # 3. The flushing of the overlying water at least twice daily in the static-renewal system may prevent the accumulation of ammonia which offers an advantage of this system over the static system. USEPA (1985) recommends a safe level of 0.02 mg/L unionized NH_3-N for all aquatic life. Monda *et al.* (1995) found that acute 96 h LC50 values for *C. riparius* were 9.4 mg/L for total ammonia and 6.6 mg/L for unionized ammonia in well water, values which approach the high level of 12 mg/L found in sediment # 3 receiving the highest ration of food. However, Schubauer-Berigan *et al.* (1995) found that the toxicity of total ammonia-N to the midge *C. tentans* was pH-dependent. The LC50 for a 10-d exposure of *C. tentans* in water with pH in the range of the overlying water in this study (pH 8.6) was 82.4 (70.0-97.0) mg/L. As survival of *C. riparius* was not below the acceptability criterion and growth was not reduced in beakers where total ammonia was higher than the literature values for LC50s for this species, it is presumed that these concentrations did not have a detrimental affect on organisms contained in the test beakers.

Dissolved oxygen. The continuous aeration provided in the static system maintained oxygen

concentrations at or near saturation (≥7.0 mg/L) throughout the 10-day exposure period. The lack of continuous aeration in the static-renewal system, and the 12-hour intervals between each water renewal may lead to periodic lower concentrations of oxygen in the static-renewal system as demonstrated in the present series of tests with *C. riparius*. The addition of food may also contribute to oxygen depletion (i.e., <40% saturation) in the overlying water when using a static-renewal system. In the present static-renewal tests, a drop in oxygen level was sometimes observed shortly after the addition of food followed by a rise with the subsequent flushing of overlying water. Ankley *et al.* (1993) recommends at least four water replacements per day in tests with *C. tentans* but USEPA (1994) allows the minimum of twice daily replacements in their test methods. The periodical decline in dissolved oxygen levels just prior to the daily replacement of overlying water did not seem to have any significant affect on the survival and growth of this species in the present study. It may require longer sustained periods of lowered levels of dissolved oxygen to have a significant impact on survival of *C. riparius*.

4.1.2 *Hyalella azteca*

Survival

Based on the high (≥80%) survival observed in each of the three "clean" field sediments, both the YCT diets (daily or 3X/week) and the Nutrafin^{R} diet are adequate to use in a 14-day whole-sediment toxicity test with *H. azteca*. In addition, neither the static nor the static-renewal system impacted survival and each system had similar precision based on the observed CVs for mean % survival. The initial age of juvenile *H. azteca* used to start the bioassays in this exposure was 3 to 10 d, younger than the juvenile *H. azteca* (7 to 14 d) recommended by USEPA (1994). These results indicate that week-old juveniles of *H. azteca* can be used successfully in the static-renewal system.

Growth

Similar to the results for *C. riparius*, choice of system, diet, and the physico-chemical characteristics of a sediment appear to influence growth measured as dry weight. For example, *H.*

azteca had better growth when fed YCT (either daily or 3X/week) in two of three sediments (sediment # 1 and sediment # 3) especially in the static system. Therefore, while both diets maintained adequate survival, the YCT diet appears to be preferable for this species when growth is the end point. YCT may be more nutritious or contain essential vitamins that are absent in the Nutrafin^{R} diet which could account for the increased biomass in *H. azteca* fed this diet. The removal of YCT during the twice-daily flushing of the overlying water in the static-renewal system may also remove ration and affect the growth of the organisms.

The physico-chemical characteristics of the sediment clearly can also play a significant role in the growth of this species as evidenced by the relatively poor growth in sediment #2. The organic content of this sediment was apparently not a determining factor since the TOC content of sediment #2 was intermediate of the three sediments tested. Sediment #2 had a relatively high percentage of clay (62.6%), which may have affected the burrowing activities of *H. azteca* adversely and contributed to their poorer growth in this sediment. *H. azteca* have been shown to spend more time in the water column of bioassay beakers when the quality and level of contamination of the sediment is detrimental to their growth and survival (Whiteman *et al.*, 1996).

Most sediment toxicity tests with *H. azteca* are either tests of lethal exposure with survival as the end point measured after 10-d exposure or sublethal exposures with survival and growth measured as end points after 28-d (Ingersoll and Nelson, 1990; USEPA, 1994). Kubitz *et al.* (1995) suggests that a 14-d *H. azteca* growth inhibition test is a definitive test of chronic exposure to toxic sediments with 3 to 10-d old juveniles used to initiate the test. In the current study, growth of 2 to 9 day-old juvenile *H. azteca* was measurable after 14-d exposures to clean sediments. Variability among replicate beakers was reasonably low for either system with CVs ranging from 6.0 to 28.6% for the static system and 12.0 to 23.5% for the static-renewal system.

Water Quality

Ammonia In contrast to the results from the intralaboratory study with *C. riparius,* levels of total ammonia in either the static or the static-renewal systems were < 0.1 mg/L for all sediments studied and for all feeding regimes and food type. The only natural sediment in common with the study using *C. riparius* was sediment # 1 from Long Point marsh, Lake Erie, which also had low levels of ammonia when used in beakers under the static system. These levels of ammonia are

well below the levels of 14.4 to 19.8 mg N/L considered toxic to *H. azteca* by Ankley *et al.* (1995). Similar values were reported by Borgmann (1994).

Dissolved oxygen Levels of dissolved oxygen were slightly lower in the static-renewal system vs. the static system for all sediments but none were below the 40% level of saturation considered essential for test acceptability by USEPA (1994).

4.1.3 Recommended Test Procedures for Interlaboratory Round-Robin

Based on the results outlined in Section 3.1, both the static and the static-renewal system were included in interlaboratory comparisons by laboratories participating in the round-robin. The diet for the 10-d *C. riparius* whole sediment toxicity tests was 60 mg of a Nutrafin^{R} slurry added four times over the course of the exposure on non-consecutive days. The diet for *H. azteca* was 3.5 mL of the YCT mixture added five times over the 14-d exposure on non-consecutive days.

4.2 Interlaboratory Round-Robins

Criterion for a valid test

One of the objectives of the interlaboratory study was to assess whether the criteria for a valid toxicity test given in Environment Canada's draft biological test method for midge larvae (*C. riparius* or *C. tentans*) (EC, 1995a) and *H. azteca* (EC, 1995b) are appropriate or need revision. Current guidance documents (USEPA, 1994; ASTM, 1995) only provide a minimum acceptable criteria for percent survival of animals in control sediment(s) *i.e.*, ≥80% survival of *H. azteca* in the 10-d survival test and ≥70% survival of *C. tentans* or *C. riparius* in the survival and growth test. An average size of *C. tentans* of 0.6 mg d.w\individual in the control sediment at the end of a survival and growth test is suggested in USEPA (1994) based on Ankley *et al.* (1993). Neither protocol has included a minimum acceptable value for average size of *H. azteca* or *C. riparius* The rational for the recommended criterion for minimum acceptable survival and growth for each species are discussed below.

Minimum acceptable survival of animals in control sediment(s) The values for minimum acceptable survival in control sediment (≥80% for *H. azteca;* ≥70% for *C. riparius*) proposed in

EC (1995a, 1995b) as tentative criterion for each species were consistent with those specified in USEPA (1994). For the thirteen 10-day tests with *C. riparius* and copper-spiked sediment completed by the various participating laboratories using both static and static-renewal test options, 85% achieved ≥70% control survival. For the eighteen 14-day tests with *H. azteca* and copper-spiked sediment performed by participating laboratories, 100% achieved ≥80% control survival. Given the differences between Environment Canada's draft test methods and those in USEPA (1994), validation of the "USEPA-1994 adopted" criterion for minimum acceptable control survival using *C. riparius* (different species; two test options) or *H. azteca* (different test duration; two test options) is warranted.

The general culture health of the test organisms for the one participating laboratory with poor survival may have accounted for their failure to achieve the acceptable control survival criterium for *C. riparius* during the course of this test. Healthy test organisms at test initiation are of critical importance, and thus the implementation of the concurrent water only reference toxicant test is relevant. Laboratory C was unable to perform the water only reference toxicant test due to high mortality in test organisms (animals died before they reached second instar indicating possible problems with the organisms used in the bioassay).

Minimum acceptable growth of animals in control sediment(s). In this study, a grand mean (± one standard deviation) was calculated for the growth of each species in each of the two test systems (static and static-renewal) for all data measured in non-contaminated sediments ("clean and control sediments) and semi-artificial formulated sediment used in both the intra- and interlaboratory studies (Appendix G). This grand mean incorporates most of the inherent variability which should occur in laboratories which utilize different operators, equipment and culture organisms and under different environmental conditions. It also takes into consideration the variability in the responses of organisms to sediments with differing natural characteristics such as organic carbon content and grain size. A conservative approach was taken in determining the minimum acceptable criterion for growth in control sediments. This level is set at two standard deviations below the grand mean for growth in the static-renewal system. Due to the limited number of tests which were conducted and laboratories which participated in the round-robin, a single value for either system is being recommended. For *C. riparius*, the minimum acceptable level of growth of larvae in a control sediment after 10-d exposure at 23 ± 1 °C is 0.5

mg d.w.\individual. For *H. azteca*, the minimum acceptable level for growth in a control sediment after 14-d exposure at 23 ± 1 °C is 0.1 mg d.w.\individual. These values can be re-evaluated and revised as more data from laboratories conducting solid-phase toxicity tests using either a static or a static-renewal system become available.

Test precision

The precision of a test method describes the closeness of agreement between test results obtained from repeated testing of a prescribed method (ASTM, 1990; ASTM, 1992). Quantitative determination of precision as well as accuracy in toxicity testing as compared to analytical (chemical) determinations is difficult or may be impossible in some cases as the true values for the toxicity of samples are not known (Burton *et al.*, 1996). However, repeatability (closeness of agreement of values when the test is repeated by a single operator with a single system using the same test material under identical test conditions) and reproducibility (variability between single test results obtained from the same sample in multiple laboratories) can be determined from intra- and interlaboratory studies respectively. Interlaboratory tests are often referred to as "round-robins" or "ring-tests". The coefficient of variation (CV) is the most commonly used statistic for evaluating test precision in round-robins involving toxicity tests. Burton *et al.* (1996) also recommends that the consistency statistics *h* and *k* suggested in ASTM (1992) be used to describe between-laboratory and within-laboratory consistency, respectively. When environmental samples are extremely toxic, CVs can be very high (>100%) yet the range of responses could be very low. For example, if there are multiple replicates with no survival and one with low survival, CVs could be as high as 256% (USEPA, 1994). Consistency statistics help to show if acceptable variation exists.

For the round-robin tests co-ordinated in this study, the grand CV's (standard deviation divided by the grand mean of all laboratories x 100) were ascertained for both species and both end points (survival and growth) and for natural sediments and sediments spiked with copper for all data which achieved the minimum acceptable criteria for survival. Additionally, grand CV's were determined for the 10-d (*C. riparius*) and 14-d (*H. azteca*) LC50's and IC25's in dose-response experiments with copper-spiked sediment.

Grand mean CVs for percent survival of *C. riparius* ranged from 2.1 to 25.1% in the static system and 7.7 to 142.9% in the static-renewal system. Grand CVs for percent survival of *H. azteca* ranged from 4.7 to 155.5% in the static system and 2.5 to 118.2% in the static-renewal system. The large range for these CVs are due to the high variability shown in replicates as samples of sediment spiked with copper increase in toxicity. These CVs compare favourably to those reported in USEPA (1994) for a solid-phase round-robin with both *C. tentans* and *H. azteca* where values ranged from 36.2 to 256% for the 10-d survival test with the amphipod and 36.7 to 233% for the 10-d survival test with the midge. Other marine and freshwater solid-phase toxicity tests have also reported that CVs are lowest in control sediments and highest in sediment dilutions of toxic sediments (Schlekat *et al.*, 1995; Burton *et al.*,1996).

Grand CVs for growth of *C. riparius* in the static system ranged from 2.6 to 56.3% and 29.2 to 75.9% in the static-renewal system. Grand CVs for growth of *H. azteca* ranged from 3.6 to 100.5% in the static system and 2.6 to 54.8% in the static-renewal system. With growth as an end point, Burton *et al.* (1996) reported CVs of 26.6% for *C. tentans* in control sediment and 31.9% for this species in moderately toxic sediment. Rue *et al.* (1988) reviewed intra- and interlaboratory variability in acute toxicity tests results for a variety of effluents and reported an average CV of 15.8% for all tests where the CV's of 0 were excluded. They suggest that the longer test periods used in sublethal toxicity tests offer greater opportunities for random physical, chemical and biological factors to affect these test results; thus, results from longer-term tests might be expected to be more variable than results for shorter-term tests.

DeGraeve *et al.* (1992) summarized intra- and interlaboratory precision data for a large number of analytical methods and found that they were similar to those found for acute and short-term chronic effluent toxicity tests. For example, the CVs for the chemical analysis of phthalate esters ranged from 1 to 80%. Higher variabilities were generally associated with analyses performed near the analytical detection limits whereas lower variabilities were associated with midrange or higher analyses. Thus, acute and chronic tests when performed according to standardized procedures using healthy organisms are as precise and reproducible as many analytical chemical methods.

The use of the consistency statistics *h* and *k* also provide a measure of precision. When looking at precision using the *h* and *k* graphs, the interlaboratory growth and survival results appeared similar

using either system. For instance, a similar number of laboratories exceeded these critical values representing precision in both systems. Additionally, at higher nominal concentrations of copper, (1000 and 2000 μg/g), where the highest CVs for survival and growth were noted, both the *h* and *k* values were either on or below the critical value line in both systems, indicating that the systems were similarly consistent in terms of variability under such conditions.

Comparison of the static vs. the static-renewal systems

Chironomus riparius

Copper-spiked sediments. In analyzing and reviewing the results for 10-day "side-by-side" tests with copper-spiked sediment which used the static system rather than the static-renewal system, the following was noted: (1) lower overall LC50s and IC25s (Table 11); (2) decreased survival at the highest nominal copper concentrations (Table 10); and (3) significant differences in growth (compared to the controls) at nominal concentrations lower than those inhibiting growth using the static-renewal system (Tables D-3 and D-4, Appendix D). All of these results suggest increased toxicity in tests conducted using the static system, relative to those using the static-renewal system. Any reduced toxicity evident in the static-renewal system is most likely due to the flushing of contaminants from the overlying water resulting from the twice-daily renewal process. This was confirmed by the higher copper concentrations found in the overlying water in the static system relative to those in the static-renewal system (Figure F-1, Appendix F).

The 10-d LC50's reported for *C. riparius* in either the static or the static-renewal systems based on nominal bulk sediment concentrations were similar to values of 857μg Cu/g d.w sediment and 1026 μg Cu/g d.w. sediment reported by Cairns *et al.* (1984) and Suedel *et al.* (1996a) for *C. tentans*. Suedel *et al.* (1996a) conclude that the responses of benthic invertebrates to copper-spiked sediment corresponds to the overlying water concentration of copper rather than the concentration of copper in the bulk sediment or pore water. However, the concentration of copper found in the overlying water is dependent ultimately on the concentration of copper in the sediment and the sediment characteristics (organic ligands, pH, organic carbon) themselves (Leckie and Davis, 1979; Malueg *et al.*, 1986; Lewis, 1992)

Overall, the intra- and interlaboratory results for survival and growth using the static system were typically less variable when only data from samples of sediments which meet the minimum acceptable level of survival are considered. The grand-mean CVs (all laboratories) for survival ranged from 2.1 to 25.1% in the static system and 2.6 to 56.3% in the static-renewal system. The grand mean CVs for growth of *C. riparius* ranged from 7.7 to 27.4% in the static system and from 29.2 to 75.9% in the static-renewal system. Undoubtably, some of the variability reported in both the static and the static-renewal systems when data from all the laboratories are summarized incorporates interlaboratory differences in the source/size/age/condition of the test organisms as well as differences in the source of the overlying water. Each of the participating laboratories used their own source of water for the tests and this water varied in hardness measured as mg/L $CaCO_3$.

In spite of our present evidence from the round-robin tests with *C. riparius* for somewhat lesser variability and greater test sensitivity using the static system, as compared to the static-renewal system, our statistical comparisons of LC50s or IC25s for side-by-side tests performed at the same laboratory do not distinguish a consistent difference in test sensitivity using either system. A lack of significant difference in grand-mean LC50s or IC25s, when all survival or growth data for participating laboratories testing the copper-spiked sediment was pooled, further supports the conclusion that either test mode (i.e., static or static-renewal), when performed according to the procedures and conditions used here, yields similar end point-toxicity results. It is also noteworthy that other sediment-toxicity tests using *C. tentans* and copper-spiked sediment have recorded LC50s for copper (i.e., 1026 µg/g, Suedel *et al*, 1995a; 857 µg/g, Cairns *et al.*, 1984) that are similar to those found in the current round-robin tests with *C. riparius*.

Field sediments. Both the static and the static-renewal systems showed a similar precision when interlaboratory results for percent survival in two samples of field-collected sediment were compared. However, growth in the static system appeared to be more precise than that in the static-renewal system (Table 10), perhaps due to the lack of flushing overlying water from the static system and the consequent greater availability of food to enable more consistent growth. Further side-by-side comparisons of growth and survival of *C. riparius* (or *C. tentans*) in a variety of field-collected sediments (clean and contaminated) using each of these two test systems are required before a clear understanding of the influence of these differing test systems on test performance is available.

Hyalella azteca

Copper-spiked sediments. As with *C. riparius*, there appears to be some indication of increased toxicity in the static system relative to the static-renewal system in side-by-side comparisons. However, no consistent and significantly different trends in lower LC50s or IC25s are evident for the static system. For instance, for two of the four pairwise comparisons of LC50s derived at each laboratory using static or static-renewal systems, the values did not differ significantly ($p < 0.05$); and none of the four pairwise comparisons of IC25s calculated using these two systems differed significantly (Table 14). Nonetheless, a trend toward lower LC50s and lower IC25s was evident from these data (see Table 14 and Tables D-3 and D-4, Appendix D). The mean LC50's reported in this study (i.e. 379.5 μg Cu/g for the static system, and 742.5 μg Cu/g for the static-renewal system) were slightly higher than the value of 247μg Cu/g d.w. sediment reported by Suedel *et al.* (1996a) for a 14-d exposure of *H. azteca* but lower than the value of 1078 μg Cu/g d.w. sediment reported by Cairns *et al.* (1984) for a 10-d test. As suggested previously, toxicity observed in copper-contaminated sediment tests was presumed by Suedel *et al.*(1996a) to be associated with the copper in the overlying water rather than the copper in the pore water or bulk sediment. Chemical analysis of the overlying water in each of the two systems showed that the overlying water in the static-renewal system had less copper at the higher concentrations of spiked-sediment which could lead to a decrease in toxicity. The toxicity of copper to *H. azteca* is also dependent on the pH of the overlying water with toxicity increasing as pH increases (Schubauer-Bergian *et al.*,1996).

As with *C. riparius*, when looking at precision for *H. azteca* using the *h* and *k* graphs, both static and static-renewal systems revealed a similar number of laboratories exceeding the critical values but general precision was good.

Field-collected sediments. The exposure of *H. azteca* simultaneously to a number of natural sediments in various laboratories and in both the static and the static-renewal systems allowed a number of key observations and comparisons to be made. First, the reproducibility of results in the two test systems could be determined by a comparison of CVs. Variability in percent survival was low using either system with CVs ranging from 3.6 to 19.6% in the static system and 2.5 to 11.0% in the static-renewal system. Data for growth were more variable in both systems with CVs for growth in the static system ranging from 28.4 to 48.8% and 26.0 to 35.7%

in the static-renewal system. As already suggested above, data which measure sublethal end points such as growth or reproduction are generally more variable and this have higher CVs due to the longer time required for exposure and the opportunities for random physical, chemical and biological factors to affect these test results (Rue *et al.*, 1988).

A second and perhaps more important observation is whether both systems provide the same ranking for the toxicity of contaminated sediments. Side-by-side comparisons of the static and the static-renewal systems within a laboratory consistently ranked the sediments in the same order of toxicity (Tables 16, 17). Ranking was also similar independent of the end point measured. It can thus be concluded that the use of either a static or a static-renewal system will provide the same results regarding the toxicity of a sediment.

third observation that can be made for these data is the consistent ranking of the toxicity of the natural sediments among the participating laboratories. With minor exceptions, all laboratories ranked sediment C as the least toxic and sediment D as the most toxic independent of whether percent survival or growth was the measured end point. Sediment C was the Long Point marsh sediment used as a reference sediment at NWRI for past five years. Sediment D was a sediment collected from the inner harbour at Montreal, Quebec.

Interlaboratory Differences in LC50s and IC25s

The significant differences in LC50s and IC25s for each test species noted between laboratories using either the static system or the static-renewal system were undoubtedly due to several factors, some of which can be discussed briefly here. First, different sources of test organisms employed in the tests were used by the various laboratories. This is especially relevant for the *H. azteca* tests, where most laboratories obtained their test organisms from independent suppliers. For the *C. riparius* test participating laboratories were supplied stock from the cultures maintained at NWRI; only one laboratory (laboratory C) obtained its animals from a different source and significant differences were noted in the LC50s and IC25s of this laboratory compared to all other laboratories (Table 11). Another factor contributing to diverse results would be the different sources of overlying water employed in the tests. Each laboratory used their own source of culture and test water which included dechlorinated municipal tap water, well water and reconstituted water. Subsequently, differences in water hardness between

laboratories were apparent and ranged from a soft water (30 mg/L as $CaCO_3$) to a hard water (260 mg/L as $CaCO_3$). Acute copper toxicity has been reported to decrease with increasing water hardness (USEPA, 1980; Suedel *et al.*, 1995a) as well as pH (Schubauer-Bergian *et al.*, 1993). Gauss *et al.* (1985) found that first instar *C. tentans* were significantly more sensitive to copper in soft (40-50 mg/L) water and medium (100-120 mg/L) water than in hard water (160-185 mg/L). However, this effect was not observed in the interlaboratory tests with *C. riparius* and *H. azteca*. In water-only reference toxicity tests with *C. riparius*, the laboratory with the hardest water (200-240 mg/L) had the lowest 96-h LC50 (Table 9). For *H. azteca*, again the laboratory with the hardest water had one of the lowest 96-h LC50s (Table 12). The significant differences noted in LC50s (*H. azteca*) and IC25s (*C. riparius*) between the two systems within the same laboratory may be due to such factors as the use of more than one operator, subtle differences in temperature and lighting between laboratories, the equipment used and its calibration and unreported deviation from the procedures outlines in the standard operating procedures provide to the participating laboratories.

5.0 CONCLUSIONS

5.1 Intralaboratory Studies

5.1.1 *Chironomus riparius*

- For the differing ration levels or feeding regimes studies, survival of *C. riparius* was unaffected and was consistently greater than the minimum acceptable criterion of ≥70% in both the static and the static-renewal systems

- The higher food ration (*i.e.*, 6 mg daily or 15 mg provided 4X during the 10-d test) resulted in greater growth. For either ration level, the pattern of feeding (daily or 4X during the test) did not appear to influence survival or growth.

- Growth can be dependent in either test system on natural sediment characteristics regardless of food provided and feeding regime. Organic carbon does not appear to be a significant modifying factor within the range of 0.6 to 8.8% TOC but particle size distribution may have some effects.

- A trend toward higher growth was evident for the static system. Flushing of food from the static-renewal system during the twice-daily exchanges of overlying water was concluded to be the likely cause of this apparent difference.

- The twice-daily renewal of overlying water in the static-renewal system and no aeration of the overlying water may not be sufficient to maintain dissolved oxygen levels above the 40% saturation criterion in certain sediments with a high sediment oxygen demand or with the higher food ration.

- Certain sediments may generate higher total ammonia concentrations in the static system than in the static-renewal system. The lower ammonia concentrations observed in the static-renewal system may be due to the flushing of ammonia resulting from the twice-daily renewal of overlying water.

- Overall, the static system produced more precise results for both survival and growth for the three samples of field sediment tested.

5.1.2 *Hyalella azteca*

- Results for 14-day tests with *H. azteca* fed YCT (daily or 3X/week) or Nutrafin^{R} (3X/week) indicate that YCT is the preferred diet, since it enabled greater growth using either the static or the static-renewal system. Feeding the same total amount of YCT during a test, either daily or 3 times/week (using larger portions/feeding) does not influence survival or growth rates in either test system.

- Higher growth rates for *H. azteca* were achieved using the static system in comparison with the static-renewal system, for all diets tested. Loss of food from the static-renewal system during the twice-daily flushing of the overlying water is concluded to be the likely explanation for this finding.

- Precision of results for survival or growth were not influenced to any detectable extent by the test system used.

- Changes in 14-day growth of *H. azteca* due to sediment type (i.e., field sediments with differing physico-chemical characteristics) were similar independent of system used. Organic content does not appear to be a significant modifying factor within the range of 0.1 to 8.1% TOC.

- The static system maintains higher and more stable dissolved oxygen levels in the overlying water than the static-renewal system, due to the continuous aeration of this water throughout the test. However, given the food rations and sediments studied here, both systems were able to maintain an acceptable (i.e., ≥40% saturation) level of dissolved oxygen in the overlying water at all times. of the food together with that of the sediment. Additionally, for each test system, food ration and feeding regime, no problems were encountered with respect to elevated ammonia concentrations in the overlying water.

5.2 Interlaboratory Studies

5.2.1 *Chironomus riparius*

- The tentative criterion for a valid 10-d solid-phase sediment toxicity test using *C. riparius* of ≥70% survival in control sediment is achievable yet discriminating based on the findings of these interlaboratory tests as well as the preceding intralaboratory studies with this species.

- For these round-robin tests with *C. riparius*, some evidence suggested that results using the static option were less variable than those using the static-renewal option, and that the former option was more sensitive in detecting sediment toxicity. However, statistical comparisons of LC50s and IC25s for side-by-side tests with copper-spiked sediment performed at the same laboratory did not distinguish a consistent difference in test sensitivity using either system.

- Results for round-robin tests, which measured and compared the performance of the static and static-renewal options using two samples of field-collected sediment, suggested similar precision for survival but somewhat greater precision using the static system

when measuring growth. Due to the minimal number of field sediments included in this side-by-side comparison (two), no conclusion can be drawn at this time regarding the relative sensitivity of these two test options in detecting or quantifying sediment toxicity.

- Based on a review of data compiled from these and earlier comparative studies for mean individual dry weights at test end, it was concluded that dry weights for controls were similar using each test option (static or static-renewal), and that a criterion for test validity of ≥0.5 mg per individual control organism would be normally attainable yet discriminatory for this species.

5.2.2 *Hyalella azteca*

- The tentative criterion for a valid 14-d solid-phase sediment toxicity test using *H. azteca* of ≥80% survival in control sediment is achievable yet discriminating, based on the findings of these interlaboratory tests as well as the preceding intralaboratory studies with this species.

- For these round-robin tests with *H. azteca*, results were similar in precision when using the static and the static-renewal options, although a trend toward lower LC50s and lower IC25s using the static option was evident in side-by-side tests with copper-spiked sediment. Statistical comparisons of LC50s and IC25s for side-by-side tests with copper-spiked sediment performed at the same laboratory did not distinguish a consistent difference in test sensitivity using either system.

- Results for round-robin tests with four diverse field sediments indicate that ranking of these sediments for relative toxicity, based on data for survival or growth, was similar for each participating laboratory and for each test system (static or static-renewal).

- Based on a review of data compiled from these and earlier comparative studies for mean individual dry weights at test end, it was concluded that dry weights for controls were similar using each test option (static or static-renewal), and that a criterion for test validity of ≥0.1 mg per individual control organism would be normally attainable yet discriminatory for this species.

6.0 RECOMMENDATIONS

1. For 10-d solid-phase sediment-toxicity tests using *C. riparius* and either static or static-renewal test options described in EC (1995b), a Nutrafin^{R} diet is recommended. The total ration fed during the test should be 60 mg dry food per test chamber, which may be offered either as 6 mg daily or 15-mg portions fed four times (non-consecutive days) during the 10-day test period.

2. For 14-day sediment-toxicity tests using *H. azteca* and either static or static-renewal test options described in EC (1995a), the YCT diet is recommended. A standard YCT suspension should be added to each test chamber at the rate of either 1.5 mL daily or 3.5 mL three times per week on non-consecutive days.

3. The tentative criteria for valid tests given in EC (1995a,b) of ≥70% survival of *C. riparius* in control sediment during 10-day tests (either option), and of ≥80% survival of H. azteca in control sediment during 14-day tests (either option) should be adopted.

4. The following additional criteria for valid sediment-toxicity tests using these species, which are based on a minimum mean dry weight of individual control organisms attained at test end, are recommended: for *C. riparius*, ≥0.5 mg; for *H. azteca*, ≥0.1 mg.

5. Both the static and static-renewal test options for measuring sediment toxicity with *C. riparius* or *H. azteca* can yield reliable and similar results; and each is recommended for inclusion in Environment Canada's biological test methods using these species.

6. Standardization and validation of Environment Canada's two-option toxicity-test methods has reached a satisfactory conclusion, and it is recommended that each document now be published.

7. Additional side-by-side comparative studies of static versus static-renewal systems with diverse samples of clean and contaminated field sediment are warranted and should be encouraged, in order to distinguish the relative sensitivity and discriminatory power of each test option.

Table 1. Summary of Test Conditions for Intralaboratory and Interlaboratory Studies with *C. riparius*.

	Intralaboratory Tests	Interlaboratory Tests
Duration of Test	10 days	10 days
Temperature	23 ± 1° C	23 ± 1° C
Photoperiod	16L:8D	16L:8D
Food Type	Nutrafin^{R} fish flakes	Nutrafin^{R} fish flakes
Feeding Rate(s)	4 mg daily 6 mg daily 10 mg fed 4 times\10d 15 mg fed 4 times\10d	15 mg fed 4 times\10d
Types of Sediment	Uncontaminated field-collected sediment with a range in TOC from low to high	Phase-I: Formulated sediment spiked with copper Phase-II: Formulated sediment spiked with copper and field-collected sediment
Test System	Static and Static-Renewal	Static and Static-Renewal
Test Chamber	300 mL beaker	300 mL beaker
Volume of Sediment	100 mL	100 mL
Volume of Overlying Water	175 mL	175 mL
Age of organism at Test Initiation	1st instar	1st instar
No. organisms/test chamber	10	10
Replication	4	3 - 4
Aeration Device (Static System)	Pasteur pipet (5¾ mm)	Plastic eppendorf tip
Endpoints	Survival and growth	Survival and growth

Table 2. Physical and Chemical Characteristics of Sediments Employed in NWRI's Intralaboratory Tests with *C. riparius*.

Characteristic	Sediment # 1 Long Point	Sediment # 2 108	Sediment # 3 WB
Organic Carbon (%)	8.8	1.9	0.6
Inorganic Carbon (%)	3.7	2.9	1.0
Total Carbon (%)	12.5	4.7	1.6
% Sand	1.8	4.21	86.9
% Silt	80.7	61.0	13.2*
% Clay	17.6	34.8	-
Total Ammonia in Porewater (ppm)	1.5	0.02	2.0
Porewater pH	7.1	7.2	7.7
Mean Water Content (%)	76.4	63.2	24.1

* Represents % silt and % clay combined.

Table 3. Summary of Test conditions for Intralaboratory and Interlaboratory Studies with *H. azteca*.

	Intralaboratory Tests	Interlaboratory Tests
Duration of Test	14 days	14 days
Temperature	23 ± 1° C	23 ± 1° C
Photoperiod	16L:8D	16L:8D
Food Type	YCT/Nutrafin® fish flakes	YCT
Feeding Rate(s)	1.5 mL YCT daily 3.5 mL YCT 3X/week 4 mg Nutrafin® 3X/week	3.5 mL YCT 3X/week
Types of Sediment	Uncontaminated field-collected sediment with a range in TOC from low to high	Phase-I: Formulated sediment spiked with copper Phase-II: Field-collected sediment
Test System	Static and Static-Renewal	Static and Static-Renewal
Test Chamber	300 mL beaker	300 mL beaker
Volume of Sediment	100 mL	100 mL
Volume of Overlying Water	175 mL	175 mL
Age of organism at Test Initiation	3 - 10 days	2 - 9 days
No. organisms/test chamber	10	10
Replication	4 - 5	3 - 4
Aeration Device (Static System)	Pasteur pipet (5¾ mm)	Plastic eppendorf tip
Endpoints	Survival and growth	Survival and growth

Table 4. Physical and Chemical Characteristics of Sediments Employed in NWRI's Intralaboratory Tests with *H. azteca*.

Characteristic	Sediment # 1 Long Point	Sediment # 2 1213	Sediment # 3 100
Organic Carbon (%)	8.1	2.1	0.1
Inorganic Carbon (%)	1.7	0.7	1.0
Total Carbon (%)	9.8	2.8	1.1
% Sand	1.8	7.9	97.3
% Silt	72.3	29.5	1.6*
% Clay	25.9	62.6	-
Total Ammonia in Porewater (ppm)	1.5	-[1]	-[1]
Porewater pH	7.1	-[1]	-[1]
Mean Water Content (%)	76.4	-[1]	-[1]

* Represents % silt and % clay combined.

[1] Data lost.

Table 5. Physical and Chemical Characteristics of Sediments Employed in Phase I and II Interlaboratory Studies with *C. riparius*.

Characteristic	Formulated Sediment Phase I	Formulated Sediment Phase II	Long Point Phase II	Hamilton Harbour Phase II
Organic Carbon (%)	1.9	1.0	7.2	0.8
Inorganic Carbon (%)	3.3	1.0	2.3	5.9
Total Carbon (%)	5.2	2.0	9.5	6.7
% Sand	41.7	33.0	2.2	22.9
% Silt	24.9	33.6	86.5	51.0
% Clay	33.4	33.5	11.3	26.1
Total Ammonia in Porewater (ppm)	0.1	0.2	2.0	0.5
Porewater pH	7.4	7.4	7.4	7.4
Mean Water Content (%)	55.4	53.9	73.2	51.8

Table 6. Physical and Chemical Characteristics of Sediments Employed in Phase I and II Interlaboratory Tests with *H. azteca*.

Characteristic	Formulated Sediment Phase I	Sediment #1 Long Point Phase II	Sediment #2 Toronto Harbour Phase II	Sediment #3 Hamilton Harbour Phase II	Sediment #4 Montreal Harbour Phase II
Organic Carbon (%)	1.0	7.2	1.2	3.1	3.1
Inorganic Carbon (%)	0.9	2.3	1.0	4.0	3.8
Total Carbon (%)	1.9	9.5	2.2	7.1	6.9
% Sand	36.1	2.2	0.9	1.4	11.2
% Silt	21.1	86.5	58.4	21.3	43.0
% Clay	42.8	11.3	40.7	77.3	45.7
Total Ammonia in Porewater (ppm)	3.0	2.0	<0.05	7.0	10.0
Porewater pH	7.2	7.4	7.3	7.2	7.5
Mean Water Content (%)	54.7	73.2	71.9	79.3	69.5

Table 7. Effect of Sediment Type on the Growth of *C. riparius* Within a Given Diet Using Static (S) or Static-Renewal (S-R) Systems.

Sediment	Diet							
	4 mg daily		10 mg 4X		6 mg daily		15 mg 4X	
	S-R	S	S-R	S	S-R	S	S-R	S
LP	.57 mg^{A}	.67 mg^{A}	.64 mg^{A}	.70 mg^{A}	.66 mg^{A}	.89 mg^{A}	.73 mg^{A}	.83 mg^{A}
108	.55 mg^{A}	.72 mg^{A}	.58 mg^{A}	.69 mg^{A}	.58 mg^{A}	.89 mg^{A}	.57 mg^{A}	.89 mg^{A}
WB	.88 mg^{B}	.95 mg^{B}	.91 mg^{B}	.94 mg^{B}	.93 mg^{A}	1.08 mg^{A}	.73 mg^{A}	1.05 mg^{B}

Values within a column having different superscripts are significantly different (Alpha<.05).

Table 8. Effect of Sediment Type on the Growth of *H. azteca* Within a Given Diet Using Static (S) or Static-Renewal (S-R) Systems.

Sediment	Diet					
	YCT daily		YCT 3x/week		Nutrafin^{R} 3x/week	
	S-R	S	S-R	S	S-R	S
LP	.21 mg^{A}	.31 mg^{A}	.24 mg^{A}	.31 mg^{A}	.14 mg^{AB}	.25 mg^{A}
1213	.12 mg^{B}	.13 mg^{B}	.11 mg^{B}	.13 mg^{B}	.10 mg^{A}	.16 mg^{A}
100	.18 mg^{AB}	.28 mg^{A}	.21 mg^{A}	.29 mg^{A}	.15 mg^{B}	.20 mg^{A}

Values within a column having different superscripts are significantly different (Alpha<.05).

Table 9. Summary of Interlaboratory Results for "Water-Only" Reference-Toxicity Tests with *C. riparius* Exposed to Copper.

Laboratory	Water Hardness Range (mg/L)	Control Survival (%)	96-h LC50* (μg/L)
A			
Round-Robin #1	160-260	100.0	626 (455 - 859)[a]
Round-Robin #2	140-180	80.0	1508 (1368 - 1662)[b]
B			
Round-Robin #1	130-140	100.0	1650 (1514 - 1798)[b]
Round-Robin #2	120-145	100.0	1030 (886 - 1198)[c]
C			
Round-Robin #1	-	--	--
Round-Robin #2	60-90	100.0	504 (339 - 675)[a]
D			
Round-Robin #1	-	--	--
Round-Robin #2	200-240	90.0	493 (332 - 733)[a]
Mean (SD)[1]	-	95.0 (8.4)	968.5 (513.5)
CV[1]	-	8.8%	53.0%
Mean (SD)[2]	-	98.0 (4.5)	860.6 (492.2)
CV[2]	-	4.6%	57.2%

* values are significantly different if designated by different letters.
[1] Calculations include all values.
[2] Calculations exclude values for which the proposed minimum acceptable control survival of ≥ 90% was not met.

Table 10. Summary of Grand Means for % Survival and Growth (mg dry wt/ind.) of *C. riparius* in 10-Day Round-Robin Tests with Copper-Spiked Formulated Sediment or Field Sediment, Using Static or Static-Renewal Systems.

	Nominal Concentration of Copper (μg/g) Round-Robin # 1						Field-Collected Sediment Round-Robin # 2	
	0	100	250	500	1000	2000	LP	HH
% Survival (SD)								
Static	94.5(5.4)	91.1(7.8)	93.9(5.7)	92.2(5.4)	72.2(18.1)	0	92.2(5.1)	95.6(2.0)
CV%	5.8	8.5	6.1	5.9	25.1	--	5.5	2.1
Static-Renewal	89.3(11.4)	86.7(10.5)	82.0(22.4)	86.0(8.3)	74.7(12.6)	16.7(23.8)	93.3	80.0
CV%	12.8	12.2	27.4	9.7	16.9	142.9	7.7	17.0
Growth (SD)								
Static	0.88(0.16)	0.86(0.17)	0.77(0.24)	0.71(0.13)	0.25(0.14)	--	0.90(0.04)	0.76(0.02)
CV%	17.9	19.6	32.5	18.5	56.3		4.4	2.6
Static-Renewal	0.84(0.37)	0.71(0.25)	0.74(0.29)	0.70(0.42)	0.56(0.41)	0.40(0.29)	0.78(0.22)	0.80(0.45)
CV%	43.4	35.3	40.4	60.0	75.9	72.1	29.2	56.2

Table 11. Summary of Endpoint Statistics (LC50, IC25) Determined by Each Laboratory in Round-Robin 10-day Tests with *C. riparius* and Copper-Spiked Sediment, Using Static or Static-Renewal Systems.

Laboratory	10-day LC50 (µg/g) % Survival		10-day IC25 (µg/g) Growth (mg dry wt/ind.)	
	Static	Static-Renewal	Static	Static-Renewal
A				
Round Robin # 1	1414 (n.c.)	2555 (n.c.)	731 (66 - 1195)[a]	85 (n.c.)
Round Robin # 2	970 (832 - 1130)[a]	783 (557 - 1101)[a]	627* (513 - 664)[a]	400* (175 - 498)[a]
B				
Round Robin # 1	1253 (1101 - 1427)[b]	1088 (949 - 1248)[ab]	259 (216 - 321)[b]	253 (108 - 584)[ab]
Round Robin # 2	1168 (1039 - 1312)[ab]	1134 (996 - 1291)[b]	407 (216 - 536)[c]	534 (363 - 604)[b]
C				
Round Robin # 1	-	-	-	-
Round Robin # 2	-	1552 (1419 - 1697)[c]	-	1168 (518 - 1714)[c]
D				
Round Robin # 1	1160 (1015 - 1324)[ab]	-	343 (170 - 403)[bc]	-
Round Robin # 2	1000 (856 - 1169)[a]	-	607 (417 - 653)[a]	-
Mean (SD)[1]	1160.8 (164.3)	1422.4 (689.8)	495.7 (185.6)	488.0 (415.3)
CV	14.2%	48.5%	37.4%	85.1%
Mean (SD)[2]	1110.2 (120.4)	1139.3 (316)	-	589 (402)
CV	10.8%	27.8%		68.4%

Values within a system are significantly different if designated by different letters.
[1]Calculation includes all values.
[2]Calculation excludes values for which confidence intervals could not be calculated.
* denotes significant difference between static vs. static-renewal systems.
n.c. = not calculable

Table 12. Summary of Interlaboratory Results for "Water-Only" 96-h Reference-Toxicity Tests with *H. azteca* Exposed to Copper.

Laboratory	Water Hardness Range (mg/L)	Control Survival (%)	96-h LC50* (μg/L)
A			
Round-Robin #1	120-160	100.0	125 (95 - 164)[a]
Round-Robin #2	100-120	80.0	120 (36 - 195)[a]
B			
Round-Robin #1	140	43.3	42 (31 - 53)[b]
Round-Robin #2	120-150	95.0	99 (80 - 123)[a]
D			
Round-Robin #1	220-250	50.0	55 (44 - 69)[b]
Round-Robin #2	220-260	60.0	146 (0.5 - 244)[ac]
E			
Round-Robin #1	140-145	100.0	293 (250 - 500)[c]
Round-Robin #2	140-145	100.0	263 (189 - 356)[c]
F			
Round-Robin #1	30-40	96.7	139 (124 - 155)[a]
Round-Robin #2	36-44	95.0	134 (113 - 158)[a]
Mean (SD)[1]	-	82.0 (22.4)	141.6 (80.1)
CV[1]	-	27.4%	56.6%
Mean (SD)[2]	-	97.8 (2.5)	175.5 (81.1)
CV[2]	-	2.6%	46.2%

*values are significantly different if designated by different letters.

[1] Calculations include all values.

[2] Calculations exclude values for which the proposed minimum acceptable control survival of ≥ 90% was not met.

Table 13. Summary of Grand Means for % Survival and Growth (mg dry wt/ind.) of *H. azteca* in 14-day Round-Robin Tests with Copper-Spiked Formulated Sediment, Using Static or Static-Renewal Systems.

	Nominal Concentration of Copper (μg/g)-Round-Robin # 1					
	0	50	125	250	500	1000
% Survival (SD)						
Static	97.3 (5.9)	96.7 (4.5)	94.0 (6.4)	73.3 (27.2)	40.7 (27.2)	16.0 (24.9)
CV%	6.1%	4.7%	6.8%	37.1%	66.9%	155.5%
Static-Renewal	93.3 (2.7)	96.7 (3.9)	96.7 (2.7)	89.2 (5.0)	57.5 (32.6)	21.7(25.6)
CV%	2.9%	4.0%	2.8%	5.6%	56.7%	118.2%
Growth (SD)						
Static	0.18 (.07)	0.17 (.07)	0.10 (.06)	0.06(.05)	0.08 (.08)	0.02 (.01)
CV%	36.8%	42.7%	53.2%	85.4%	100.5%	-
Static-Renewal	0.15 (.05)	0.14(.04)	0.13 (.07)	0.10(.05)	0.06(.03)	0.06(.03)
CV%	31.6%	29.4%	54.8%	46.0%	43.4%	50.4%

Table 14. Summary of Endpoint Statistics (LC50, IC25) Determined by Each Laboratory in Round-Robin 14-Day Tests with *H. azteca* and Copper-Spiked Sediment, Using Static or Static-Renewal Systems.

Laboratory	14-day LC50 (µg/g) % Survival		14-day IC25 (µg/g) Growth (mg dry wt/ind.)	
	Static	Static-Renewal	Static	Static-Renewal
A	393 (329- 471)[a]	454 (402 - 513)[a]	31 (22 - 90)[a]	114 (42 - 370)[abc]
B	338* (257 - 413)[a]	849* (648 - 1314)[b]	140 (39 - 182)[b]	189 (91 - 255)[c]
D	568 (483 - 670)[b]	-	84 (79 - 95)[ac]	-
E	219* (181 - 263)[c]	414* (365 - 469)[a]	74 (62 - 78)[ac]	97 (84 - 135)[b]
F	1415 (n.c.)	1253 (840 - 4743)[ab]	120 (42 - 270)[bc]	259 (202 - 354)[c]
Mean (SD)[1] CV	586.6 (479.8) 81.8%	742.5 (392.9) 52.9%	89.8 (42.3) 89.8%	164.8 (74.5) 45.2%
Mean (SD)[2] CV	379.5 (145.1) 38.2%	-	-	-

Values within a system are significantly different if designated by different letters.
[1]Calculation includes all values.
[2]Calculation excludes values for which confidence intervals could not be calculated
* denotes significant difference in values between static vs. static-renewal systems.
n.c. = not calculable

Table 15. Summary of Grand Means for % Survival and Growth (mg dry wt/ind.) of *H. azteca* in 14-Day Round-Robin Tests with Field-Collected Sediment Using Static or Static-Renewal Systems.

	Field-Collected Sediment - Round-Robin # 2			
	SED A	SED B	SED C	SED D
% Survival (SD)				
Static	88.0 (6.9)	78.5 (10.2)	94.0 (3.4)	66.3 (13.0)
CV%	7.9%	13.1%	3.6%	19.6%
Static-Renewal	96.9 (2.4)	89.4 (9.9)	97.5 (3.5)	85.0 (8.7)
CV%	2.5%	11.0%	3.6%	10.2%
Growth (SD)				
Static	0.109 (.05)	0.084 (.02)	0.202 (.1)	0.086 (.03)
CV%	48.8%	28.4%	48.6%	36.7%
Static-Renewal	0.133 (.04)	0.119 (.04)	0.177 (.05)	0.089 (.03)
CV%	33.5%	35.7%	26.0%	29.0%

Table 16. By-Laboratory Ranking of Survival Data for Field-Collected Sediments (*H. azteca* Interlaboratory Study: Phase II)[1].

Sediment		Laboratory				
		Lab A	Lab B	Lab D	Lab E	Lab F
A	Static	2	1	2	2.5	2
	Renewal	2	1	-	3	2
B	Static	3	3	3	2.5	3.5
	Renewal	3.5	3	-	2	1
C	Static	1	2	1	1	1
	Renewal	1	2	-	1	3.5
D	Static	4	4	-	4	3.5
	Renewal	3.5	4	-	4	3.5

[1]A within-laboratory ranking of "1" represents the highest survival rate; whereas "4" represents the lowest survival rate. Fractions are assigned in instances where equivalent results are obtained for two sediments.

Table 17. By-Laboratory Ranking of Growth Data for Field-Collected Sediments (*H. azteca* Interlaboratory Study: Phase II)[1].

Sediment		Laboratory				
		Lab A	Lab B	Lab D	Lab E	Lab F
A	Static	3	2	3	2	2
	Renewal	2	3	-	2	2
B	Static	4	3	2	4	3
	Renewal	3	2	-	3	3
C	Static	1	1	1	1	1
	Renewal	1	1	-	1	1
D	Static	2	4	-	3	4
	Renewal	4	4	-	4	4

[1]A within-laboratory ranking of "1" represents the greatest mean dry weight at test end; whereas "4" represents the least dry weight at test end.

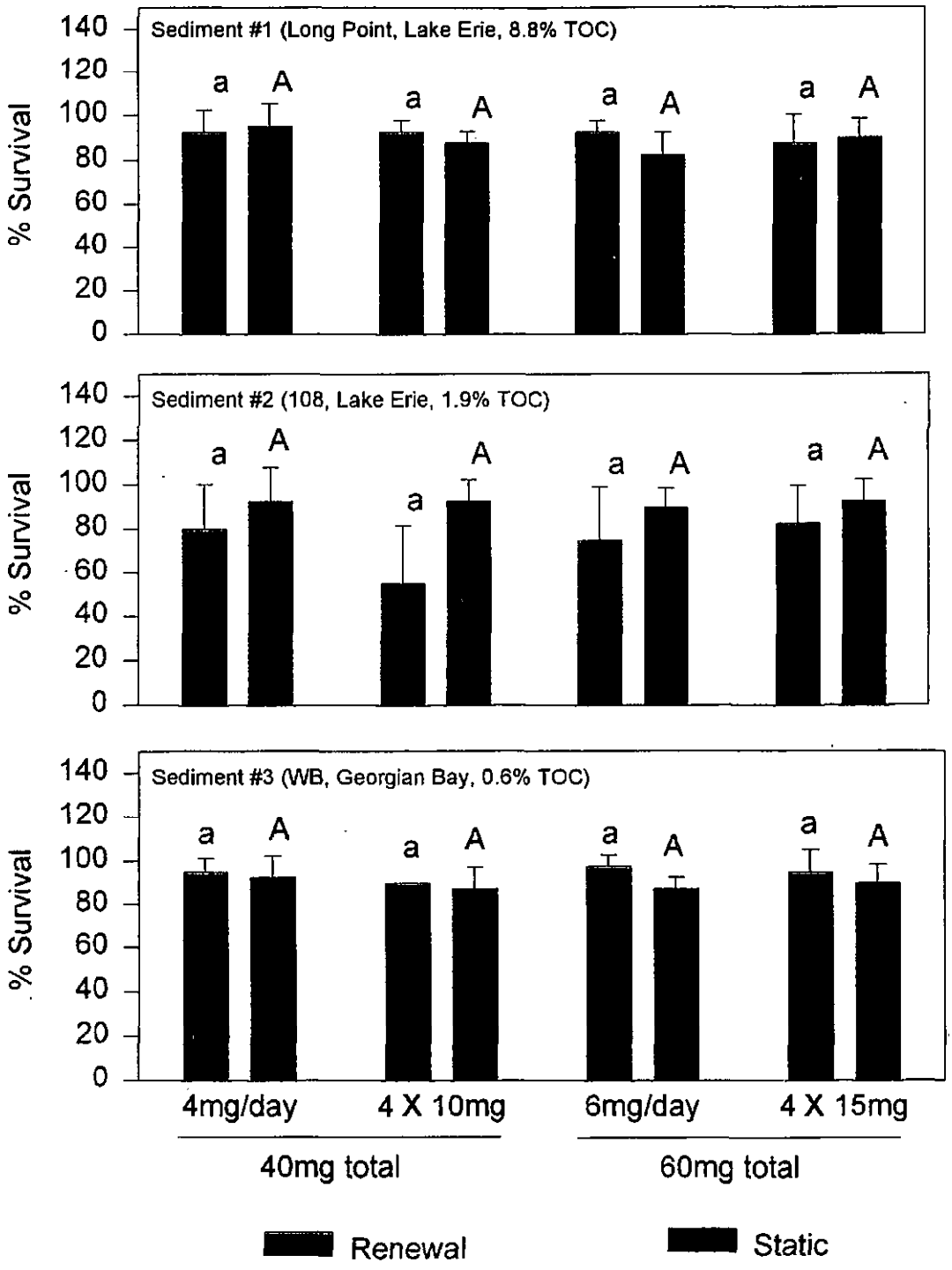


Figure 1. Effect of Diet on Survival of *C. riparius* in a 10-day Whole Sediment Toxicity Test with 3 Clean Field Sediments of Varying Organic Carbon Content.

similar coloured bars within the same graph are significantly different (alpha< 0.05) from each other if designated by different letters.

*above adjacent bars indicates a significant difference (alpha<0.05) between those bars.

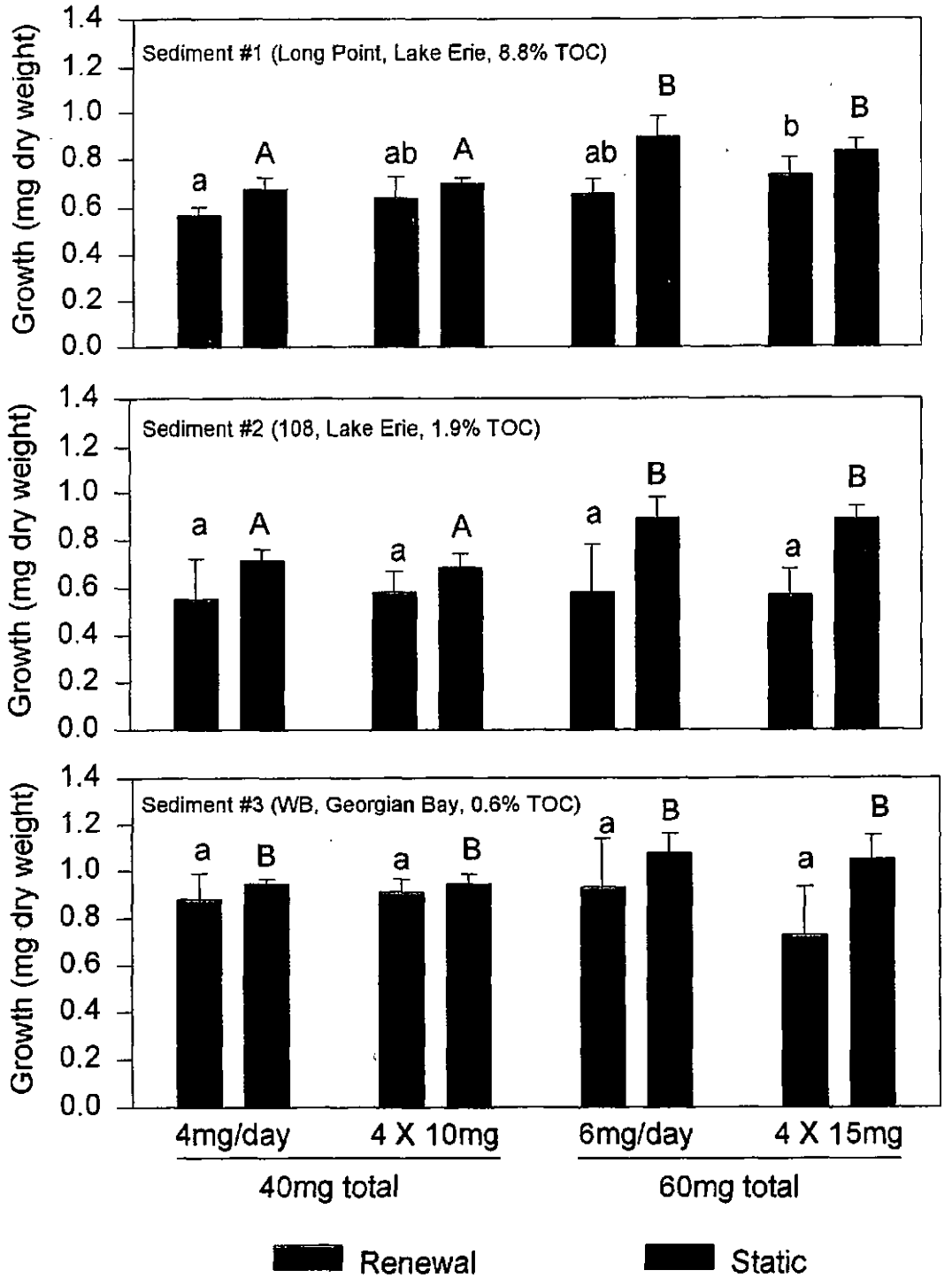


Figure 2. Effect of Diet on Growth of *C. riparius* in a 10-day Whole Sediment Toxicity Test with 3 Clean Field Sediments of Varying Organic Carbon Content.

similar coloured bars within the same graph are significantly different (alpha< 0.05) from each other if designated by different letters.

*above adjacent bars indicates a significant difference (alpha<0.05) between those bars.

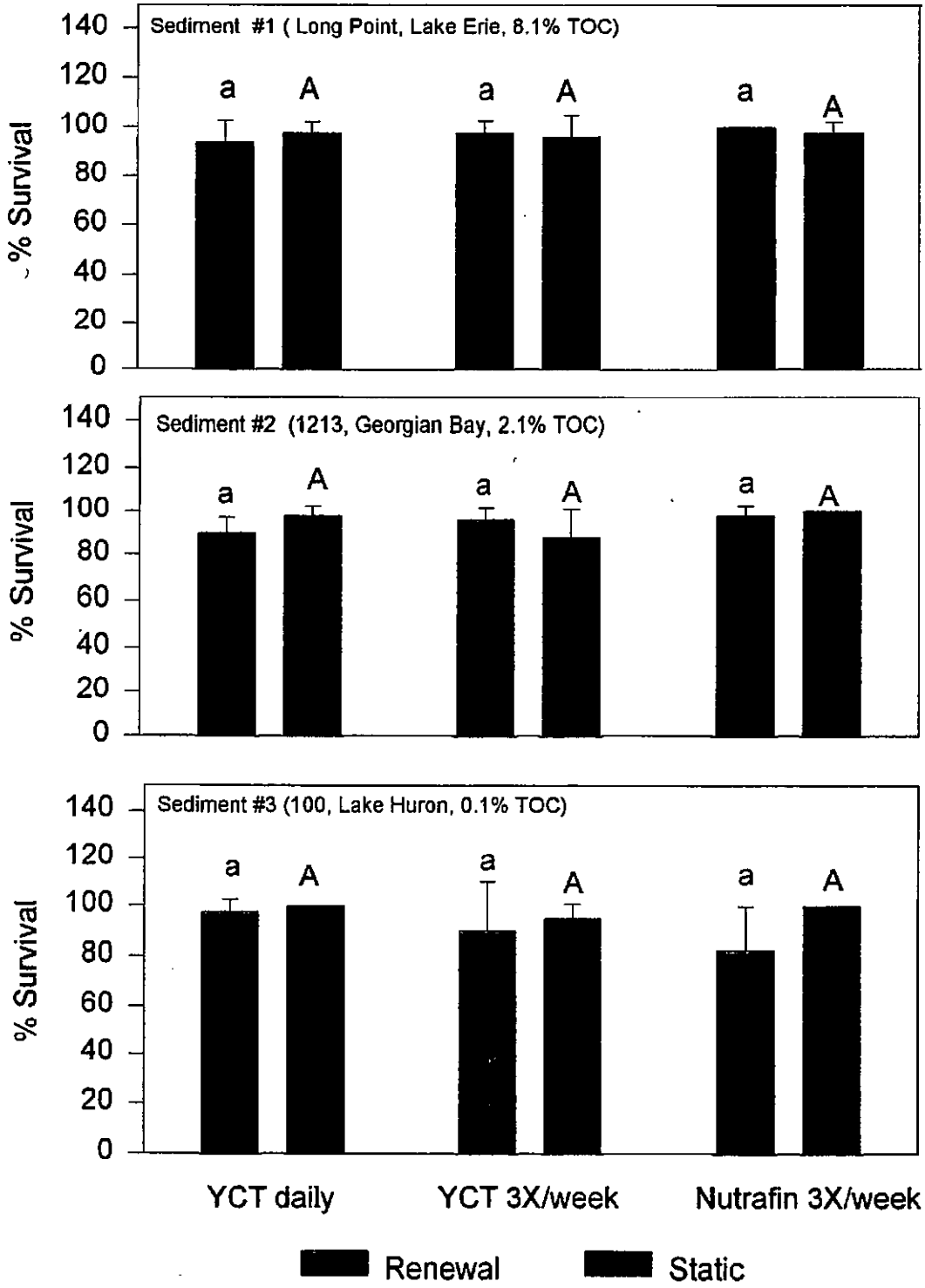


Figure 3. Effect of Diet on Survival of *H. azteca* in a 14-day Whole Sediment Toxicity Test with 3 Clean Field Sediments of Varying Organic Carbon Content.

similar coloured bars within the same graph are significantly different (alpha< 0.05) from each other if designated by different letters.

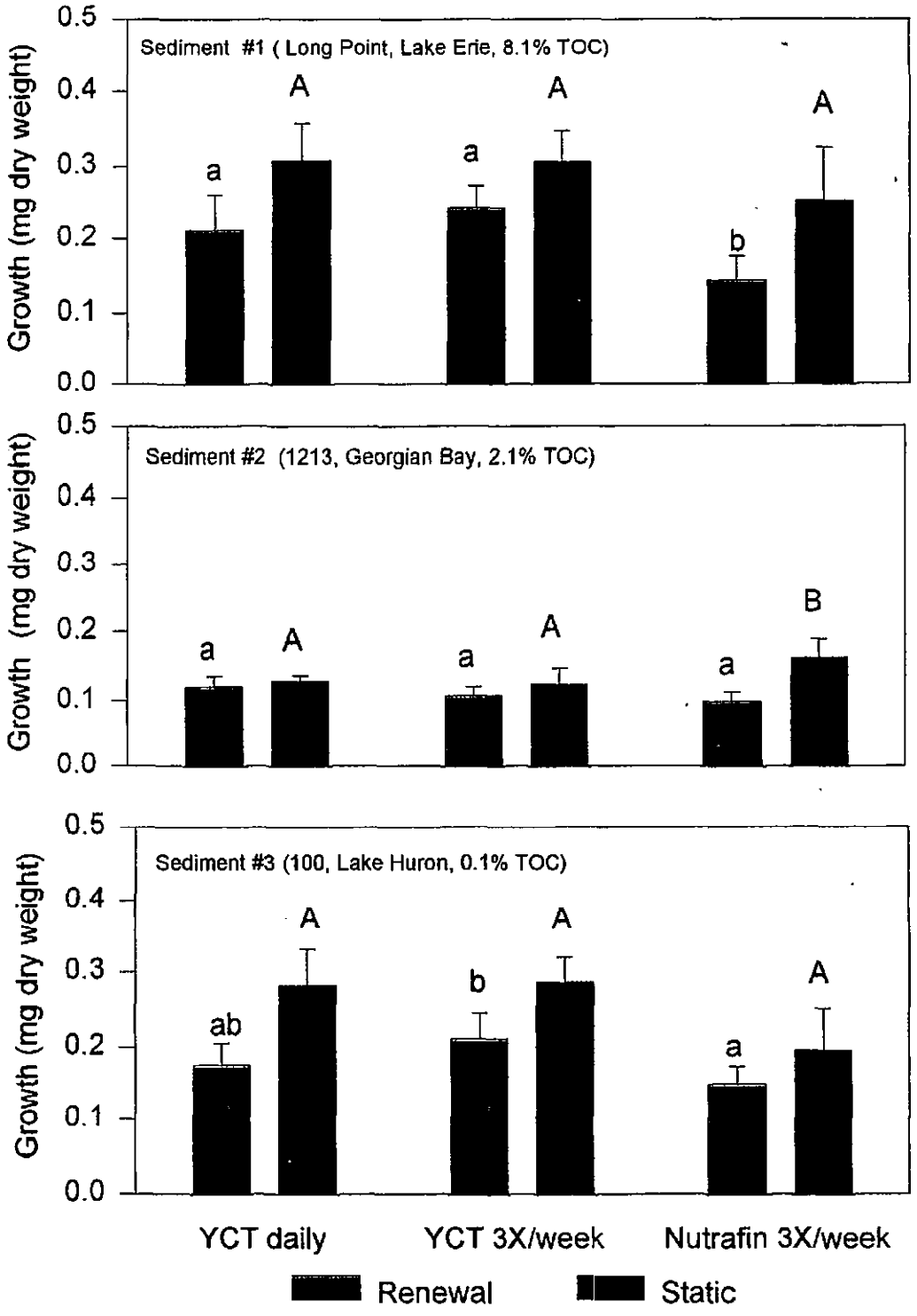


Figure 4. Effect of Diet on Growth of *H. azteca* in a 14-day Whole Sediment Toxicity Test with 3 Clean Field Sediments of Varying Organic Carbon Content.

similar coloured bars within the same graph are significantly different (alpha< 0.05) from each other if designated by different letters.

*above adjacent bars indicates a significant difference (alpha<0.05) between those bars.

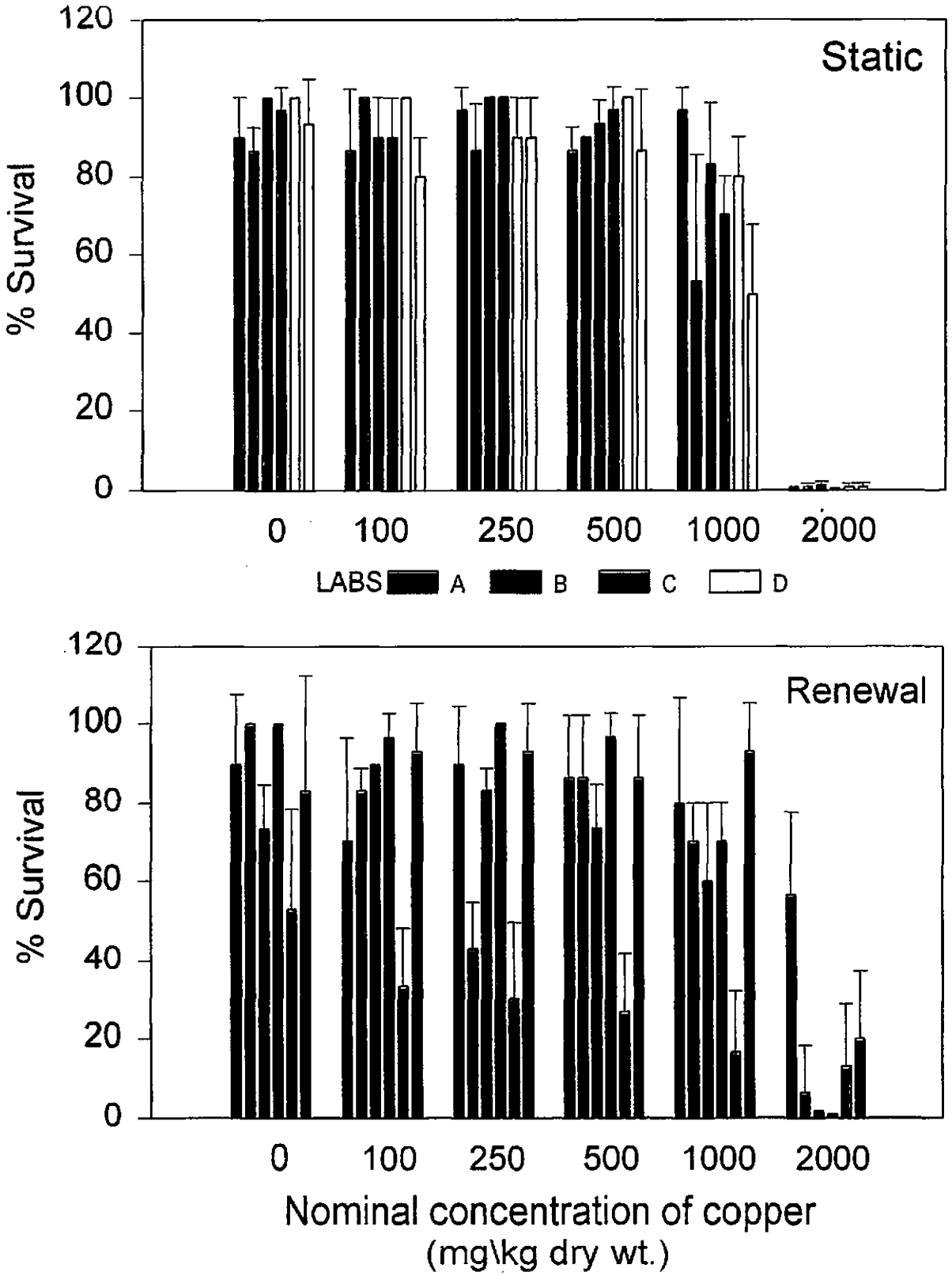
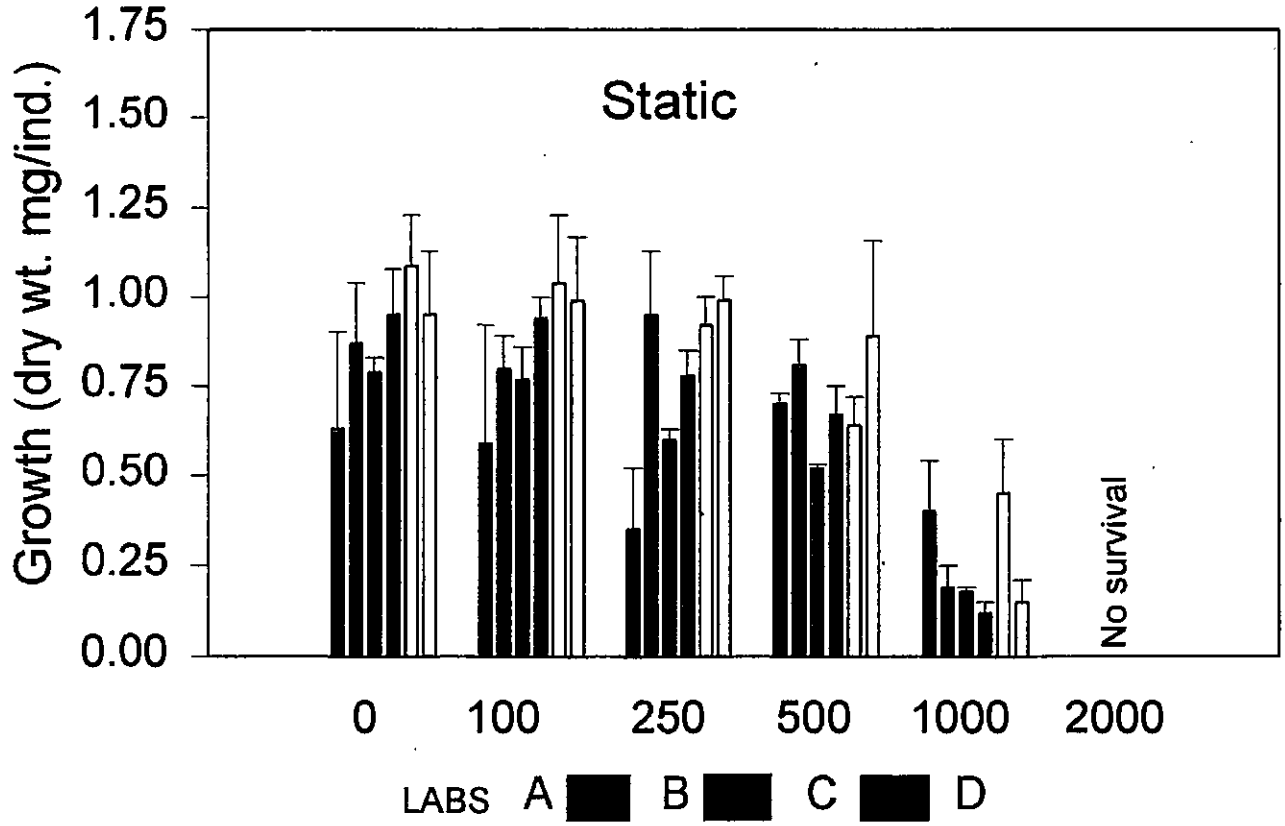


Figure 5. Mean Percent Survival of *C. riparius* in Sediment Spiked with Copper.



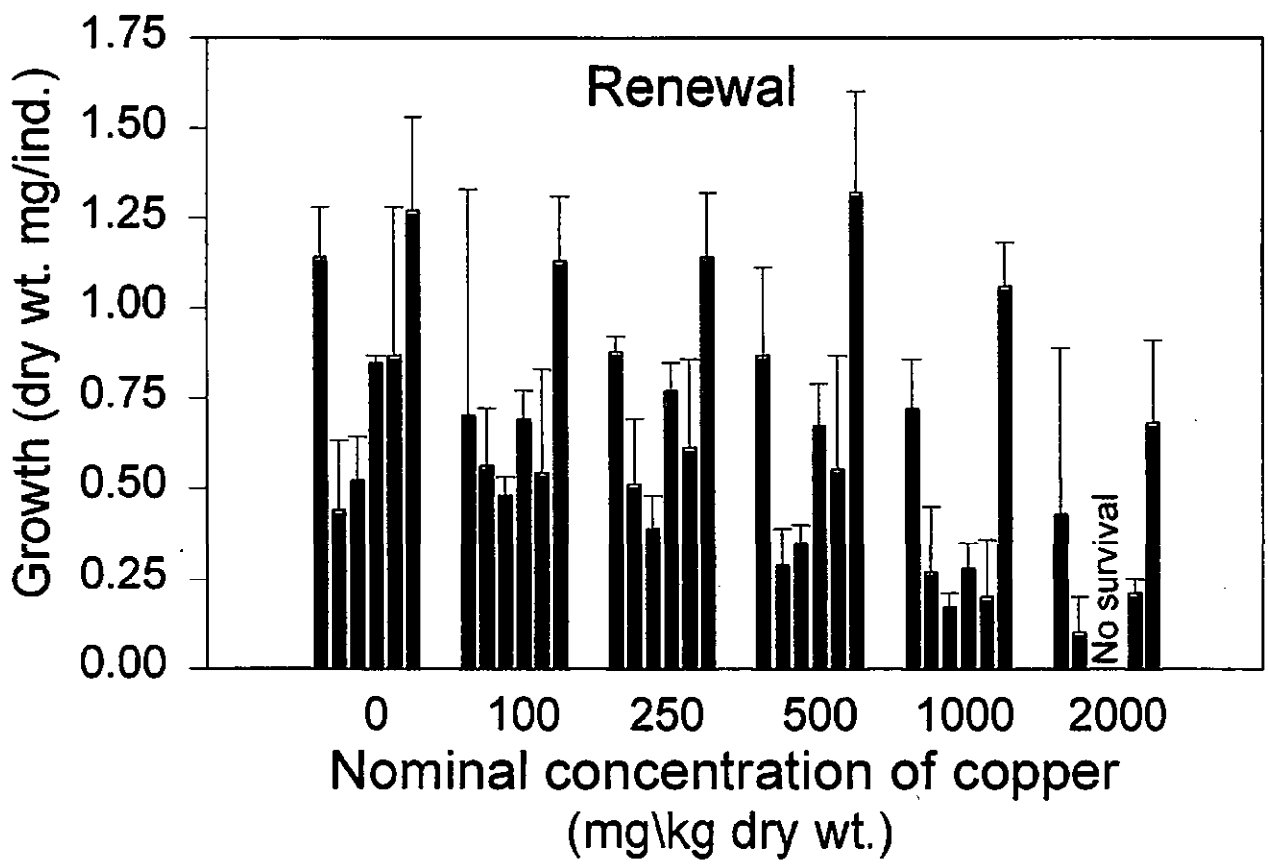


Figure 6. Mean Growth of *C. riparius* in Sediment Spiked with Copper

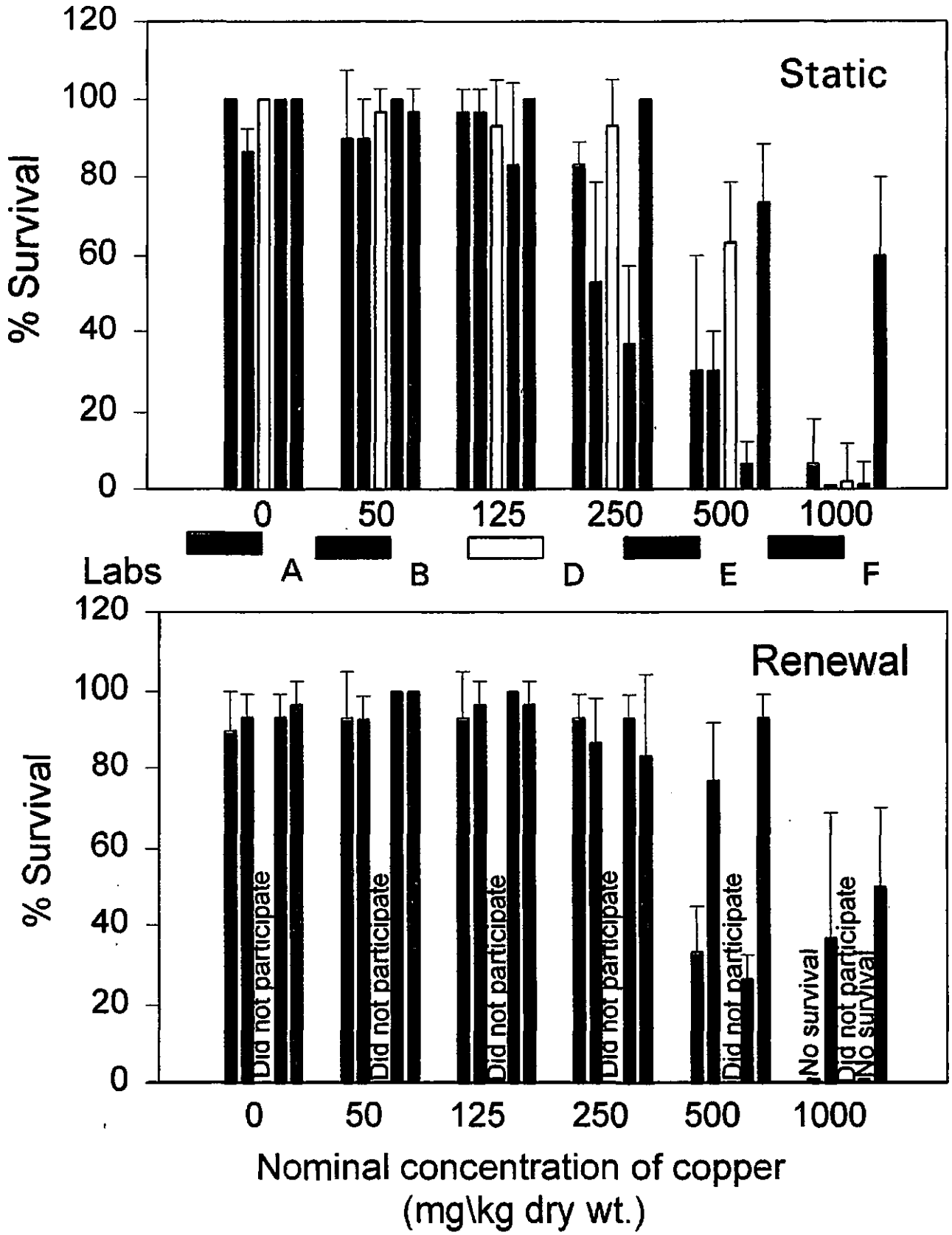


Figure 7. Mean Percent Survival of *H. azteca* in Sediment Spiked with copper.

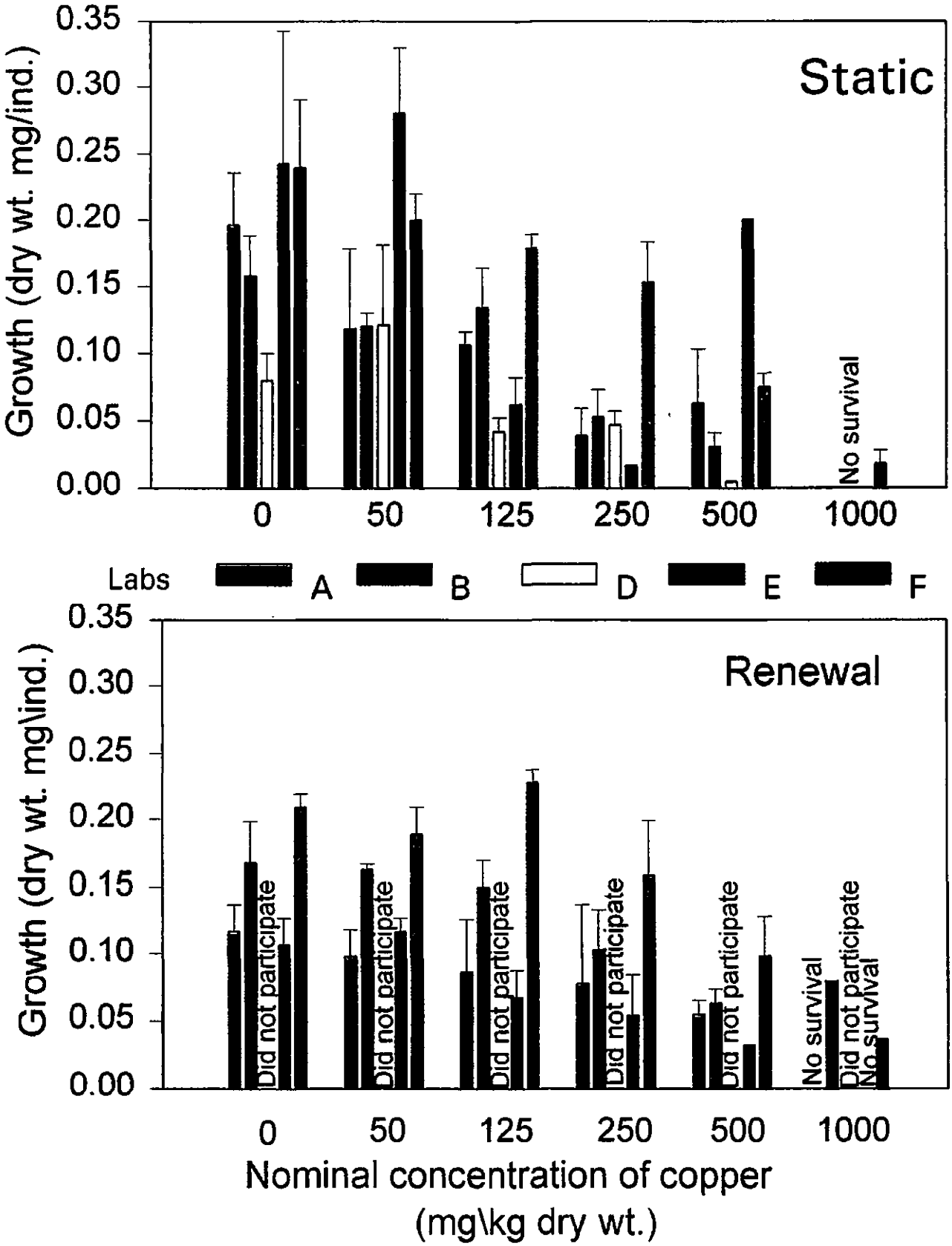


Figure 8. Mean Growth of *H. azteca* in Sediment Spiked with Copper

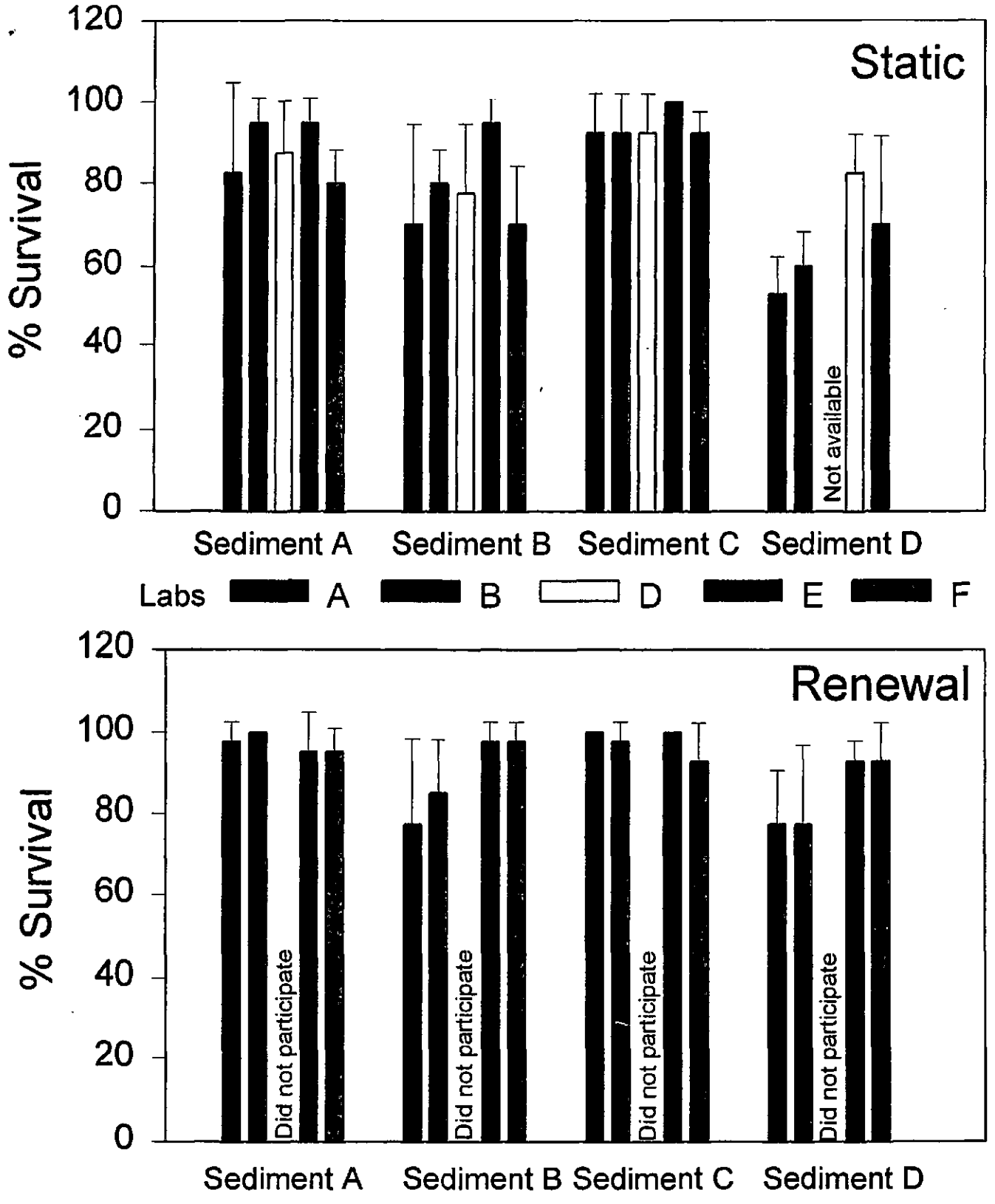
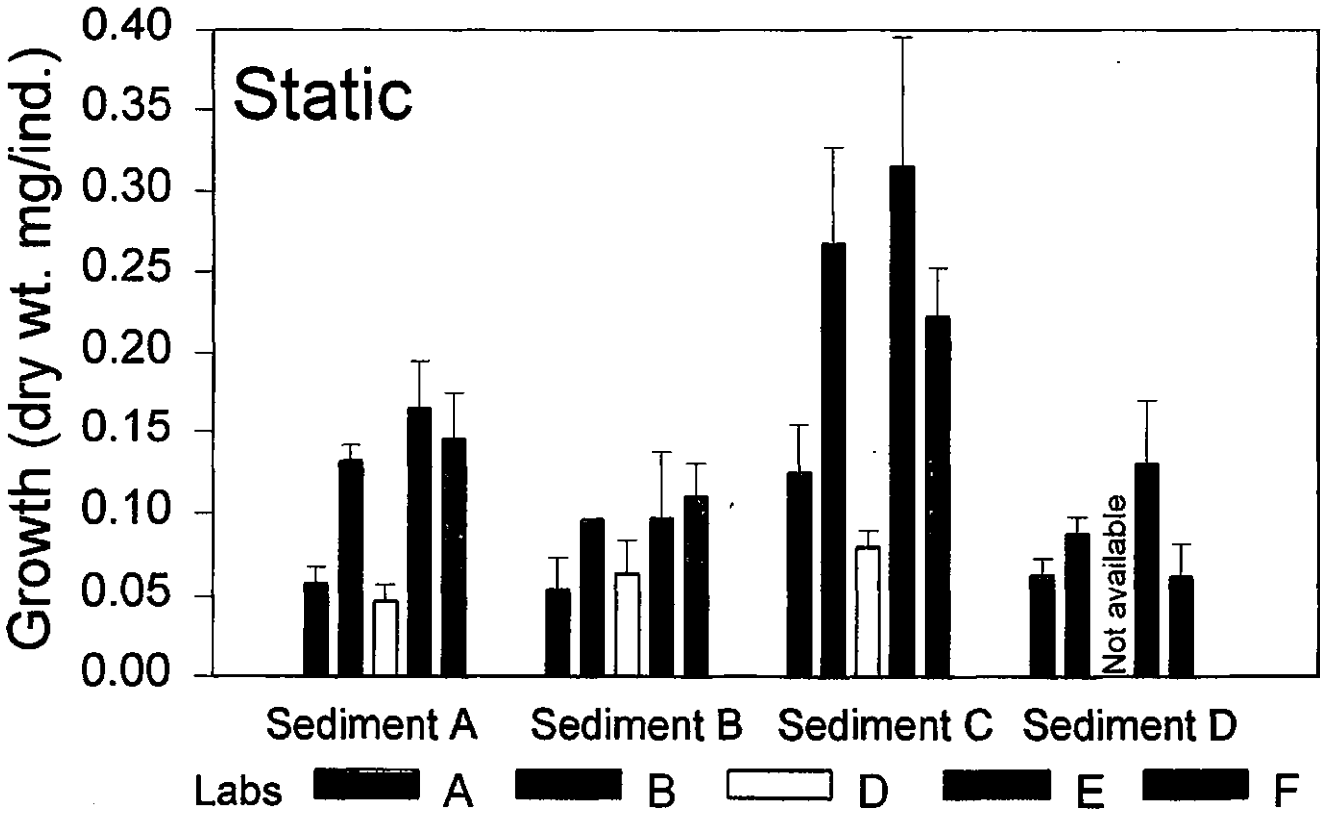


Figure 9. Mean Percent Survival of *H. azteca* in Field Collected Sediments.



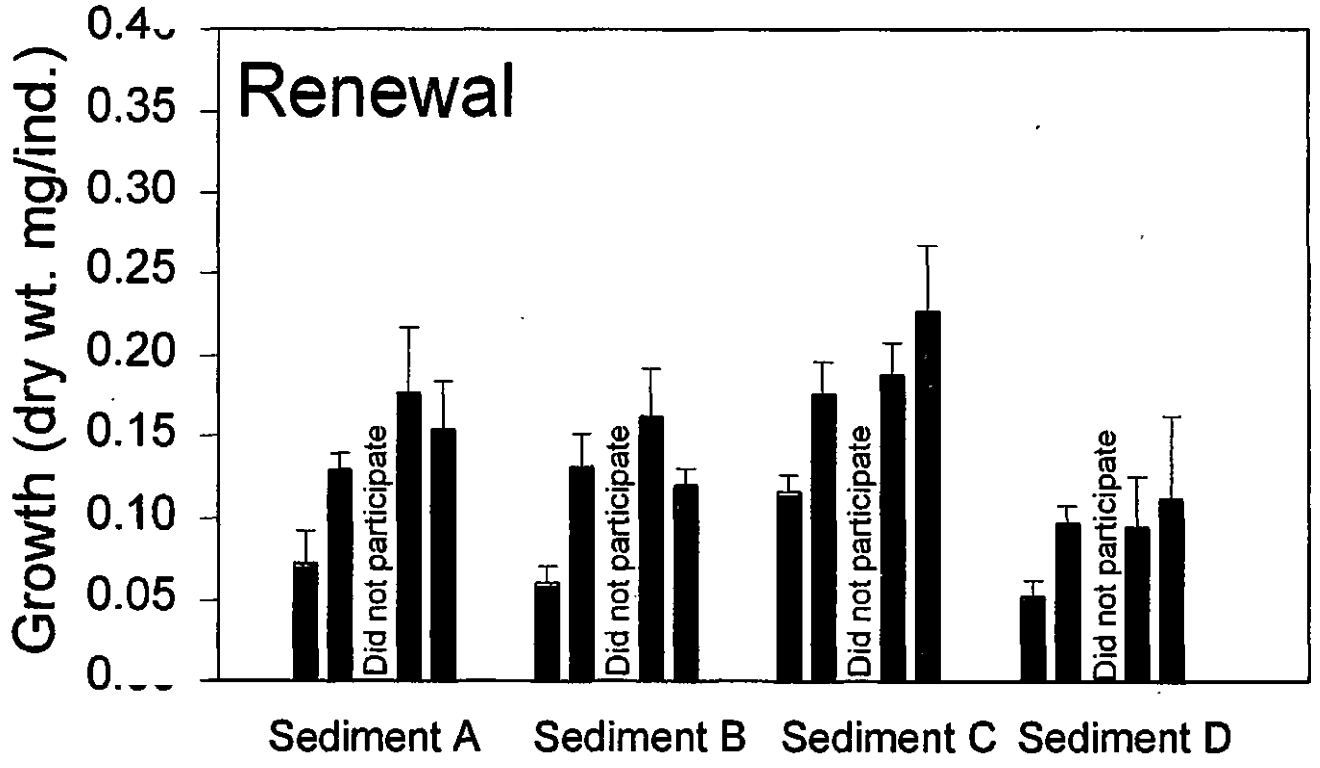


Figure 10. Mean Growth of *H. azteca* in Field-Collected Sediments.

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APPENDIX A

Standard Operating Procedures for Interlaboratory Studies with

C. riparius or *H. azteca*

CHIRONOMUS RIPARIUS ROUND-ROBIN OPERATING PROCEDURES
PHASE I: COPPER-SPIKED FORMULATED SEDIMENT

DATE: OCTOBER 13 - 23, 1995

1. Setting up Proper Test Conditions
2. Storing of Sediment/Food Upon Arrival
3. Allocating Sediment and Adding Overlying Water to Test Chambers
4. Equilibrating
5. Examining Egg Masses (Day -2 and Day -1)
6. Removing Test Chambers from Cold Room (Day -1)
7. Randomizing Test Chambers (Day -1)
8. Aerating Static Test Chambers (Day -1)
9. Measuring Water Quality (Day 0)
10. Adding Test Organisms (Day 0)
11. Feeding
12. Monitoring Throughout the Test
13. Taking Down Test

Step 1. **Setting up Proper Test Conditions**

a) Temperature - The test must be run at 23±1° C, either in a temperature-controlled chamber or with the use of a water bath.

b) Lighting - Use broad spectrum fluorescent lights in the range of 500-1000 lux.

c) Photoperiod - 16L:8D

Step 2. **Storing of Sediment/Food Upon Arrival**

You will be receiving 6 containers with 500 mL sediment per container. These will be labelled as A,B,C,D,&E. You will only need 300 ml of the 1L to run the test.

a) Store sediment immediately upon arrival at 4° C until ready for use.

b) The sediment must be allocated to test chambers within 1 week of receiving it.

c) Store food at 4° C upon arrival and over the course of the test.

Step 3. **Allocating Sediment and Adding Overlying Water to Test Chambers**

a) Within 1 week of receiving the sediment, remove from cold room and thoroughly homogenize.

b) Allocate 100 ml of each sediment to each 300 mL test chamber (3 reps per concentration).

c) Add 175 ml of overlying reconstituted water, pouring slowly along the side of the test chambers to minimize disturbance of the sediment.

Step 4. **Equilibrating**

a) Cover all test chambers with plastic petri dishes (supplied if necessary) and place in 4°C cold room for two weeks minus 1 day.

Step 5. **Examining Egg Masses on Day -2 and Day -1**

a) Two days before the test is to begin, examine all egg masses that we have sent you under a dissecting microscope.

b) Gently shake off or remove all other organisms that are swimming around or attached to the egg masses themselves, then place all egg masses in a beaker containing 100-150 mL reconstituted water (it is not necessary to aerate this water).

c) Examine these egg masses the next day under a dissecting microscope and separate a minimum of three that have hatched (while the eggs are hatched, the organisms should still be attached to the egg mass). Place these hatched egg masses together in a beaker containing reconstituted water. It is not necessary to feed these hatched egg masses set aside for the test.

Step 6. **Removing Test Chambers From Cold Room on Day -1**

a) The day before the test is to commence, remove the test chambers from the cold room and let them warm up to the test temperature.

Step 7. **Randomizing Test Chambers on Day -1**

a) Once the test chambers have warmed to the test temperature, tape the plastic petri lids with airline holes (supplied) to the test chambers using duct tape.

b) Number the test chambers randomly and mark the number on each test chamber (write it on the tape if you wish). Place the test chambers in this order in testing location.

Step 8. **Aerating Static Chambers on Day -1**

a) Aerate static test chambers overnight.

b) Aerate continuously and minimally (2-3 bubbles/sec), using plastic eppendorf tips provided (over aerating could resuspend sediments and test organisms after they have been added).

Step 9. **Measuring Water Quality on Day 0**

a) Measure D.O., pH, conductivity and temperature in all test chambers and record data on the water quality sheets provided.

b) Take an overlying water sample for hardness and/or alkalinity and ammonia analysis. Remove an appropriate amount of overlying water from a combination of the 3 reps from

each concentration. Replace water removed with test water by slowly running it down the side of the test chamber.

Step 10. **Adding Test Organisms**

a) Pour contents of beaker containing hatched test organisms that you have separated the day previous into a glass petri dish.

b) Pipet (5 3/4") up to 5 organisms at a time and release below the water line into the test chamber until 10 per test chamber is achieved.

c) Rinse your pipet in test water between transfers.

d) Choose organisms that appear healthy (ie. actively swimming, not stuck to side of the dish).

Step 11. **Feeding**

a) Feed each test chamber 3.75 mL of the prepared food suspension (warm to test temperature) immediately after adding the test organisms and then 3 more times over the course of the test, for a total of 15.0 mL for each system (feed on non-consecutive days).

b) Shake the food suspension immediately before taking each aliquot of food.

c) Do not allow the pipet to touch the overlying water when adding the aliquots.

Step 12. **Monitoring Throughout the Test**

a) Measure D.O. and temperature ≥3 times/week in at least 1 rep from each concentration.

b) Make note of any irregularities, ie. a lot of algal/fungal growth etc.

Step 13. **Taking Down Test**

a) On day 10, measure D.O., temperature, pH, and conductivity in all test chambers and record values on the water quality sheets provided.

b) Remove an appropriate amount of overlying water from each concentration for hardness and/or alkalinity and ammonia analysis.

c) Sieve contents of each test chamber through a 250μm sieve and lightly spray organisms back into the test chamber until ready for wet weighing (If you do not have a sprayer, pick animals out with the use of a sorting tray).

d) Record survival and wet weights of organisms on the summary sheets provided.

e) Dry organisms at 60°C for 24 hours, then record dry weights on the summary sheets.

C. RIPARIUS ROUND-ROBIN OPERATING PROCEDURES
PHASE II: FIELD-COLLECTED SEDIMENT

NOVEMBER 17 - 29, 1995

1. Setting up Proper Test Conditions
2. Recalibrating Renewal System
3. Storing of Sediment/Food Upon Arrival
4. Separating/Examining Egg Masses (Day -3, Day -2, and Day -1)
5. Allocating Sediment and Adding Overlying Water to Test Chambers (Day -1)
6. Randomizing Test Chambers (Day -1)
7. Aerating Static Test Chambers (Day -1)
8. Starting Renewal System (Day -1)
9. Measuring Water Quality (Day 0)
10. Adding Test Organisms (Day 0)
11. Feeding
12. Monitoring Throughout the Test
13. Taking Down Test

Step 1. **Setting up Proper Test Conditions**

a) Temperature - The test must be run at 23±1° C, either in a temperature-controlled chamber or with the use of a water bath.

b) Lighting - Use broad spectrum fluorescent lights in the range of 500-1000 lux.

c) Photoperiod - 16L:8D

Step 2. **Recalibrating Renewal System**

Your automatic renewal system should still be calibrated from the first round-robin test last month. However, please recheck the calibration to be sure, and adjust if necessary.

a) Starting day 0, set your timers to start the renewal system twice daily, at 12 hour intervals.

Step 3. **Storing of Sediment/Food Upon Arrival**

You will be receiving 3 containers with 1.5 L of sediment per container. These will be labelled as Sediments A, B & C. You will need 1.2 L of each sediment to run the test. Use proper safety equipment when handling the sediment.

a) Store sediment immediately upon arrival at 4° C until ready for use.

b) Store food at 4° C upon arrival and over the course of the test.

Step 4. **Separating/Examining Egg Masses on Day -3, Day -2 and Day -1**

a) 2-3 days before the test is to begin, remove all egg masses from your cultures.

b) Gently shake off or remove all other organisms that are swimming around or attached to the egg masses themselves, then place all egg masses in a beaker containing 100-150 mL culture water (it is not necessary to aerate this water).

c) Examine these egg masses the next days under a dissecting microscope, and separate a minimum of three when they have hatched (while the eggs are hatched, the organisms should still be attached to the egg mass). Place these hatched egg masses together in a beaker containing culture water. It is not necessary to feed these hatched egg masses set aside for the test.

Step 5. **Allocating Sediment and Adding Overlying Water to Test Chambers on Day -1**

a) Remove sediment from cold room and thoroughly homogenize.

b) Allocate 100 ml of each sediment to each 300 mL test chamber (6 reps per sediment type for both the static test and renewal test).

c) Add 175 ml of overlying culture water, pouring slowly along the side of the test chambers to minimize disturbance of the sediment.

Step 6. **Randomizing Test Chambers on Day -1**

Static Chambers

a) Once the test chambers have warmed to the test temperature, tape the plastic petri lids with airline holes to the test chambers using duct tape.

b) Number the test chambers randomly and mark the number on each test chamber (write it on the tape if you wish). Place the test chambers in this order in testing location.

Renewal Chambers

a) Number test chambers randomly, then place below automatic renewal system in this order.

Step 7. **Aerating Static Chambers on Day -1**

a) Aerate static test chambers overnight.

b) Aerate continuously and minimally (2-3 bubbles/sec), using plastic eppendorf tips provided (over-aerating could resuspend sediments and test organisms after they have been added).

Step 8. **Starting Renewal System on Day -1**

a) Start the automated renewal system, manually if desired (ie. add water directly to the header tank, rather than having it start automatically from the carboys).

Step 9. **Measuring Water Quality on Day 0**

a) Start the renewal system before taking water quality parameters.

b) Measure D.O., pH, conductivity and temperature in all test chambers (static and renewal) and record data on the water quality sheets provided.

c) Take an overlying water sample for hardness and/or alkalinity and ammonia analysis. Remove an appropriate amount of overlying water from a combination of the 6 reps from each sediment type.

Step 10. **Adding Test Organisms**

a) Pour contents of beaker containing hatched test organisms that you have separated the day previous into a glass petri dish.

b) Pipet (5 3/4") up to 5 organisms at a time and release below the water line into the test chamber until 10 per test chamber is achieved.

c) Rinse your pipet in test water between transfers.

d) Choose organisms that appear healthy (ie. actively swimming, not stuck to side of the dish).

Step 11. **Feeding**

a) Feed each test chamber (static and renewal) 3.75 mL of the prepared food suspension (warm to test temperature) immediately after adding the test organisms and then 3 more times over the course of the test, for a total of 15.0 mL for each system (feed on non-consecutive days).

b) Shake the food suspension immediately before taking each aliquot of food.

c) Do not allow the pipet to touch the overlying water when adding the aliquots.

Step 12. **Monitoring Throughout the Test**

a) Measure D.O. and temperature ≥3 times/week in at least 1 rep from each sediment type (make sure this is done prior to a water renewal).

b) Make note of any irregularities, ie. a lot of algal/fungal growth etc.

Step 13. **Taking Down Test**

a) On day 10, measure D.O., temperature, pH, and conductivity in all test chambers and record values on the water quality sheets provided.

b) Remove an appropriate amount of overlying water from each sediment type from both systems for hardness and/or alkalinity and ammonia analysis.

c) Sieve contents of each test chamber through a 250μm sieve and lightly spray organisms back into the test chamber until ready for wet weighing. If the sediment does not pass through the 250 μm sieve, pour the contents of the test chamber into a sorting tray and collect organisms with a pipet.

d) Record survival and wet weights of organisms on the summary sheets provided.

e) Dry organisms at 60°C for 24 hours, then record dry weights on the summary sheets.

H. AZTECA ROUND-ROBIN OPERATING PROCEDURES
PHASE I: COPPER-SPIKED FORMULATED SEDIMENT

DATE: JANUARY 25 - FEBRUARY 8, 1996

1. Setting up Proper Test Conditions
2. Calibrating Renewal System
3. Storing of Sediment/Food Upon Arrival
4. Allocating Sediment and Adding Overlying Water to Test Chambers
5. Equilibrating
6. Separating Young (Day -2)
7. Thawing YCT (Day -2)
8. Removing Test Chambers from Cold Room (Day -1)
9. Randomizing Test Chambers (Day -1)
10. Aerating Static Chambers (Day -1)
11. Triggering Renewal System (Day -1)
12. Measuring Water Quality (Day 0)
13. Adding Test Organisms (Day 0)
14. Feeding
15. Monitoring Throughout the Test
16. Taking Down Test

Step 1. **Setting up Proper Test Conditions**

a) Temperature - The test must be run at 23±1° C, either in a temperature-controlled chamber or with the use of a water bath.

b) Lighting - Use broad spectrum fluorescent lights in the range of 500-1000 lux.

c) Photoperiod - 16L:8D

Step 2. **Calibrating Renewal System**

The renewal system should be calibrated to deliver 175 mL ± 10%. Set the timers to trigger the system twice daily at 12 hour intervals.

Step 3. **Storing of Sediment/Food Upon Arrival**

You will be receiving 6 containers with 650-700 mL sediment per container. These will be labelled as A, B, C, D, E & F.

a) Store sediment immediately upon arrival at 4° C until ready for use.

b) Store YCT in the freezer upon arrival.

Step 4. **Allocating Sediment and Adding Overlying Water to Test Chambers**

a) On Thursday January 11, remove the sediment from the cold room and thoroughly homogenize (shake vigorously).

b) Allocate 100 mL of each sediment to each 300 mL test chamber (3 reps per concentration for both the static test and renewal test).

c) Add 175 mL of overlying culture water, by slowly pouring along the side of the test chambers to minimize disturbance of the sediment.

Step 5. **Equilibrating**

a) Cover all test chambers with plastic petri dishes and place in 4°C cold room for two weeks minus 1 day.

Step 6. **Separating Young on Day -2**

Two days before the test is to begin you must separate your *Hyalella* young to ensure that there will be enough organisms of a similar age (i.e. 2-9 days old on Day 0) to start the test.

Step 7. **Thawing YCT on Day -2**

a) Two days before the test is to commence, remove the YCT from the freezer and thaw.

b) Once thawed, store at 4° C for the remainder of the test.

Step 8. **Removing Test Chambers From Cold Room on Day -1**

a) The day before the test is to commence, remove the test chambers from the cold room and let them warm up to the test temperature.

Step 9. **Randomizing Test Chambers on Day -1**

a) Static Chambers
Tape the plastic petri lids (with airline holes) to the test chambers using duct tape. Number the test chambers randomly (write the number on the tape). Place the test chambers in this order in testing location overnight.

b) Renewal Chambers
Number test chambers randomly, then place below renewal system in this order overnight.

Step 10. **Aerating Static Chambers on Day -1**

a) Aerate static test chambers overnight.

b) Aerate continuous and minimal (2-3 bubbles/sec), using plastic eppendorf tips provided (over aerating could resuspend sediments and test organisms after they have been added).

Step 11. **Triggering Renewal System on Day -1**

a) Trigger the automated renewal system once, at the end of the day.

Step 12. **Measuring Water Quality on Day 0**

a) Trigger the renewal system first thing in the morning.

b) Measure dissolved oxygen, pH, conductivity and temperature in all test chambers (static and renewal) and record data on the water quality sheets provided.

c) Take an overlying water sample for hardness and/or alkalinity and ammonia analysis. Remove an appropriate amount of overlying water from a combination of the 3 reps from each concentration.

Step 13. **Adding Test Organisms**

a) Set out 18 or 36 small plastic weigh boats and fill to halfway with culture water.

b) Pour the jar containing the young that you have separated 2 days previous into several glass petri dishes. Dislodge animals from the gauze by gently shaking and/or spraying gauze with a water bottle into petri dishes.

c) Using a glass pipet, transfer test organisms (2-4 at a time) to the weigh boats until 10 per boat is reached (choose animals that appear healthy, ie.actively swimming).

d) Gently pour contents of weigh boats into each test chamber. Using a water bottle, spray the boat (do not spray test organisms directly) if test organisms are stuck.

e) After all test organisms have been added, check each test chamber for floaters. Pop down floaters with the use of a pipet (add a drop of culture water directly to floater).

f) Double check for floaters. If there are still floaters after you have already popped them down, replace them.

Step 14. **Feeding**

a) Remove as much YCT as you will need for the days feeding and warm to test temperature.

b) Feed each test chamber 3.5 mL of YCT after adding the animals on day 0, and 5 more times over the course of the test for a total of 21 mL (feed 3 times per week on non-consecutive days).

c) Shake the food suspension immediately before taking **each** aliquot of food.

d) Do not allow the pipet to touch the overlying water when adding the aliquots.

Step 15. **Monitoring Throughout the Test**

a) Measure dissolved oxygen and temperature ≥3 times/week in at least 1 rep from each concentration (make sure this is done prior to a water renewal).

b) Make note of any irregularities, ie. a lot of algal/fungal growth etc.

Step 16. **Taking Down Test**

a) On day 14, measure dissolved oxygen, temperature, pH, and conductivity in all test chambers and record values on the water quality sheets provided.

b) Remove an appropriate amount of overlying water from each concentration from both systems for hardness and/or alkalinity and ammonia analysis.

c) Sieve contents of each test chamber through a 250 μm sieve and lightly spray organisms back into the test chamber until ready for wet weighing (If you do not have a sprayer, pick animals out with the use of a sorting tray).

d) Record survival and wet weights of organisms on the summary sheets provided.

e) Dry organisms at 60°C for 24 hours, then record dry weights on the summary sheets.

H. AZTECA ROUND-ROBIN OPERATING PROCEDURES
PHASE II: FIELD-COLLECTED SEDIMENT

DATE: FEBRUARY 15 - 29, 1996

1. Setting up Proper Test Conditions
2. Calibrating Renewal System
3. Storing of Sediment/Food Upon Arrival
4. Separating Young (Day -2)
5. Thawing YCT (Day -2)
6. Allocating Sediment and Adding Overlying Water to Test Chambers (Day -1)
7. Randomizing Test Chambers (Day -1)
8. Aerating Static Chambers (Day -1)
9. Triggering Renewal System (Day -1)
10. Measuring Water Quality (Day 0)
11. Adding Test Organisms (Day 0)
12. Feeding
13. Monitoring Throughout the Test
14. Taking Down Test

Step 1. **Setting up Proper Test Conditions**

a) Temperature - The test must be run at 23±1° C, either in a temperature-controlled chamber or with the use of a water bath.

b) Lighting - Use broad spectrum fluorescent lights in the range of 500-1000 lux.

c) Photoperiod - 16L:8D.

Step 2. **Calibrating Renewal System**

The renewal system should be calibrated to deliver 175 mL ± 10%. Set the timers to trigger the system twice daily at 12 hour intervals.

Step 3. **Storing of Sediment/Food Upon Arrival**

You will be receiving 4 containers with ~900 mL sediment per container. These will be labelled as A, B, C & D.

a) Store sediment immediately upon arrival at 4° C until ready for use.

b) Store YCT in the freezer upon arrival.

Step 4. **Separating Young on Day -2**

Two days before the test is to begin you must separate your *Hyalella* young from the adults to ensure that there will be enough organisms of a similar age (i.e., 2 - 9 days old on Day 0) to start the test (Please see preview-to-final manuscript for details on how to obtain young of proper age).

Step 5. **Thawing YCT on Day -2**

a) Two days before the test is to commence, remove the YCT from the freezer and thaw.

b) Once thawed, store at 4° C for the remainder of the test.

Step 6. **Allocating Sediment and Adding Overlying Water to Test Chambers on Day -1**

a) Remove sediment from cold room and thoroughly homogenize (shake vigorously).

b) Allocate 100 mL of each sediment to each 300 mL test chamber (4 reps per sediment type for both the static test and renewal test).

c) Add 175 mL of overlying culture water, by pouring slowly along the side of the test chambers to minimize disturbance of the sediment.

Step 7. **Randomizing Test Chambers on Day -1**

a) Static Chambers
Tape the plastic petri lids with airline holes to the test chambers using duct tape. Number the test chambers randomly (write the number on the tape). Place the test chambers in this order in testing location overnight.

b) Renewal Chambers
Number test chambers randomly, then place below renewal system in this order overnight.

Step 8. **Aerating Static Chambers on Day -1**

a) Aerate static test chambers overnight.

b) Aerate continuous and minimal (2-3 bubbles/sec), using plastic eppendorf tips provided (over aerating could resuspend sediments and test organisms after they have been added).

Step 9. **Triggering Renewal System on Day -1**

a) Trigger the renewal system once, at the end of the day.

Step 10. **Measuring Water Quality on Day 0**

a) Trigger the renewal system first thing in the morning.

b) Measure dissolved oxygen, pH, conductivity and temperature in all test chambers (static and renewal) and record data on the water quality sheets provided.

c) Take an overlying water sample for hardness and/or alkalinity and ammonia analysis. Remove 25 mL overlying water from each replicate beaker from each sediment type.

Step 11. **Adding Test Organisms**

a) Set out 18 (1-mode) or 36 (2-modes) small plastic weigh boats and fill to halfway with culture

water.

b) Pour the jar containing the young that you have separated 2 days previous into several glass petri dishes. Dislodge animals from the gauze by gently shaking and/or spraying gauze with a water bottle into petri dishes.

c) Using a glass pipet, transfer test organisms (2 - 4 at a time) to the weigh boats until 10 per boat is reached (choose animals that appear healthy, ie. actively swimming).

d) Gently pour contents of weigh boats into test chambers. Using a water bottle, spray the boat (do not spray test organisms directly) if any test organisms are stuck.

e) After all test organisms have been added, check each test chamber for floaters. Pop down floaters with the use of a pipet (add a drop of culture water directly to floater).

f) Double check again for floaters. If there are still floaters after you have already popped them down once, replace them.

Step 12. **Feeding**

a) Remove as much YCT as you will need for the days feeding and warm to test temperature.

b) Feed each test chamber 3.5 mL of YCT immediately after adding the animals on day 0, and 5 more times over the course of the test for a total of 21 mL (feed 3 times per week on non-consecutive days).

c) Shake the food suspension immediately before taking **each** aliquot of food.

d) Do not allow the pipet to touch the overlying water when adding the aliquot.

Step 13. **Monitoring Throughout the Test**

a) Measure dissolved oxygen and temperature ≥3 times/week in at least 1 rep from each sediment type (make sure this is done prior to a water renewal).

b) Make note of any irregularities, ie. a lot of algal/fungal growth etc.

Step 14. **Taking Down Test**

a) On day 14, measure dissolved oxygen, temperature, pH, and conductivity in all test chambers and record values on the water quality sheets provided.

b) Remove an appropriate amount of overlying water from each sediment type from both systems for hardness and/or alkalinity and ammonia analysis.

c) Sieve contents of each test chamber through a 250 μm sieve and lightly spray organisms back into the test chamber until ready for wet weighing (if you do not have a sprayer, pick animals out with the use of a sorting tray).

d) Record survival and wet weights of organisms on the summary sheets provided.

e) Dry organisms at 60°C for 24 hours, then record dry weights on the summary sheets.

OPERATING PROCEDURES FOR 96-H *C. RIPARIUS* COPPER REFERENCE TEST

2nd instar animals are used for *C. riparius* reference tests; therefore, it will be necessary to place the remaining test organisms from the copper-spiked sediment test (Dec. 8) into an aquarium for 3-5 days before starting the reference test.

Follow steps 1 - 7. For further test conditions, please see the preview-to-final-manuscript for *C. riparius* that I have sent you; Section 4.8, table 4, p.55.

You will be supplied with:
1. $CuCl_2$ stock solution (100 ppm)
2. Food suspension
3. Silica sand
4. Data sheets

1. Preparing For Test

a) Place the remaining test organisms from the sediment copper-spiked test in an aquarium for 3-5 days, as you would normally to initiate a new culture (silica sand substrate). Feed tank as normal.

2. Addition of Copper

Table 1. Copper Concentrations

Concentration (ppb)	Amout $CuCl_2$ to add (mL)	Amount Culture Water to add (mL)
0	0	200.0
250	0.5	199.5
500	1.0	199.0
1000	2.0	198.0
1500	3.0	197.0
2000	4.0	196.0
2500	5.0	195.0

a) Label seven 250 mL beakers (acid-rinsed) as follows: 0, 250, 500, 1000, 1500, 2000, & 2500 ppb.

b) Add a monolayer of silica sand to each beaker (~2 mL).

c) Starting from lowest concentration, add respective amount of $CuCl_2$, according to table 1, to a 250 mL graduated cylinder.

d) Top up with culture water to 200 mL, then add to respective beaker.

3. **Parameters (Day 0)**

a) Measure dissolved oxygen, temperature, pH, conductivity, hardness and alkalinity on day 0, and record on the sheet provided.

4. **Addition of Test Organisms**

a) Dislodge the 2nd instar animals from their cases by gently swirling the water just above the silica sand with an aquarium net.

b) Net out the animals and place in a petri dish filled with culture water.

c) Set out 7 weigh boats, and add a drop of culture water to each.

d) Pipet test organisms into weigh boats until 10 per boat is reached (choose animals of uniform size). Once you have reached 10 per boat, try to remove as much of the water as possible from the boat with a pipet.

e) Add contents of 1 weigh boat to each beaker (if the animals get stuck on the weigh boat, dislodge by running the test solution over the boat with the use of a pipet).

5. **Feeding (Day 0 and Day 2)**

a) Feed each beaker the amount indicated on the food bottle on day 0 and on day 2 (suspension equates to 4 mg dry solids).

6. **Monitoring Throughout the Test**

a) Check dissolved oxygen and temperature daily in each beaker. Record on sheet.

b) Check test organisms daily, and record number of dead or moribund (you may not be able to tell if the animals are in their cases).

7. **Taking Down Test**

a) Measure dissolved oxygen, temperature, pH, conductivity, hardness and alkalinity and record on the sheet provided.

b) Record the number of dead organisms. Swirl water in beaker to dislodge animals from the silica sand or remove animals from their cases with the use of a probe, or:

c) Pour contents of beaker into a sorting tray to facilitate counting of dead organisms.

OPERATING PROCEDURES FOR 96-H *H. AZTECA* COPPER REFERENCE TEST

You will need to use 2 - 9 day old test organisms; therefore, the reference test should be started the same day as the sediment test (Jan 25).

Follow steps 1 - 7. For further test conditions, please see the preview-to-final-manuscript for *H. azteca,* section 4.9, table 3, p.53.

You will be supplied with:
1. $CuCl_2$ stock solution - use same stock from previous reference test
2. Food
3. Data sheets
4. Gauze

NOTE: Procedures are outlined for setting up 1 rep per concentration; however you may be adding extra reps dependant on time and animal restrictions.

1. **Preparing For Test**

Presoak seven 2.5 cm X 2.5 cm strips of the supplied gauze in culture water for 24 hours prior to starting the reference test.

2. **Addition of Copper**

Table 1. Copper Concentrations

Concentration (ppb)	Amount $CuCl_2$ to add (μL)	Amount Culture Water to add (mL)
0	0	200.00
25	50	199.95
50	100	199.90
100	200	199.80
250	500	199.50
500	1000	199.00
1000	2000	198.00

a) Label seven 300 mL beakers (acid-rinsed) as follows: 0, 25, 50, 100, 250, 500, & 1000 ppb.

b) Add a 2.5 cm x 2.5 cm strip of the presoaked gauze to each beaker.

c) Starting from lowest concentration, add respective amount of $CuCl_2$, according to table 1, to a 250 mL graduated cylinder.

d) Top up with culture water to 200 mL, then add to respective beaker.

3. **Parameters (Day 0)**

a) Measure dissolved oxygen, temperature, pH, conductivity, hardness and alkalinity on day 0, and record on the sheet provided.

4. **Addition of Test Organisms**

a) Set out 7 weigh boats, and add ~1 mL culture water to each.

b) Pipet test organisms into weigh boats until 10 per boat is reached.

c) Add contents of 1 weigh boat to each beaker. Try to remove as much of the water as you can prior to adding the organisms to the beakers (if the animals get stuck on the weigh boat, dislodge by running the test solution over the boat with the use of a pipet).

d) Check several times for floaters. Pop down floaters by adding a drop of test solution directly to floater. Replace animal if floating persists.

5. **Feeding (Day 0 and Day 2)**

a) Feed each test chamber 0.5 mL YCT on day 0 and day 2.

6. **Monitoring Throughout the Test**

a) Check dissolved oxygen and temperature daily in each beaker. Record on sheet.

b) Check test organisms daily, and record number of dead or moribund.

7. **Taking Down Test**

a) Measure dissolved oxygen, temperature, pH, conductivity, hardness and alkalinity and record on the sheet provided.

b) Record the number of dead organisms. Shake/spray gauze to dislodge test organisms with the use of a probe, or:

c) Pour contents of beaker into a sorting tray/petri dish to facilitate counting of dead organisms.

APPENDIX B

Data from Intralaboratory Feeding Trials with

C. riparius or *H. azteca*

Appendix B , Table B-1. Survival and Growth Data for Intralaboratory Studies with C. riparius.

Static-Renewal System

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
LP - 4mg	1	1	0.925	0.096	10.35	0.577	0.569	0.03	5.98
	2	1				0.544			
	3	0.8				0.54			
	4	0.9				0.613			
LP - 6mg	1	0.9	0.925	0.05	5.405	0.646	0.655	0.06	9.12
	2	0.9				0.614			
	3	1				0.618			
	4	0.9				0.742			
LP - 10mg/4x	1	1	0.925	0.05	5.405	0.576	0.64	0.08	13.2
	2	0.9				0.758			
	3	0.9				0.581			
	4	0.9				0.644			
LP - 15mg/4x	1	0.9	0.875	0.126	14.38	0.716	0.734	0.07	9.67
	2	0.7				0.733			
	3	1				0.657			
	4	0.9				0.828			
108 - 4mg	1	0.9	0.8	0.2	25	0.482	0.554	0.17	30
	2	0.9				0.416			
	3	0.5				0.794			
	4	0.9				0.523			
108 - 6mg	1	0.8	0.75	0.238	31.74	0.744	0.582	0.2	34
	2	0.4				0.673			
	3	0.9				0.296			
	4	0.9				0.614			
108 - 10mg/4x	1	0.3	0.55	0.265	48.1	0.707	0.583	0.09	14.9
	2	0.4				0.573			
	3	0.9				0.51			
	4	0.6				0.54			
108 - 15mg/4x	1	0.9	0.825	0.171	20.7	0.636	0.571	0.11	19.1
	2	1				0.508			
	3	0.6				0.688			
	4	0.8				0.453			
WB - 4mg	1	0.9	0.95	0.058	6.077	0.794	0.88	0.11	12.2
	2	1				0.986			
	3	1				0.782			
	4	0.9				0.959			
WB - 6mg	1	1	0.975	0.05	5.128	0.744	0.929	0.21	22.4
	2	1				1.136			
	3	0.9				1.081			
	4	1				0.755			
WB - 10mg/4x	1	0.9	0.9	0	0	0.933	0.908	0.06	6.1
	2	0.9				0.862			
	3	0.9				0.974			
	4	0.9				0.862			
WB - 15mg/4x	1	1	0.95	0.1	10.53	0.735	0.728	0.2	27.9
	2	1				0.626			
	3	1				0.541			
	4	0.8				1.008			

Static System

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
LP - 4mg	1	1	0.95	0.1	10.5	0.707	0.674	0.05	7.19
	2	0.8				0.718			
	3	1				0.657			
	4	1				0.613			
LP - 6mg	1	0.9	0.825	0.1	11.6	0.863	0.893	0.09	10.3
	2	0.7				1.024			
	3	0.9				0.809			
	4	0.8				0.874			
LP - 10mg/4x	1	0.9	0.875	0.05	5.71	0.729	0.697	0.02	3.22
	2	0.9				0.677			
	3	0.8				0.69			
	4	0.9				0.691			
LP - 15mg/4x	1	1	0.9	0.08	9.07	0.814	0.834	0.05	6.09
	2	0.9				0.822			
	3	0.8				0.791			
	4	0.9				0.907			
108 - 4mg	1	1	0.925	0.15	16.2	0.696	0.716	0.04	6.29
	2	1				0.679			
	3	0.7				0.781			
	4	1				0.707			
108 - 6mg	1	1	0.9	0.08	9.07	0.874	0.893	0.09	9.99
	2	0.9				0.937			
	3	0.8				0.983			
	4	0.9				0.777			
108 - 10mg/4x	1	0.8	0.925	0.1	10.4	0.738	0.687	0.06	8.27
	2	1				0.606			
	3	1				0.695			
	4	0.9				0.708			
108 - 15mg/4x	1	0.8	0.925	0.1	10.4	0.888	0.89	0.05	6.03
	2	0.9				0.963			
	3	1				0.873			
	4	1				0.835			
WB - 4mg	1	1	0.925	0.1	10.4	0.935	0.945	0.02	1.91
	2	1				0.937			
	3	0.9				0.972			
	4	0.8				0.936			
WB - 6mg	1	0.9	0.875	0.05	5.71	1.031	1.075	0.08	7.86
	2	0.9				1.064			
	3	0.9				1.197			
	4	0.8				1.008			
WB - 10mg/4x	1	0.9	0.875	0.1	10.9	0.949	0.944	0.04	4.21
	2	0.8				0.998			
	3	1				0.913			
	4	0.8				0.915			
WB - 15mg/4x	1	0.8	0.9	0.08	9.07	0.969	1.049	0.1	9.9
	2	0.9				1.2			
	3	0.9				1.03			
	4	1				0.996			

Appendix B, Table B-2. Overlying Water Chemistry During Intralaboratory Studies with C. riparius.

Ammonia (ppm)

Day 0

	Static	Renewal
Long Point	<0.01	nd
Stn 108	nd	nd
Stn WB	<0.01	nd

Day 10

	Static	Renewal
Long Point:		
4 mg daily	<.01	0.175
6 mg daily	<.05	0.4
10mg/4x	<.05	0.175
15mg/4x	0.1	0.44
Stn 108:		
4 mg daily	<.05	0.3
6 mg daily	0.125	0.425
10mg/4x	<.05	0.15
15mg/4x	<.05	0.6
Stn WB:		
4 mg daily	5.8	0.7
6 mg daily	9	1.5
10mg/4x	6	0.69
15mg/4x	12	1.8

Hardness (mg/L)

Day 0

	Static	Renewal
Long Point	140	140
Stn 108	130	105
Stn WB	120	120

Day 10

	Static	Renewal
Long Point:		
4 mg daily	165	140
6 mg daily	145	150
10mg/4x	145	130
15mg/4x	190	120
Stn 108:		
4 mg daily	195	120
6 mg daily	200	115
10mg/4x	170	120
15mg/4x	200	130
Stn WB:		
4 mg daily	140	120
6 mg daily	145	110
10mg/4x	140	120
15mg/4x	140	135

Alkalinity (mg/L)

Day 0

	Static	Renewal
Long Point	93	76
Stn 108	85	71
Stn WB	94	70

Day 10

	Static	Renewal
Long Point:		
4 mg daily	115	110
6 mg daily	115	95
10mg/4x	110	100
15mg/4x	80	100
Stn 108:		
4 mg daily	90	80
6 mg daily	80	90
10mg/4x	90	85
15mg/4x	100	85
Stn WB:		
4 mg daily	125	100
6 mg daily	130	100
10mg/4x	130	90
15mg/4x	140	95

Dissolved Oxygen (mg/L) - lowest value over 10 days

	Static	Renewal	Avg -Ren.
Long Point:			
4 mg daily	>7.0	4	6.2
6 mg daily	>7.0	3.6	5.39
10mg/4x	>7.0	4.5	5.97
15mg/4x	>7.0	3.9	6
Stn 108:			
4 mg daily	>7.0	3.9	5.9
6 mg daily	>7.0	4.3	5.89
10mg/4x	>7.0	5	6.64
15mg/4x	>7.0	3.1	5.46
Stn WB:			
4 mg daily	>7.0	4.9	6.14
6 mg daily	>7.0	2.3	4.74
10mg/4x	>7.0	4.5	5.95
15mg/4x	>7.0	2.8	2.79

Temperature range over 10 days

	Static	Renewal
Long Point:		
4 mg daily	22.6-23.7	22.2-23.2
6 mg daily	22.4-23.2	22.0-23.5
10mg/4x	22.4-23.1	22.0-23.8
15mg/4x	22.4-23.4	22.2-23.0
Stn 108:		
4 mg daily	22.1-23.0	21.9-23.1
6 mg daily	22.5-23.1	22.3-23.4
10mg/4x	22.0-23.4	21.7-22.8
15mg/4x	22.4-23.0	22.0-22.7
Stn WB:		
4 mg daily	22.6-23.1	22.0-23.2
6 mg daily	22.1-22.9	22.4-23.2
10mg/4x	22.2-23.2	22.0-23.2
15mg/4x	22.6-23.2	22.1-22.8

Conductivity range over 10 days

	Static	Renewal
Long Point:		
4 mg daily	327-403	268-325
6 mg daily	327-420	263-323
10mg/4x	325-421	267-326
15mg/4x	325-425	264-332
Stn 108:		
4 mg daily	320-438	264-317
6 mg daily	323-453	253-323
10mg/4x	318-457	267-321
15mg/4x	319-464	267-318
Stn WB:		
4 mg daily	307-362	252-321
6 mg daily	313-386	254-324
10mg/4x	311-396	250-323
15mg/4x	313-412	257-327

ph range over 10 days

	Static	Renewal
Long Point:		
4 mg daily	7.99-8.52	7.64-8.71
6 mg daily	8.25-8.47	7.83-8.61
10mg/4x	8.21-8.58	7.66-8.50
15mg/4x	8.24-8.53	7.74-8.67
Stn 108:		
4 mg daily	7.90-8.35	7.89-8.23
6 mg daily	8.19-8.32	7.86-8.80
10mg/4x	8.33-8.43	7.88-8.81
15mg/4x	8.21-8.35	7.70-8.55
Stn WB:		
4 mg daily	8.35-8.49	7.85-8.39
6 mg daily	8.35-8.53	7.93-8.12
10mg/4x	8.34-8.61	7.91-8.41
15mg/4x	8.35-8.60	7.77-8.02

Appendix B, Table B-3. Survival and Growth Data for Intralaboratory Studies with H. azteca.

Static-Renewal System

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
LP 1x	1	1	0.94	0.089	9.52	0.151	0.21	0.05	23.57
	2	1				0.192			
	3	1				0.281			
	4	0.9				0.239			
	5	0.8				0.194			
LP 3x	1	0.9	0.975	0.05	5.13	0.203	0.24	0.03	12.66
	2	1				0.234			
	3	1				0.266			
	4								
	5	1				0.268			
LP N	1	1	1	0	0	0.192	0.14	0.03	22.74
	2	1				0.155			
	3	1				0.109			
	4	1				0.122			
	5	1				0.135			
1213 1x	1	0.9	0.9	0.071	7.86	0.139	0.12	0.01	12.03
	2	0.9				0.106			
	3	0.8				0.131			
	4	1				0.121			
	5	0.9				0.107			
1213 3x	1	1	0.96	0.055	5.71	0.121	0.11	0.01	11.61
	2	1				0.121			
	3	1				0.1			
	4	0.9				0.106			
	5	0.9				0.093			
1213 N	1	1	0.98	0.045	4.56	0.102	0.1	0.01	13.48
	2	1				0.092			
	3	1				0.109			
	4	1				0.08			
	5	0.9				0.113			
100 1x	1	1	0.975	0.05	5.13	0.131	0.18	0.03	17.4
	2	1				0.182			
	3	1				0.201			
	4	0.9				0.186			
100 3x	1	1	0.95	0.1	10.5	0.248	0.21	0.03	16.22
	2	1				0.173			
	3	0.8				0.194			
	4	1				0.232			
100 N	1	0.8	0.825	0.171	20.7	0.164	0.15	0.02	16.22
	2	1				0.113			
	3	0.9				0.154			
	4	0.6				0.163			

Static System

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
LP 1x	1	1	0.98	0.045	4.56	0.327	0.31	0.049	16.01
	2	1				0.269			
	3	1				0.279			
	4	0.9				0.387			
	5	1				0.28			
LP 3x	1	1	0.96	0.089	9.32	0.25	0.31	0.041	13.42
	2	0.8				0.324			
	3	1				0.313			
	4	1				0.359			
	5	1				0.285			
LP N	1	1	0.98	0.045	4.56	0.266	0.25	0.072	28.39
	2	0.9				0.249			
	3	1				0.135			
	4	1				0.324			
	5	1				0.292			
1213 1x	1	1	0.98	0.045	4.56	0.123	0.13	0.008	6.051
	2	1				0.131			
	3	1				0.139			
	4	0.9				0.134			
	5	1				0.12			
1213 3x	1	0.8	0.88	0.13	14.8	0.129	0.12	0.022	17.88
	2	0.9				0.15			
	3	1				0.14			
	4	0.7				0.11			
	5	1				0.095			
1213 N	1	1	1	0	0	0.185	0.16	0.026	16.14
	2	1				0.182			
	3	1				0.157			
	4	1				0.12			
	5	1				0.167			
100 1x	1	1	1	0	0	0.312	0.28	0.048	16.82
	2	1				0.263			
	3	1				0.226			
	4	1				0.331			
100 3x	1	0.9	0.95	0.058	6.08	0.257	0.29	0.032	11.23
	2	1				0.294			
	3	1				0.329			
	4	0.9				0.267			
100 N	1	1	1	0	0	0.123	0.2	0.056	28.69
	2	1				0.182			
	3	1				0.228			
	4	1				0.25			

Appendix B, Table B-4. Overlying Water Chemistry During Intralaboratory Studies with H. azteca.

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Ammonia (ppm)

Day 0

	Static	Renewal
Long Point	-	-
Stn 1213	-	-
Stn 100	-	-

Day 14

	Static	Renewal
Long Point:		
YCT-Daily	<.05	<.05
YCT-3X/Week	<.05	-
Nutrafin-3X/Week	<.05	<.05
Stn 1213:		
YCT-Daily	<.05	<.05
YCT-3X/Week	<.1	<.1
Nutrafin-3X/Week	<.05	<.1
Stn 100:		
YCT-Daily	<.05	<.05
YCT-3X/Week	nd	<.05
Nutrafin-3X/Week	<.05	<.05

Hardness (mg/L)

Day 0

	Static	Renewal
Long Point	156	-
Stn 1213	132	-
Stn 100	144	-

Day 14

	Static	Renewal
Long Point:		
YCT-Daily	175	148
YCT-3X/Week	217	133
Nutrafin-3X/Week	179	120
Stn 1213:		
YCT-Daily	123	121
YCT-3X/Week	127	125
Nutrafin-3X/Week	135	124
Stn 100:		
YCT-Daily	140	121
YCT-3X/Week	151	136
Nutrafin-3X/Week	142	120

Alkalinity (mg/L)

Day 0

	Static	Renewal
Long Point	95	-
Stn 1213	51	-
Stn 100	81	-

Day 14

	Static	Renewal
Long Point:		
YCT-Daily	144	101
YCT-3X	143	75
Nutrafin	95	78
Stn 1213:		
YCT-Daily	22	72
YCT-3X	21	76
Nutrafin	30	-
Stn 100:		
YCT-Daily	73	85
YCT-3X	81	82
Nutrafin	68	81

Dissolved Oxygen (mg/L) - lowest value over 14 days

	Static	Renewal	Avg -Ren.
Long Point:			
YCT-Daily	7.4	5.2	6.7
YCT-3X/Week	7.4	5.6	6.8
Nutrafin-3X/Week	7.4	5.9	7
Stn 1213:			
YCT-Daily	7.3	5.9	7.2
YCT-3X/Week	7	5.3	6.9
Nutrafin-3X/Week	7.4	5.8	7.2
Stn 100:			
YCT-Daily	7.4	6.6	7.6
YCT-3X/Week	7.5	6.6	7.4
Nutrafin-3X/Week	7.5	7.1	7.8

Temperature range over 14 days

	Static	Renewal
Long Point:		
YCT-Daily	22.0-23.1	21.5-23.2
YCT-3X/Week	22.1-23.4	21.1-23.1
Nutrafin-3X/Week	21.8-23.1	21.3-23.1
Stn 1213:		
YCT-Daily	22.1-23.6	21.4-23.0
YCT-3X/Week	22.3-23.3	21.2-23.0
Nutrafin-3X/Week	21.4-23.3	21.2-22.6
Stn 100:		
YCT-Daily	22.0-23.3	21.1-22.5
YCT-3X/Week	22.0-23.0	21.2-23.0
Nutrafin-3X/Week	21.2-23.1	21.1-22.4

Conductivity range over 14 days

	Static	Renewal
Long Point:		
YCT-Daily	342-435	305-324
YCT-3X/Week	342-506	289-328
Nutrafin-3X/Week	341-441	297-326
Stn 1213:		
YCT-Daily	294-343	275-313
YCT-3X/Week	292-325	278-314
Nutrafin-3X/Week	292-328	267-315
Stn 100:		
YCT-Daily	328-433	288-318
YCT-3X/Week	310-377	294-318
Nutrafin-3X/Week	295-367	286-313

pH range over 14 days

	Static	Renewal
Long Point:		
YCT-Daily	7.81-8.53	7.80-8.65
YCT-3X/Week	7.85-8.51	7.52-8.75
Nutrafin-3X/Week	7.84-8.54	7.83-8.66
Stn 1213:		
YCT-Daily	7.57-8.48	7.78-8.70
YCT-3X/Week	7.53-8.43	7.67-8.84
Nutrafin-3X/Week	7.49-8.27	7.31-8.77
Stn 100:		
YCT-Daily	7.50-8.73	7.87-8.55
YCT-3X/Week	7.56-8.45	7.71-8.53
Nutrafin-3X/Week	7.62-8.43	7.93-8.68

APPENDIX C

Biological and Water Quality Data from Interlaboratory Studies with

C. riparius or *H. azteca*

Appendix C, Table C-1. Data from C. riparius Interlaboratory Study # 1

Static System

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	90	10.00	11.11	0.771	0.625	0.272	43.57
	2	80				0.794			
	3	100				0.311			
100	1	90	86.667	15.28	17.63	0.481	0.59	0.326	55.27
	2	100				0.332			
	3	70				0.956			
250	1	90	96.667	5.77	5.97	0.157	0.353	0.17	48.23
	2	100				0.44			
	3	100				0.463			
500	1	90	86.667	5.77	6.66	0.721	0.696	0.033	4.72
	2	90				0.709			
	3	80				0.659			
1000	1	100	96.667	5.77	5.97	0.509	0.404	0.142	35.04
	2	100				0.243			
	3	90				0.46			
2000	1	0	0	0	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100	0	0	0.75	0.793	0.04	5.00
	2	100				0.828			
	3	100				0.801			
100	1	90	90	10	11.11	0.851	0.77	0.08	11.00
	2	80				0.778			
	3	100				0.682			
250	1	100	100	0	0	0.607	0.598	0.03	5.52
	2	100				0.625			
	3	100				0.561			
500	1	100	93.333	5.77	6.186	0.504	0.517	0.01	2.28
	2	90				0.527			
	3	90				0.52			
1000	1	70	83.333	15.28	18.33	0.186	0.182	0.01	7.39
	2	100				0.167			
	3	80				0.193			
2000	1	0	0	0	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY C

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	20	40	43.59	108.97	0.62	0.536	0.08	15
	2	90				0.527			
	3	10				0.46			
100	1	10	50	34.64	69.28	1.28	1.935	0.585	30.24
	2	70				2.12			
	3	70				2.406			
250	1	40	40	20	50	0.995	0.696	0.339	48.68
	2	20				0.765			
	3	60				0.328			
500	1	10	33.33	40.41	121.24	1.9	1.063	0.73	68.72
	2	80				0.558			
	3	10				0.73			
1000	1	20	23.33	25.17	107.85	0.33	0.196	0.19	96.69
	2	0							
	3	50				0.062			
2000	1	0	0	0	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY D

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100	0	0	1.243	1.087	0.137	12.58
	2	100				0.988			
	3	100				1.03			
100	1	100	100	0	0	0.836	1.039	0.188	18.09
	2	100				1.073			
	3	100				1.207			
250	1	90	90	0	0	0.973	0.917	0.079	8.64
	2	90				0.861			
	3								
500	1	100	100	0	0	0.594	0.644	0.079	12.30
	2	100				0.602			
	3	100				0.735			
1000	1	90	80	10	12.5	0.558	0.451	0.147	32.64
	2	80				0.511			
	3	70				0.283			
2000	1	0	0	0	-	-	-	-	-
	2	0				-			
	3	0				-			

Static-Renewal System

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	90	17.32	19.245	1.051	1.1437	0.14	12.19
	2	70				1.304			
	3	100				1.076			
100	1	60	70	26.46	37.7964	1.227	0.7043	0.63	89.53
	2	50				0.004			
	3	100				0.882			
250	1	80	90	14.14	15.7135	0.909	0.883	0.04	4.16
	2								
	3	100				0.857			
500	1	100	86.667	15.28	17.6253	0.848	0.8657	0.24	27.81
	2	90				0.836			
	3	70				1.113			
1000	1	100	80	26.46	33.0719	0.875	0.7147	0.14	19.44
	2	50				0.638			
	3	90				0.631			
2000	1	40	56.667	20.82	36.7353	0.15	0.4343	0.46	106.68
	2	50				0.184			
	3	80				0.969			

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	80	73.333	11.55	15.7459	0.548	0.5237	0.12	22.42
	2	80				0.396			
	3	60				0.627			
100	1	90	90	0	0	0.459	0.475	0.05	9.51
	2	90				0.44			
	3	90				0.526			
250	1	90	83.333	5.774	6.9282	0.472	0.3933	0.09	22.91
	2	80				0.413			
	3	80				0.295			
500	1	80	73.333	11.55	15.7459	0.315	0.3503	0.05	14.60
	2	60				0.327			
	3	80				0.409			
1000	1	80	60	20	33.3333	0.215	0.1707	0.04	24.49
	2	60				0.132			
	3	40				0.165			
2000	1	0	0	0	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY C

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	50	53.33	25.17	47.19	0.494	0.872	0.41	46.46
	2	30				1.3			
	3	80				0.823			
100	1	50	33.33	15.28	45.83	0.874	0.535	0.29	54.81
	2	20				0.375			
	3	30				0.357			
250	1	50	30	20	66.67	0.756	0.611	0.25	40.26
	2	10				0.75			
	3	30				0.327			
500	1	10	26.67	15.28	57.28	0.92	0.553	0.32	57.69
	2	30				0.39			
	3	40				0.348			
1000	1	20	16.67	15.28	91.65	0.31	0.2	0.16	77.78
	2	0							
	3	30				0.09			
2000	1	10	13.33	15.28	114.56	0.24	0.214	0.04	17.55
	2	0							
	3	30				0.187			

Appendix C, Table C-2. Overlying Water Chemistry Data from C. riparius Interlaboratory Study # 1

Static System

HARDNESS (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	200	-	230	-
100	145	-	248	-
250	200	-	338	-
500	200	-	354	-
1000	200	-	487	-
2000	200	-	670	-
Day 10				
0 (ppm)	>200	300	300	-
100	>200	340	320	-
250	190	390	340	-
500	>200	400	400	-
1000	>200	760	580	-
2000	200	1000	760	-

ALKALINITY (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	100	-	128	138
100	101	-	131	144
250	110	-	134	129
500	100	-	131	141
1000	112	-	128	180
2000	97	-	122	135
Day 10				
0 (ppm)	125	137	104	150
100	100	154	102	234
250	105	146	101	240
500	94	148	106	243
1000	110	160	152	177
2000	125	160	105	159

AMMONIA (ppm)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	0.12	0.35	1	0.46
100	0.21	0.15	0.8	0.5
250	0.35	0.7	1.5	0.67
500	1	1.4	1.5	1.4
1000	0.75	1.45	1.5	1.3
2000	0.8	1.2	2.5	0.84
Day 10				
0 (ppm)	<.05	0.3	0.6	14.2
100	<.05	0.5	2.5	22.2
250	<.05	0.63	9	19.7
500	<.05	0.7	8	19.7
1000	9	0.1	10	40.3
2000	9	0.7	7	23.7

Static-Renewal System

HARDNESS (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	140	-	152	-
100	140	-	216	-
250	150	-	177	-
500	140	-	200	-
1000	140	-	255	-
2000	140	-	250	-
Day 10				
0 (ppm)	130	-	180	-
100	125	-	180	-
250	140	-	160	-
500	150	-	160	-
1000	140	-	200	-
2000	140	-	180	-

ALKALINITY (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	82	-	84	105
100	76	-	81	162
250	90	-	88	99
500	85	-	84	174
1000	82	-	100	132
2000	92	-	78	99
Day 10				
0 (ppm)	80	-	152	147
100	85	-	152	159
250	90	-	191	105
500	91	-	165	120
1000	91	-	178	150
2000	82	-	133	138

AMMONIA (ppm)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	0.07	-	0.8	0.1
100	0.07	-	0.8	0.68
250	0.095	-	0.8	0.27
500	0.2	-	1	0.73
1000	0.18	-	1	0.74
2000	0.185	-	1	0.04
Day 10				
0 (ppm)	<.05	-	0.1	2.8
100	<.05	-	0.3	2.4
250	<.05	-	0	2.5
500	<.05	-	1	2.4
1000	0.23	-	1.5	3.5
2000	0.72	-	1	2.5

Appendix C, Table C-2 (cont'd). Overlying Water Chemistry Data from C. riparius Interlaboratory Study # 1

Static System

DISSOLVED OXYGEN RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	7.7 - 8.2	6.6 - 8.6	7.8 - 9.4	5.8 - 7.1
100	7.7 - 8.3	6.7 - 8.6	7.5 - 9.2	6.0 - 7.6
250	7.7 - 8.3	7.5 - 8.4	7.2 - 9.4	5.9 - 7.2
500	7.7 - 8.2	7.4 - 8.6	8.1 - 9.2	5.6 - 7.2
1000	7.6 - 8.2	7.2 - 8.5	8.0 - 9.8	5.8 - 6.7
2000	7.7 - 8.2	7.8 - 8.7	7.5 - -9.1	5.7 - 7.4

CONDUCTIVITY RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	410-538	593-699	660-830	410-541
100	465-599	587-674	740-890	646-770
250	548-668	656-742	810-1030	591-675
500	685-812	796-914	970-1090	704-894
1000	964-1149	1148-1401	1300-1380	740-1157
2000	1481-1936	1789-1988	1860-2100	1567-1990

pH RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	7.90-8.39	8.0-8.5	8.0-8.4	8.0-8.48
100	7.88-8.42	8.0-8.5	7.8-8.3	8.0-8.30
250	7.92-8.35	8.1-8.5	7.9-8.3	7.5-8.32
500	7.91-8.28	8.1-8.4	7.8-8.4	8.00-8.22
1000	7.87-8.48	8.2-8.5	7.8-8.2	6.92-8.32
2000	7.8-8.12	8.3-8.5	7.9-8.2	7.68-8.36

TEMPERATURE RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	22.6-23.0	22.5-23.0	22.5-23.5	21.0-24.7
100	22.5-23.0	22.5-23.0	22.5-23.5	21.5-24.9
250	22.0-23.1	22.5-23.0	22.0-23.5	21.0-24.9
500	22.7-23.0	22.5-23.0	22.5-23.5	21.0-24.9
1000	22.7-23.2	22.5-23.0	22.5-23.5	21.0-25.2
2000	22.6-23.1	22.5-23.0	22.0-23.5	21.0-24.7

Static-Renewal System

DISSOLVED OXYGEN RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	6.8 - 11.2	-	8.5 - 9.2	2.9 - 6.4
100	6.6 - 10.2	-	6.8 - 9.6	4.2 - 6.1
250	6.6 - 11.0	-	8.1 - 9.5	3.1 - 6.4
500	6.5 - 11.0	-	6.8 - 9.1	2.9 - 5.8
1000	6.2 - 11.1	-	6.2 - 8.6	2.6 - 6.1
2000	6.5 - 8.4	-	5.6 - 9.1	4.0 - 6.2

CONDUCTIVITY RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	292-312	-	470-610	340-436
100	293-324	-	590-640	378-562
250	283-344	-	580-620	420-779
500	291-380	-	540-660	405-592
1000	299-443	-	610-710	540-951
2000	322-610	-	660-830	480-557

pH RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	8.05-8.52	-	7.6-8.4	7.00-8.09
100	7.96-8.56	-	7.5-8.5	6.99-7.99
250	7.93-8.63	-	7.5-8.4	7.00-7.99
500	7.90-8.70	-	7.6-8.2	7.00-7.92
1000	7.84-8.67	-	7.4-8.3	6.50-7.60
2000	7.74-8.45	-	7.5-8.3	6.91-8.02

TEMPERATURE RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	21.5-22.4	-	22.0-24.5	22.0-23.9
100	21.6-22.3	-	22.5-24.5	21.9-23.7
250	21.4-22.3	-	22.0-24.5	21.9-23.7
500	21.5-22.2	-	22.0-24.5	22.0-23.7
1000	21.5-22.2	-	22.5-24.5	21.9-23.7
2000	21.4-22.2	-	22.0-24.5	21.7-23.5

Appendix C, Table C-3. Data from C. riparius Interlaboratory Study # 2.

Static System

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	86.67	5.77	6.66	0.729	0.869	0.17	19.93
	2	90				0.816			
	3	80				1.063			
100	1	100	100.00	0.00	0.00	0.883	0.799	0.09	11.42
	2	100				0.702			
	3	100				0.813			
250	1	80	86.67	11.55	13.32	1.066	0.945	0.18	19.34
	2	100				0.735			
	3	80				1.035			
500	1	90	90.00	0.00	0.00	0.878	0.812	0.07	8.19
	2	90				0.814			
	3	90				0.745			
1000	1	40	53.33	32.15	60.27	0.223	0.188	0.06	29.97
	2	30				0.123			
	3	90				0.218			
2000	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			
Long Point	1	100	96.67	5.77	5.97	0.901	0.85	0.15	17.82
	2	90				0.68			
	3	100				0.97			
Stelco	1	90	93.33	5.77	6.19	0.726	0.736	0.13	17.57
	2	100				0.612			
	3	90				0.87			

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	96.67	5.77	5.97	1.06	0.949	0.13	13.27
	2	90				0.974			
	3	100				0.812			
100	1	100	90.00	10.00	11.11	0.898	0.94	0.06	6.35
	2	90				0.913			
	3	80				1.008			
250	1	100	100.00	0.00	0.00	0.708	0.781	0.07	8.78
	2	100				0.844			
	3	100				0.791			
500	1	100	96.67	5.77	5.97	0.591	0.67	0.08	11.37
	2	100				0.743			
	3	90				0.676			
1000	1	70	70.00	10.00	14.29	0.094	0.122	0.03	25.39
	2	80				0.116			
	3	60				0.155			
2000	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			
Long Point	1	90	93.33	5.77	6.19	0.959	0.922	0.08	9.06
	2	100				0.98			
	3	90				0.826			
Stelco	1	100	96.67	5.77	5.97	0.863	0.755	0.14	18.22
	2	90				0.6			
	3	100				0.801			

LABORATORY D

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	93.33	11.55	12.37	0.762	0.954	0.17	18.32
	2	80				1.104			
	3	100				0.995			
100	1	90	80.00	10.00	12.50	1.189	0.994	0.18	18.32
	2	70				0.966			
	3	80				0.828			
250	1	90	90.00	10.00	11.11	0.913	0.987	0.07	6.65
	2	100				1.038			
	3	80				1.01			
500	1	90	86.67	15.28	17.63	1.191	0.891	0.27	30.39
	2	70				0.664			
	3	100				0.819			
1000	1	70	50.00	17.32	34.64	0.221	0.152	0.06	39.04
	2	40				0.118			
	3	40				0.118			
2000	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			
Long Point	1	90	86.67	5.77	6.66	0.998	0.921	0.07	7.91
	2	90				0.913			
	3	80				0.853			
Stelco	1	100	96.67	5.77	5.97	0.709	0.77	0.11	13.65
	2	90				0.891			
	3	100				0.709			

Static-Renewal System

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0	0	0.384	0.438	0.19	42.24
	2	100				0.286			
	3	100				0.644			
100	1	80	83.33	5.774	6.9282	0.651	0.562	0.16	28.95
	2	80				0.374			
	3	90				0.66			
250	1	30	43.33	11.55	26.647	0.72	0.513	0.18	35.84
	2	50				0.448			
	3	50				0.37			
500	1	100	86.67	15.28	17.625	0.21	0.294	0.10	35.54
	2	70				0.411			
	3	90				0.261			
1000	1	60	70.00	10	14.286	0.175	0.268	0.18	66.67
	2	70				0.474			
	3	80				0.155			
2000	1	0	6.67	11.55	173.21		0.105	-	-
	2	0							
	3	20				0.105			
Long Point	1	100	96.67	5.774	5.9726	0.509	0.559	0.06	11.34
	2	100				0.537			
	3	90				0.63			
Stelco	1	100	76.67	20.82	27.152	0.295	0.381	0.20	52.93
	2	60				0.612			
	3	70				0.237			

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0	0	0.858	0.852	0.02	2.61
	2	100				0.87			
	3	100				0.827			
100	1	90	96.67	5.774	5.9726	0.742	0.694	0.08	11.12
	2	100				0.605			
	3	100				0.735			
250	1	100	100.00	0	0	0.825	0.765	0.08	9.81
	2	100				0.681			
	3	100				0.79			
500	1	100	96.67	5.774	5.9726	0.533	0.666	0.12	18.14
	2	100				0.769			
	3	90				0.696			
1000	1	80	70.00	17.32	24.744	0.251	0.276	0.05	19.43
	2	80				0.338			
	3	50				0.24			
2000	1	0	0.00	0	-	-	-	-	-
	2	0				-			
	3	0				-			
Long Point	1	100	90.00	10	11.111	0.811	0.804	0.11	13.52
	2	90				0.692			
	3	80				0.909			
Stelco	1	80	83.33	5.774	6.9282	0.75	0.749	0.04	4.94
	2	90				0.712			
	3	80				0.786			

LABORATORY C

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	83.33	28.87	34.641	1.089	1.274	0.26	20.45
	2	50				1.572			
	3	100				1.161			
100	1	100	93.33	11.55	12.372	1.26	1.126	0.18	16.20
	2	80				1.199			
	3	100				0.918			
250	1	100	93.33	11.55	12.372	0.957	1.142	0.18	15.70
	2	80				1.315			
	3	100				1.155			
500	1	100	86.67	15.28	17.625	1.399	1.321	0.28	20.97
	2	70				1.55			
	3	90				1.013			
1000	1	80	93.33	11.55	12.372	1.0913	1.058	0.12	11.49
	2	100				0.923			
	3	100				1.159			
2000	1	0	20.00	17.32	86.603		0.675	0.23	34.57
	2	30				0.51			
	3	30				0.84			
Long Point	1	90	80.00	17.32	21.651	0.939	0.986	0.04	4.34
	2	90				1.023			
	3	60				0.995			
Stelco	1	80	76.67	25.17	32.825	1.339	1.282	0.15	11.32
	2	50				1.39			
	3	100				1.117			

Appendix C, Table C-4. Overlying Water Chemistry from C. riparius Interlaboratory Study # 2.

Static System

HARDNESS (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	240	290	195	-
100	280	290	245	-
250	200	310	245	-
500	500	380	375	-
1000	600	570	455	-
2000	600	970	725	-
Long Point	150	280	170	-
Stelco	150	250	150	-
Day 10				
0 (ppm)	280	290	340	-
100	280	380	420	-
250	300	345	320	-
500	400	380	400	-
1000	450	580	580	-
2000	400	980	1080	-
Long Point	150	295	280	-
Stelco	140	260	300	-

ALKALINITY (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	125	-	-	-
100	100	-	-	-
250	125	-	-	-
500	200	-	-	-
1000	95	-	-	-
2000	120	-	-	-
Long Point	110	-	-	-
Stelco	90	-	-	-
Day 10				
0 (ppm)	100	-	-	-
100	200	-	-	-
250	95	-	-	-
500	85	-	-	-
1000	125	-	-	-
2000	95	-	-	-
Long Point	110	-	-	-
Stelco	90	-	-	-

AMMONIA (ppm)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	0.51	0.75	0.8	-
100	0.7	0.85	0.9	-
250	1.1	1.13	1.5	-
500	1.6	1.87	2	-
1000	1.5	1.73	2	-
2000	1.62	2.08	3	-
Long Point	0.27	1.17	0.8	-
Stelco	0.14	1.07	0.6	-
Day 10				
0 (ppm)	0.085	1.12	0.3	-
100	<0.05	0.47	0.2	-
250	0.62	1.04	1	-
500	0.07	9.5	5.5	-
1000	16.5	9.05	8.1	-
2000	15	7.75	6.4	-
Long Point	0.078	0.34	0.2	-
Stelco	0.19	4.7	0.2	-

Static-Renewal System

HARDNESS (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	140	-	150	-
100	140	-	165	-
250	180	-	130	-
500	140	-	145	-
1000	180	-	145	-
2000	200	-	200	-
Long Point	140	-	175	-
Stelco	140	-	145	-
Day 10				
0 (ppm)	135	-	180	120
100	105	-	180	117
250	150	-	180	105
500	135	-	160	102
1000	135	-	160	105
2000	135	-	200	126
Long Point	140	-	160	105
Stelco	150	-	200	120

ALKALINITY (mg/L)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	80	-	-	84
100	85	-	-	66
250	90	-	-	81
500	85	-	-	81
1000	90	-	-	69
2000	75	-	-	69
Long Point	75	-	-	72
Stelco	80	-	-	75
Day 10				
0 (ppm)	100	-	108	-
100	95	-	100	-
250	90	-	101	-
500	100	-	103	-
1000	100	-	107	-
2000	90	-	100	-
Long Point	98	-	112	-
Stelco	90	-	101	-

AMMONIA (ppm)

	Lab B	Lab D	Lab A	Lab C
Day 0				
0 (ppm)	0.12	-	0	0.13
100	0.15	-	0	0.16
250	0.21	-	0	0.16
500	0.35	-	0	0.31
1000	0.35	-	0	0.32
2000	0.4	-	0	0.24
Long Point	0.13	-	0	0.21
Stelco	0.085	-	0	0.18
Day 10				
0 (ppm)	0.4	-	0.4	4.2
100	<0.01	-	0.6	3.04
250	0.34	-	0.6	2.01
500	0.41	-	0.6	3.18
1000	0.22	-	0.4	4.2
2000	0.51	-	0	1.35
Long Point	0.28	-	1	2.11
Stelco	0.15	-	0.6	1.9

Appendix C, Table C-4 (cont'd). Overlying Water Chemistry from C. riparius Interlaboratory Study # 2.

Static System

DISSOLVED OXYGEN RANGE OVER 10 DAYS (AVG)

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	7.3 - 8.4 (7.8)	7.3 - 8.6 (8.1)	7.9 - 10.0 (8.8)	-
100	7.2 - 8.2 (7.7)	7.3 - 8.3 (7.8)	8.7 - 10.0 (9.2)	-
250	7.3 - 8.4 (7.8)	6.9 - 8.8 (8.1)	8.5 - 9.9 (9.1)	-
500	7.1 - 8.2 (7.7)	6.8 - 8.6 (8.1)	8.2 - 9.8 (8.9)	-
1000	7.2 - 8.2 (7.7)	6.9 - 8.8 (8.3)	8.2 - 10.3 (9.0)	-
2000	7.2 - 8.2 (7.8)	7.9 - 8.6 (8.3)	8.3 - 10.1 (8.9)	-
Long Point	7.3 - 8.4 (7.8)	8.3 - 8.7 (8.5)	8.1 - 10.4 (9.0)	-
Stelco	7.3 - 8.4 (7.7)	7.3 - 8.7 (7.9)	6.7 - 10.0 (8.6)	-

CONDUCTIVITY RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	437-578	537-600	700-900	-
100	500-648	589-663	700-1000	-
250	590-763	648-755	800-1070	-
500	723-883	770-940	1000-1230	-
1000	1115-1430	1247-1425	1400-1600	-
2000	1625-2070	1965-2230	1800-2400	-
Long Point	361-518	508-610	600-760	-
Stelco	336-507	493-619	600-700	-

TEMPERATURE RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	22.7-23.3	23.0-23.0	22.5-23.0	-
100	22.8-23.3	23.0-23.0	22.5-23.5	-
250	22.8-23.3	23.0-23.0	22.5-23.5	-
500	22.9-23.3	23.0-23.0	22.5-23.5	-
1000	22.7-23.3	23.0-23.0	22.5-23.5	-
2000	22.9-23.3	23.0-23.5	22.5-23.5	-
Long Point	22.8-23.3	23.0-23.0	22.5-23.0	-
Stelco	22.9-23.3	23.0-23.5	22.5-23.5	-

Static-Renewal Ayatem

DISSOLVED OXYGEN RANGE OVER 10 DAYS (AVG)

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	5.1 - 7.1 (6.3)	-	6.1 - 9.6 (8.5)	1.8 - 7.4 (4.9)
100	6.3 - 7.5 (6.9)	-	5.2 - 9.7 (8.3)	2.2 - 7.4 (4.9)
250	5.7 - 7.2 (6.4)	-	7.2 - 10.1(8.9)	4.7 - 7.5 (6.1)
500	5.9 - 7.5 (6.8)	-	7.6 - 9.6 (8.8)	2.0 - 7.6 (5.5)
1000	6.3 - 8.7 (7.2)	-	6.1 - 9.2 (8.5)	3.0 - 7.3 (5.1)
2000	6.4 - 7.4 (6.9)	-	8.2 - 9.5 (8.8)	4.2 - 7.5 (6.0)
Long Point	4.4 - 7.0 (6.1)	-	5.2 - 9.5 (8.2)	3.2 - 7.0 (5.2)
Stelco	4.8 - 7.0 (6.2)	-	5.3 - 9.2 (7.9)	1.6 - 7.2 (4.5)

CONDUCTIVITY RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	298-334	-	500-610	2570-2900
100	305-321	-	500-610	2430-3180
250	322-343	-	500-590	2750-3010
500	314-389	-	500-610	2620-3200
1000	320-487	-	570-640	2730-3880
2000	335-685	-	600-700	2910-4570
Long Point	275-331	-	500-590	2900-3330
Stelco	273-326	-	500-610	2760-3150

TEMPERATURE RANGE OVER 10 DAYS

	Lab B	Lab D	Lab A	Lab C
0 (ppm)	21.0-22.4	-	19.5-24.5	21.5-24.1
100	21.0-22.4	-	19.0-24.5	22.2-23.8
250	21.0-22.4	-	21.0-24.5	21.8-23.8
500	21.0-22.4	-	20.0-24.5	21.5-23.7
1000	21.0-22.3	-	18.5-24.5	21.5-24.0
2000	21.0-22.3	-	19.5-24.5	21.6-23.9
Long Point	21.0-22.7	-	19.5-24.5	21.4-23.8
Stelco	21.0-22.5	-	20.0-24.5	21.4-24.0

Appendix C, Table C-5. Data from H. azteca Interlaboratory Study # 1

Static System

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	80	86.67	5.77	6.66	0.158	0.158	0.03	18.71
	2	90				0.187			
	3	90				0.128			
50	1	80	90.00	10.00	11.11	0.124	0.12	0.01	6.51
	2	100				0.125			
	3	90				0.111			
125	1	100	96.67	5.77	5.97	0.165	0.134	0.03	20.30
	2	90				0.118			
	3	100				0.118			
250	1	80	53.33	25.17	47.19	0.073	0.053	0.02	40.86
	2	50				0.056			
	3	30				0.03			
500	1	30	30.00	10.00	33.33	0.04	0.031	0.01	25.60
	2	20				0.025			
	3	40				0.028			
1000	1	0	0.00	0.00	-	-	-	-	-
	2	0							
	3	0							

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0.00	0.00	0.172	0.196	0.04	18.47
	2	100				0.238			
	3	100				0.179			
50	1	70	90.00	17.32	19.25	0.071	0.118	0.06	47.81
	2	100				0.181			
	3	100				0.103			
125	1	100	96.67	5.77	5.97	0.11	0.106	0.01	13.26
	2	90				0.09			
	3	100				0.117			
250	1	90	83.33	5.77	6.93	0.042	0.039	0.02	47.90
	2	80				0.056			
	3	80				0.019			
500	1	60	30.00	30.00	100.00	0.033	0.063	0.04	67.34
	2	30				0.093			
	3	0							
1000	1	0	6.67	11.55	173.21	-	-	-	-
	2	20				nd			
	3	0				-			

LABORATORY D

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0.00	0.00	0.062	0.080	0.02	21.36
	2	100				0.083			
	3	100				0.096			
50	1	90	96.67	5.77	5.97	0.183	0.121	0.06	45.61
	2	100				0.105			
	3	100				0.076			
125	1	100	93.33	11.55	12.37	0.049	0.042	0.01	16.59
	2	100				0.043			
	3	80				0.035			
250	1	80	93.33	11.55	12.37	0.056	0.047	0.01	23.01
	2	100				0.035			
	3	100				0.05			
500	1	50	63.33	15.28	24.12	nd	0.005	-	-
	2	60				nd			
	3	80				0.005			
1000	1	10	10.00	10.00	100.00	nd	-	-	-
	2	20				nd			
	3	0				-			

LABORATORY E

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0.00	0.00	0.13	0.243	0.10	42.18
	2	100				0.27			
	3	100				0.33			
50	1	100	100.00	0.00	0.00	0.23	0.280	0.05	16.37
	2	100				0.32			
	3	100				0.29			
125	1	60	83.33	20.82	24.98	0.033	0.060	0.02	39.79
	2	90				0.078			
	3	100				0.07			
250	1	20	36.67	20.82	56.77	nd	0.017	-	-
	2	60				0.017			
	3	30				nd			
500	1	10	6.67	5.77	86.60	0.2	0.200	-	-
	2	10				nd			
	3	0				-			
1000	1	0	3.33	5.77	173.21	-	-	-	-
	2	10				nd			
	3	0				-			

LABORATORY F

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	100	100.00	0.00	0.00	0.227	0.240	0.05	20.75
	2	100				0.198			
	3	100				0.295			
50	1	90	96.67	5.77	5.97	0.214	0.200	0.02	8.89
	2	100				0.18			
	3	100				0.206			
125	1	100	100.00	0.00	0.00	0.187	0.179	0.01	4.76
	2	100				0.179			
	3	100				0.17			
250	1	100	100.00	0.00	0.00	0.129	0.153	0.03	16.68
	2	100				0.18			
	3	100				0.151			
500	1	90	73.33	15.28	20.83	0.071	0.075	0.01	9.69
	2	70				0.083			
	3	60				0.07			
1000	1	60	60.00	20.00	33.33	0.03	0.018	0.01	61.05
	2	40				0.01			
	3	80				0.013			

Appendix C, Table C-5 (cont'd). Data from H. azteca Interlaboratory Study # 1

Static-Renewal System

LABORATORY A

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	96.667	5.77	5.97	0.209	0.209	0.01	3.83
	2	100				0.201			
	3	100				0.217			
50	1	100	100.000	0.00	0.00	0.198	0.189	0.02	12.78
	2	100				0.208			
	3	100				0.162			
125	1	100	96.667	5.77	5.97	0.216	0.228	0.01	5.05
	2	100				0.228			
	3	90				0.239			
250	1	60	83.333	20.82	24.98	0.12	0.159	0.04	25.52
	2	90				0.156			
	3	100				0.201			
500	1	90	93.333	5.77	6.19	0.126	0.098	0.03	34.85
	2	90				0.109			
	3	100				0.06			
1000	1	50	50.000	20.00	40.00	0.032	0.037	0.01	24.44
	2	30				0.047			
	3	70				0.031			

LABORATORY B

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	90.000	10.00	11.11	0.143	0.117	0.02	20.11
	2	100				0.109			
	3	80				0.098			
50	1	80	93.333	11.55	12.37	0.113	0.098	0.02	15.37
	2	100				0.083			
	3	100				0.097			
125	1	80	93.333	11.55	12.37	0.044	0.086	0.04	43.87
	2	100				0.096			
	3	100				0.117			
250	1	90	93.333	5.77	6.19	0.033	0.077	0.06	81.25
	2	90				0.148			
	3	100				0.049			
500	1	40	33.333	11.55	34.64	0.05	0.055	0.00	9.09
	2	40				0.055			
	3	20				0.06			
1000	1	0	0.000	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY C

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	93.333	5.77	6.19	0.111	0.107	0.02	14.95
	2	100				0.12			
	3	90				0.089			
50	1	100	100.000	0.00	0.00	0.11	0.117	0.01	9.90
	2	100				0.11			
	3	100				0.13			
125	1	100	100.000	0.00	0.00	0.06	0.067	0.02	31.22
	2	100				0.09			
	3	100				0.05			
250	1	90	93.333	5.77	6.19	0.025	0.054	0.03	46.59
	2	90				0.067			
	3	100				0.07			
500	1	20	26.667	5.77	21.65	nd	0.033	-	-
	2	30				0.033			
	3	30				nd			
1000	1	0	0.000	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			

LABORATORY D

Treatment	Rep	Survival	Mean	SD	CV	Dry Wt	Mean	SD	CV
0 (ppm)	1	90	93.333	5.77	6.19	0.149	0.17	0.03	18.567
	2	90				0.151			
	3	100				0.204			
50	1	90	93.333	5.77	6.19	0.167	0.16	0.00	2.4845
	2	100				0.162			
	3	90				0.159			
125	1	90	96.667	5.77	5.97	0.169	0.15	0.02	12.125
	2	100				0.133			
	3	100				0.147			
250	1	100	86.667	11.55	13.32	0.118	0.10	0.03	25.427
	2	80				0.119			
	3	80				0.073			
500	1	60	76.667	15.28	19.92	0.068	0.06	0.01	17.577
	2	80				0.07			
	3	90				0.05			
1000	1	60	36.667	32.15	87.67	0.077	0.08	0.00	0.9124
	2	50				0.078			
	3	0							

Appendix C, Table C-6. Overlying Water Chemistry from Interlaboratory Studies with H. azteca.

Static System

HARDNESS (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	240	200	270	263	192
50	260	160	270	245	184
125	300	200	290	231	200
250	280	150	340	294	220
500	360	230	380	363	268
1000	520	400	500	507	364
Day 14					
0 (ppm)	300	220	310		248
50	300	220	300		236
125	320	280	330		272
250	340	200	350		280
500	400	240	410		296
1000	540	400	540		376

ALKALINITY (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	116	110	-		96
50	112	115	-		96
125	120	110	-		100
250	113	120	-		100
500	108	100	-		100
1000	100	100	-		96
Day 14					
0 (ppm)	109	100	-		110
50	117	110	-		106
125	123	95	-		112
250	112	110	-		110
500	112	110	-		104
1000	145	110	-		144

AMMONIA (ppm)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	0.6	0.14	0.46	0.351	0.24
50	0.8	0.14	0.28	0.256	0
125	0.8	0.2	0.55	0.374	0
250	1	0.3	0.76	0.516	0.4
500	1.5	0.7	1.24	1.17	0
1000	1.5	0.8	1.55	1.11	0.5
Day 14					
0 (ppm)	0.6	<.05	0.55		0.21
50	0.8	<.05	0.45		0
125	0.2	<.05	0.45		0.12
250	0.8	nd	0.55		0.08
500	0.6	nd	0.6		0
1000	10	2.3	7.3		1.29

Static-Renewal System

HARDNESS (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	180	180	-	143	180
50	140	150	-	142	168
125	160	140	-	156	200
250	160	150	-	145	200
500	180	180	-	173	208
1000	180	170	-	228	300
Day 14					
0 (ppm)	120	130	-		44
50	140	110	-		52
125	140	150	-		56
250	120	160	-		44
500	120	130	-		72
1000	140	120	-		40

ALKALINITY (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	73	100	-		92
50	72	90	-		96
125	72	105	-		92
250	69	100	-		92
500	75	100	-		100
1000	75	100	-		96
Day 14					
0 (ppm)	59	70	-		28
50	59	70	-		32
125	59	80	-		32
250	59	80	-		32
500	52	70	-		32
1000	59	75	-		32

AMMONIA (ppm)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
0 (ppm)	0.2	<.05	-	0.106	0.19
50	0.1	<.05	-	<.002	0.14
125	0.2	<.05	-	0.006	0.11
250	0.2	0.25	-	0.004	0.16
500	0.4	0.1	-	0.105	0.46
1000	0.4	0.1	-	0.135	0.45
Day 14					
0 (ppm)	0.1	nd	-		0.04
50	0.1	nd	-		0.12
125	0.1	nd	-		0.04
250	0.2	0	-		0.06
500	0	nd	-		0.08
1000	0.1	<.05	-		0

Appendix C, Table C-6 (cont'd). Overlying Water Chemistry from Interlaboratory Studies with H. azteca.

Static System

DISSOLVED OXYGEN RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	8.1 - 9.9	6.9 - 8.0	7.5 - 8.5	6.8 - 8.2	6.5 - 9.1
50	8.2 - 10.3	6.9 - 7.9	7.1 - 8.4	6.3 - 8.2	6.8 - 8.9
125	8.3 - 10.1	6.9 - 8.1	7.2 - 8.4	6.9 - 8.3	6.9 - 8.8
250	8.3 - 9.8	6.1 - 8.1	7.0 - 8.3	7.1 - 8.2	5.9 - 8.7
500	8.2 - 10.1	6.9 - 9.7	6.6 - 8.5	7.0 - 8.3	6.5 - 8.5
1000	8.0 - 10.0	6.6 - 7.8	7.5 - 8.6	6.7 - 8.3	6.0 - 8.8

Static-Renewal System

DISSOLVED OXYGEN RANGE OVER 10 DAYS (AVG)

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	8.2 - 9.8	5.6 - 8.6	-	5.9 - 7.9	5.9 - 8.4
50	8.6 - 9.9	5.7 - 9.8	-	5.8 - 8.0	4.1 - 8.7
125	8.6 - 9.8	5.5 - 10.3	-	missing	5.8 - 8.5
250	8.7 - 9.9	5.5 - 10.1	-	6.0 - 8.2	5.7 - 8.7
500	8.7 - 10.3	5.6 - 10.2	-	6.0 - 8.2	5.2 - 8.5
1000	8.6 - 9.9	5.6 - 11.3	-	5.8 - 8.0	5.7 - 8.4

CONDUCTIVITY RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	600-710	437-555	546-623	550-700	350-550
50	590-750	455-579	552-646	600-700	350-500
125	650-840	509-642	624-696	625-950	400-550
250	640-900	576-693	350-782	700-850	400-600
500	890-1060	702-789	808-908	850-1000	500-650
1000	1220-1350	986-1117	1136-1289	1100-130	700-1050

CONDUCTIVITY RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	390-420	297-352	-	380-525	180-300
50	420-440	291-358	-	390-500	180-400
125	390-420	301-375	-	-	180-350
250	390-470	280-378	-	390-500	180-350
500	420-500	291-430	-	450-550	180-500
1000	420-570	285-517	-	575-600	180-700

pH RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	8.2-8.4	8.51-8.58	8.0-8.3	8.2-8.4	7.5-8.3
50	8.0-8.5	8.54-8.58	7.9-8.3	8.1-8.2	7.5-8.0
125	8.0-8.4	8.54-8.57	8.0-8.3	8.2-8.4	7.6-8.1
250	8.1-8.5	8.09-8.67	7.9-8.3	8.2-8.3	7.4-7.8
500	7.9-8.4	8.47-8.70	7.8-8.2	8.2-8.4	7.5-8.0
1000	8.0-8.5	8.26-8.48	8.0-8.3	7.9-8.3	7.5-8.2

pH RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	7.7-8.3	8.26-8.64	-	7.6-7.9	7.4-7.8
50	7.5-8.3	8.27-8.58	-	7.6-7.9	7.4-7.8
125	7.7-8.4	8.33-8.58	-	-	7.3-7.8
250	7.7-8.5	8.34-8.70	-	7.6-7.9	7.5-8.0
500	7.7-8.8	8.27-8.70	-	7.6-7.8	7.4-7.8
1000	7.7-8.5	8.19-8.86	-	7.6-7.8	7.4-7.8

TEMPERATURE RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	22.0-24.0	22.9-23.8	23.0-24.0	24.1-25.0	22.0-23.0
50	22.0-24.0	23.1-23.6	23.0-24.0	24.1-24.5	22.0-23.0
125	22.0-24.0	22.8-23.7	23.0-24.0	24.3-25.1	22.0-23.0
250	22.0-24.0	23.1-23.6	23.0-24.0	24.2-25.2	22.0-23.0
500	22.0-24.0	23.3-23.7	23.0-24.0	24.2-25.0	22.0-23.0
1000	22.0-24.0	23.1-23.7	23.0-24.0	24.2-24.9	22.0-23.0

TEMPERATURE RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
0 (ppm)	22.0-24.0	21.0-22.5	-	20.8-21.8	22.0-23.0
50	21.0-23.5	21.1-22.6	-	20.9-21.9	22.0-23.0
125	21.5-23.5	20.8-22.6	-	-	22.0-23.0
250	21.0-23.5	21.0-22.6	-	20.9-22.1	22.0-23.0
500	21.0-23.5	21.0-22.6	-	21.0-22.0	22.0-23.0
1000	21.0-24.0	21.0-22.7	-	20.9-21.7	22.0-23.0

Appendix C, Table C-7. Data from H. azteca Interlaboratory Study # 2

Static system

LABORATORY B

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	100	95	5.77	6.08	0.124	0.131	0.01	5.35
(Toronto Harbour)	2	90				0.132			
	3	90				0.138			
	4	100							
Sediment B:	1	70	80	8.16	10.21	0.090	0.096	0.00	4.36
(Hamilton Harbour)	2	80				0.098			
	3	90				0.099			
	4	80				0.098			
Sediment C:	1	100	92.5	9.57	10.35	0.226	0.267	0.06	23.39
(Long Point Control)	2	80				0.341			
	3	100				0.296			
	4	90				0.206			
Sediment D:	1	60	60	8.16	13.61	0.093	0.088	0.01	9.60
(Moulin A Vent)	2	70				0.086			
	3	50				0.096			
	4	60				0.077			

LABORATORY A

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	90	82.5	22.17	26.88	0.049	0.058	0.01	19.29
(Toronto Harbour)	2	90				0.073			
	3	100				0.050			
	4	50				0.058			
Sediment B:	1	60	70	24.49	34.99	0.054	0.054	0.02	37.52
(Hamilton Harbour)	2	90				0.069			
	3	90				0.066			
	4	40				0.025			
Sediment C:	1	100	92.5	9.57	10.35	0.089	0.124	0.03	28.00
(Long Point Control)	2	100				0.105			
	3	90				0.133			
	4	80				0.168			
Sediment D:	1	60	52.5	9.57	18.24	0.047	0.063	0.01	20.26
(Moulin A Vent)	2	40				0.063			
	3	60				0.062			
	4	50				0.078			

LABORATORY E

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	90	95	5.77	6.08	0.133	0.165	0.03	15.49
(Toronto Harbour)	2	100				0.180			
	3	90				0.156			
	4	100				0.190			
Sediment B:	1	90	95	5.77	6.08	0.067	0.097	0.04	39.79
(Hamilton Harbour)	2	100				0.070			
	3	90				0.100			
	4	100				0.150			
Sediment C:	1	100	100	0.00	0.00	0.350	0.315	0.08	24.38
(Long Point Control)	2	100				0.200			
	3	100				0.350			
	4	100				0.360			
Sediment D:	1	90	82.5	9.57	11.61	0.078	0.129	0.04	28.76
(Moulin A Vent)	2	90				0.156			
	3	80				0.125			
	4	70				0.157			

LABORATORY D

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	100	87.5	12.58	14.38	0.064	0.047	0.01	24.28
(Toronto Harbour)	2	90				0.038			
	3	70				0.044			
	4	90				0.043			
Sediment B:	1	60	77.5	17.08	22.04	0.048	0.064	0.02	25.40
(Hamilton Harbour)	2	70				0.083			
	3	80				0.071			
	4	100				0.053			
Sediment C:	1	100	92.5	9.57	10.35	0.077	0.080	0.01	9.79
(Long Point Control)	2	90				0.070			
	3	80				0.085			
	4	100				0.087			
Sediment D:	1	0	0	0.00	-	-	-	-	-
(Moulin A Vent)	2	0				-			
	3	0				-			
	4	0				-			

LABORATORY F

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	90	80	8.16	10.21	0.170	0.145	0.03	20.31
(Toronto Harbour)	2	80				0.119			
	3	70				0.171			
	4	80				0.120			
Sediment B:	1	70	70	14.14	20.20	0.130	0.110	0.02	18.54
(Hamilton Harbour)	2	90				0.098			
	3	60				0.125			
	4	60				0.088			
Sediment C:	1	90	92.5	5.00	5.41	0.257	0.222	0.03	12.16
(Long Point Control)	2	90				0.208			
	3	100				0.195			
	4	90				0.228			
Sediment D:	1	70	70	21.60	30.86	0.053	0.062	0.02	31.88
(Moulin A Vent)	2	90				0.089			
	3	80				0.063			
	4	40				0.043			

Appendix C, Table C-7 (cont'd). Data from H. azteca Interlaboratory Study # 2

Static-Renewal System

LABORATORY B

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	100	100	0.00	0.00	0.120	0.129	0.01	7.43
(Toronto Harbour)	2	100				0.129			
	3	100				0.124			
	4	100				0.142			
Sediment B:	1	100	85	12.91	15.19	0.152	0.131	0.02	12.32
(Hamilton Harbour)	2	80				0.133			
	3	70				0.113			
	4	90				0.127			
Sediment C:	1	100	97.5	5.00	5.13	0.149	0.176	0.02	13.82
(Long Point Control)	2	100				0.203			
	3	90				0.163			
	4	100				0.188			
Sediment D:	1	50	77.5	18.93	24.43	0.086	0.098	0.01	9.39
(Moulin A Vent)	2	80				0.096			
	3	90				0.107			
	4	90				0.103			

LABORATORY A

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	100	97.5	5	5.128	0.096	0.0733	0.0172	23.524
(Toronto Harbour)	2	100				0.056			
	3	90				0.076			
	4	100				0.065			
Sediment B:	1	100	77.5	20.62	26.6	0.07	0.0608	0.0149	24.466
(Hamilton Harbour)	2	80				0.056			
	3	50				0.042			
	4	80				0.075			
Sediment C:	1	100	100	0	0	0.115	0.1158	0.0067	5.7901
(Long Point Control)	2	100				0.125			
	3	100				0.114			
	4	100				0.109			
Sediment D:	1	80	77.5	12.58	16.24	0.048	0.052	0.0146	28.044
(Moulin A Vent)	2	90				0.07			
	3	60				0.035			
	4	80				0.055			

LABORATORY E

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	80	95	10.00	10.53	0.138	0.177	0.04	24.90
(Toronto Harbour)	2	100				0.140			
	3	100				0.220			
	4	100				0.210			
Sediment B:	1	100	97.5	5.00	5.13	0.190	0.162	0.03	18.89
(Hamilton Harbour)	2	90				0.178			
	3	100				0.120			
	4	100				0.160			
Sediment C:	1	100	100	0.00	0.00	0.170	0.188	0.02	12.60
(Long Point Control)	2	100				0.220			
	3	100				0.190			
	4	100				0.170			
Sediment D:	1	90	92.5	5.00	5.41	0.089	0.095	0.03	28.05
(Moulin A Vent)	2	100				0.070			
	3	90				0.089			
	4	90				0.133			

LABORATORY F

Site	Rep	Survival	Mean	SD	CV	Dry Wt.	Mean	SD	CV
Sediment A :	1	100	95	5.77	6.08	0.193	0.154	0.03	17.22
(Toronto Harbour)	2	90				0.150			
	3	90				0.138			
	4	100				0.136			
Sediment B:	1	90	97.5	5.00	5.13	0.108	0.120	0.01	11.49
(Hamilton Harbour)	2	100				0.118			
	3	100				0.140			
	4	100				0.115			
Sediment C:	1	80	92.5	9.57	10.35	0.265	0.227	0.04	15.75
(Long Point Control)	2	90				0.250			
	3	100				0.197			
	4	100				0.196			
Sediment D:	1	80	92.5	9.57	10.35	0.180	0.112	0.05	40.59
(Moulin A Vent)	2	100				0.094			
	3	100				0.088			
	4	90				0.086			

Appendix C, Table C-8. Overlying Water Chemistry from H. azteca Interlaboratory Study # 2

Static System

HARDNESS (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	160	130	220	-	100
Sediment B	140	120	230	-	104
Sediment C	160	120	250	-	104
Sediment D	120	120	210	-	84
Day 14					
Sediment A	220	140	-	-	172
Sediment B	200	140	-	-	164
Sediment C	240	160	-	-	188
Sediment D	200	160	-	-	152

ALKALINITY (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	95	80	-	91.1	60
Sediment B	75	70	-	77.5	60
Sediment C	87	90	-	96.2	136
Sediment D	87	80	-	79.2	64
Day 14					
Sediment A	124	120	-	-	116
Sediment B	97	75	-	-	112
Sediment C	136	120	-	-	140
Sediment D	101	85	-	-	108

AMMONIA (ppm)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	0.6	0.5	1.55	0.735	0.55
Sediment B	2	1.5	6.1	1.71	0.66
Sediment C	0.6	0.33	2.3	0.551	0.42
Sediment D	3	2.5	6.88	2.61	0.86
Day 14					
Sediment A	0.3	nd	0.33	-	0.16
Sediment B	0.3	<.05	0.58	-	0.17
Sediment C	0.2	nd	0.31	-	0.36
Sediment D	0.1	nd	0.36	-	0.14

Renewal System

HARDNESS (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	140	120	-	-	56
Sediment B	140	120	-	-	52
Sediment C	140	120	-	-	56
Sediment D	140	120	-	-	48
Day 14					
Sediment A	100	140	-	-	44
Sediment B	120	140	-	-	40
Sediment C	140	140	-	-	44
Sediment D	120	120	-	-	40

ALKALINITY (mg/L)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	75	90	-	71.1	44
Sediment B	77	80	-	65.7	36
Sediment C	73	75	-	73.5	28
Sediment D	75	80	-	68.4	28
Day 14					
Sediment A	83	100	-	-	24
Sediment B	73	95	-	-	32
Sediment C	72	90	-	-	36
Sediment D	71	85	-	-	32

AMMONIA (ppm)

	Lab A	Lab B	Lab D	Lab E	Lab F
Day 0					
Sediment A	0.2	0.19	-	0.344	<.01
Sediment B	0.6	0.58	-	0.751	0.24
Sediment C	0.1	0.08	-		<.01
Sediment D	1	1	-	1.17	0.31
Day 14					
Sediment A	0.1	<.05	-	-	0.33
Sediment B	0.05	nd	-	-	0.36
Sediment C	0.05	nd	-	-	0.19
Sediment D	0.05	nd	-	-	0.18

Appendix C, Table C-8 (cont'd). Overlying Water Chemistry from H. azteca Interlaboratory Study # 2

Static System

DISSOLVED OXYGEN RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	7.9 - 9.3	7.3 - 7.9	4.4 - 8.5	7.2 - 7.8	3.9 - 8.4 (7.5)
Sediment B	8.0 - 9.5	7.3 - 8.1	5.2 - 8.7	7.1 - 7.8	6.7 - 8.5
Sediment C	8.5 - 10.1	7.3 - 8.1	6.6 - 9.0	7.2 - 8.1	6.6 - 8.5
Sediment D	7.5 - 9.9	7.1 - 7.8	6.6 - 8.4	6.8 - 8.0	6.6 - 5.5

CONDUCTIVITY RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	400-870	335-442	476-495	470-600	240-440
Sediment B	400-620	326-414	459-474	460-525	250-420
Sediment C	370-770	341-476	472-571	470-600	240-460
Sediment D	380-550	323-384	399-488	450-500	240-370

pH RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	8.1-8.3	8.3-8.5	7.8-8.4	7.8-8.0	7.1-7.9
Sediment B	8.0-8.4	8.3-8.4	7.9-8.3	7.8-8.0	7.1-7.8
Sediment C	8.0-8.5	8.3-8.6	8.0-8.4	8.0-8.3	7.4-8.2
Sediment D	7.8-8.3	8.3-8.5	7.8-8.4	7.7-8.0	7.5-7.7

TEMPERATURE RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	22.0-24.0	23.4-23.9	22.5-23.0	24.4-25.3	22.0-23.0
Sediment B	22.0-24.5	23.2-23.8	22.5-23.0	24.5-25.2	22.0-23.0
Sediment C	22.0-24.0	23.3-23.9	22.5-23.0	24.6-25.3	22.0-23.0
Sediment D	22.0-24.0	23.3-23.9	22.5-23.0	24.5-25.3	22.0-23.0

Renewal System

DISSOLVED OXYGEN RANGE OVER 10 DAYS (AVG)

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	8.3 - 9.7	6.0 - 7.5	-	6.0 - 7.8	4.5 - 8.1
Sediment B	8.3 - 10.0	5.4 - 8.9	-	5.7 - 7.3	5.0 - 8.1
Sediment C	8.3 - 10.0	5.6 - 7.7	-	5.8 - 7.8	5.2 - 8.2
Sediment D	8.0 - 9.0	5.6 - 7.7	-	5.4 - 7.6	5.1 - 8.4

CONDUCTIVITY RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	460-480	304-315		430-500	170-200
Sediment B	450-470	291-310		430-500	170-200
Sediment C	470-500	297-316		430-500	180-200
Sediment D	460-490	298-310		425-500	170-200

pH RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	7.7-8.7	7.5-8.0	-	7.6-7.8	7.2-7.5
Sediment B	7.8-8.3	7.8-8.1	-	7.4-7.8	7.1-7.6
Sediment C	8.0-8.5	7.9-8.1	-	7.6-7.9	7.3-7.6
Sediment D	7.8-8.4	8.0-8.1	-	7.4-7.7	7.0-7.6

TEMPERATURE RANGE OVER 14 DAYS

	Lab A	Lab B	Lab D	Lab E	Lab F
Sediment A	19.0-24.0	22.4-22.9	-	21.3-22.3	22.0-24.0
Sediment B	19.0-24.0	22.4-22.7	-	21.4-22.1	22.0-23.0
Sediment C	19.0-24.0	22.3-22.8	-	21.6-24.4	22.0-23.0
Sediment D	22.0-24.0	22.3-22.7	-	21.4-22.5	22.0-24.0

APPENDIX D

Means and Standard Deviations for Survival and Growth of

C. riparius or *H. azteca*

During Interlaboratory Studies

Appendix D

Table D-1. Mean Percent Survival (SD) of *C. riparius* in Copper-Spiked Sediment and Field-Collected Sediment (Interlaboratory Study -Phase I and Phase II: Static System).

Laboratory	Nominal Concentration of Copper (μg/g)						Field-Collected	
	0	100	250	500	1000	2000	Long Point	HH
A								
Round-Robin # 1	90 (10)	86.7 (15.3)	96.7 (5.8)	86.7 (5.8)	96.7 (5.8)	0*(0)	-	-
Round-Robin # 2	86.7 (5.8)	100 (0)	86.7 (11.6)	90 (0)	53.3 (32.2)	0*(0)	96.7 (5.8)	93.3 (5.8)
B								
Round-Robin # 1	100 (0)	90 (10)	100 (0)	93.3 (5.8)	83.3 (15.3)	0*(0)	-	-
Round-Robin # 2	96.7 (5.8)	90 (10)	100 (0)	96.7 (5.8)	70 (10)	0*(0)	93.3 (5.8)	96.7 (5.8)
C								
Round-Robin # 1	40 (43.6)	50 (34.6)	40 (20)	33.3 (40.4)	23.3 (25.2)	0*(0)		
Round-Robin # 2	-	-	-	-	-	-	-	-
D								
Round-Robin # 1	100 (0)	100 (0)	90 (10)	100 (0)	80 (10)	0*(0)	-	-
Round-Robin # 2	93.3 (11.5)	80 (10)	90 (10)	86.7 (15.2)	50 (17.3)	0*(0)	86.7 (5.8)	96.7 (5.8)
Mean (SD)[1]	86.7 (21.2)	85.2 (17.1)	86.2 (21.0)	83.8(22.8)	65.2 (24.8)	0 (0)	92.2 (5.1)	95.6 (2.0)
C.V.[1]	24.4%	20.0%	24.4%	27.2%	38.0%	-	5.5%	2.1%
Mean (SD)[2]	94.5 (5.4)	91.1 (7.8)	93.9 (5.7)	92.2 (5.4)	72.2 (18.1)	0 (0)	-	-
C.V.[2]	5.8%	8.5%	6.1%	5.9%	25.1%	-	-	-

* Denotes significant difference from control.

[1] Calculation includes all values.

[2] Calculation excludes all values for which the proposed (EC,1995b) minimum acceptable control survival of ≥ 70% was not met.

Appendix D

Table D-2. Mean Percent Survival (SD) of *C. riparius* in Copper-Spiked Formulated Sediment and Field-Collected Sediment (Interlaboratory Study - Phase I and Phase II: Static-Renewal System).

Laboratory	Nominal Concentration of Copper (μg/g)						Field-Collected	
	0	100	250	500	1000	2000	Long Point	Hamilton Harbour
A								
Round-Robin # 1	90 (17.3)	70 (26.5)	90 (14.1)	86.7 (15.3)	80 (26.5)	56.7 (20.8)	-	-
Round-Robin # 2	100 (0)	83.3 (5.8)	43.3 (11.6)	86.7 (15.3)	70* (10)	6.7* (11.6)	96.7 (5.8)	76.7 (20.8)
B								
Round-Robin # 1	73.3 (11.6)	90 (0)	83.3 (5.8)	73.3 (11.6)	60 (20)	0* (0)	-	-
Round-Robin # 2	100 (0)	96.7 (5.8)	100 (0)	96.7 (5.8)	70 (10)	0* (0)	90 (10)	83.3 (5.8)
C								
Round-Robin # 1	53.3 (25.2)	33.3 (15.3)	30 (20)	26.7(15.3)	16.7 (15.3)	13.3 (15.3)	-	-
Round-Robin # 2	83.3 (28.9)	93.3 (11.6)	93.3 (11.6)	86.7 (15.3)	93.3 (11.6)	20 (17.3)	80 (17.3)	76.7 (25.2)
Mean (SD)[1]	83.3 (17.9)	77.8 (23.7)	73.3 (29.2)	76.1 (25.3)	65 (26.2)	16.1 (21.3)	88.9 (8.4)	78.9 (3.8)
C.V.[1]	21.5%	30.5%	39.8%	33.3%	40.3%	132.4%	9.5%	4.8%
Mean (SD)[2]	89.3 (11.4)	86.7 (10.5)	82.0 (22.4)	86.0 (8.3)	74.7 (12.6)	16.7 (23.8)	-	-
C.V.[2]	12.8%	12.2%	27.4%	9.7%	16.9%	142.8%	-	-

* Denotes significant difference from control.

[1] Calculations include all values.

[2] Calculations exclude the values for which the proposed (EC,1995b) minimum acceptable control survival of ≥70% was not met.

Appendix D

Table D-3. Mean Growth (mg dry wt/ind.) (SD) of *C. riparius* in Copper-Spiked Sediment and Field-Collected Sediment (Interlaboratory Study -Phase I and Phase II: Static System).

Laboratory	Nominal Concentration of Copper (μg/g)						Field Sediment	
	0	100	250	500	1000	2000	Long Point	HH
A								
Round-Robin # 1	0.63 (0.27)	0.59 (0.33)	0.35 (0.17)	0.70 (0.03)	0.40 (0.14)	-	-	-
Round-Robin # 2	0.87 (0.17)	0.80 (0.09)	0.95 (0.18)	0.81 (0.07)	0.19* (0.06)	-	0.85 (0.15)	0.74 (0.13)
B								
Round-Robin # 1	0.79 (0.04)	0.77 (0.09)	0.60* (0.03)	0.52* (0.01)	0.18* (0.01)	-	-	-
Round-Robin # 2	0.95 (0.13)	0.94 (0.06)	0.78* (0.07)	0.67* (0.08)	0.12* (0.03)	-	0.92 (0.08)	0.76 (0.14)
C								
Round-Robin # 1	0.54 (0.08)	1.94 (0.59)	0.70 (0.34)	1.06 (0.73)	0.19 (0.19)	-		
Round-Robin # 2	-	-	-	-	-	-	-	-
D								
Round-Robin # 1	1.09 (0.14)	1.04 (0.19)	0.92 (0.08)	0.64* (0.08)	0.45* (0.15)	-	-	-
Round-Robin # 2	0.95 (0.18)	0.99 (0.18)	0.99 (0.07)	0.89 (0.27)	0.15* (0.06)	-	0.92 (0.07)	0.77 (0.11)
Mean (SD)[1]	0.83 (0.19)	1.01 (0.44)	0.76 (0.23)	0.76 (0.18)	0.24 (0.13)	-	0.90 (0.04)	0.76 (0.02)
CV[1]	23.2%	43.3%	30.2%	23.7%	54.0%	-	4.5%	2.0%
Mean (SD)[2]	0.88 (0.16)	0.86 (0.17)	0.77 (0.25)	0.71 (0.13)	0.25 (0.14)	-	-	-
CV[2]	17.9%	19.6%	32.5%	18.5%	56.3%	-	-	-

* Denotes significant difference from control.

[1] Calculations include all values.

[2] Calculations exclude values for which the proposed (EC,1995b) minimum acceptable control survival of ≥ 70% was not met.

Appendix D

Table D-4. Mean Growth (mg dry wt/ind.) (SD) of *C. riparius* in Copper-Spiked Sediment and Field-Collected Sediment (Interlaboratory Study - Phase I and Phase II: Static-Renewal System).

Laboratory	Nominal Concentration of Copper (μg/g)						Field-Collected	
	0	100	250	500	1000	2000	Long Point	Hamilton Harbour
A								
Round-Robin # 1	1.14 (0.14)	0.70 (0.63)	0.88 (0.04)	0.87 (0.24)	0.72 (0.14)	0.43 (0.46)	-	-
Round-Robin # 2	0.44 (0.19)	0.56 (0.16)	0.51 (0.18)	0.29 (0.10)	0.27 (0.18)	0.10	0.56 (0.06)	0.38 (0.20)
B								
Round-Robin # 1	0.52 (0.12)	0.48 (0.05)	0.39 (0.09)	0.35* (0.05)	0.17* (0.04)	-	-	-
Round-Robin # 2	0.85 (0.02)	0.69 (0.08)	0.77 (0.08)	0.67* (0.12)	0.28* (0.07)	-	0.80 (0.11)	0.75 (0.04)
C								
Round-Robin # 1	0.87 (0.41)	0.54 (0.29)	0.61 (0.25)	0.55 (0.32)	0.20 (0.16)	0.21 (0.04)	-	-
Round-Robin # 2	1.27 (0.26)	1.13 (0.18)	1.14 (0.18)	1.32 (0.28)	1.06 (0.12)	0.68 (0.23)	0.99 (0.04)	1.28*(0.15)
Mean (SD)[1]	0.85 (0.33)	0.68 (0.24)	0.72 (0.27)	0.68 (0.38)	.045 (0.36)	0.36 (0.26)	0.78 (0.22)	0.80 (0.45)
CV[1]	38.7%	34.4%	37.9%	56.4%	80.2%	72.2%	27.5%	56.3%
Mean (SD)[2]	0.84 (0.37)	0.71 (0.25)	0.74 (0.30)	0.70 (0.42)	0.50 (0.38)	0.40 (0.29)	-	-
CV[2]	43.4%	35.3%	40.4%	60.0%	75.9%	72.1%	-	-

* Denotes significant difference from control.
[1] Calulation includes all values.
[2] Calculation excludes values for which the proposed (EC, 1995b) minimum acceptable control survival of ≥ 70% was not met.

Appendix D

Table D-5. Mean Percent Survival (SD) of *H. azteca* in Copper-Spiked Sediment (Interlaboratory Study - Phase I: Static System).

Laboratory	Nominal Concentration of Copper (μg/g)-Round-Robin # 1					
	0	50	125	250	500	1000
A	100.0(0)	90.0(17.3)	96.7(5.8)	83.3(5.8)	30.0(30)*	6.7(11.5)*
B	86.7(5.8)	90.0(10)	96.7(5.8)	53.3(25.2)*	30.0(10)*	0.0(0)*
D	100.0(0)	96.7(5.8)	93.3(11.6)	93.3(11.6)	63.3(15.3)*	10.0(10)*
E	100.0(0)	100.0(0)	83.3(20.8)	36.7(20.8)*	6.7(5.8)*	3.3(5.8)*
F	100.0(0)	96.7(5.8)	100.0(0)	100.0(0)	73.3(15.3)	60.0(20)
Grand Mean (SD)	97.3 (5.9)	94.7 (4.5)	94.0 (6.4)	73.3 (27.2)	40.7 (27.2)	16.0 (24.9)
CV	6.1%	4.7%	6.8%	37.1%	66.9%	155.5%

* Denotes significant difference from control.

Appendix D

Table D-6. Mean Percent Survival (SD) of *H. azteca* in Copper-Spiked Sediment (Interlaboratory Study - Phase I: Static-Renewal System).

Laboratory	Nominal Concentration of Copper (μg/g)-Round-Robin # 1					
	0	50	125	250	500	1000
A	90.0(10.0)	93.3(11.5)	93.3(11.5)	93.3(5.8)	33.3(11.5)*	0.0(0)*
B	93.3(5.8)	93.3(5.8)	96.7(5.8)	86.7(11.5)	76.7(15.3)	36.7(32.1)*
E	93.3(5.8)	100(0)	100(0)	93.3(5.8)	26.7(5.8)*	0(0)*
F	96.7(5.8)	100(0)	96.7(5.8)	83.3(20.8)	93.3(5.8)	50.0(20.0)*
Grand Mean (SD)	93.3 (2.7)	96.7 (3.9)	96.7 (2.7)	89.2 (5.0)	57.5 (32.6)	21.7 (25.6)
CV	2.9%	4.0%	2.8%	5.6%	56.7%	118.2%

* Denotes significant difference from control.

Appendix D

Table D-7. Mean Growth (mg dry weight) (SD) of *H. azteca* in Copper-Spiked Sediment (Interlaboratory Study - Phase I: Static System).

Laboratory	Nominal Concentration of Copper (μg/g)-Round-Robin # 1					
	0	50	125	250	500	1000
A	.196(.04)	.118(.06)	.106(.01)	.039(.02)*	.063(.04)*	-
B	.158(.03)	.120(.01)	.134(.03)	.053(.02)*	.031(.01)*	-
D	.080(.02)	.121(.06)	.042(.01)	.047(.01)	.005(-)	-
E	.243(.10)	.280(.05)	.060(.02)	.017(-)	.200(-)	-
F	.240(.05)	.200(.02)	.179(.01)*	.153(.03)*	.075(.01)*	.018(.01)*
Grand Mean (SD)	0.183 (.07)	0.168 (.07)	0.104 (.06)	0.062 (.05)	0.075 (.08)	0.018 (-)
CV	36.8%	42.7%	53.2%	85.4%	100.5%	-

* Denotes significant difference from control.

Appendix D

Table D-8. Mean Growth (mg dry weight) (SD) of *H. azteca* in Copper-Spiked Sediment (Interlaboratory Study - Phase I: Static-Renewal System).

Laboratory	Nominal Concentration of Copper (μg/g)-Round-Robin # 1					
	0	50	125	250	500	1000
A	.117(.02)	.098(.02)	.086(.04)	.077(.06)	.055(.01)	-
B	.168(.03)	.163(.004)	.150(..02)	.103(.03)*	.063(.01)*	.078(.001)*
E	.107(.02)	.117(.01)	.067(.02)	.054(.03)*	.033(-)*	-
F	.209(.01)	.189(.02)	.228(.01)	.159(.04)	.098(.03)*	.037(.01)*
Grand Mean (SD)	0.150 (.05)	0.142 (.04)	0.133 (.07)	0.098 (.05)	0.062 (.03)	0.058 (.03)
CV	31.6%	29.4%	54.8%	46.0%	43.4%	50.4%

* Denotes significant difference from control.

Appendix D

Table D-9. Mean Percent Survival (SD) of *H. azteca* in Field-Collected Sediment (Interlaboratory Study - Phase II: Static System).

Laboratory	Field-Collected Sediment-Round-Robin # 2			
	SED A	SED B	SED C	SED D
A	82.5(22.2)	70.0(24.5)	92.5(9.6)	52.5(9.6)*
B	95.0(5.8)	80.0(8.2)	92.5(9.6)	60.0(8.2)*
D	87.5(12.6)	77.5(17.1)	92.5(9.6)	-
E	95.0(5.8)	95.0(5.8)	100.0(0)	82.5(9.6)*
F	80.0(8.2)	70.0(14.1)	92.5(5)	70.0(21.6)
Grand Mean (SD)	88.0 (6.9)	78.5 (10.2)	94.0 (3.4)	66.3 (13.0)
CV	7.9%	13.1%	3.6%	19.6%

* Denotes significant difference from control.

Table D-10. Mean Percent Survival (SD) of *H. azteca* in Field-Collected Sediment (Interlaboratory Study - Phase II: Static-Renewal System).

Laboratory	Field-Collected Sediment-Round-Robin # 2			
	SED A	SED B	SED C	SED D
A	97.5(5.0)	77.5(20.6)*	100(0)	77.5(12.6)*
B	100(0)	85.0(12.9)	97.5(5.0)	77.5(18.9)
E	95.0(10.0)	97.5(5.0)	100(0)	92.5(5.0)
F	95.0(5.8)	97.5(5.0)	92.5(9.6)	92.5(9.6)
Grand Mean (SD)	96.9 (2.4)	89.4 (9.9)	97.5 (3.5)	85.0 (8.7)
CV	2.5%	11.0%	3.6%	10.2%

* Denotes significant difference from control.

Appendix D

Table D-11. Mean Growth (mg dry weight) (SD) of *H. azteca* in Field-Collected Sediment (Interlaboratory Study - Phase II: Static System).

Laboratory	Field-Collected Sediment-Round-Robin # 2			
	SED A	SED B	SED C	SED D
A	.058(.01)*	.054(.02)*	.124(.03)	.063(.01)*
B	.131(.01)*	.096(.00)*	.267(.06)	.088(.01)*
D	.047(.01)*	.064(.02)	.080(.01)	-
E	.165(.03)*	.097(.04)*	.315(.08)	.129(.04)*
F	.145(.03)*	.110(.02)*	.222(.03)	.062(.02)*
Grand Mean (SD)	0.109 (.05)	0.084 (.02)	0.202 (.1)	0.086 (.03)
CV	48.8%	28.4%	48.6%	36.7%

* Denotes significant difference from control.

Table D-12. Mean Growth (mg dry weight) (SD) of *H. azteca* in Field-Collected Sediment (Interlaboratory Study - Phase II: Static-Renewal System).

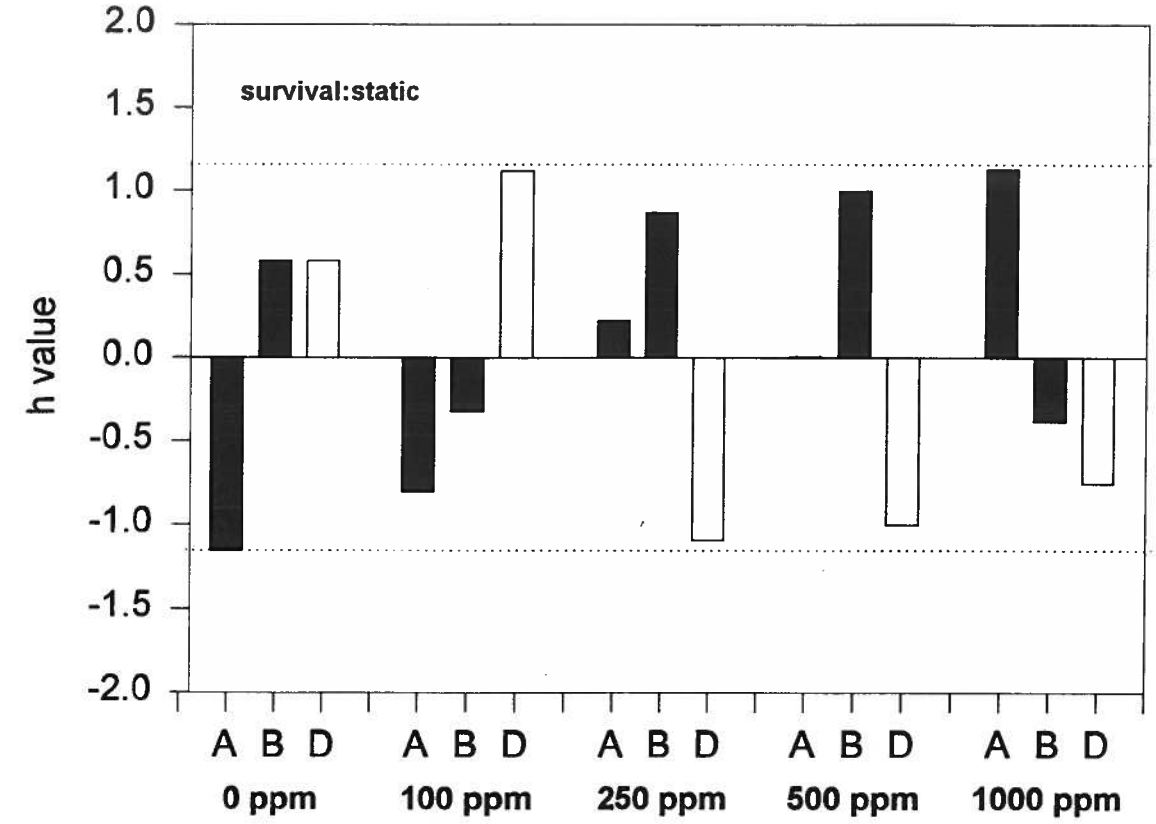
Laboratory	Field-Collected Sediment-Round-Robin # 2			
	SED A	SED B	SED C	SED D
A	.073(.02)*	.061(.01)*	.116(.01)	.052(.01)*
B	.129(.01)*	.131(.02)*	.176(.02)	.098(.01)*
E	.177(.04)	.162(.03)	.188(.02)	.095(.03)*
F	.154(.03)*	.120(.01)*	.227(.04)	.112(.05)*
Grand Mean (SD)	0.133 (.04)	0.119 (.04)	0.177 (.05)	0.089 (.03)
CV	33.5%	35.7%	26.0%	29.0%

* Denotes significant difference from control.

APPENDIX E

h and *k* Consistency Statistic Graphs for

C. riparius or *H. azteca* Interlaboratory Studies



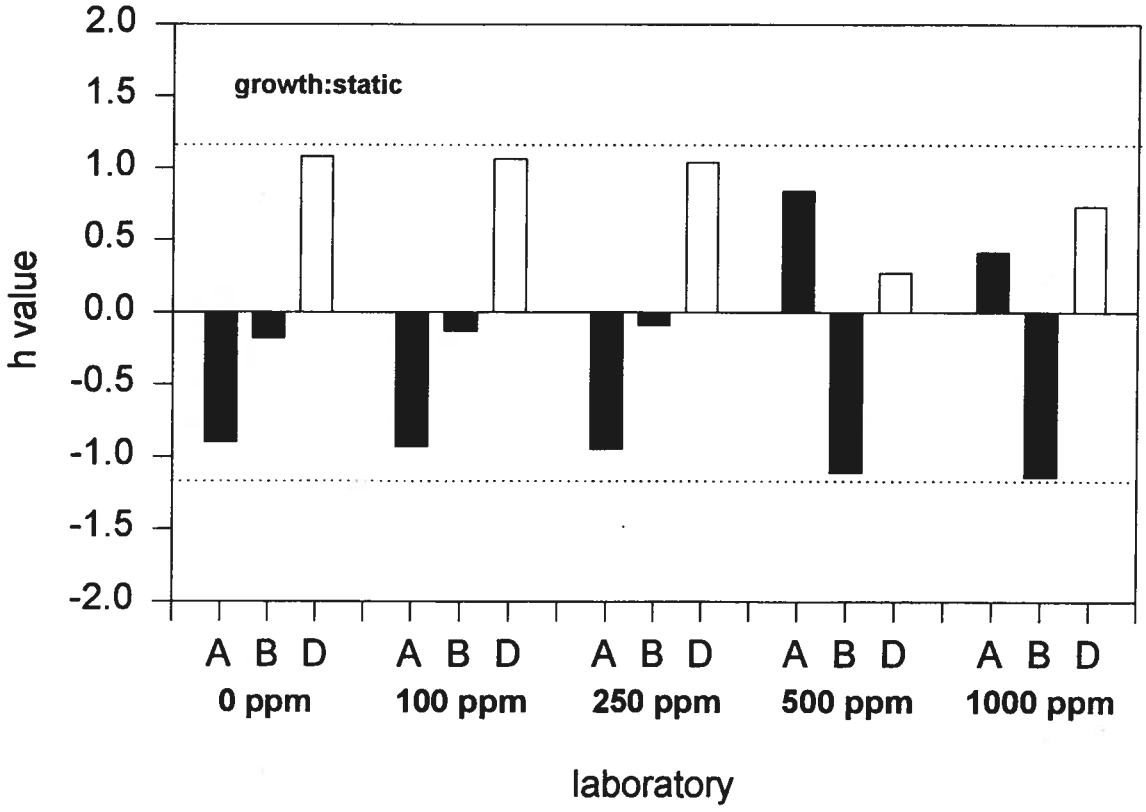


Figure E-1. Interlaboratory Comparison of h-values for *Chironomus riparius* in Phase I.

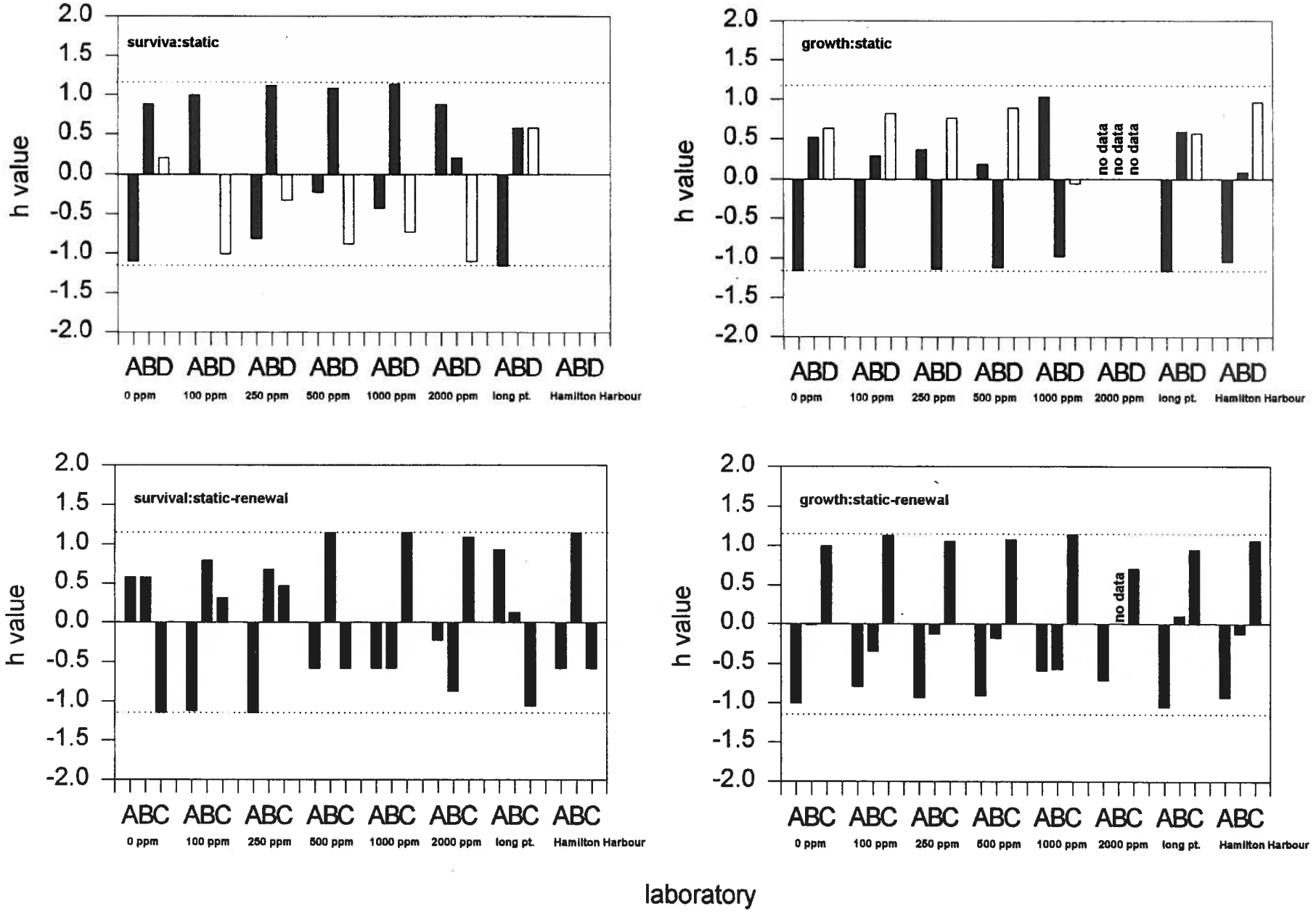


Figure E-2. Interlaboratory Comparison of h-values for *Chironomus riparius* in Phase II.

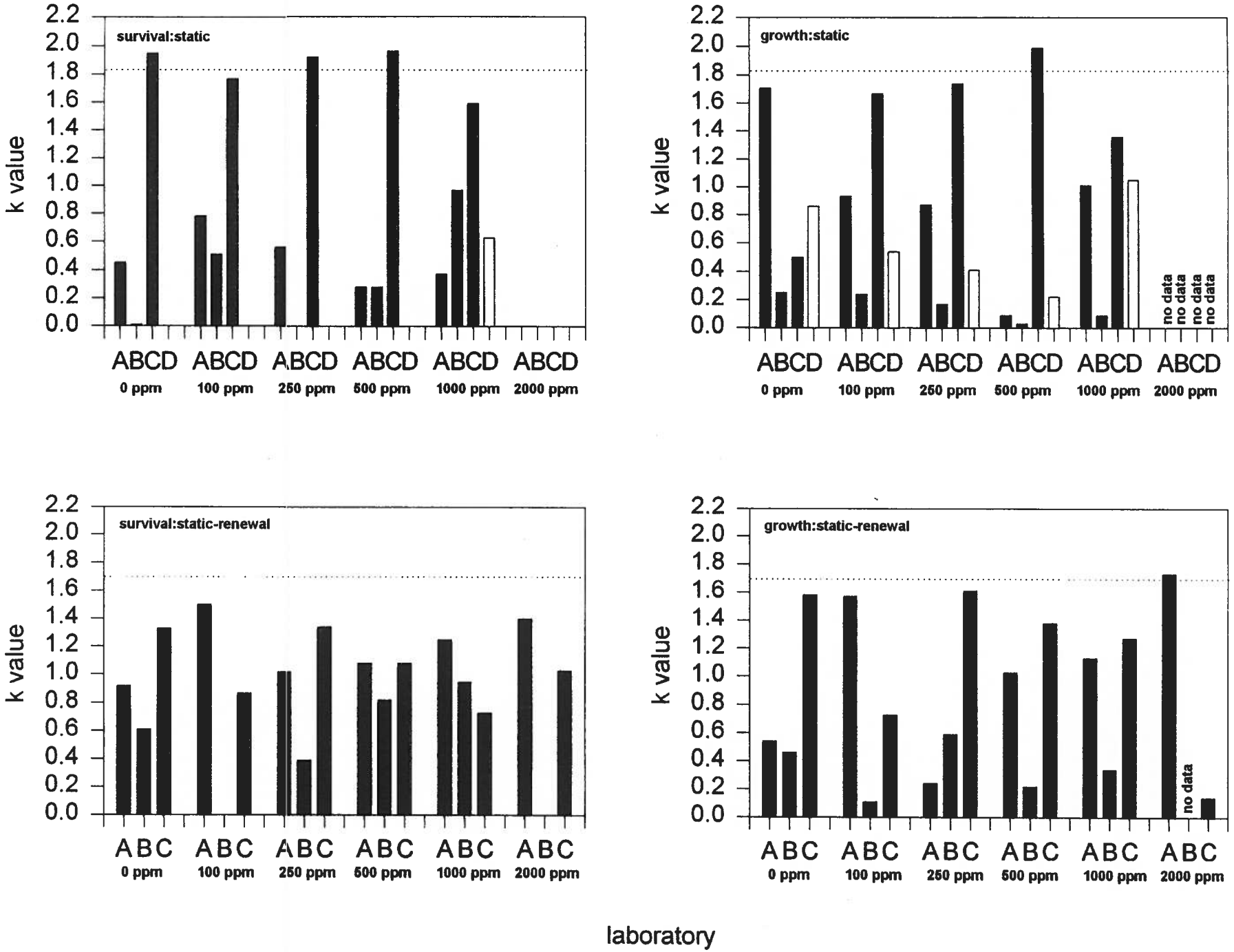


Figure E-3. Interlaboratory Comparison of k-values for *Chironomus riparius* in Phase I.

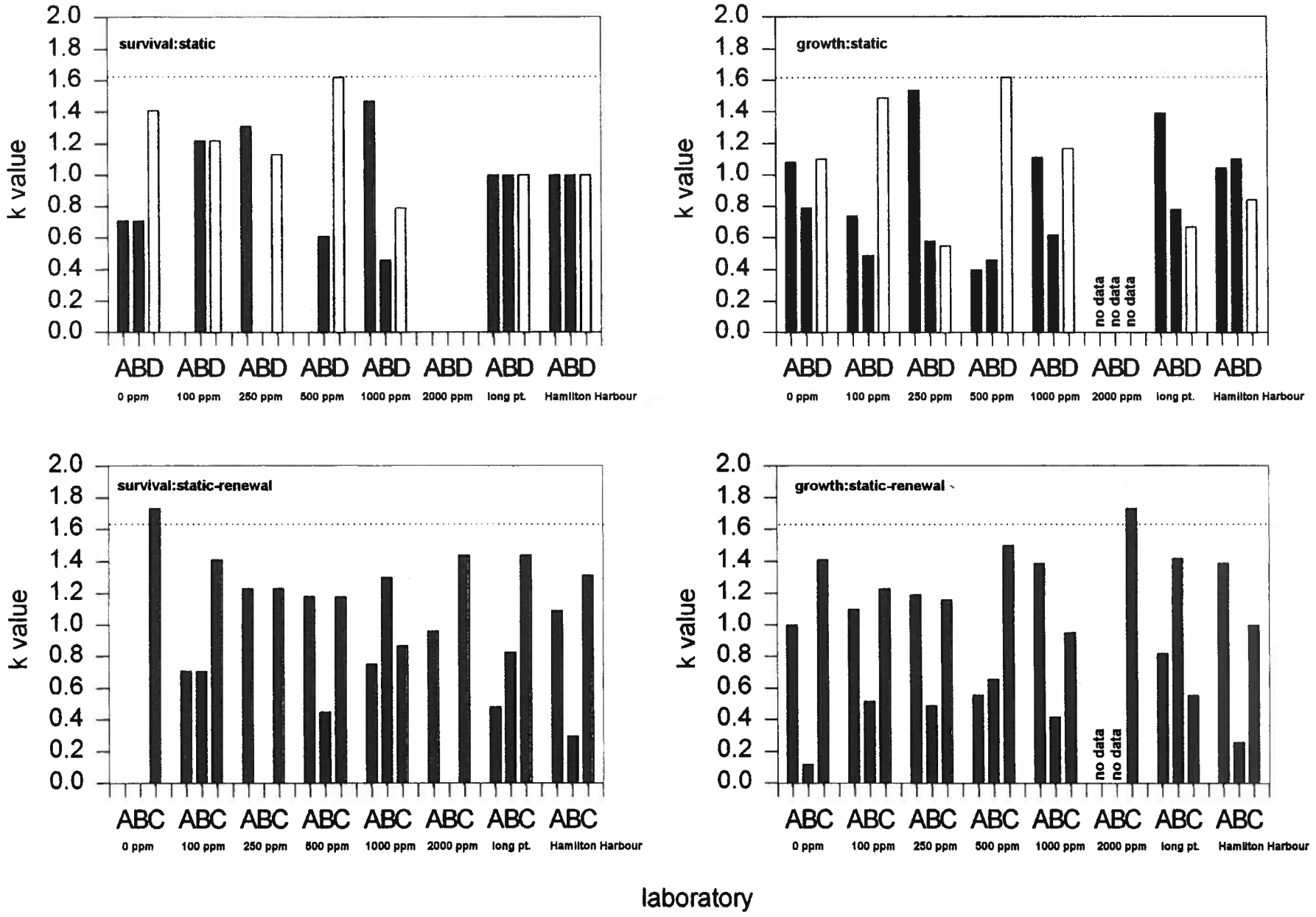


Figure E-4. Interlaboratory Comparison of k-values for *Chironomus riparius* in Phase II.

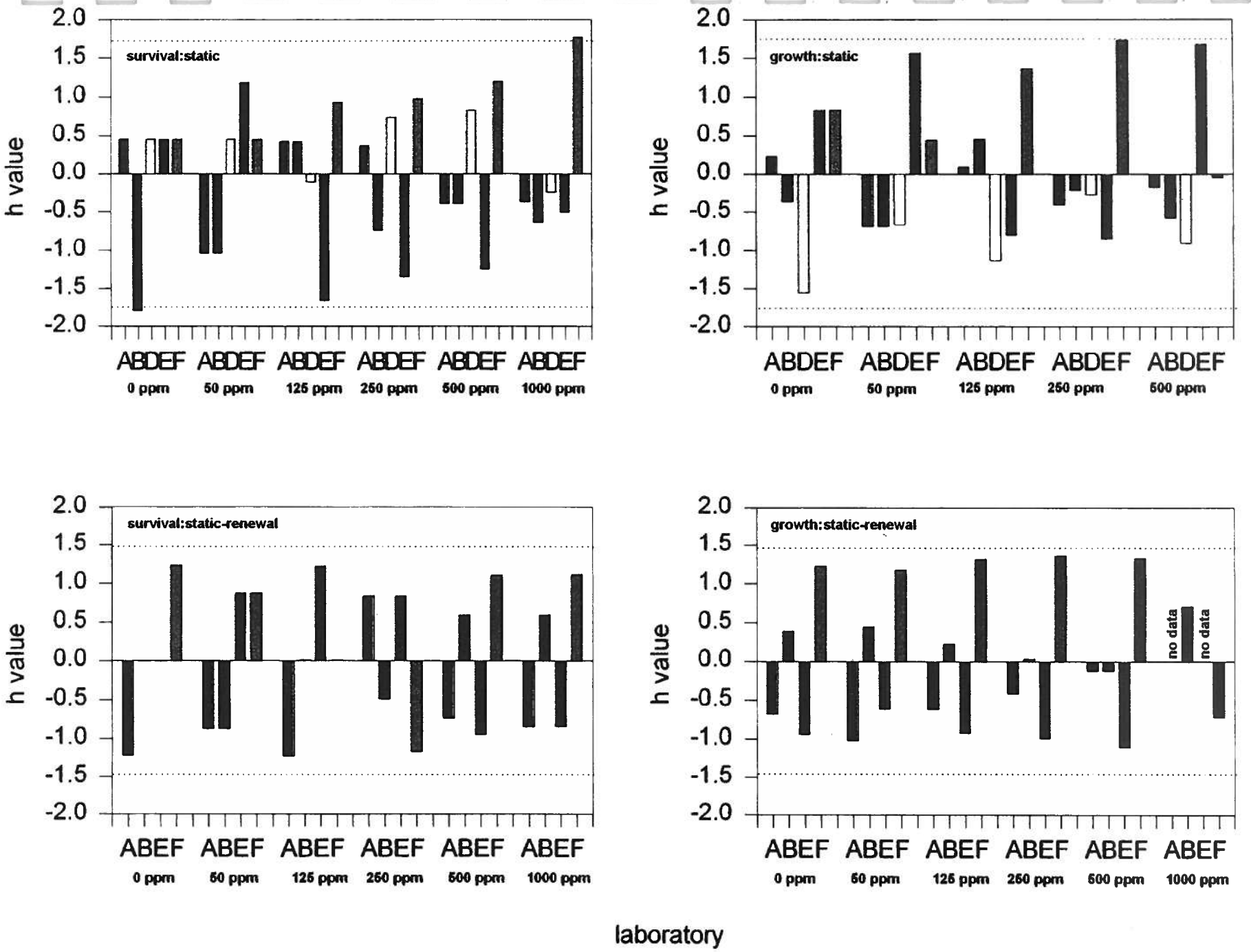


Figure E-5. Interlaboratory Comparison of h-values for *Hyalella azteca* in Phase I.

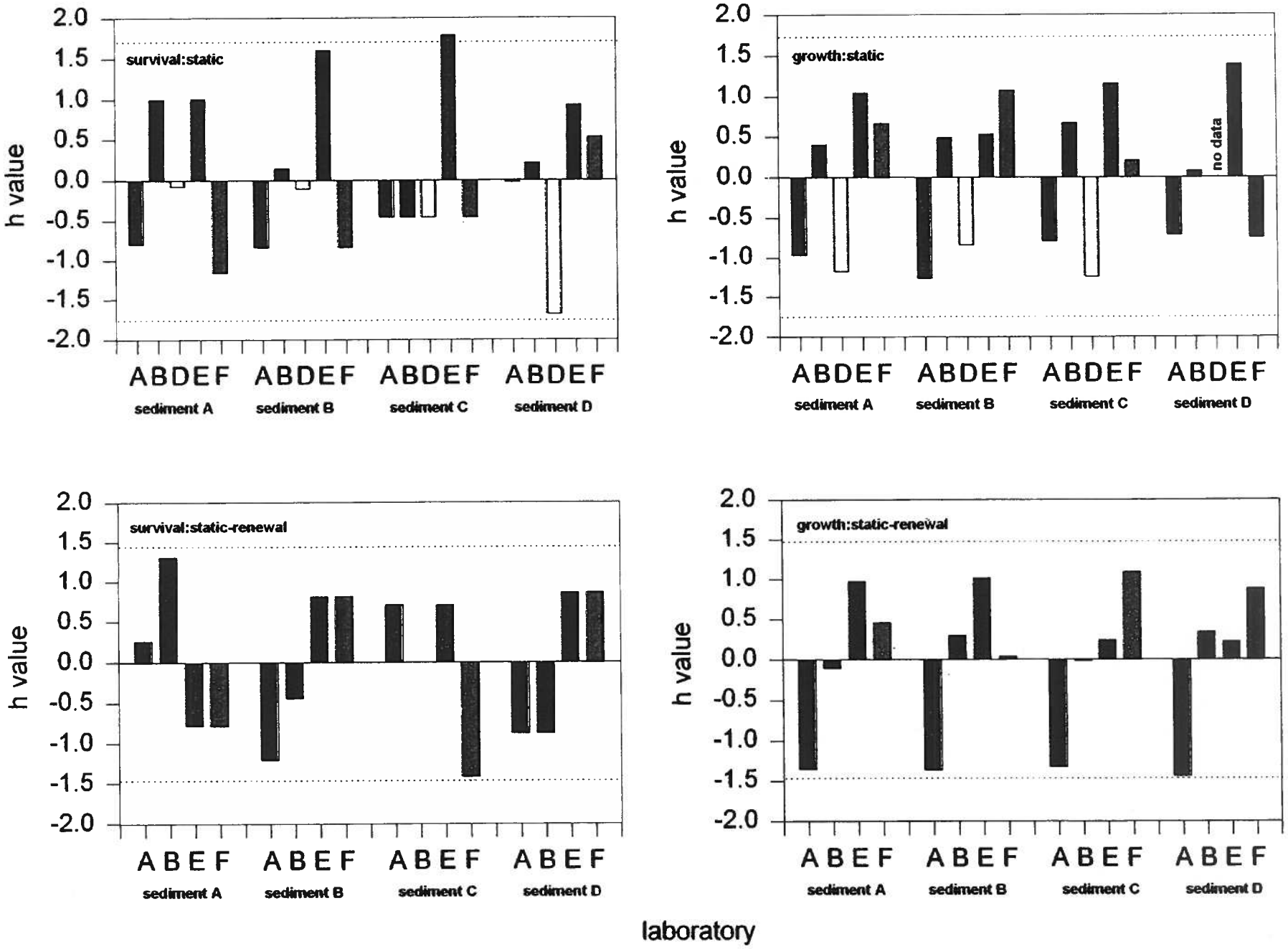


Figure E-6. Interlaboratory Comparison of h-values for *Hyalella azteca* in Phase II.

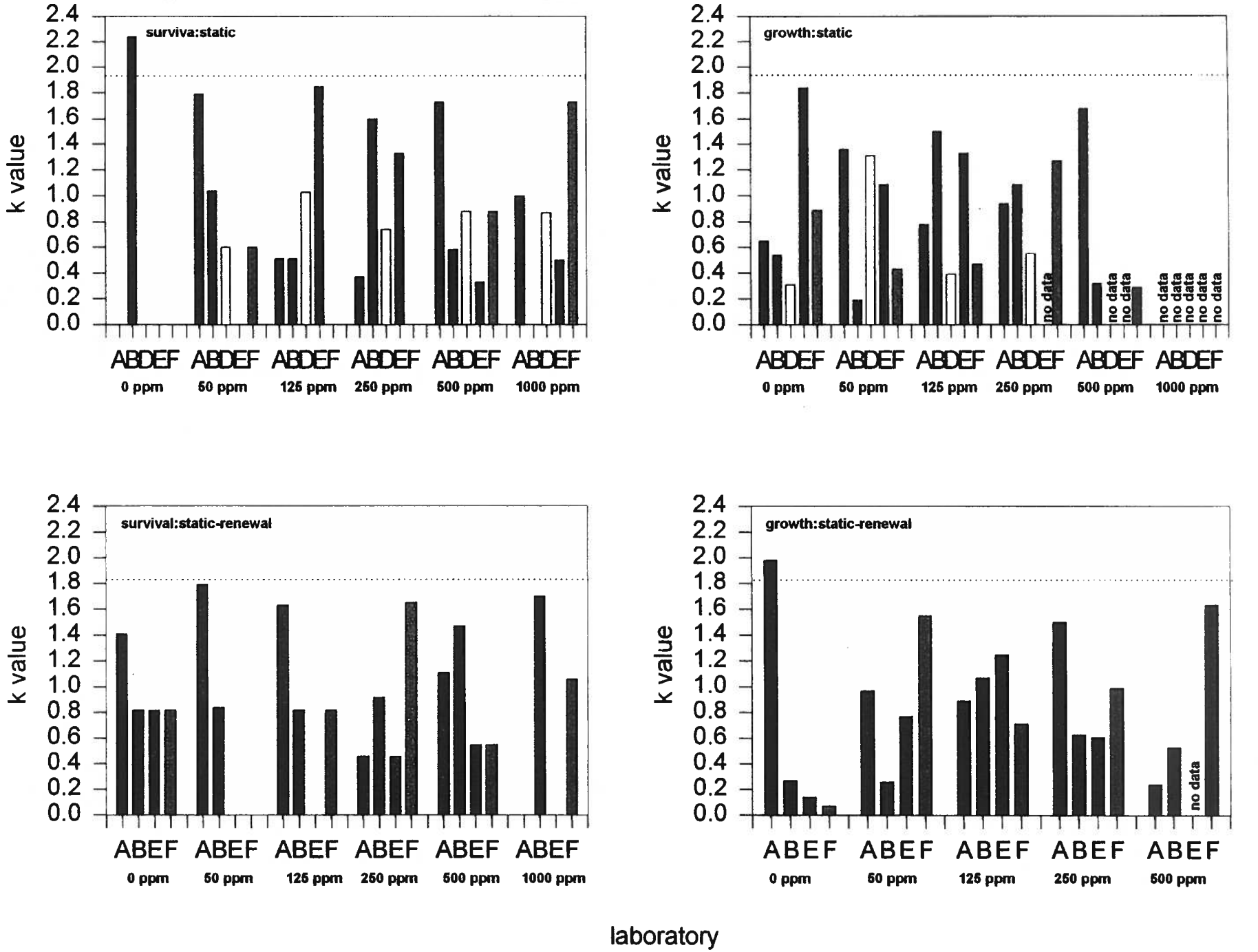


Figure E-7. Interlaboratory Comparison of k-values for *Hyalella azteca* in Phase I.

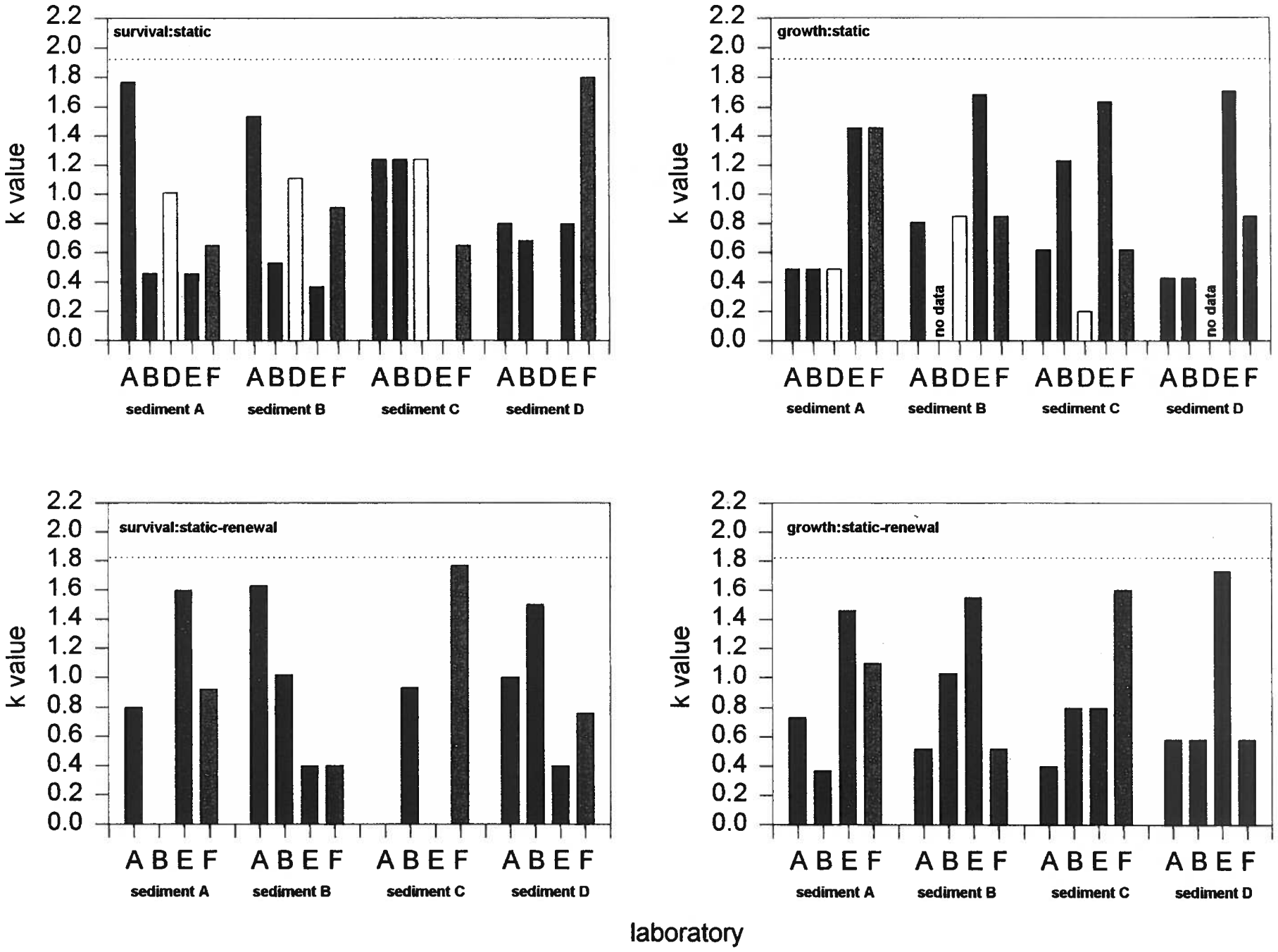


Figure E-8. Interlaboratory Comparison of k-values for *Hyalella azteca* in Phase II.

APPENDIX F

Copper Analysis for

C. riparius or *H. azteca* Interlaboratory Studies

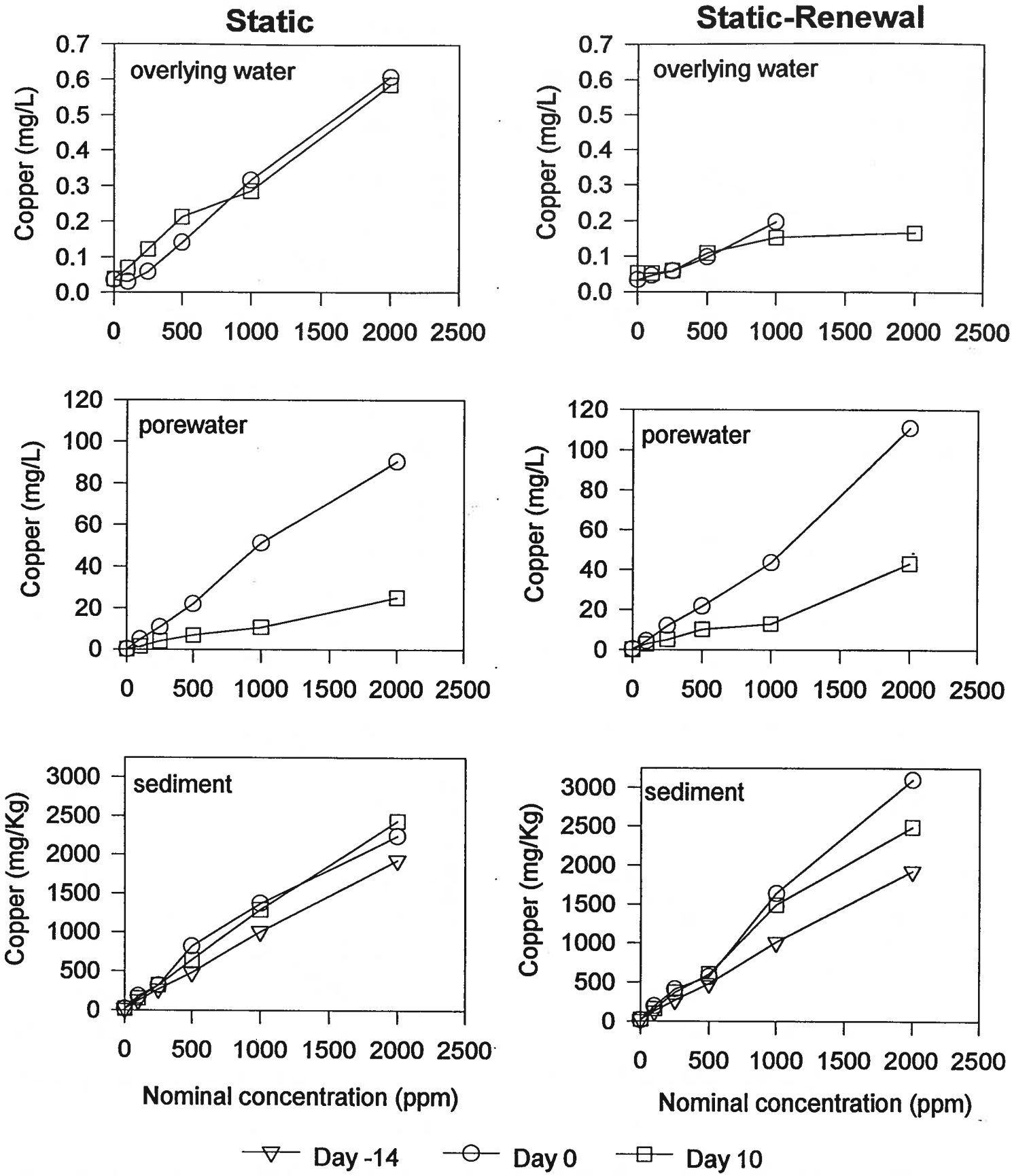


Fig. F-1. Overlying Water, Porewater and Bulk Sediment Copper Concentrations from *C. riparius* Phase I Interlaboratory Test with Static and Static-Renewal Systems.

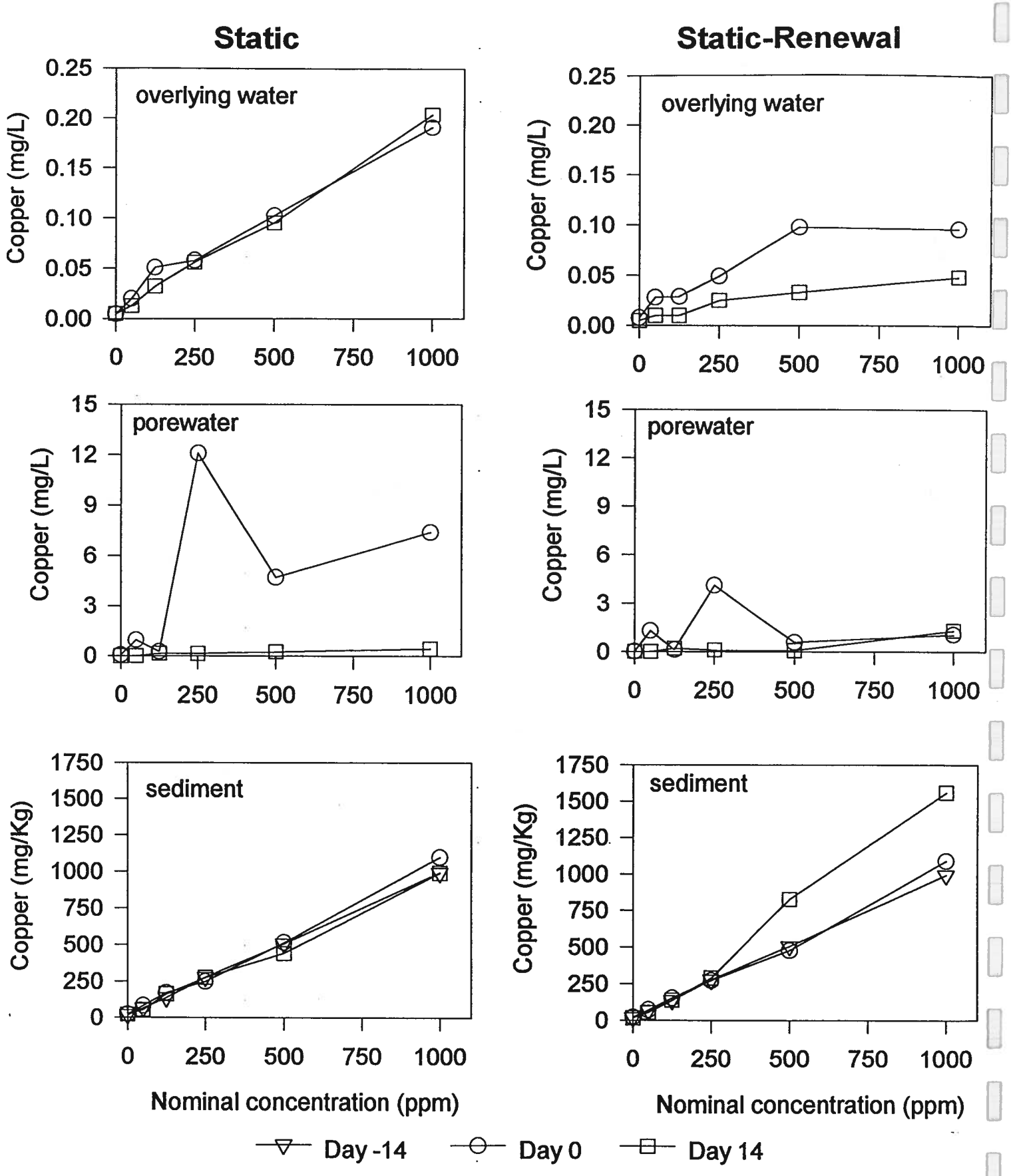


Fig. F-2. Overlying Water, Porewater and Bulk Sediment Copper Concentrations from *H.azteca* Phase I Interlaboratory Test with Static and Static-Renewal Systems.

APPENDIX G

Data Showing Derivation of Minimum Acceptable Dry Weights for Control

for *C. riparius* or *H. azteca* at Test End

Appendix G, Table G-1. Minimum Acceptable Level of Growth for C. riparius in Control Sediment

Static System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
Long Point	8.57	10	0.86
	8.62	9	0.96
	7.61	8	0.95
	7.43	9	0.83
	7.61	9	0.85
	7.87	9	0.87
	7.46	10	0.75
	6.18	8	0.77
	4.19	5	0.84
	5.60	7	0.80
	5.38	9	0.60
Long Point	7.77	9	0.86
	7.17	7	1.02
	7.28	9	0.81
	6.99	8	0.87
	8.14	10	0.81
	7.40	9	0.82
	6.33	8	0.79
	8.16	9	0.91
Long Point	8.63	9	0.96
	9.80	10	0.98
	7.43	9	0.83
Long Point	8.98	9	1.00
	8.22	9	0.91
	6.82	8	0.85
Long Point	9.91	11	0.90
	6.12	9	0.68
	10.67	11	0.97
108	8.74	10	0.87
	8.43	9	0.94
	7.86	8	0.98
	6.99	9	0.78
	7.10	8	0.89
	8.67	9	0.96
	8.73	10	0.87
	8.35	10	0.84

Static-Renewal System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
Long Point	5.81	9	0.65
	5.53	9	0.61
	6.18	10	0.62
	6.68	9	0.74
	6.44	9	0.72
	5.13	7	0.73
	6.57	10	0.66
	7.45	9	0.83
Long Point	8.11	10	0.81
	6.23	9	0.69
	7.27	8	0.91
Long Point	5.09	10	0.51
	5.37	10	0.54
	5.67	9	0.63
Long Point	8.45	9	0.94
	9.21	9	1.02
	5.97	6	1.00
108	5.95	8	0.74
	2.69	4	0.67
	2.66	9	0.30
	5.53	9	0.61
	5.72	9	0.64
	5.08	10	0.51
	4.13	6	0.69
	3.62	8	0.45
WB	7.44	10	0.74
	11.36	10	1.14
	9.73	9	1.08
	7.55	10	0.76
	7.35	10	0.74
	6.26	10	0.63
	5.95	11	0.54
	8.06	8	1.01
Form sed.	5.97	8	0.75
	2.60	6	0.43
	6.40	10	0.64

Appendix G, Table G-1 (cont'd). Minimum Acceptable Level of Growth for C. riparius in Control Sediment

Static System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
WB	9.28	9	1.03
	9.58	9	1.06
	10.77	9	1.20
	8.06	8	1.01
	7.75	8	0.97
	10.80	9	1.20
	9.27	9	1.03
	9.96	10	1.00
Form sed.	8.29	10	0.83
	6.60	10	0.66
	7.72	9	0.86
Form sed.	7.50	10	0.75
	8.28	10	0.83
	8.01	10	0.80
Form sed.	12.43	10	1.24
	9.88	10	0.99
	10.30	10	1.03
Form sed.	6.94	9	0.77
	6.35	8	0.79
	3.11	10	0.31
Form sed.	10.60	10	1.06
	8.77	9	0.97
	8.12	10	0.81
Form sed.	7.62	10	0.76
	8.83	8	1.10
	9.95	10	1.00
Form sed.	6.56	9	0.73
	7.34	9	0.82
	8.50	8	1.06

	Overall Mean	SD	CV
Long Point	0.86	0.10	0.11
108	0.89	0.07	0.08
WB	1.06	0.09	0.08
Form sed.	0.87	0.20	0.23
Grand Mean	0.92	0.10	0.10

- 2SD (.19) ***= 0.73 mg dry wt/individual***

Static-Renewal System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
Form sed.	4.38	8	0.55
	3.17	8	0.40
	3.76	6	0.63
Form sed.	11.56	11	1.05
	9.13	7	1.30
	10.76	10	1.08
Form sed.	8.58	10	0.86
	8.70	10	0.87
	8.27	10	0.83
Form sed.	3.84	10	0.38
	2.86	10	0.29
	6.44	10	0.64
Form sed.	10.89	10	1.09
	7.86	5	1.57
	11.61	10	1.16

	Overall Mean	SD	CV
Long Point	0.74	0.15	0.21
108	0.58	0.15	0.26
WB	0.83	0.22	0.26
Form sed.	0.81	0.35	0.44
Grand Mean	0.74	0.11	0.15

- 2 SD (0.23) ***= 0.51 mg dry wt/individual***

Appendix G, Table G-2. Minimum Acceptable Level of Growth for H. azteca in Control Sediment

Static System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
Long Point	1.33	10	0.13
	1.92	10	0.19
	1.97	10	0.20
	1.66	8	0.21
	3.19	9	0.35
	1.56	9	0.17
	1.13	9	0.13
Long Point	3.27	10	0.33
	2.69	10	0.27
	2.51	9	0.28
	3.48	9	0.39
	2.80	10	0.28
	2.25	9	0.25
	2.59	8	0.32
	3.13	10	0.31
	3.59	10	0.36
	2.85	10	0.29
Long Point	2.26	10	0.23
	2.73	8	0.34
	2.96	10	0.30
	1.85	9	0.21
Long Point	0.89	10	0.09
	1.05	10	0.11
	1.20	9	0.13
	1.34	8	0.17
Long Point	0.85	11	0.08
	0.63	9	0.07
	0.68	8	0.09
	0.87	10	0.09
Long Point	3.50	10	0.35
	2.00	10	0.20
	3.50	10	0.35
	3.60	10	0.36
Long Point	2.31	9	0.26
	1.87	9	0.21
	1.95	10	0.20
	2.05	9	0.23
1213	1.23	10	0.12
	1.18	9	0.13
	1.39	10	0.14
	1.21	9	0.13
	1.20	10	0.12

Static-Renewal System

Sediment	Dry wt (mg)	No. Animals	Wt/individual
Long Point	1.51	10	0.15
	1.92	10	0.19
	2.81	10	0.28
	2.15	9	0.24
	1.55	8	0.19
	1.83	9	0.20
	2.34	10	0.23
	2.66	10	0.27
	2.68	10	0.27
Long Point	1.49	10	0.15
	2.03	10	0.20
	1.47	9	0.16
	1.88	10	0.19
Long Point	1.27	11	0.12
	1.25	10	0.13
	1.14	10	0.11
	1.09	10	0.11
Long Point	1.70	10	0.17
	2.20	10	0.22
	1.90	10	0.19
	1.70	10	0.17
Long Point	2.12	8	0.27
	2.25	9	0.25
	1.97	10	0.20
	1.96	10	0.20
1213	1.25	9	0.14
	0.95	9	0.11
	1.05	8	0.13
	1.21	10	0.12
	0.96	9	0.11
	1.21	10	0.12
	1.21	10	0.12
	1.00	10	0.10
	0.95	9	0.11
	0.84	9	0.09
100	1.31	10	0.13
	1.82	10	0.18
	2.01	10	0.20
	1.67	9	0.19
	2.48	10	0.25
	1.73	10	0.17
	1.36	7	0.19

Appendix G, Table G-2 (cont'd). Minimum Acceptable Level of Growth for H. azteca in Control Sediment

Static System

Sediment			
1213	1.03	8	0.13
	1.35	9	0.15
	1.40	10	0.14
	0.77	7	0.11
	0.95	10	0.10
100	3.12	10	0.31
	2.63	10	0.26
	2.26	10	0.23
	3.31	10	0.33
	2.32	9	0.26
	2.94	10	0.29
	3.29	10	0.33
	2.40	9	0.27
Form sed.	2.29	10	0.23
	2.07	9	0.23
	2.58	10	0.26
Form sed.	1.26	8	0.16
	1.68	9	0.19
	1.15	9	0.13
Form sed.	1.72	10	0.17
	2.38	10	0.24
	1.79	10	0.18
Form sed.	0.62	10	0.06
	0.83	10	0.08
	1.06	11	0.10
Form sed.	1.30	10	0.13
	2.70	10	0.27
	3.30	10	0.33
Form sed.	2.27	10	0.23
	1.98	10	0.20
	2.95	10	0.30

	Overall Mean	SD	CV
Long Point	0.23	0.09	0.41
Sed 1213	0.13	0.02	0.13
Sed 100	0.28	0.04	0.13
Form sed.	0.19	0.07	0.39
Grand Mean	0.21	0.07	0.32

- 2SD (.14) ***= 0.080 mg dry wt/individual***

Static-Renewal System

Sediment			
100	2.32	10	0.23
Form sed.	1.34	9	0.15
	1.36	9	0.15
	2.04	10	0.20
Form sed.	1.29	9	0.14
	1.09	10	0.11
	0.78	8	0.10
Form sed.	1.00	9	0.11
	1.20	10	0.12
	0.80	9	0.09
Form sed.	1.88	9	0.21
	2.01	10	0.20
	2.17	10	0.22

	Overall Mean	SD	CV
Long Point	0.19	0.05	0.26
Sed 1213	0.11	0.01	0.13
Sed 100	0.19	0.04	0.19
Form sed.	0.150	0.047	0.312
Grand Mean	0.16	0.04	0.24

- 2 SD (.08) ***= 0.51 mg dry wt/individual***