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EXECUTIVE SUMMARY

A two-part study was conducted to investigate the contribution of selenium and other trace metals to toxicity in San Diego Creek. The first part of the study included the analysis of dry and wet weather samples from San Diego Creek for toxicity assessment, toxicity identification, and metals concentrations. The objective of this portion of the study was to determine the relative contribution of metals to the direct toxicity of San Diego Creek. The second part of the study consisted of a laboratory study to study the effects of selenium bioaccumulation on larval rainbow trout. The objective of the bioaccumulation study was to determine the relationship between selenium exposure and impacts on endpoints of significance to fish populations.

Water samples from San Diego Creek were frequently toxic to either the water flea (*Ceriodaphnia dubia*) or a green alga (*Selenastrum capricornutum*). Toxicity was present in six of 10 samples, which were collected on five dates between March 2002 and February 2003 and included both wet and dry weather flow conditions. The samples were also analyzed for alkalinity, hardness, conductivity, total and dissolved metals, and organophosphate pesticides. Partial TIE results and the analytical chemistry data indicated that trace metals were not the likely cause of most of the toxicity. Trace metal concentrations were usually far below both TMDL numeric targets and median effect concentrations for both species. In addition, there was no obvious relationship between the patterns of variation in metal concentration and toxicity.

Other potential causes of toxicity in the San Diego Creek samples may include dissolved solids such as chlorides and unidentified organics. Many of the creek samples contained elevated conductivity values that were indicative of toxic conditions for *Ceriodaphnia*. Toxicity in some of the samples was unstable in storage, which suggests a nonmetal toxicant is present. Chemical analyses reported nondetectable or low (i.e., nonotoxic) concentrations of 18 organophosphate pesticides. These compounds were therefore unlikely to have caused the observed toxicity. Other organic contaminants, such as pyrethroid pesticides, may be present in the samples as a result of changing pesticide usage patterns and contribute to the observed toxicity. No analyses for these compounds were made, as it was beyond the scope of this study. The relative contribution of other pesticides to the direct toxicity in San Diego Creek cannot be determined from these data.

Elevated selenium (Se) concentrations in water (>5 μg/L) have been measured in San Diego Creek and there is concern that selenium exposure may impair fish populations. A 90-day laboratory experiment was conducted to determine the effects of selenomethionine (SeMe) exposure on the growth, survival and whole body Se accumulation in larval rainbow trout. The reduced and oxidized glutathione and thiobarbituric acid reactive substance levels were also measured in the livers of the trout to assess oxidative damage caused by Se. Se was administered orally using food spiked with SeMe to contain 9.2, 16.6 and 22.6 μg/g (dry weight) of Se.

Fish exposed to SeMe through their food for 90-days exhibited a reduction in body weight and fork length at all levels of exposure when compared to control samples. Whole body total Se concentrations significantly increased in fish fed 22.6 μg/g SeMe after 60-days when compared to controls. A significant increase in the whole body Se concentration was also observed after 90 days at all levels of exposure. Lipid peroxidation and GSH:GSSG ratios were unchanged by the Se treatments. Based on reduced growth after 90 days, a dietary Se LOEC value of 9.2 μg/g was obtained, as well as a body burden LOEC of 0.51 μg/g (dry weight).

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INTRODUCTION

This study was conducted to determine whether selenium and other trace metals are a significant cause of water quality impairment in San Diego Creek. The results of this study will be used in the TMDL development process to help select and prioritize constituents of concern.

San Diego Creek, which discharges into upper Newport Bay, has been listed as an impaired water body. The listing was due to the presence of toxic chemicals in animal tissues and toxicity of creek water during both dry and wet periods. Multiple constituents appear to be responsible for the toxicity of San Diego Creek and its tributaries. An extensive study of toxicity throughout the San Diego Creek watershed found that the organophosphorus pesticides diazinon and chlorpyrifos (and occasionally the pesticide carbaryl) were present in most of the samples and that approximately one-half of the toxicity measured could be accounted for by the concentrations of these compounds (Lee and Taylor 2001). Approximately 50% of the toxic samples contained unidentified toxicants; toxicity identification studies indicated that pyrethroid pesticides and possibly other types of nonpolar toxicants were present in these samples, but the identity of the toxicants could not be confirmed (Lee and Taylor 2001).

Elevated concentrations of trace metals may also be contributing to water quality impairment in San Diego Creek. Selenium concentrations above the 5 µg/L objective set in the California Toxics Rule are often found. Concentrations of copper, zinc, and occasionally other metals are also elevated in San Diego Creek. Although many samples from the creek have been tested for toxicity, few toxicity identification studies have been conducted to assess the influence of metals on toxicity (Lee and Taylor 2001).

The objective of this study was to determine the relative contribution of trace metals to the toxicity of water from San Diego Creek. Samples were collected during both wet and dry weather conditions. All samples were tested for toxicity using the *Ceriodaphnia* 7-day survival and reproduction test. Wet weather samples were also tested with the freshwater algae *Selenastrum* 96 hr growth test. Samples exhibiting a strong level of toxicity were to be analyzed using EPA toxicity evaluation methods designed to identify the presence of metal-caused toxicity.

METHODS

STUDY DESIGN

Samples were collected from San Diego Creek during five time periods, three storm events and two dry weather periods (Table 1). For each collecting event, two discreet samples were to be taken. The objective of the wet weather sampling was to collect one sample during the initial first flush and to collect a second sample during peak flow. During dry weather collection, again two discreet samples were to be taken, several hours apart. For all collections except the last wet weather event, samples were taken for measurement of total and dissolved metals, organophosphate pesticides, toxicity, and total suspended solids. For the final wet weather collection, only toxicity samples were collected. Partial Phase I toxicity identification evaluations (TIE) were conducted on some of the wet weather samples and a full Phase I analysis was conducted on a sample from the final wet weather event.

SAMPLING

For wet weather, samples were collected from the Campus Drive bridge. To take the samples, a metal davit was attached to the bridge. From the davit a torpedo sampler was lowered to the creek. Inside the sampler was a 1 gallon glass bottle. At each sampling period, multiple drops were made and the water was transferred to a 5 gallon polycarbonate bottle until it was full. Between samples, the bottle was rinsed with tap water, then methanol, followed by 10% nitric acid and rinsed with DIW. Samples for all analyses were then aliquoted from this composite. During the sample transfer, the 5 gallon bottle was swirled to keep the particle load suspended. Samples for dissolved metals were taken from the composite by an ISCO sampler with a 0.45 micron filter attached using EPA suggested clean sampling techniques. Samples for total and dissolved metals analysis were transferred to new polystyrene or cleaned Teflon 500 ml or 1 liter bottles. The cleaning procedure for the Teflon bottles consisted of washing with soap and water then rinsed with DI water. Next, they were rinsed with 2% nitric acid solution and then soaked with 2% HCl for 24 hours, rinsed with DI water and double bagged in ziplock bags until used. Samples for toxicity testing were transferred to 10 liter cubitaners. Samples for organic analysis were transferred to clean 1 gallon amber glass bottles with zero headspace. Total suspended solids aliquots were put into 1 liter plastic bottles. Creek flow data was not available during sampling. Thus, the two sampling times were selected on the basis of rainfall amount and water level observations to capture first flush and peak flow conditions. The time interval between the samples ranged from 3 hours to 34 hours. For the February wet weather event, no samples were taken for chemical analysis.

For dry weather, samples were collected directly from the creek bed at the base of the bridge using an ISCO sampler. For each collection event, a sample was taken in the morning and then another was taken 4 to 5 hours later. The samples for metals analysis went straight from the creek into their respective containers (either through the filter or not). The remaining samples were taken from a composite made in a 5 gallon polycarbonate bottle. All sample containers were the same as described above.

The samples were stored on ice until transported back to SCCWRP where they were stored at 5 °C. Samples for chemical analysis were delivered to the chemistry lab within 24 hrs of collection. Toxicology samples were shipped out on ice the day of collection for delivery to the laboratory by the next morning.

CHEMICAL MEASUREMENTS

All creek samples were analyzed for trace metals directly without extraction. The samples were analyzed by means of EPA Method 200.8 on an HP 4500 Inductively Coupled Plasma Mass Spectrometer (ICPMS). All chemical analyses were conducted by CRG Marine Laboratories (Torrance, CA).

Total suspended solids was measured by Cal Science (Garden Grove, CA) or CRG Marine Laboratories using EPA method 160.2. Organophosphate pesticides were measured using methylene chlorine separatory funnel extraction (EPA method 3510C) and GCMS analysis (EPA method 8270). The detection limits for all analyses are shown in Table 2.

TOXICITY MEASUREMENTS

All toxicity tests were conducted by AMEC Earth and Environmental (San Diego, CA). Samples from both the dry and wet weather periods were tested for toxicity using the *Ceriodaphnia dubia* chronic survival and reproduction bioassay. Test procedures followed EPA methods (U.S. EPA 1994). Tests were conducted in 30 ml polystyrene containers with 15 ml of test solution. Each chamber had one neonate *C. dubia* added at test initiation. Each sample was tested at 50 and 100% concentrations with a control consisting of laboratory dilution water (four parts Nanopure to one part Perrier). For each concentration, 10 replicate chambers were tested. Tests were conducted at 25 °C with a 16:8 hour light:dark cycle. The animals were fed daily with vitamin enriched YCT and *Selenastrum*. Each chamber was monitored daily for offspring production, mortality, sublethal effects and water quality parameters. Test duration was 7 days. Test endpoints are survival and number of offspring produced. A reference toxicant test with copper was conducted within 14 days of each runoff test.

Samples from all three wet weather events were tested for toxicity using the fresh water algae, *Selenastrum capricornutum*, chronic cell growth test. Test procedures followed the EPA protocol (U.S. EPA 1994). The test was conducted in 125 ml Erlenmeyer flasks containing 50 ml of sample. The test duration was 96 hrs at a temperature of 25 °C. At the beginning of the test, *Selenastrum* from a laboratory culture was added to the flasks to a concentration of approximately 10,000 cells per ml. Due to the presence of native algae, the runoff sample was passed through a 45 µm glass fiber filter prior to testing. Filtered samples were then tested at 100% and 50% concentrations. A sample of 100% unfiltered sample was also tested for comparison purposes. Four replicates were tested of each concentration. Fluorescent lights suspended above the test vessels provided illumination. At test termination, chlorophyll-A fluorescence was measured using a Turner Model TD 700 fluorometer. Cell counts were derived from the fluorescence using a standard curve. Control results were verified microscopically. A reference toxicant test with copper was conducted within 14 days of each runoff test.

TOXICITY IDENTIFICATION

For samples from the second wet weather event and both dry weather events, a set of samples for the *C. dubia* test had Ethylenediaminetraacetic acid (EDTA) added to them to order to remove toxicity caused by metals. EDTA chelates metals, thus rendering them unavailable to cause toxicity. For the first dry weather sample, EDTA was added to achieve a concentration of 60 mg/L. This concentration proved to be toxic and 8 mg/L was used for subsequent tests.

For the final wet weather collection, a complete suite of Phase I TIE treatments were performed on one of the samples and then tested for toxicity using *Selenastrum* (Figure 1). Phase I TIE testing uses chemical or physical manipulations of the sample to attempt the reduction or elimination of toxicity associated with broad groups of chemical classes (e.g. EDTA chelation of cationic metals). The treatments consisted of addition of EDTA, at a concentration of 60 mg/L; addition of sodium thiosulfate, which reduces anionic constituents, such as chlorine, at a concentration of 100 mg/L; a one hour aeration treatment; filtration to 0.45 μM; solid phase extraction through a C-18 column to remove nonpolar organic chemicals; and solid phase extraction through a cation exchange column to remove cationic metals.

DATA ANALYSIS

Comparisons between control survival and each test concentration for the *Ceriodaphnia* tests was performed using Fisher's Exact Test. Comparisons between control reproduction and each test concentration for the *Ceriodaphnia* tests and for cell growth for the *Selenastrum* tests were performed using Dunnett's Test. All statistical testing was done using ToxCalc Statistical Software version 5.0.

RESULTS

HYDROLOGY

Samples of wet weather runoff were successfully collected from three storm events, on March 7, 2002, November 8, 2002 and February 11, 2003. The first storm dropped 0.36 in of rain at John Wayne Airport. The first sample for this storm was taken just before the peak flow of the creek and the second was taken as the flow began to decrease (Figure 2). The base flow for this storm was 15 cfs with a peak flow of 238 cfs.

The second storm was a larger event with rain occurring over three days. The sample was taken during the first day when 0.54 in of rain was recorded at the airport. The first sample for this storm was taken a couple of hours after the rain had started (Figure 3). Flow had noticeably increased. The second sample was taken three hours later after a period without rain. Subsequently, another 0.54 in fell on November 9 and 0.07 in on the 10th. The base flow at the start of this storm was 7.1 cfs with a peak of 2350 cfs.

The third storm was also a large event with rain falling on four consecutive days. The initial sample was taken during the initial rise in creek flow on the first day of the storm (February 11, 2003), on which 0.64 in of rain was recorded at the airport (Figure 4). Flow was above the center channel of the creek at the time of this sample. The second sample was collected during the period of peak flow on the following day, on which an additional 0.68 in of rain were recorded. Subsequently, 0.46 in were recorded on February 13 and 0.11 in on the 14th. The base flow for this storm was 4.9 cfs with a flow of 2610 cfs.

Samples of dry weather flow were collected on May 10 and August 20, 2002. In each case, the first sample was taken at approximately 0900 hrs and a second sample at approximately 1400 hrs.

TOXICITY

Wet weather

For the March 2002 wet weather event, no toxicity was observed in the first sample for *Ceriodaphnia* (Table 3 and 4 and Figure 5). However, the second sample caused complete mortality of the *Ceriodaphnia*. It was noted that this mortality occurred after four days of exposure. In order to test the persistence of the toxicity, an acute *Ceriodaphnia* test was started six days after the sample had been collected. There was no significant mortality after four days in this test; the test was extended to 7 days after which the survival had dropped to 20%. No TIE was conducted on the March samples, due to the degradation of toxicity. For the *Selenastrum* test, no toxicity was observed in either sample (Figure 5). The cell counts in all of the filtered concentrations were more than twice control values, while the unfiltered samples were very similar to the controls (Table 5). This may have been caused by the filtered samples having nutrients in the runoff, but competing native algae were removed by filtration, thus increasing cell production. The unfiltered samples also would have the added nutrients, but competition the native species may have kept the *Selenastrum* counts from increasing.

For the November 2002 storm, no toxicity was observed in either sample for the *Ceriodaphnia* test (Table 4). The results of the concurrent EDTA addition were therefore inconclusive regarding the influence of metals, as no toxicity was present. Toxicity was present in the *Selenastrum* test of 100% filtered sample from both time points, with cell counts being approximately 25% of the control value (Table 5). Toxicity was not present in the 50% filtered samples, indicating that dilution of the filtered sample with dilution water reduced the concentration of toxic constituents below the threshold for toxicity to *Selenastrum*. The 100% unfiltered samples from each timepoint were not toxic. Unfortunately, the results of this test were not provided in sufficient time to implement TIE procedures on the sample. It is not known why the filtered samples were toxic, but no toxicity was detected in the unfiltered samples. This pattern of results may indicate that the toxicity was due to a constituent of relatively low water solubility that partitioned onto the additional particulate matter present in the unfiltered sample, thus reducing the bioavailable concentration of the toxicant.

A third storm event was sampled in February 2003 in an attempt to verify the toxicity observed in November and perform a TIE. This sample was tested for *Ceriodaphnia* survival (no reproduction) and *Selenastrum* growth. No toxicity to *Ceriodaphnia* was detected in either February sample, but the first sample (0906 hrs) was found to be toxic to *Selenastrum* (Tables 4 and 5). The magnitude of response of *Selenastrum* in the filtered 100% portion of the 0906 hrs sample (34% of control cell count) was similar to that measured in both of the filtered November 2002 samples (22% and 25% of control, Figure 5). A toxic response (31% of control cell count) was also present in the 100% unfiltered and 50% filtered portions of the 0906 hrs sample, a pattern that differed from that observed for the November 2002 samples (no toxicity detected in these portions).

A *Selenastrum* TIE on the first sample from February 2003 event was initiated one week after sample collection. In addition to the treatments described in the methods section, a portion of unfiltered sample to which no TIE treatments were performed was tested to determine the baseline amount of toxicity. The TIE results were inconclusive because the baseline test results indicated that the sample was no longer toxic (Figure 6). Algae growth in the baseline and all treatments were greater than the respective control values (data shown in Appendix).

Dry weather

No toxicity to *Ceriodaphnia* was found in either of the samples from the May 2002 dry weather collection (Figure 5). In anticipation of finding toxicity, subsamples of creek water were treated with EDTA at a concentration of 60 mg/L. It was found that this concentration of EDTA greatly reduced reproduction of the control animals (Table 4). The samples treated with EDTA had somewhat greater reproduction than the controls. The reduction in the controls was likely caused by the fact that the EDTA itself is toxic at high concentrations. The EDTA likely reacted with the matrix of constituents in the creek samples, thus reducing the toxicity of the EDTA.

Neither of the August 2002 dry weather samples was toxic to *Ceriodaphnia* survival (Figure 5 and Table 4). Survival in the second sample was 80% and less than the control value (100%), but the difference was not statistically significant. Both of the samples caused a statistically significant decrease in reproduction relative to the controls, however. Treatment with EDTA reduced the toxicity of both samples. Survival in the

EDTA-treated second sample increased to 100%. *Ceriodaphnia* reproduction in both of the EDTA-treated samples was greater than the corresponding baseline (untreated) samples and greater than the EDTA control, but still less than the dilution water control (Figure 6). The EDTA treatment results indicate that metals may have been partially responsible for the toxicity detected in the August 2002 dry weather samples. Reproduction in the EDTA control was reduced relative to the dilution water control, which is another indication of the partial inherent toxicity of EDTA that is occasionally detected when this compound is added to dilution water. Because of this effect, comparison of the results of the EDTA-treated sample to the dilution water control usually provides a more reliable measure of the effectiveness of the EDTA treatment than does comparison to the EDTA control.

Toxicity Q/A Assessment

All of the tests performed using *Ceriodaphnia dubia* met test acceptability criteria for control survival and number of offspring produced. The reference toxicant test associated with each runoff test fell within the control chart limits, indicating that each test was comparable in sensitivity.

The March 2002 *Selenastrum* test met test acceptability criteria for number of cells in the controls. For the November testing, the cell counts in the controls were below the test acceptability criteria of 200,000 (Table 5). However, an analysis of the last 20 reference toxicant tests performed by the laboratory indicated that there was no relationship between control cell counts and test sensitivity to copper. Therefore, the observed toxicity should be considered valid. The reference toxicant test associated with each runoff test fell within the control chart limits.

CHEMISTRY

Substantial differences in several general constituents were present among the March 2002 wet weather samples and the other three sets of samples collected in 2002. Alkalinity, hardness and conductivity concentrations in the March samples were often <50% of the values reported for the other samples (Table 6). These relatively low values are typical of the effect expected when the normal dry weather creek flow is greatly diluted by rainfall runoff. The alkalinity, hardness, and conductivity concentrations for the November 2002 wet weather samples were similar to those of the May and August dry weather samples. These results indicate that the November samples, which were collected at the beginning of the storm runoff event, were probably composed primarily of dry weather flow.

Total and dissolved metals measurements were made on all samples collected in 2002. A summary of the results for the metals of concern for TMDL development (i.e., Cd, Cu, Pb, Se, and Zn) is shown in Table 6. The concentrations of other metals are reported in the Appendix.

Total Cd concentrations were below the TMDL chronic toxicity limit of 0.64 µg/L for all samples. The average concentration of total cadmium for each sampling event ranged from 0.16 µg/L in March and May to 0.12 in August (Table 6). There were no statistically significant differences among the means. No detectable dissolved Cd was present in any of the samples (Table 6).

Cu was detected in all of the samples (Table 6). The dissolved Cu concentration ranged from 10.5 µg/L (March) to 1.66 µg/L (August) and accounted for 43-68% of the total value. Average total Cu concentrations were significantly different among the sampling dates (Table 7). The average concentration of total Cu in the March samples (15.7 µg/L) was significantly greater than the average concentration for the three other sampling events. All of the dissolved Cu measurements were below the draft TMDL chronic targets of 29.3 µg/L and 15.5 µg/L for base and large flows, respectively (U.S. EPA 2002). The measured concentrations were also well below published toxicity median effects values for the two test species (Figure 7 and Table 8).

The concentrations of Zn were also highest in the two March wet weather samples, relative to all the other samples (Table 6). The average concentration for the March samples was significantly greater than the average concentrations for the other three sampling events (Table 7). Between 30 and 56% of the total Zn was present in the dissolved form in the March samples. The dissolved portion accounted for 30-40% of the total Zn for the May, August, and November samples. The measured dissolved Zn concentrations were all well below the most conservative TMDL chronic target of 203.5 µg/L proposed for San Diego Creek (large flow value). All measured Zn concentrations were also below published median effect concentrations for *Ceriodaphnia* and *Selenastrum* that were obtained using similar test methods (Figure 7 and Table 8).

Total Pb concentrations were relatively low in all samples, ranging from 2.52 µg/L in March to 0.86 µg/L in August (Table 6). Analysis of variance detected a significant difference overall among the average concentrations for each sampling event but none of the means were significantly different from each other (Table 7). Most of the lead (79-88%) was associated with the particulate fraction of the March and May samples. There were no detectable concentrations of dissolved Pb in samples collected in August and November. All dissolved Pb concentrations were far below the most conservative chronic TMDL target of 5.0 µg/L proposed for San Diego Creek (Figure 8).

Total Se concentrations in the creek samples were highest (up to 25.1 µg/L) in the low flow samples collected in May, August, and November 2002 (Table 6). There was a significant overall difference in the mean concentrations among sampling events and the mean for November was significantly greater than the March mean. Nearly all of the Se was present in the dissolved phase. In a few cases, the dissolved value is reported as being slightly higher than the total concentration. This is due to the variability inherent in the analysis and those samples should be viewed as having 100% of the Se in the dissolved phase. Except for the second March wet weather sample, all of the measured total Se concentrations exceeded the proposed TMDL target value of 5 µg/L (Figure 8). The measured Se concentrations were all well below the median effect concentration for *Ceriodaphnia* reproduction of 870 µg/L determined by AMEC in previous experiments.

The organophosphate pesticide diazinon was detected in both of the November 2002 samples at concentrations (34.9 and 33.2 ng/L) approximately one tenth of that known to cause strong toxicity to *Ceriodaphnia* . No toxicity to *Ceriodaphnia* was present in these samples, however. Another organophosphate pesticide, trichloronate, was detected at a concentration of 25.5 ng/L in the first of the November 2002 samples. The samples were

examined for 16 other organophosphate pesticides, but no detectable concentrations (>10 ng/L) were found.

DISCUSSION

This project was successful in obtaining toxicity and metal concentration data for San Diego Creek under a variety of conditions. Samples of wet weather flow were obtained from three storm events and tested with two freshwater species, a water flea (*Ceriodaphnia dubia*) and a green alga (*Selenastrum capricornutum*). These samples included both the initial rising flow of the creek (e.g., November 2002 and February 2003), where contaminant concentrations may be highest, and peak flows that represent the majority of the volume discharged (e.g., March 2002 and February 2003). Samples of dry weather flow were also analyzed.

Toxicity was detected frequently in the samples. Of the 10 separate grab samples tested, 6 were toxic to either *Ceriodaphnia or Selenastrum*. The toxicity was not stable in storage and had different patterns of expression that limited the usefulness of the TIE experiments. For example, the toxicity to *Selenastrum* present in one of the February 2003 samples had disappeared before the TIE experiment was conducted and thus the cause of the toxicity could not be investigated.

In spite of the limited success of the TIE effort, information has been obtained that indicates that metal contamination is an unlikely cause of direct toxicity in San Diego Creek. Four aspects of the results that are useful for evaluating the cause of toxicity in San Diego Creek: 1) concentration of dissolved metals in the samples; 2) patterns of toxic response between the test species; 3) stability of the toxicity; and 4) TIE results.

Concentration of metals

The concentrations of Cu and Zn, the two metals most commonly associated with direct toxicity from urban creek flows, were below levels known to be toxic to the test organisms. In addition, the variation in concentration of Cu, Zn, and the other detectable metals among the samples did not correspond with variations in toxicity, further indicating that metals were not a significant cause of the toxicity observed. For example, the first March 2002 sample was not toxic, while the second sample was highly toxic to *Ceriodaphnia*. None of the metals measured in the second March 2002 sample were substantially elevated relative to the first sample and therefore were unlikely to be a cause of toxicity.

Pattern of toxic response

Three distinct patterns of toxic response were observed between the two test species. One of the March 2002 samples was acutely toxic to *Ceriodaphnia*, but produced no effect on *Selenastrum*. In contrast, the November 2002 samples were toxic to *Selenastrum*, yet produced no effect on *Ceriodaphnia* survival or reproduction. A third pattern of response was obtained for the August 2002 samples; these samples reduced *Ceriodaphnia* reproduction, but had no effect on survival. The same toxicity test procedures were used for each sample, so it can be assumed that any species-specific differences to individual contaminants (e.g., zinc or diazinon) would be similar among the five batches of tests. If all of the variation in toxicity observed in this study were due to changes in the concentration of the same toxicant in each sample, then a consistent pattern (e.g., *Ceriodaphnia* response always greater than *Selenastrum* response) would be expected to occur whenever toxicity was present. The presence of three very distinct patterns of relative toxic response suggests that multiple causes of toxicity were present.

Stability of toxicity
A strong decline in the magnitude of toxicity within a week of storage time was observed for the March 2002 and February 2003 samples. Metals are relatively stable in solution for a few days. Thus, the relatively rapid decline in toxicity observed for some samples suggests that the principal toxicant was degradable and/or relatively hydrophobic. These characteristics are more typical of organics, rather than the types of metals usually associated with short-term toxic effects.

TIE results
The partial TIE treatment (EDTA addition) applied to the August 2002 samples resulted in a reduction in reproductive toxicity to *Ceriodaphnia*. These results are suggestive of at least a partial role for metals in the toxicity of the two August 2002 samples, but the analytical chemistry data do not support this conclusion. Concentrations of metals were relatively low in both of these samples.

Two other potential causes of toxicity in San Diego Creek may be present: elevated dissolved solids and unmeasured pesticides. All of the low flow samples from San Diego Creek contained relatively high conductivities of 2440-3110 µS, possibly due to chlorides and other anions. Elevated conductivity (>2000 µS) in San Diego Creek has been observed in previous studies and associated as a contributor to the dry weather toxicity present in some samples from the watershed (Lee and Taylor 2001). Recent SCCWRP research in the Malibu Creek watershed has measured toxicity to *Ceriodaphnia* reproduction that appears to be related to similar conductivities (Brown and Bay 2003). *Ceriodaphnia* is relatively sensitive to dissolved solids. A laboratory experiment conducted as part of the Malibu Creek watershed study showed that a strong reduction in *Ceriodaphnia* reproduction, but little effect on survival, occurred at conductivities of >1890 µS. There is no consistent relationship between toxicity and conductivity in the San Diego Creek samples, but elevated conductivity may have contributed to the toxicity present in the August 2002 samples; these samples had conductivities of 2950 µS and 2940 µS.

There is ample evidence that pesticides other than diazinon and chlorpyrifos may have been present in the San Diego Creek samples. Monthly monitoring by the California Department of Pesticide Regulation (CDPR) as part of the Red Imported Fire Ant (RIFA) control project since March 1999 has detected a number of pesticides in San Diego Creek and its tributaries (www.cdpr.ca.gov/docs/rifa/reports.htm). These studies have detected bifenthrin, malathion, dimethoate, and fonofos in samples from San Diego Creek; concentrations of these compounds were at levels likely to cause toxicity to *Ceriodaphnia* in some samples. Toxicity identification studies conducted by Lee and Taylor (2001) concluded that the unknown toxicants in some samples from San Diego Creek were nonpolar and possessed characteristics indicative of pyrethroids.

The results of the present study are in general agreement with prior studies by Lee and Taylor (2001) and indicate that metals are not likely to be a substantial cause of short-term aquatic life toxicity in San Diego Creek. This study found less toxicity to *Ceriodaphnia* and lower concentrations of organophosphate pesticides compared to previous analyses by Lee and Taylor (2001) and CDPR. Prior monitoring has detected diazinon and chlorpyrifos in the majority of the dry and wet weather samples from San

Diego Creek, with diazinon concentrations in the range of 100-400 ng/L. This trend may be evidence that load reduction efforts are having an effect.

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TABLE 1. Summary of sampling and analysis activities at San Diego Creek. Two samples were collected for analysis on each of the five dates.

Analysis	Wet Weather			Dry Weather	
	3/7/2002	11/8/2002	2/11/2003	5/2/2002	8/12/2002
Ceriodaphnia toxicity	X	X	X	X	X
Ceriodaphnia TIE (EDTA)		X		X	X
Selenastrum toxicity	X	X	X		
Selenastrum TIE (Phase I)			X		
Total and dissolved metals	X	X		X	X
Diazinon and other op pesticides	X	X		X	X

TABLE 2. Constituents measured in samples from San Diego Creek.

Constituent	Units	MDL	Constituent	Units	MDL
Metals			**OP Pesticides**		
Aluminum	µg/L	5.0	Bolstar (Sulprofos)	ng/L	10
Antimony	µg/L	0.5	Chlorpyrifos	ng/L	10
Arsenic	µg/L	0.5	Coumaphos	ng/L	10
Barium	µg/L	0.5	Demeton	ng/L	10
Beryllium	µg/L	0.5	Diazinon	ng/L	10
Cadmium	µg/L	0.1	Disulfoton	ng/L	10
Chromium	µg/L	0.5	Ethoprop (Ethoprofos)	ng/L	10
Cobalt	µg/L	0.5	Fenchlorophos (Ronnel)	ng/L	10
Copper	µg/L	0.5	Fensulfothion	ng/L	10
Iron	µg/L	5.0	Fenthion	ng/L	10
Lead	µg/L	0.5	Guthion	ng/L	10
Manganese	µg/L	0.5	Merphos	ng/L	10
Mercury	µg/L	0.1	Methyl Parathion	ng/L	10
Molybdenum	µg/L	0.5	Mevinphos (Phosdrin)	ng/L	10
Nickel	µg/L	0.5	Phorate	ng/L	10
Selenium	µg/L	0.5	Tetrachlovinphos (Stirofos)	ng/L	10
Silver	µg/L	0.1	Tokuthion	ng/L	10
Strontium	µg/L	1.0	Trichloronate	ng/L	10
Thallium	µg/L	0.5			
Tin	µg/L	0.5			
Titanium	µg/L	0.5			
Vanadium	µg/L	0.5			
Zinc	µg/L	0.5			
Water Quality					
TSS	mg/L				
Alkalinity	mg/L				
Hardness	mg/L				
pH					

TABLE 3. Summary of toxicity results from sampling activities at San Diego Creek. Samples are indicated as toxic if at least one concentration was found to be significantly reduced from the control. Two samples were collected for analysis on each of the five dates and are listed in the table as 3 and 4.

Analysis	Wet Weather						Dry Weather			
	3/7/2002		11/8/2002		2/11/2003		5/2/2002		8/12/2002	
	1	2	1	2	1	2	1	2	1	2
Ceriodaphnia survival	NT	TOX	NT	NT	NT	NT	NT	NT	NT	NT
Ceriodaphnia reproduction	NT	TOX	NT	NT	NA	NA	NT	NT	TOX	TOX
Selenastrum cell growth	NT	NT	TOX	TOX	TOX	NT	NA	NA	NA	NA

NT= Not toxic; TOX= Toxic; NA= Not analyzed

TABLE 4. Results of *Ceriodaphnia dubia* survival and reproduction tests on wet and dry weather samples from San Diego Creek. All tests results are for chronic, 7 day toxicity testing, unless otherwise noted. Reproduction was not measured on the February, 2003 samples.

	Survival	Number of Offspring	
Sample	Mean	Mean	SD
March 7, 2002 (Wet)			
Control	100	16	6.6
1100 hrs 50%	100	28#	5.1
1100 hrs 100%	100	30#	3.5
1425 hrs 50%	100	31#	7.7
1425 hrs 100%	0*	4*	2.9
Control (Acute, 7 days)	95	na	
1425 hrs 100% (Acute, 7 days)	20*	na	
May 2, 2002 (Dry)			
Control	100	31	6.4
0900 hrs 50%	90	28	6.3
0900 hrs 100%	100	29	5.6
1405 hrs 50%	100	30	5.1
1405 hrs 100%	100	26	6.9
EDTA Control 60 mg/L	80	0	0.0
0900 hrs 50% EDTA 60 mg/L	90	2	2.2
0900 hrs 100% EDTA 60 mg/L	100	16	8.2
1405 hrs 50% EDTA 60 mg/L	100	1	1.5
1405 hrs 100% EDTA 60 mg/L	100	8	5.9
August 12, 2002 (Dry)			
Control	100	40	4.5
0922 hrs 50%	90	26*	12.9
0922 hrs 100%	100	25*	8.0
1400 hrs 50%	90	31*	12.9
1400 hrs 100%	80	27*	14.2
EDTA Control 8 mg/L	100	34	6.7
0922 hrs 50% EDTA 8 mg/L	100	38	4.3
0922 hrs 100% EDTA 8 mg/L	100	33	7.0
1400 hrs 50% EDTA 8 mg/L	100	36	4.1
1400 hrs 100% EDTA 8 mg/L	100	36	4.7
November 8, 2002 (Wet)			
Control	100	33	3.0
0100 hrs 50%	100	38#	2.6
0100 hrs 100%	100	34	4.9
0430 hrs 50%	100	39#	3.8
0430 hrs 100%	100	34	2.1
EDTA Control 8 mg/L	100	22	8.1
0100 hrs 50% EDTA 8 mg/L	100	38#	4.2
0100 hrs 100% EDTA 8 mg/L	100	39#	4.6
0430 hrs 50% EDTA 8 mg/L	100	39#	3.7
0430 hrs 100% EDTA 8 mg/L	100	37#	3.2
February 11, 2003			
Control	100		
0906 hrs 50%	80		
0906 hrs 100%	95		
February 12, 2003			
Control	95		
1910 hrs 50%	90		
1910 hrs 100%	100		

*Significantly reduced from control ($p<0.05$).
#Significantly greater than the control ($p<0.05$).

TABLE 5. Results of *Selenastrum capricornutum* phytoplankton growth tests on wet weather samples from San Diego Creek.

Sample	Mean Cell Counts	SD
March 7, 2002		
Control	1762500	184640
1100 hrs 50% Filtered	4540000	3068041
1100 hrs 100% Filtered	4807500#	136725
1100 hrs 100% Unfiltered	1807500	78048
Control	1762500	273046
1425 hrs 50% Filtered	3735000#	357440
1425 hrs 100% Filtered	3862500#	570530
1425 hrs 100% Unfiltered	1775000	122617
November 8, 2002		
Control	174000	18384
0100 hrs 50% Filtered	242750	80559
0100 hrs 100% Filtered	37750*	9105
0100 hrs 100% Unfiltered	182500	34346
Control	137000	23452
0430 hrs 50% Filtered	127500	44784
0430 hrs 100% Filtered	34500*	5196
0430 hrs 100% Unfiltered	168750	30891
February 11, 2003		
Control	1600000	341000
0906 hrs 50% Filtered	500000*	168000
0906 hrs 100% Filtered	540000*	346000
0906 hrs 100% Unfiltered	500000*	114000
February 12, 2003		
Control	800000	89000
1910 hrs 50% Filtered	1810000#	92000
1910 hrs 100% Filtered	2050000#	434000
1910 hrs 100% Unfiltered	2150000#	177000

*Significantly reduced from control ($p<0.05$).
#Significantly greater than the control ($p<0.05$).

TABLE 6. Concentrations of constituents of concern in San Diego Creek samples.

Constituent	Units	MDL	3/7/2002 (wet)		5/2/2002 (dry)		8/12/2002 (dry)		11/8/2002 (wet)	
			1100 hrs	1425 hrs	0900 hrs	1405 hrs	0922 hrs	1400 hrs	0100 hrs	0430 hrs
Total Suspended Solids	mg/L		62	38	80	83	61	41	48.9	55.3
Alkalinity	mg/L		129	85	261	232	282	293	293	292
Hardness	mg/L		393	183	681	666	524	692	768	783
Conductivity	µS		1508	970	2440	2450	2950	2940	3110	3060
Cadmium - total	µg/L	0.1	0.15	0.18	0.15	0.17	0.14	0.10	0.12	0.15
Cadmium - dissolved	µg/L	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.10	<0.1	<0.1
Chromium – total	µg/L	0.5	5.01	2.22	2.16	2.16	1.16	<0.5	1.72	1.80
Chromium - dissolved	µg/L	0.5	3.86	1.57	0.72	0.70	<0.5	<0.5	0.86	1.06
Copper - total	µg/L	0.5	16.0	15.4	4.67	6.26	3.68	3.07	3.50	3.57
Copper - dissolved	µg/L	0.5	10.4	10.5	2.89	2.71	1.66	1.98	1.85	1.79
Lead – total	µg/L	0.5	2.00	2.52	1.49	1.64	1.16	0.86	1.22	1.22
Lead - dissolved	µg/L	0.5	0.31[a]	0.52	0.20[a]	0.20[a]	<0.5	<0.5	<0.5	<0.5
Nickel – total	µg/L	0.5	5.07	4.71	4.01	3.94	3.31	3.12	3.84	3.97
Nickel - dissolved	µg/L	0.5	4.15	3.89	3.09	2.98	2.84	2.98	3.24	3.22
Selenium - total	µg/L	0.5	10.70	4.09	18.5	19.6	17.7	16.3	24.5	25.1
Selenium - dissolved	µg/L	0.5	10.20	4.13	18.6	18.5	16.8	16.5	23.2	24.1
Zinc - total	µg/L	0.5	54.6	50.0	10.80	12.7	6.66	7.12	9.68	10.3
Zinc - dissolved	µg/L	0.5	30.5	27.8	4.33	4.36	2.10	2.16	3.26	3.09
Diazinon	ng/L	10	<10	<10	<10	<10	<10	<10	34.9	33.2
Trichloronate	ng/L	10	<10	<10	<10	<10	<10	<10	25.5	<10

[a] Reported concentration is below stated detection limit, but appears to be a valid value.

TABLE 7. Comparison of average total metal concentration in San Diego Creek samples among sampling times. Results are shown for a one-way analysis of variance test of the means for each metal and a multiple comparison test of the means. Within each metal, means with a different letter designation are significantly different (Tukey multiple comparison using alpha=0.01)

Constituent	ANOVA p	Average Concentration (µg/L) and Group Designation			
		3/7/2002	5/2/2002	8/12/2002	11/8/2002
Cadmium - total	0.272	0.16 A	0.16 A	0.12 A	0.14 A
Chromium - total	0.181	3.62 A	2.16 A	0.70 A	1.76 A
Copper - total	<0.001	15.7 A	5.5 B	3.4 B	3.5 B
Lead - total	0.017	2.26 A	1.56 A	1.01 A	1.22 A
Nickel - total	0.002	4.89 A	3.98 A B	3.22 B	3.90 A B
Selenium - total	0.009	7.40 B	19.05 A B	17.00 A B	24.80 A
Zinc - total	<0.001	52.3 A	11.8 B	6.9 B	10.0 B

TABLE 8. Previously determined LC50 and EC50 concentrations for selected metals for *Selenastrum* and *Ceriodaphnia*.

Metal	Mean or Median Concentration (µg/L)*	Range (mg/L)	Data Source
Zn			
Selenastrum	60	30-70	Toussaint et al. 1995
Ceriodaphnia	76	-	Toussaint et al. 1995
Cu			
Selenastrum	40	6-70	Toussaint et al. 1995
Selenastrum	155	96-214 (+/- 1 SD)	AMEC
Ceriodaphnia	27	10-30	Toussaint et al. 1995
Ceriodaphnia	80	48-112 (+/- 1 SD)	AMEC
Cd			
Selenastrum	40	40-57	Toussaint et al. 1995
Ceriodaphnia	110	30-180	Toussaint et al. 1995

*Note that methods and duration of tests may not be identical between literature values and those used for the current study.

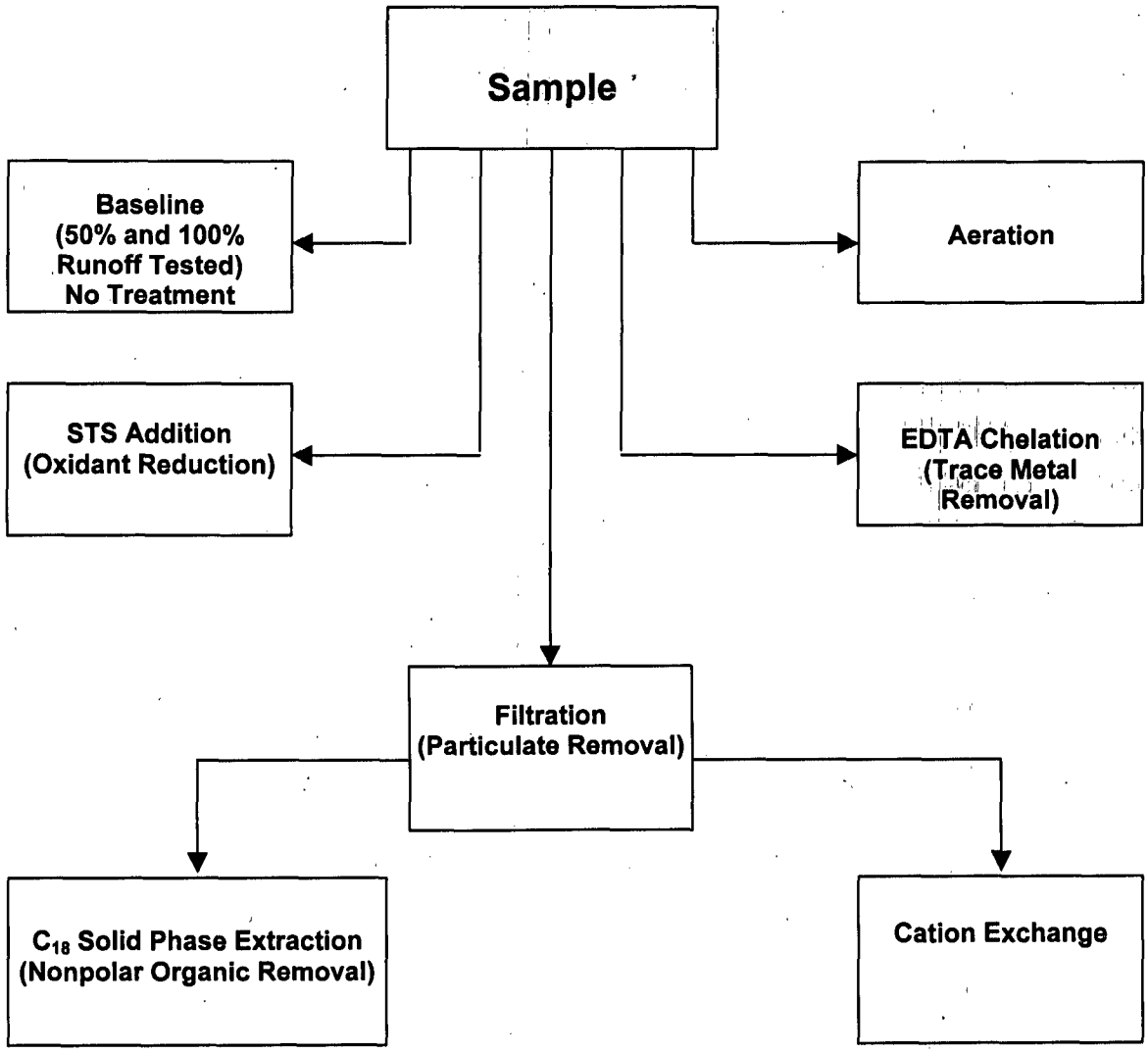

FIGURE 1. Schematic diagram of Phase I TIE sample fractionations for San Diego Creek. The shaded boxes indicate the treatments applied concurrently with initial testing with *Ceriodaphnia*.

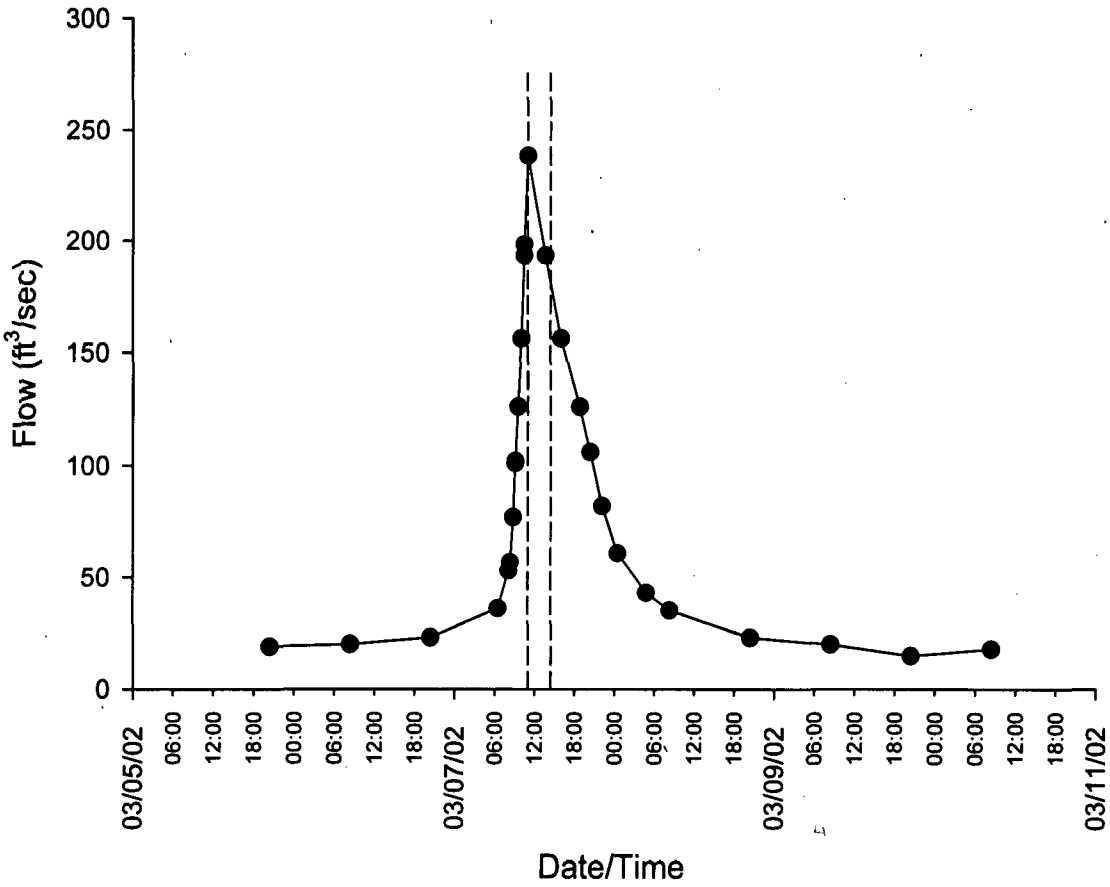

FIGURE 2. Hydrograph of San Diego Creek at Campus Drive for wet weather event in March 2002. Dashed lines indicate when samples were taken.

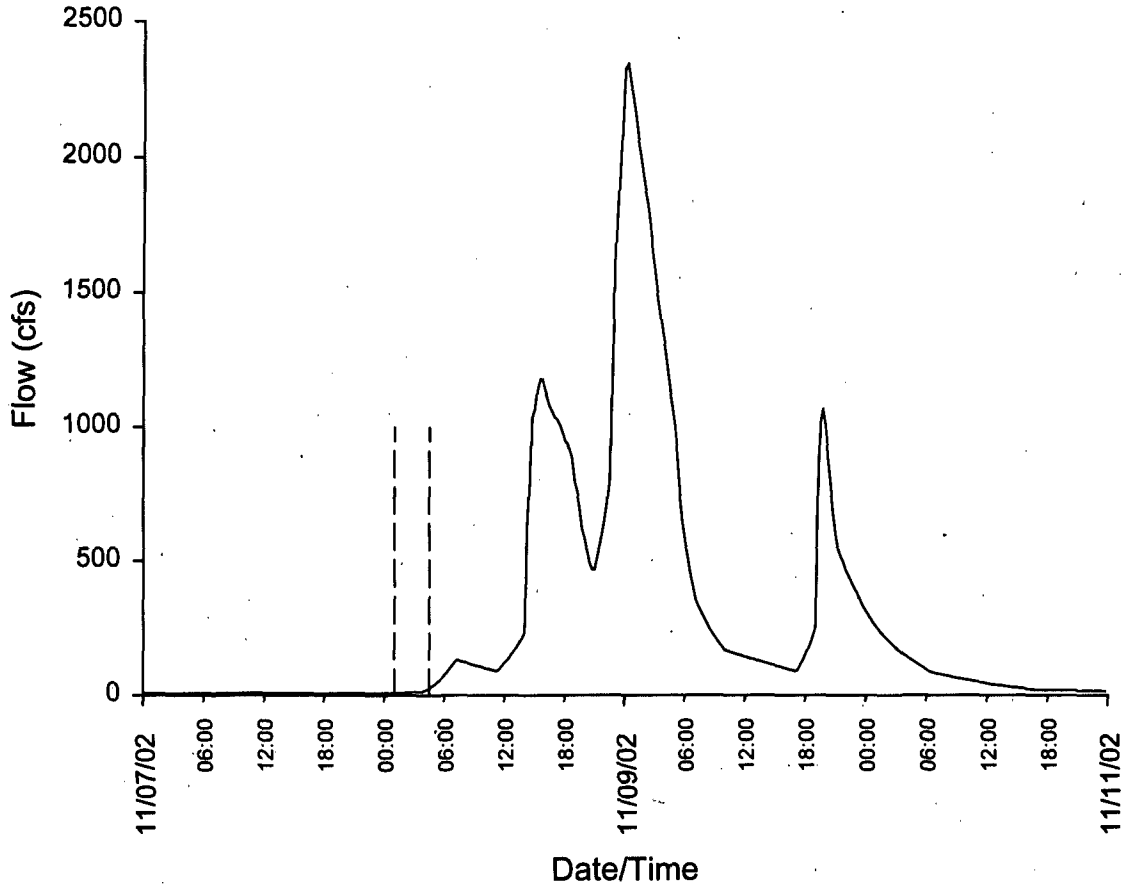

FIGURE 3. Hydrograph of San Diego Creek at Campus Drive for wet weather event in November 2002. Dashed lines indicate when samples were taken.

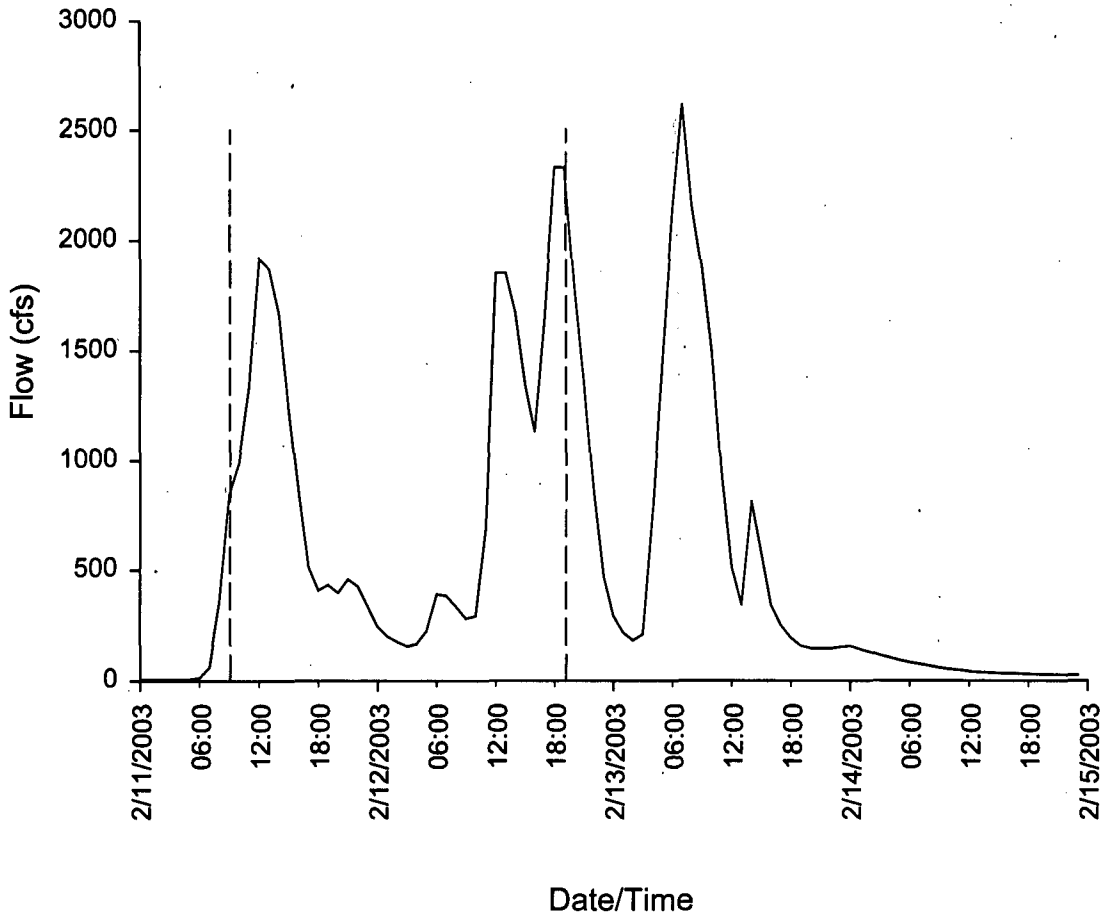

FIGURE 4. Hydrograph of San Diego Creek at Campus Drive for wet weather event in February 2003. Dashed lines indicate when samples were taken.

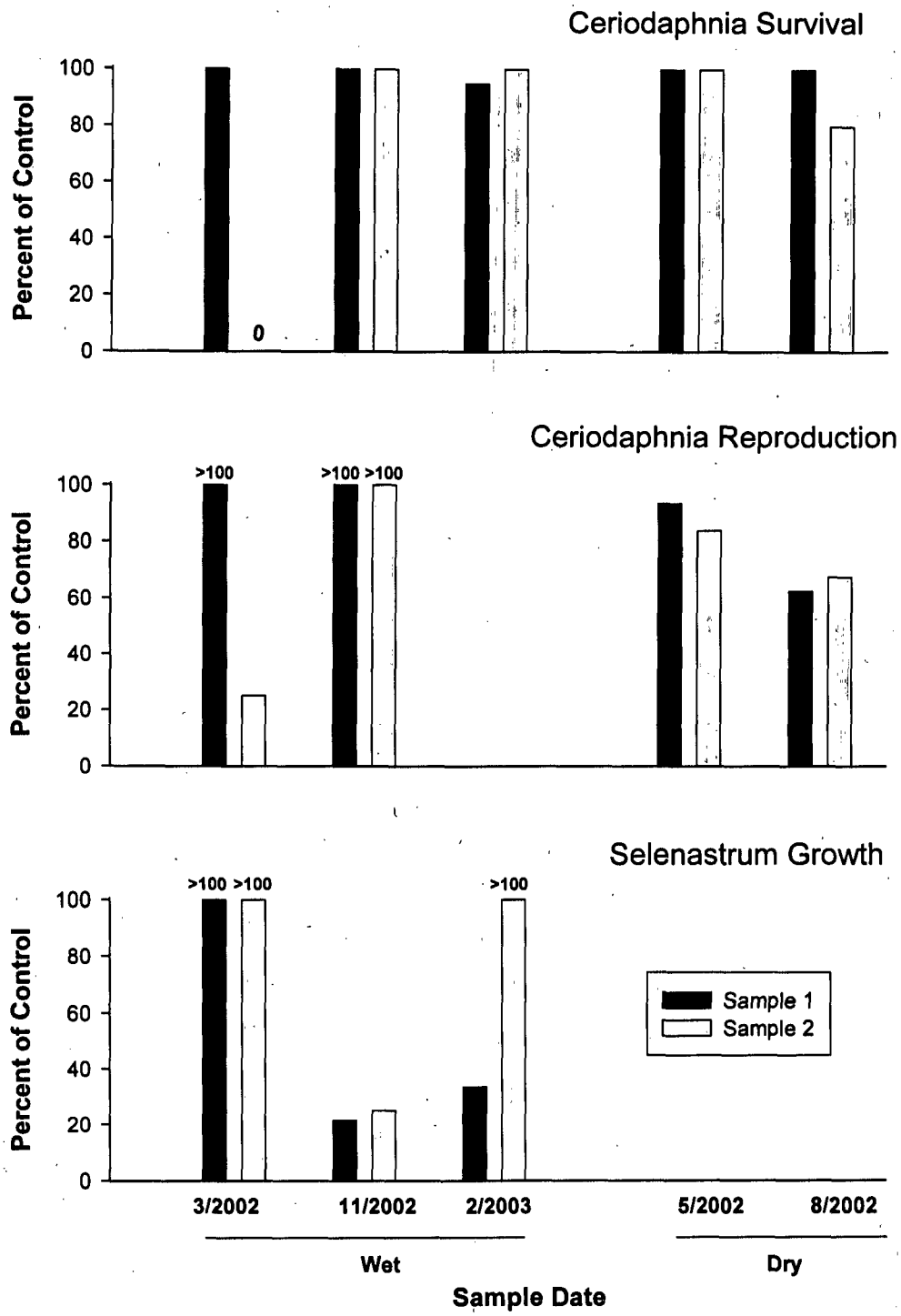

FIGURE 5. Toxicity test results for samples of wet and dry weather runoff from San Diego Creek. Results are shown for the 100% sample concentration and are expressed as a percentage of the control value. *Ceriodaphnia* reproduction was not measured for the February 2003 sample and the *Selenastrum* test was not conducted on the dry weather samples.

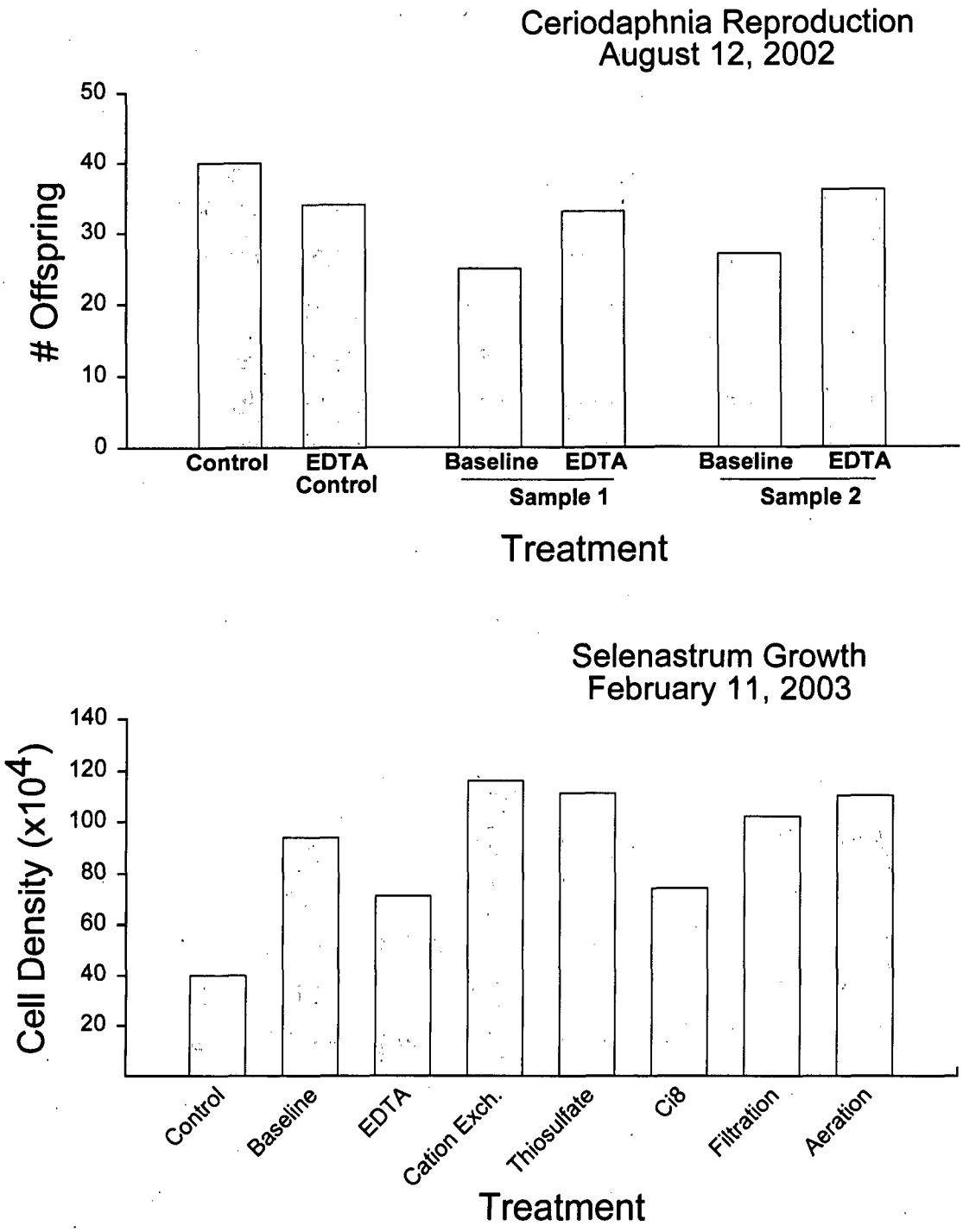

FIGURE 6. TIE results for August 2002 dry weather and February 2003 wet weather samples from San Diego Creek.

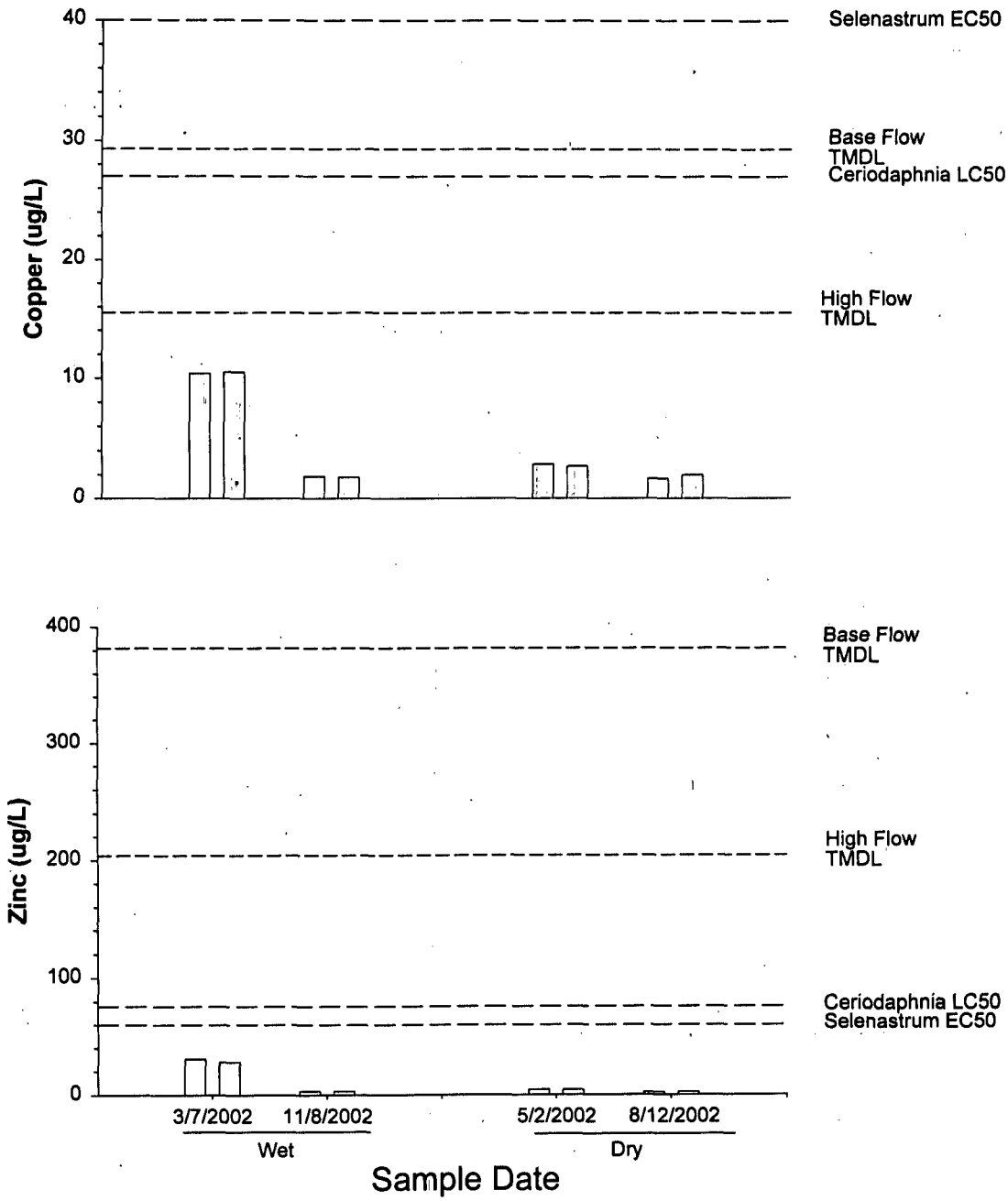

FIGURE 7. Concentrations of dissolved copper and zinc in San Diego Creek wet and dry weather samples. Dashed lines are TMDL target values (U.S. EPA 2002) or median effect concentrations for the test species derived from the literature (Toussaint *et al.* 1995)

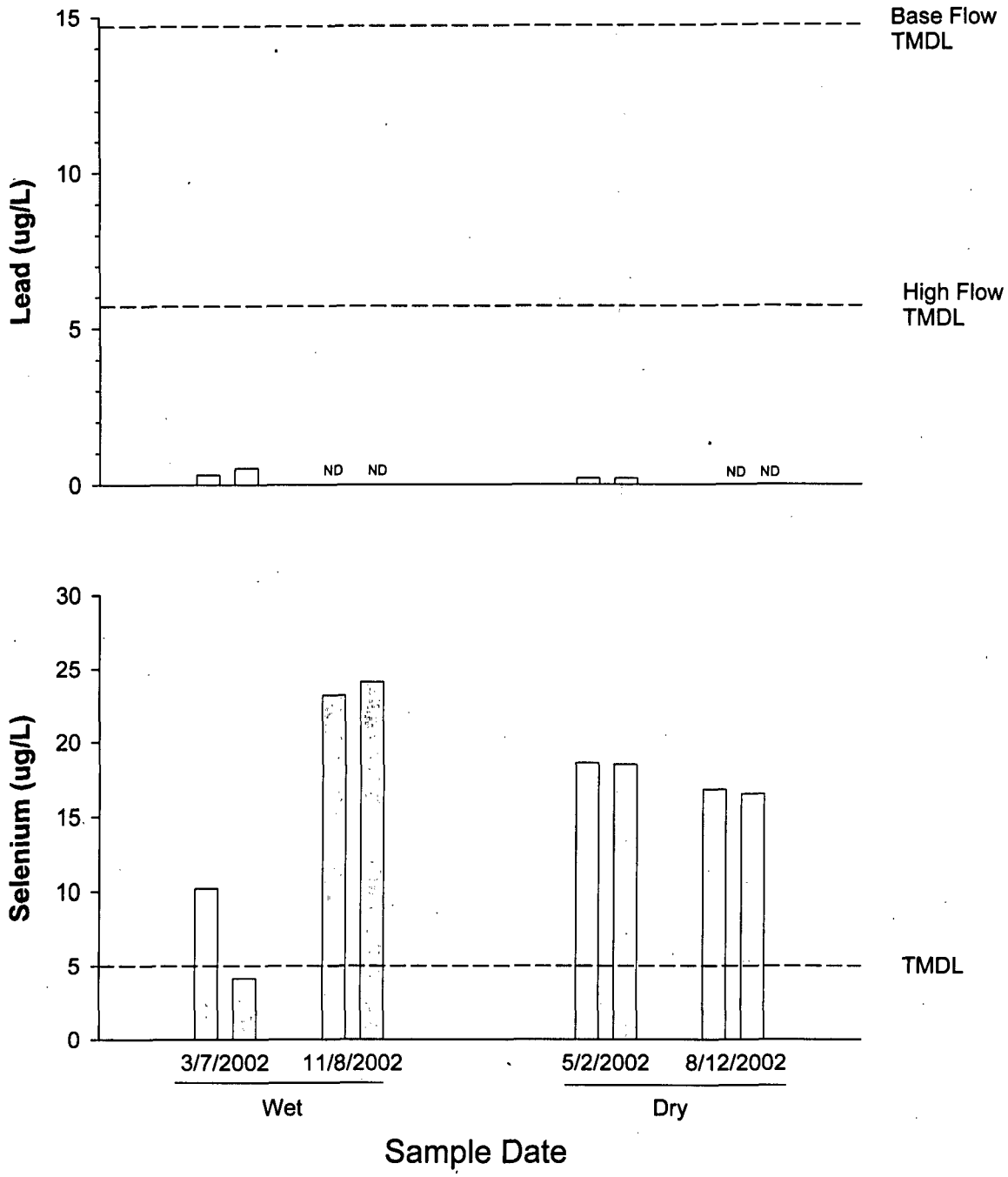

FIGURE 8. Concentrations of dissolved lead and selenium in San Diego Creek wet and dry weather samples. Dashed lines are TMDL target values.

APPENDIX

Chemistry and Toxicity Analysis Results for San Diego Creek Samples.

Concentrations of total and dissolved metals from San Diego Creek wet weather runoff samples collected March 7, 2002. All metals data is expressed in μg/L.

		1100 Sample		1425 Sample	
Element	MDL	Total	Dissolved	Total	Dissolved
Aluminum	5.0	1250	157	857	259
Antimony	0.5	1.6	1.39	2.12	1.88
Arsenic	0.5	4.35	3.77	3.88	3.61
Barium	0.5	46.1	33.9	35	24.8
Beryllium	0.5	<0.5	<0.5	<0.5	<0.5
Cadmium	0.1	0.15	<0.1	0.18	<0.1
Chromium	0.5	5.01	3.86	2.22	1.57
Cobalt	0.5	0.17	<0.5	<0.5	<0.5
Copper	0.5	16.0	10.4	15.4	10.5
Iron	5.0	991	203	789	234
Lead	0.5	2.00	0.31	2.52	0.52
Manganese	0.5	115	83.0	63.7	42.2
Mercury	0.1	<0.1	<0.1	<0.1	<0.1
Molybdenum	0.5	17.7	21.4	8.77	10.1
Nickel	0.5	5.07	4.15	4.71	3.89
Selenium	0.5	10.70	10.20	4.09	4.13
Silver	0.1	<0.1	<0.1	<0.1	<0.1
Strontium	1.0	862	841	379	408
Thallium	0.5	<0.5	<0.5	<0.5	<0.5
Tin	0.5	0.11	<0.5	0.16	<0.5
Titanium	0.5	45.3	11.4	42.1	15.6
Vanadium	0.5	12.70	10.10	10.30	8.93
Zinc	0.5	54.6	30.5	50.0	27.8
TSS mg/L		62		38	
Alkalinity (mg/L)		129		85	
Hardness (mg/L)		393		183	
pH		7.66		7.57	

Concentrations of total and dissolved metals from San Diego Creek dry weather runoff samples collected May 2, 2002. All metals data is expressed in µg/L.

	0900 Sample		1405 Sample	
Element	Total	Dissolved	Total	Dissolved
Aluminum	1080	204	1090	196
Antimony	1.32	1.45	1.33	1.47
Arsenic	6.46	5.30	6.40	5.22
Barium	74.0	58.7	73.8	58.2
Beryllium	<0.5	<0.5	<0.5	<0.5
Cadmium	0.15	<0.10	0.17	<0.10
Chromium	2.16	0.72	2.16	0.70
Cobalt	0.87	<0.50	0.91	<0.50
Copper	4.67	2.89	6.26	2.71
Iron	1340	281	1390	279
Lead	1.49	0.20	1.64	0.20
Manganese	130	15.2	121	19
Mercury	<0.1	<0.1	<0.1	<0.1
Molybdenum	36.7	45.8	37.1	45.8
Nickel	4.01	3.09	3.94	2.98
Selenium	18.5	18.6	19.6	18.5
Silver	<0.1	<0.1	<0.1	<0.1
Strontium	1510	1520	1490	1500
Thallium	<0.5	<0.5	<0.5	<0.5
Tin	0.16	0.15	0.16	0.13
Titanium	53.1	13.5	65.7	12.7
Vanadium	16.8	11.6	16.6	11.7
Zinc	10.80	4.33	12.70	4.36
TSS mg/L	80		83	
Alkalinity (mg/L)	261		232	
Hardness (mg/L)	681		666	
pH	8.03		8.24	

Concentrations of total and dissolved metals from San Diego Creek dry weather runoff samples collected August 12, 2002. All metals data is expressed in µg/L.

	0922 Sample		1400 Sample	
Element	Total	Dissolved	Total	Dissolved
Aluminum	596	<5.0	388	<5.0
Antimony	1.09	1.07	1.08	1.12
Arsenic	9.06	7.70	8.17	8.08
Barium	53.0	41.9	52.7	42.4
Beryllium	<0.5	<0.5	<0.5	<0.5
Cadmium	0.14	<0.10	0.10	<0.10
Chromium	1.16	<0.50	<0.50	<0.50
Cobalt	0.71	0.54	0.64	0.51
Copper	3.68	1.66	3.07	1.98
Iron	794	118	541	114
Lead	1.16	<0.50	0.86	<0.50
Manganese	90.2	22.3	69.9	19.5
Mercury	<0.1	<0.1	<0.1	<0.1
Molybdenum	54.0	57.2	51.2	55.8
Nickel	3.31	2.84	3.12	2.98
Selenium	17.7	16.8	16.3	16.5
Silver	<0.1	<0.1	<0.1	<0.1
Strontium	1630	1650	1570	1610
Thallium	<0.5	<0.5	<0.5	<0.5
Tin	0.45	0.38	0.38	0.35
Titanium	30.1	0.66	21.1	0.57
Vanadium	18.3	16.0	17.9	17.2
Zinc	6.66	2.10	7.12	2.16
TSS mg/L	61		41	
Alkalinity (mg/L)	282		293	
Hardness (mg/L)	524		692	
pH	8.35		8.56	

Concentrations of total and dissolved metals from San Diego Creek wet weather runoff samples collected November 8, 2002. All metals data is expressed in µg/L.

	0100 Sample		0430 Sample	
Element	Total	Dissolved	Total	Dissolved
Aluminum	378	20.3	460	19.3
Antimony	1.02	1.21	1.03	1.21
Arsenic	5.75	4.80	5.74	5.06
Barium	53.6	49.1	52.6	47.7
Beryllium	<0.5	<0.5	<0.5	<0.5
Cadmium	0.12	<0.1	0.15	<0.1
Chromium	1.72	0.86	1.80	1.06
Cobalt	0.66	<0.5	0.64	<0.5
Copper	3.50	1.85	3.57	1.79
Iron	669	203	744	197
Lead	1.22	<0.5	1.22	<0.5
Manganese	49.9	11.7	54.4	11.9
Mercury	<0.1	<0.1	<0.1	<0.1
Molybdenum	53.4	60.8	49.4	59.4
Nickel	3.84	3.24	3.97	3.22
Selenium	24.5	23.2	25.1	24.1
Silver	<0.1	<0.1	<0.1	<0.1
Strontium	1780	1690	1730	1660
Thallium	<0.5	<0.5	<0.5	<0.5
Tin	<0.5	<0.5	<0.5	<0.5
Titanium	18.1	2.09	16.7	2.45
Vanadium	15.9	13.0	15.9	13.0
Zinc	9.68	3.26	10.3	3.09
TSS mg/L	48.9		55.3	
Alkalinity (mg/L)	293		292	
Hardness (mg/L)	768		783	
pH	8.16		8.20	

Concentrations in of organophosphorus pesticides in runoff from San Diego Creek samples collected November 8, 2002. All concentrations are expressed in ng/L.

		3/7/2002		5/2/2002		8/12/2002		11/8/2002	
	MDL	1100 hrs	1425 hrs	0900 hrs	1405 hrs	0922 hrs	1400 hrs	0100 hrs	0430 hrs
Bolstar (Sulprofos)	10	nd[a]	nd	nd	nd	nd	nd	nd	nd
Chlorpyrifos	10	nd	nd	nd	nd	nd	nd	nd	nd
Coumaphos	10	nd	nd	nd	nd	nd	nd	nd	nd
Demeton	10	nd	nd	nd	nd	nd	nd	nd	nd
Diazinon	10							34.9	33.2
Disulfoton	10	nd	nd	nd	nd	nd	nd	nd	nd
Ethoprop (Ethoprofos)	10	nd	nd	nd	nd	nd	nd	nd	nd
Fenchlorophos (Ronnel)	10	nd	nd	nd	nd	nd	nd	nd	nd
Fensulfothion	10	nd	nd	nd	nd	nd	nd	nd	nd
Fenthion	10	nd	nd	nd	nd	nd	nd	nd	nd
Guthion	10	nd	nd	nd	nd	nd	nd	nd	nd
Merphos	10	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Parathion	10	nd	nd	nd	nd	nd	nd	nd	nd
Mevinphos (Phosdrin)	10	nd	nd	nd	nd	nd	nd	nd	nd
Phorate	10	nd	nd	nd	nd	nd	nd	nd	nd
Tetrachlovinphos (Stirofos)	10	nd	nd	nd	nd	nd	nd	nd	nd
Tokuthion	10	nd	nd	nd	nd	nd	nd	nd	nd
Trichloronate	10	nd	nd	nd	nd	nd	nd	25.5	nd

[a] Not detected in sample

Project: San Diego Creek TIE
Sample Collected: 3/7/02
Test Initiated: 3/8/02 Test Ended: 3/15/02
Experiment Number: 0203-036
Test Method: Ceriodaphnia 7 day Chronic
Species: *Ceriodaphnia dubia* Laboratory: AMEC
Supervising Technician: Chris Stransky

Survival Test

Sample	Mean Survival (%)	Number Counted	Significantly Reduced From Control
Control	100	10	
Tox 1100 hrs 50%	100	9	
Tox 1100 hrs 100%	100	9	
Tox 1425 hrs 50%	100	10	
Tox 1425 hrs 100%	0	10	*

Tox 1100 hrs NOEC>=100%
Tox 1425 hrs NOEC=50%

Reproduction Test

Sample	Mean Reproduction (# of neonates)	Standard Deviation	Number Counted	Significantly Reduced From Control
Control	16	6.6	10	
Tox 1100 hrs 50%	28	5.1	9	
Tox 1100 hrs 100%	30	3.5	9	
Tox 1425 hrs 50%	31	7.7	10	
Tox 1425 hrs 100%	4	2.9	10	*

Test met acceptability criteria for control survival (>80%) and neonate production (>15).
The reference toxicant EC50 fell within control chart limits.

Tox 1100 hrs NOEC>=100%
Tox 1425 hrs NOEC=50%

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 1100 hrs				
Test Min.	7.53	7.9	201	25.1
Test Max.	8.42	8.5	1508	25.9
Tox 1425 hrs				
Test Min.	7.47	7.9	201	25.1
Test Max.	8.16	8.8	970	25.9

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 3/7/02
Test Initiated: 3/8/02 Test Ended: 3/12/02

Experiment Number: 0203-035

Test Method: Phytoplankton Growth
Species: *Selenastrum capricornutum* Laboratory: AMEC
Supervising Technician: Chris Stransky

Cell Density (Cells/mL)

Sample	Mean Final Cell Density	Standard Deviation	Number Counted	Significantly Reduced From Control
Control	1762500	184640	4	
Tox 1100 hrs 50% Filtered	4540000	3068041	4	
Tox 1100 hrs 100% Filtered	4807500	136725	4	
Tox 1100 hrs 100% Unfiltered	1807500	78048	4	
Tox 1425 hrs 50% Filtered	3735000	357440	4	
Tox 1425 hrs 100% Filtered	3862500	570530	4	
Tox 1425 hrs 100% Unfiltered	1775000	122617	4	

Tests met acceptability criteria for control cell denisity (>200,000 cells/ml).

Tox 1100 hrs NOEC>=100%
Tox 1425 hrs NOEC>=100%

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 1100 hrs				
Test Min.	7.17	7.4	400	24.8
Test Max.	8.64	8.5	1749	27.1
Tox 1425 hrs				
Test min.	7.17	7.5	400	24.8
Test max.	8.12	8.4	1136	27.1

Project: San Diego Creek TIE
Sample Description: San Diego Creek Dry Weather Runoff
Sample Collected: 5/2/02
Test Initiated: 5/3/02 Test Ended: 5/10/02

Experiment Number: 0205-002 to 0205-005

Test Method: Ceriodaphnia 7 day Chronic
Species: *Ceriodaphnia dubia*
Supervising Technician: Chris Stransky

Laboratory: AMEC

Survival Test

Sample	Mean Survival (%)	Number Counted	Significantly Reduced From Control
Control	100	10	
Tox 0900 hrs 50%	90	10	
Tox 0900 hrs 100%	100	10	
Tox 1405 hrs 50%	100	9	
Tox 1405 hrs 100%	100	10	
EDTA Blank 60 mg/L	80	10	
Tox 0900 hrs 50% EDTA 60 mg/L	90	10	
Tox 0900 hrs 100% EDTA 60 mg/L	100	10	
Tox 1405 hrs 50% EDTA 60 mg/L	100	10	
Tox 1405 hrs 100% EDTA 60 mg/L	100	10	

Tox 0900 Hrs NOEC >= 100%
Tox 1405 Hrs NOEC >= 100%

Reproduction Test

Sample	Mean Reproduction (# of neonates)	Standard Deviation	Number Counted	Significantly Reduced From Control
Control	31	6.4	10	
Tox 0900 hrs 50%	28	6.3	10	
Tox 0900 hrs 100%	29	5.6	9	
Tox 1405 hrs 50%	30	5.1	9	
Tox 1405 hrs 100%	26	6.9	10	
EDTA Blank 60 mg/L	0	0	10	
Tox 0900 hrs 50% EDTA 60 mg/L	2	2.2	10	
Tox 0900 hrs 100% EDTA 60 mg/L	16	8.2	10	
Tox 1405 hrs 50% EDTA 60 mg/L	1	1.5	10	
Tox 1405 hrs 100% EDTA 60 mg/L	8	5.9	10	

Test met acceptability criteria for control survival (>80%) and neonate production (>15). The reference toxicant EC50 fell within control chart limits. No significance testing was performed on EDTA treated samples due to poor reproduction on the control animals.

Tox 0900 Hrs NOEC >= 100%
Tox 1405 Hrs NOEC >= 100%

Project: San Diego Creek TIE
Sample Description: San Diego Creek Dry Weather Runoff
Sample Collected: 5/2/02
Test Initiated: 5/3/02 Test Ended: 5/10/02

Experiment Number: 0205-002 to 0205-005

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 0900 hrs.				
Test Min.	8.15	7.2	1191	24
Test Max.	8.3	11.2	2440	25
Tox 1405 hrs.				
Test Min.	8.17	7.3	1216	24
Test Max.	8.7	11.6	2450	25.3

Project: San Diego Creek TIE
Sample Description: San Diego Creek Dry Weather Runoff
Sample Collected: 8/12/02
Test Initiated: 8/13/02 Test Ended: 8/20/02

Experiment Number: 0208-062 to 0208-065

Test Method: Ceriodaphnia 7 day Chronic
Species: *Ceriodaphnia dubia* Laboratory: AMEC
Supervising Technician: Chris Stransky

Survival Test

Sample	Mean Survival (%)	Number Counted	Significantly Reduced from Control
Control	100	10	
Tox 0922 hrs 50%	90	10	
Tox 0922 hrs 100%	100	10	
Tox 1400 hrs 50%	90	10	
Tox 1400 hrs 100%	80	10	
EDTA Blank 8 mg/L	100	10	
Tox 0922 hrs 50% EDTA 8 mg/L	100	10	
Tox 0922 hrs 100% EDTA 8 mg/L	100	10	
Tox 1400 hrs 50% EDTA 8 mg/L	100	10	
Tox 1400 hrs 100% EDTA 8 mg/L	100	10	

Tox 0922 Hrs NOEC >= 100%
Tox 1400 Hrs NOEC >= 100%

Reproduction Test

Sample	Mean Reproduction (# of neonates)	Standard Deviation	Number Counted	Significantly Reduced from Control
Control	40	4.5	10	
Tox 0922 hrs 50%	26	12.9	10	
Tox 0922 hrs 100%	25	8	10	
Tox 1400 hrs 50%	31	12.9	10	*
Tox 1400 hrs 100%	27	14.2	10	*
EDTA Blank 8 mg/L	34	6.7	10	
Tox 0922 hrs 50% EDTA 8 mg/L	38	4.3	10	
Tox 0922 hrs 100% EDTA 8 mg/L	33	7	10	
Tox 1400 hrs 50% EDTA 8 mg/L	36	4.1	10	
Tox 1400 hrs 100% EDTA 8 mg/L	36	4.72	10	

Test met acceptability criteria for control survival (>80%) and neonate production (>15).
The reference toxicant EC50 fell within control chart limits.

Tox 0922 Hrs NOEC >= 100%
Tox 1400 Hrs NOEC < 50%

Project: San Diego Creek TIE
Sample Description: San Diego Creek Dry Weather Runoff
Sample Collected: 8/12/02
Test Initiated: 8/13/02 Test Ended: 8/20/02

Experiment Number: 0208-062 to 0208-065

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 0922 hrs				
Test Min.	8.13	6.4	1374	24.1
Test Max.	8.79	9.7	2950	25.7
Tox 1400 hrs				
Test min.	8.12	6.3	1546	24.2
Test max.	8.72	9.7	2940	25.7

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 11/8/02
Test Initiated: 11/9/02 Test Ended: 11/16/02
Experiment Number: 0211-052 to 0211-056

Test Method: Ceriodaphnia 7 day Chronic
Species: *Ceriodaphnia dubia* Laboratory: AMEC
Supervising Technician: Chris Stransky

Survival Test

Sample	Mean Survival (%)	Number Counted	Significantly Reduced From Control
Control	100	10	
Tox 0100 hrs 50%	100	10	
Tox 0100 hrs 100%	100	10	
Tox 0430 hrs 50%	100	10	
Tox 0430 hrs 100%	100	10	
EDTA Blank 8 mg/L	100	10	
Tox 0100 hrs 50% EDTA 8 mg/L	100	10	
Tox 0100 hrs 100% EDTA 8 mg/L	100	10	
Tox 0430 hrs 50% EDTA 8 mg/L	100	10	
Tox 0430 hrs 100% EDTA 8 mg/L	100	10	

Tox 0100 Hrs NOEC >= 100%
Tox 0430 Hrs NOEC >= 100%

Reproduction Test

Sample	Mean Reproduction (# of Neonates)	Standard Deviation	Number Counted	Significantly Reduced From Control
Control	33	3.0	10	
Tox 0100 hrs 50%	38	2.6	10	
Tox 0100 hrs 100%	34	4.9	10	
Tox 0430 hrs 50%	39	3.8	10	
Tox 0430 hrs 100%	34	2.1	10	
EDTA Blank 8 mg/L	22	8.1	10	
Tox 0100 hrs 50% EDTA 8 mg/L	38	4.2	10	
Tox 0100 hrs 100% EDTA 8 mg/L	39	4.6	10	
Tox 0430 hrs 50% EDTA 8 mg/L	39	3.7	10	
Tox 0430 hrs 100% EDTA 8 mg/L	37	3.2	10	

Test met acceptability criteria for control survival (>80%) and neonate production (>15).
The reference toxicant EC50 fell within control chart limits.

Tox 0100 Hrs NOEC >= 100%
Tox 0430 Hrs NOEC >= 100%

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 11/8/02
Test Initiated: 11/9/02 Test Ended: 11/16/02

Experiment Number: 0211-052 to 0211-056

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 0100 hrs				
Test Min.	7.91	7.2	1418	24
Test Max.	8.8	9.8	3110	25.9
Tox 0430 hrs				
Test min.	7.85	7.1	1506	24
Test max.	8.62	10.2	3060	25.8

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 11/8/02
Test Initiated: 11/9/02 Test Ended: 11/13/02

Experiment Number: 0211-052

Test Method: Phytoplankton Growth
Species: *Selenastrum capricornutum* Laboratory: AMEC
Supervising Technician: Chris Stransky

Cell Density (Cells/ml)

Sample	Mean	Standard Deviation	Number Counted	Significantly Reduced from Control
Control	174000	18384	4	
Tox 0100 hrs 50% Filtered	242750	80559	4	
Tox 0100 hrs 100% Filtered	37750	9105	4	*
Tox 0100 hrs 100% Unfiltered	182500	34346	4	
Control	137000	23452	4	
Tox 0430 hrs 50% Filtered	127500	44784	4	
Tox 0430 hrs 100% Filtered	34500	5196	4	*
Tox 0430 hrs 100% Unfiltered	168750	30891	4	

Tests did not meet acceptability criteria for control cell density (>200,000 cells/ml).

Tox 0100 hrs NOEC = 50%
Tox 0430 hrs NOEC = 50%

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 0100 hrs				
Test Min.	7.19	6.7	367	25.8
Test Max.	7.99	10.5	2950	26.3
Tox 1430 hrs				
Test min.	7.18	6.5	345	25.8
Test max.	8	10.3	2910	26.3

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 2/11/03 and 2/12/03
Test Initiated: 2/12/03 and 2/13/03 Test Ended: 2/19/03 and 2/20/03
Experiment Number: 0302-053 and 0302-077

Test Method: Ceriodaphnia 7 day Chronic
Species: *Ceriodaphnia dubia* Laboratory: AMEC
Supervising Technician: Chris Stransky

Survival Test

Sample	Mean Survival (%)	Number Counted	Significantly Reduced From Control
Control	100	4	
Tox 2/11/03 0906 hrs 50%	80	4	
Tox 2/11/03 0906 hrs 100%	95	4	
Control	95	4	
Tox 2/12/03 1910 hrs 50%	90	4	
Tox 2/12/03 1910 hrs 100%	100	4	

Tox 2/11/03 0906 hrs NOEC >= 100%
Tox 2/12/03 1710 hrs NOEC >= 100%

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 2/11/03 0906 hrs				
Test Min.	7.62	6.1	188	23.8
Test Max.	8.28	8.6	844	25.1
Tox 2/12/03 1910 hrs				
Test min.	7.80	6.0	182	24.1
Test max.	8.56	9.2	301	24.9

Project: San Diego Creek TIE
Sample Description: San Diego Creek Wet Weather Runoff
Sample Collected: 2/11/03 and 2/12/03
Test Initiated: 2/12/03 and 2/13/03 Test Ended: 2/19/03 and 2/20/03
Experiment Number: 0302-054 and 0302-078
Test Method: Phytoplankton Growth
Species: *Selenastrum capricornutum* Laboratory: AMEC
Supervising Technician: Chris Stransky

Cell Density (Cells/ml)

Sample	Mean	Standard Deviation	Number Counted	Significantly Reduced from Control
Control	1596667	340777	3	
Tox 2/11/03 0906 50% Filtered	500000	168325	4	*
Tox 2/11/03 0906 100% Filtered	543333	345880	3	*
Tox 2/11/03 0906 100% Unfiltered	495000	113870	4	*
Control	795000	88881	4	
Tox 2/12/03 1910 50% Filtered	1810000	92020	4	
Tox 2/12/03 1910 100% Filtered	2050000	434436	4	
Tox 2/12/03 1910 100% Unfiltered	2152500	177280	4	

Tests met acceptability criteria for control cell density (>200,000 cells/ml).

Tox 2/11/03 0906 NOEC = <50%
Tox 2/12/03 1710 NOEC = >100%

Sample characteristics (range among treatments during test):

Sample	pH	Dissolved Oxygen (mg/L)	Conductivity (µS)	Temp (°C)
Tox 2/11/03 0906				
Test Min.	7.23	6.7	314	24.1
Test Max.	7.74	10.0	1098	26.7
Tox 2/12/03 1910				
Test min.	7.02	6.3	377	24.8
Test max.	8.02	8.8	559	26.3

Results of TIE conducted on San Diego Creek runoff from February 11, 2003 using *Selenastrum*.

Sample	Mean Cell Counts	SD
Baseline		
Control	400000	29000
50%	810000	96000
100%	940000	94000
Sodium Thiosulfate		
Control	440000	38000
100%	1110000	119000
EDTA		
Control	30000	2000
100%	710000	86000
Filtration (0.45 um)		
Control	280000	15000
50%	990000	88000
100%	1020000	91000
Aeration		
Control	460000	58000
100%	1100000	113000
C18 Extraction		
Control	330000	24000
100%	740000	94000
Cation Exchange Extraction		
Control	430000	44000
100%	1160000	73000

EFFECTS OF SELENIUM ACCUMULATION ON LARVAL RAINBOW TROUT

INTRODUCTION

Selenium (Se) is an element that occurs naturally in many soils and sediments, and is an essential micronutrient for fish, birds, humans, and many microorganisms. In experiments preformed with rats and chickens, Se has been shown to have a role in preventing dietary hepatic necrosis and exudative diathesis (Schwarz *et al.*, 1957) and its deficiency is characterized by cardiomyopathy and muscular weakness (Chen *et al.*, 1980). Despite the fact of being a required micronutrient, Se is toxic at concentrations only slightly greater than the nutritional requirements (Hilton *et al.*, 1980). Se can cause adverse effects on aquatic organisms including fish and birds that eat contaminated prey since it bioaccumulates through the food chain (Sappington, 2002).

Elevated Se water concentrations (>10 μg/L) have been found in San Diego Creek (Orange County). These concentrations exceed the 5 μg/L criteria for fresh water systems established by the U.S. EPA (CRWQCB 2001). Se levels in San Diego Creek sediment, which range between 0.2 and 3 μg/g, are similar to the values established for sediment quality that range between 2 and 4 μg/g (CRWQCB 2001). Se levels in fish from San Diego Creek vary between 2 and 7 μg/g and do not exceed the values established for the protection of human health (20 μg/g wet). However, these levels are similar to the level of concern (>4 μg/g) for toxicological effects on wildlife (CRWQCB 2001).

Se undergoes a biogeochemical cycle analogous to the sulfur cycle (Shrift, 1973). SeO_4^{2-} and SeO_3^{2-} are the most common Se oxyanions found in soil and natural waterways (Dungan and Frankenberger, 1999). Se can also undergo four types of transformations: reduction (assimilatory and dissimilatory), oxidation, methylation, and demethylation (Dungan and Frankenberger, 1999). Se exists in many natural soil and water environments around the world, but anthropogenic activities such as irrigated agriculture on Se-laden soils has created environmental problems with respect to this element.

Inorganic Se, along with other salts, can leach out of the soil and enter aquatic habitats via agricultural drainages and ground water discharges. For example, irrigation drainage waters of the San Joaquin Valley often have Se values of 140-1400 μg/L, with some local values reaching as high as 4,200 μg/L (Presser and Barnes, 1985). Once in the waterways, microbial organisms can reduce SeO_4^{2-} as a terminal electron acceptor and incorporate it into organic compounds, such as the amino acid conjugates selenomethionine and selenocysteine (Weiss *et al.*, 1965). Se passes through the food chain primarily in organic forms, such as selenomethionine and selenocysteine (Fan *et al.*, 2002). Exposure of fish to free-amino acid SeMe has been shown to closely mimic the toxicity of Se produced by the consumption of Se-contaminated prey species, thus SeMe is a good model for naturally incorporated Se in the aquatic food chain (Hamilton *et al.*, 1990). The contamination of food chain organisms with Se can have adverse

effects on fish populations (Maier and Knight 1994; Lemly 1993; Hamilton *et al.*, 1990). Reduced fish growth, whole body concentrations of Se and survival were strongly correlated with Se in diets of Chinook salmon (Hamilton *et al.*, 1990).

Despite previous research the mechanism of selenium toxicity in fish is poorly understood. It is known that flavin-containing monooxygenases (FMO) play a role in the activation of organoselenides to selenoxides in mammals initiating the oxidation and depletion of glutathione (GSH) (Chen *et al.*, 1994). GSH is a low molecular weight thiol-containing tripeptide, which has an important role in the maintenance of cell membrane integrity, drug and chemical metabolism, and protection from oxidative stress (Rogers and Hunter, 2001).

Depletion of hepatic GSH and the induction of lipid peroxidation have been observed in numerous mammalian tissues following treatment with organoselenides (Hoffman, 2002). Measurement of the ratio of reduced GSH to oxidized GSH (GSSG) and the relative amount of lipid peroxidation are sublethal endpoints that can be used to provide an indication of oxidative stress within organisms (Baker *et al.*, 1990 Jentzsch *et al.*, 1996).

Quantification of malondialdehyde (MDA) has been used in the past decades as a marker of lipid peroxidation. One of the most widely used methods involves the use of 2-thiobarbituric acid (TBA or 4,6-dihydroxy-2-mercapto-pyrimidine). The adduct formed during the reaction can be measured by spectrophotometry and by spectrofluorometry (Jentzsch *et al.*, 1996). Biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. The concentration of TBARS returns to normal levels over time, depending upon the presence of anti-oxidants. In practice, TBARS concentration is expressed in terms of malonaldehyde (MDA) equivalents (Kwon and Watts, 1964).

Organoselenides are good substrates for FMO oxidation (Goeger *et al.*, 1993). Moreover, the primary uptake of selenium in fish is through the food (Sandholm *et al.*, 1973). In addition, exposure of fish to free-amino acid SeMe has been shown to closely mimic the toxicity of Se produced by the consumption of Se-contaminated prey species, thus SeMe is a good model for naturally incorporated Se in the aquatic food chain (Hamilton *et al.*, 1990). SeMe is commonly found in aquatic animal foods (Spallholz *et al.*, 2002).

The objective of this study was to determine whether the accumulation of Se in a model fish species could potentially cause a significant impairment of fish populations in San Diego Creek (Orange County). The present study addressed this objective by exposing larval rainbow trout to dietary SeMe in a 90-day toxicity test, with food as the primary route of exposure to:

- Determine the effects of SeMe on the survival of the fish
- Determine the effects of s SeMe on larval growth

- Determine SeMe accumulation in the fish

- Assess whether SeMe exposure caused oxidative liver damage by measuring reduced and oxidized glutathione (GSH; GSSG) and thiobarbituric acid reactive substance (TBARS) levels as an endpoint of lipid peroxidation

By determining the effects of SeMe on larval trout, this project will help to demonstrate the effects that elevated concentrations of selenium could potentially have on resident fish populations in San Diego Creek.

METHODS

FISH EXPOSURE

Larval rainbow trout (24-day old) were obtained from the Mojave Hatchery (California Department of Fish and Game). Selenium (Se) spiked food was prepared by thoroughly mixing dry fish food (provided by the hatchery) with appropriate amounts of selenomethionine (SeMe) in a methanol solution to produce Se concentrations of 4.6, 12, and 18 μg/g (dry weight). The control treatment contained (fish food with no additional SeMe) contained 0.23 μg/g of Se. Each treatment had five replicates and each replicate contained 12-16 larval trout. The total number of fish used in the experiment was 300. Each fish was fed an average of 10 mg of fish food daily for 30 days, between 30 and 60 days of exposure they were fed 25 mg per fish, and fed 40 mg per fish thereafter. The analysis of the fish diet showed that other elements in the food were present at typical concentrations (Table 9).

The study took place in September to November (2001) and was conducted in aerated, replicate ten-gallon aquaria, which were partially submerged in a refrigerated water bath (at 10±2°C). Each replicate received >1 liter of 12±2°C replacement water every minute. To reduce water borne accumulation of SeMe from fish excretory products and dissolution of diet, one third of the water in each bath was replaced with filtered water on a daily basis. Se concentration in the water column was below levels of detection (0.05-0.1 μg/L) throughout the study. Water quality (pH, DO, conductivity, ammonia, and temperature) was measured weekly. Water parameters remained within normal ranges during the exposure (Table 10). Ammonia values ranged between 0 to 1.0 mg/L, pH values ranged from 7.0-8.4, and conductivity values ranged from 0.01 to 1.11 μS/cm. Temperature was constant (10±1°C).

The glass aquaria had glass lids and were exposed to partially shaded, natural light (12/12 light regime). Fish were acclimated for three days before exposure began. Wet weight and fork length of a sample of three to five fish from each aquarium were measured as controls before the exposure began.

Ten fish from each treatment (two fish per aquarium) were sampled after 30, 60 and 90 days of exposure. Their fork length, body weight, tissue Se concentration and hepatic GSH and TBARS were measured. All of the fish were euthanized with MS-222 (3-amino-benzoic acid ethyl ether, Sigma). Daily observations of mortality were made.

SELENIUM ANALYSIS

The control diet was analyzed by Calscience Environmental Laboratories for 18 elements including Se following EPA 6010B and EPA 7471A methods. Fish tissue analyses were conducted at the University of California, Riverside. Samples of trout tissue (0.5-3.5 g) were digested in 4 ml nitric acid, 4 ml hydrogen peroxide, and 2 mL DI water at 170°C and ~200 psi in a microwave digester for 30 minutes. In order to change the oxidative

state of Se to Se (VI), the samples were heated at 90°C for 20 minutes in 12 ml HCl and 0.2 ml of 0.2 M $K_2S_2O_8$ to a final HCl concentration of 6 N. The samples were analyzed in a Perkin-Elmer atomic absorption spectrophotometer. The calibration standard concentrations used were 0, 5, 10, and 15 µg/L.

The tissues from the 30 and 60-day exposure times were analyzed without the liver (preliminary studies demonstrated that they were no differences in whole body Se concentration between the samples with and without the liver). The 90-day exposure samples were analyzed as whole-body.

For quality assurance and quality control, a dried tomato leaf reference material (National Institute of Standards and Technology) and matrix spikes were analyzed with the samples. The percent recovery for the tomato leaves ranged from 68% to 85%. The percent recovery for the spiked samples was >79%. The detection limit for the Se analysis ranged from 0.003 to 0.017 µg/g. All metal concentrations from fish were reported on a wet weight basis, whereas food was measured as dry weight.

HEPATIC LIPID PEROXIDATION AND HEPATIC GSH: GSSG

Total GSH and GSSG were measured using the method of Baker *et al.,* (1990). Trout liver tissue samples were homogenized in 1:9 v/v 5 % 5-sulfosalicyclic acid (SSA) on ice. The homogenate was centrifuged and triplicate 10 µL samples of supernatant (~1.0 mg of tissue) or standard was added to a 96-well microplate. Two hundred µL of reaction buffer were then added to the wells for measurement of GSH. Reaction buffer consisted of 5.40 mL NaH_2PO_4/EDTA buffer, 2.80 mL 1 mM DTNB (0.15mM final), 3.75 mL of 1 mM NADPH (0.2mM final) and 0.050 mL of 12U GSSG reductase (1 U per mL final). The microplate was read at 10-second intervals over two minutes at 405 nm at room temperature (25°C). The GSSG assay was similar to the above, except that the assay system included 20 µL triethanolamine (10%) and 2 µL of 2-vinylpyridine (97%).

Malondialdehyde (MDA), a byproduct of lipid peroxidation was measured utilizing the thiobarbituric acid reactive substance (TBARS) assay (Jentzsch *et al.,* 1996). Trout liver tissue was homogenized in buffer containing 0.1 M Tris, 0.15 M KCl and 1 M EDTA. A 40 µL sample of homogenate (6.67 mg/ml of tissue), 60 µL cold DI water, 600 µL of 1 % phosphoric acid and 200 µL of 0.9 % thiobarbituric acid were added. The samples were then flushed with nitrogen to remove oxygen from the reaction (to prevent formation of lipid peroxides). Samples were cooled for five minutes and then extracted with 900 µL of n-butanol. A spectrophotometer was used to measure the absorption of the n-butanol fraction at 535 nm and 520 nm (to correct for the baseline absorption). MDA concentration was calculated by subtracting the absorption of each sample at 520 nm, from the absorbance of the samples at 535 nm, and dividing the result by the slope of the standard curve.

DATA ANALYSIS

To determine whether or not the health of larval rainbow trout was adversely affected by SeMe exposure, the condition index (CI) was calculated. The index was calculated from both body weight and fork length measurements as follows:

$$CI = \text{body weight}/\text{fork length}^3$$

Trout body weight, fork length, condition index, tissue Se concentration, GSH:GSSG ratio, and TBARS data were analyzed with analysis of variance (ANOVA) to determine the statistical differences between the treatments ($p<0.05$). Dunnet's test was performed if significance among different means was observed, to determine which different groups were significantly different from the control.

The Lowest Observable Effects Concentration (LOEC), was determined as the lowest food or tissue concentration producing a statistically significant adverse effect.

RESULTS

FISH SURVIVAL AND GROWTH

Most mortalities (except two) occurred in the first 17-days of exposure (Figure 9). Thirty percent of the fish died in the control treatment by the end of the experiment. The lowest selenium (Se) treatment of 4.6 μg/g presented the same 30% cumulative mortality as the control treatment. Cumulative fish mortality in the two highest treatments of 12 and 18 μg/g was lower, 6 and 13% respectively. The fish mortality did not show a significant relationship with the Se concentrations used in this experiment.

Fish growth was clearly impacted after 90 days at all Se treatments when compared to control fish. A two-way ANOVA analysis showed that both body weight and fork length were affected by the Se concentration and also by the length of exposure (concentration significance $p= 0.062$; time of exposure significance $p <0.0001$; exposure vs. Se interaction $p= 0.025$). Due to the significant interaction between exposure time and concentration, a one-way ANOVA analysis was done for each different exposure time to determine if the treatments had significant differences in body weight and fork length.

Control fish at the end of the experiment had an average fork length of 7.70 cm while the length for SeMe exposed fish ranged between 6.84 and 7.37 cm. A variable relationship between fork length and time was observed. Fish fork length increased throughout the experiment, but the rate of increase slowed markedly after 60 days in all SeMe exposed treatments (Figure 10). Fork length was significantly reduced ($p <0.05$) after 90 days in all SeMe exposed fish (Table 11).

Trout body weight at the end of the experiment averaged 5.17 g for control fish and ranged between 3.45 and 3.82 g for exposed fish (Table 11). One-way ANOVA analysis demonstrated that there were significant differences in weight after 90 days at the concentrations of 4.6, 12 and 18 μg/g of SeMe exposure ($p =0.0022$). Fish in all treatments exhibited a similar rate of weight gain during the experiment (Figure 10).

The weight of the larval fish in the two highest treatments was significantly greater than the control weight at the beginning of the experiment. To reduce the influence of these initial size differences and to improve the detection from SeMe induced growth effects, net body weight gain was calculated by subtracting the initial (day 0) average body weight of a sample of fish in each exposure from the body weight of the sampled fish at 30, 60 and 90 days of exposure. The net weight gain results showed a similar pattern of effect as for the total weight data; a significant reduction in net weight was present at 90 days for all groups of SeMe exposed fish (Figure 11).

SeMe effects on fish growth were also assessed using the calculation of the condition index (calculated from the body weight and fork length). Condition index values ranged between 0.00741 and 0.01329 g/cm^3 during the study. Significant effects were found at time 0 for the two highest concentrations of 12 and 18 μg/g, which had a higher condition

index than the control ($p < 0.05$) due to size variability among the fish populations (Table 11). The condition index decreased in SeMe exposed fish during the first 60 days and increased thereafter (Figure 12). The control fish also showed a marked increase in condition between 60 and 90 days of exposure.

Significant effects (reduced condition index relative to the control) were observed for fish in the 12 and 18 μg/g treatments after 30 days ($p < 0.0001$). However no significant differences were observed for any of the treatments at 60 days when compared to control fish ($p = 0.19$). All of the treatments were significantly different from the controls after 90 days of exposure ($p < 0.05$) (Table 11). When the condition index data within each treatment were analyzed over time, the results showed that index values at 60 days were significantly reduced compared to all the other exposure times (ANOVA: $p < 0.0001$; Dunnet's $t = 2.178$, $p = 0.05$).

Se ACCUMULATION

A positive relationship between Se dose and accumulation was observed at each sampling time (Table 12, Figure 13). Total tissue Se accumulation was not significantly different from the control fish after 30 days, although mean Se concentrations were two to four times higher than the control ($p > 0.05$). After 60 days fish exposed to the highest SeMe treatment contained a significantly higher concentration of Se in their tissue (ANOVA: $p = 0.027$; Dunnet's $t = 2.449$, $p < 0.05$). Significantly higher concentrations of Se were also measured in fish from all SeMe exposed treatments after 90 days (ANOVA: $p < 0.0001$; Dunnet's $t = 2.449$, $p < 0.05$).

The average concentration of Se in the fish tissue appeared to decrease between 60 and 90 days in all treatment groups including controls (Figure 13). Statistical analysis of the data for the control fish showed that the Se concentration in tissue at 60 days was significantly greater than the controls at 30 and 90 days. There was no significant difference between the 30 and 90 days controls. A reanalysis of the 90-day tissue samples confirmed that the values for SeMe exposed fish had decreased when compared to previous sampling periods. The 90-day reanalysis results also showed a significant dose response relative to the control; tissue concentrations in all three Se exposure groups were significantly different from the control.

HEPATIC LIPID PEROXIDATION AND HEPATIC GSH:GSSG

Lipid peroxidation and GSH:GSSG ratios were unchanged by SeMe treatment (Table 13). Lipid peroxidation after 90-days exposure ranged from 0.03 to 0.08 nmol/ml MDA Eq among the treatments. GSH: GSSG ratio ranged from 1.71 to 2.04 after 90 days of exposure. These differences were not statistically significant.

DISCUSSION

The fish survived well in the different selenium (SeMe) exposure concentrations. Most of the mortalities were registered during the first days of the study and were not proportional to the Se exposure level, suggesting that these mortalities were due to preexisting conditions or perhaps initial stress from the laboratory environment.

The GSH:GSSG ratio and the quantification of lipid peroxidation yielded no significant differences, indicating that the Se concentrations used did not cause oxidative stress. The lack of response of these sublethal endpoints indicates that the observed growth impacts were due to mechanisms other than changes in the GSH:GSSG ratio or lipid peroxidation. Although prone to selenoxide formation and GSH oxidation via flavin monoxygenase activation in vitro, GSH did not appear to be depleted in the livers of any of the exposed animals indicating either redox cycling did not occur or other cellular defenses against oxidative stress were available to prevent injury. These results contrast studies in avian species where GSH:GSSG ratios have been shown to be reduced by Se (Hoffman et al. 1989; Spallholz and Hoffman 2002; Hoffman 2002). It is well recognized that Se induces GSH peroxidase, which catabolizes lipid hydroperoxides to the corresponding lipid hydroxide (for review see Burk 1997). Therefore, one may speculate protection against lipid peroxidation may occur in Se-treated animals. However, the lack of GSH consumption in the current study is unclear and suggests the induction of other feedback loops enhancing either reducing equivalent (i.e. NADPH) or GSH synthesis. Clearly, more work is needed to understand the molecular mechanisms of selenium toxicity in non-mammalian organisms.

This study demonstrated that both the length and concentration of dietary SeMe exposure negatively impacted larval trout growth. After 90 days the fish showed reduced growth at the 4.6, 12 and 18 g concentrations, resulting in a dietary threshold for effects (LOEC) of 4.6 g. These results coincide with other studies, which have shown that rainbow trout fed 13 μg/g of selenite for 20 weeks exhibited reduced growth and reduced survival (Hilton *et al.*, 1980). Another study with rainbow trout showed that fish fed 11 μg/g (dry diet) for 16 weeks had reduced body weight when compared to those that were fed 7 μg/g of selenite (Hilton and Hodson, 1983). Hamilton *et al.* (1990) showed that dietary Se was toxic to fish in the range of 3 to 5 μg/g. A value of 4 μg/g for invertebrates has been recommended as a threshold for a hazing program for birds by the California Department of Fish and game (see Hamilton 2002 for review).

The results of the present study showed a direct relationship between Se tissue accumulation and exposure concentration. The relationship between Se accumulation and time of exposure was not as clear. The fish contained lower concentrations of total Se after 90 days when compared to tissue samples from previous exposure times. A similar trend was also seen in the controls, although there was no change in tissue Se concentration between 30 and 90 days. The data trends indicate that a physiological change may have occurred in the fish during the latter half of the experiment. Evidence

of such a change is shown by the condition index and length data, which showed abrupt changes in the control and treatment groups between 60 and 90 days of exposure.

Larval rainbow trout mature into juveniles between the sizes of 2.5 to 13 cm (Morrow, 1980), the same size range of the fish used in this experiment. Furthermore, young trout experience body elongation at 60-90 days (Westers, 2001), which becomes evident in the results from the growth condition index. The divergences in condition index and Se body burden observed between 60 and 90 days may have been the result of a change in Se uptake or excretion produced by the fish becoming juveniles during that period of time. Other researchers have observed that essential metals accumulated in fish decreased after a certain time of constant chronic exposure. Perkins *et al.* (1997) observed that hepatic concentrations of copper in catfish exposed to aqueous copper sulfate for 10 weeks reached a maximum concentration at 6 weeks and then declined. Hamilton *et al.* (1990) observed a similar phenomenon with Chinook salmon demonstrating a 20% reduction of total Se in control animals after 90 days. Since Se is an essential nutrient, its rate of uptake, metabolism, or excretion may have been influenced by physiological changes in the fish.

The growth results for the 90-day experiment indicate a body burden threshold (LOEC) of 0.51 μg/g (wet weight basis). This value is substantially below tissue residue thresholds reported by others (for review see Hamilton 2002). De Forest *et al.* (1999) reported a whole body Se tissue threshold of 3.3-4 μg/g (dry weight) for effects on early life stage rainbow trout. The threshold value obtained in the present experiment is also less than the value of 4 μg/g recently proposed as a tissue-based criteria for adverse effects in fish (Maier and Knight 1994; Hamilton 2002). The lower body burden effects concentration obtained in the present experiment may have been due to reduction of Se uptake or enhanced Se elimination at the 60-90 day period of development. Other possibilities for the lower threshold include the use of younger fish, an ambient light:dark cycle, a lack of Se in the gut contents of fish during whole body measurement (although feeding behavior was never impaired throughout the study), or the use of a wet weight determination of total Se.

Recent studies of fish tissue Se in San Diego Creek and other channels indicate that resident fish species may be impacted by selenium. Tissue Se concentrations for red shiner, measured by the Toxic Substances Monitoring Program (TSMP), ranged from 0.4 to 1.6 μg/g (wet weight), which is above the tissue effects threshold obtained for larval trout (Figure 14). The TSMP data represent adult fish of a different species, which may be more tolerant than the larval trout used in this experiment. The use of the data from this experiment to estimate the risk from Se in San Diego Creek would be aided by studies that include the measurement of Se in the larval stages and diet of resident fish.

In summary, rainbow trout experience sublethal effects on growth, weight and condition following 90 days of dietary exposure to SeMe. The dietary LOEC was 4.6 μg/g Se. The body-burden threshold value for adverse effects was 0.51 μg/g Se. Adverse effects were not related to oxidative stress as measured by hepatic lipid peroxidation and GSH:GSSG ratios indicating other mechanisms may be involved in the toxicity of Se in fish. More

studies are needed to examine the uptake and disposition of Se in its various organic forms in fish at different stages of development to better understand its toxicity in wildlife.

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TABLE 9. Trace metal analysis of the unspiked fish food used in the experiment.

Metal (µg/g)	Result	Reporting Limit
Antimony	1.90	0.75
Arsenic	ND	0.75
Barium	5.80	0.50
Beryllium	ND	0.25
Cadmium	ND	0.50
Chromium	1.13	0.25
Cobalt	0.42	0.25
Copper	21.1	0.50
Lead	ND	0.50
Mercury	ND	0.08
Molybdenum	1.76	0.25
Nickel	1.57	0.25
Selenium	0.23	0.75
Silver	ND	0.25
Thallium	ND	0.75
Vanadium	1.23	0.25
Zinc	85.0	1.00

ND= Not detected.

TABLE 10. Water quality parameters in the Se exposure aquaria. Ranges are shown in the table.

Time (Week)	pH	Ammonia (mg/L)	Conductivity (µS/cm)
1	7.0-8.2	0.0-0.50	0.01-0.05
2	7.7-8.2	0.0-1.00	0.04-0.09
3	7.8-8.3	0.0-1.00	0.03-0.09
4	7.9-8.4	0.0-1.00	0.03-0.09
5	7.7-8.2	0.0-0.05	0.02-1.00
6	7.8-8.1	0.0-0.50	0.03-1.00
7	7.7-8.1	0.0-0.50	0.03-1.00
8	7.6-8.0	0.0-0.05	0.01-0.09
9	7.7-8.1	0.0-0.00	0.01-0.12
10	7.4-7.9	0.0-0.00	0.03-0.09
12	7.5-7.8	0.0-0.05	0.03-1.00
13	7.5-8.0	0.0-0.05	0.01-1.11

TABLE 11. Rainbow trout growth after four different selenomethionine exposures (SeMe as μg/g). Average values for results at 0, 30, 60 and 90-days. Standard deviation in parenthesis.

Time (Day)	Treatment (μg/g)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)
0	Control	0.37 (0.30)	3.14 (0.41)	0.01138 (0.0004)
	4.6	0.41 (0.06)	3.17 (0.12)	0.01283 (0.0007)
	12	0.54 (0.01) *	3.44 (0.14) *	0.01329 (0.0013) *
	18	0.55 (0.05) *	3.40 (0.24)	0.01321 (0.0009) *
30	Control	1.33 (0.92)	4.66 (0.41)	0.01283 (0.0008)
	4.6	1.25 (0.21)	4.84 (0.29)	0.01085 (0.0005)
	12	1.33 (0.30)	5.09 (0.46)	0.00992 (0.0008) *
	18	1.31 (0.37)	4.97 (0.50)	0.01045 (0.0011) *
60	Control	2.96 (0.92)	6.91 (0.56)	0.00864 (0.0018)
	4.6	2.33 (0.63)	6.69 (0.67)	0.00747 (0.0002)
	12	2.52 (0.38)	6.88 (0.35)	0.00767 (0.0005)
	18	2.59 (0.24)	6.92 (0.24)	0.00741 (0.0005)
90	Control	5.17 (1.09)	7.70 (0.33)	0.01137 (0.0012)
	4.6	3.45 (0.35) *	6.93 (0.19) *	0.01020 (0.0002) *
	12	3.45 (0.35) *	6.84 (0.68) *	0.01016 (0.0002) *
	18	3.82 (0.62) *	7.37 (0.62) *	0.01019 (0.0004) *

* Statistically significant difference from control ($p \leq 0.05$)

TABLE 12. Total Se tissue concentration in µg/g wet basis (standard deviation in parenthesis).

Treatment (µg/g)	30-day	60-day	90-day	90-day Rerun
Control	0.46 (0.20)	1.24 (0.54)	0.29 (0.14)	0.32 (0.26)
4.6	1.05 (0.77)	1.70 (0.72)	0.51 (0.19) *	0.66 (0.22) *
12	1.81 (1.04)	1.83 (0.94)	1.08 (0.16) *	1.31 (0.25) *
18	1.60 (0.93)	2.62 (1.22) *	1.06 (0.19) *	1.77 (0.35) *

* Statistically significant difference from control ($p \leq 0.05$).

TABLE 13. Quantification of hepatic lipid peroxidation (TBARS) nmol/ml MDA Eq and hepatic GSH:GSSG ratio in SeMe exposed larval rainbow trout at 60 and 90 days. Standard deviation in parenthesis.

Treatment	Hepatic Lipid Peroxidation nmol/ml		Hepatic GSH: GSSG	
(µg/g)	60-day	90-day	60-day	90-day
Control	0.18 (0.13)	0.08 (0.07)	1.38 (0.31)	2.04 (0.34)
4.6	0.27 (0.05)	0.07 (0.09)	1.31 (0.37)	1.71 (0.28)
12	0.17 (0.08)	0.03 (0.02)	1.28 (0.31)	1.91 (0.07)
18	0.25 (0.28)	0.06 (0.06)	1.00 (0.04)	1.88 (0.03)

FIGURE 9. Percent mortality of rainbow trout after 90 days of exposure.

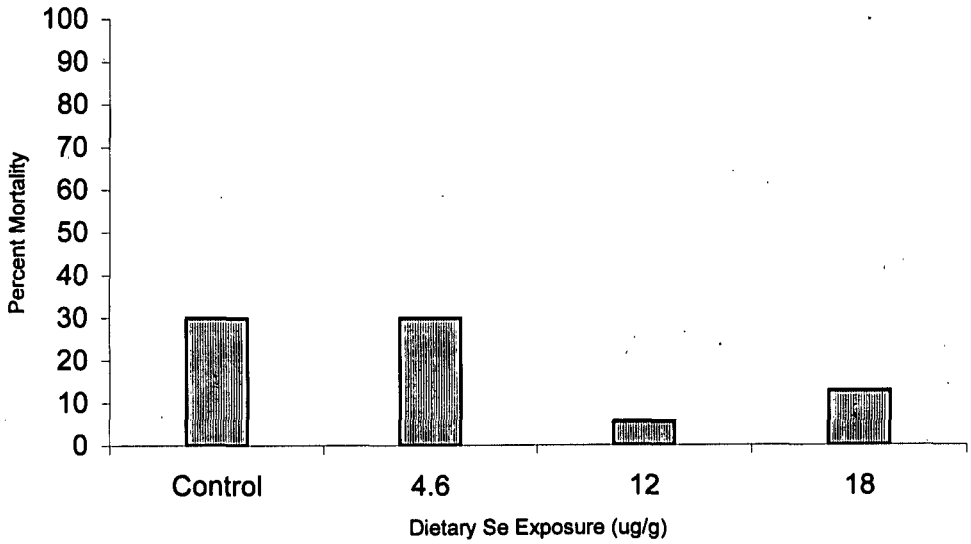


FIGURE 10. Change in rainbow trout weight (g) and fork length (cm) during 90 days for each treatment.

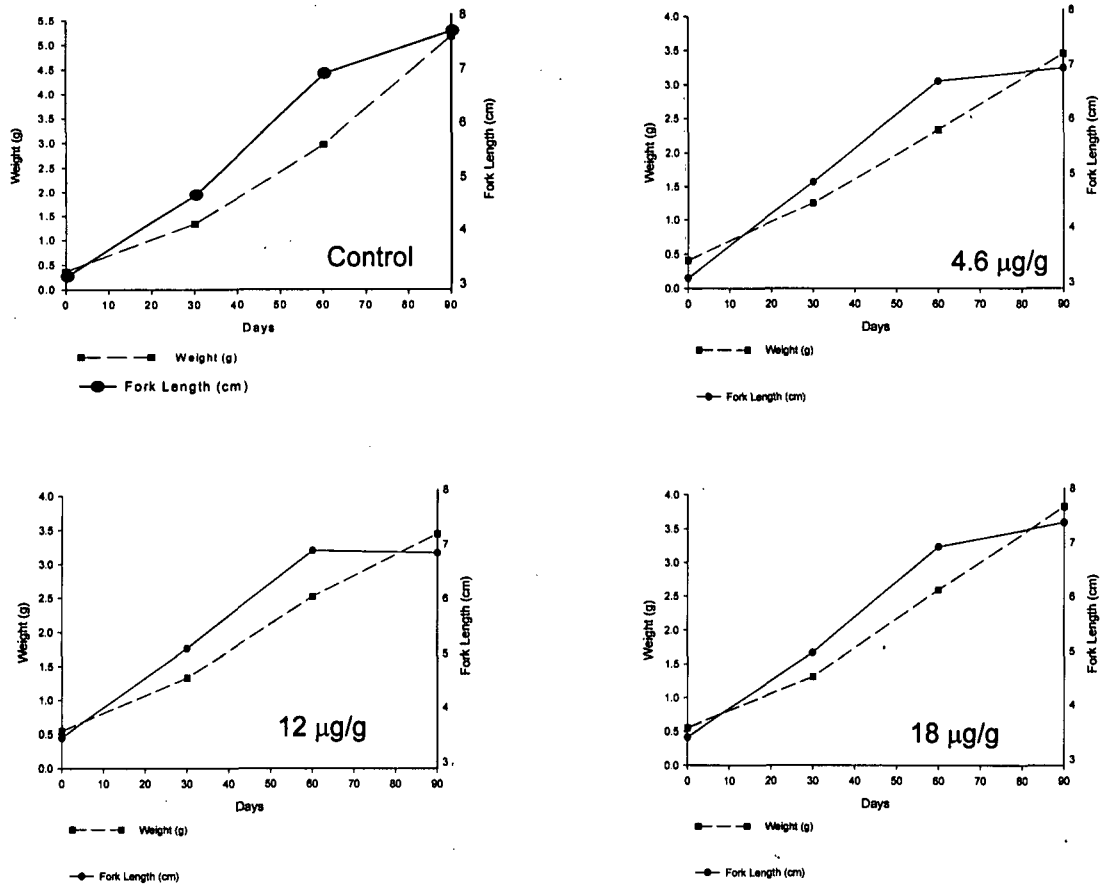
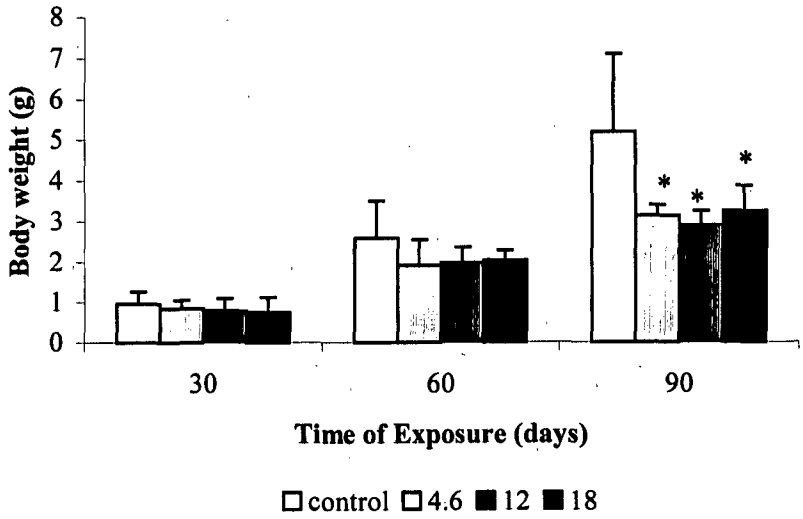


FIGURE 11. Trout growth as net change in individual body weight at different exposure lengths after exposure to Control, 4.6, 12and 18 µg/g of dietary SeMe (error bars represent the standard deviation).



*= Statistically significant difference from control ($p \leq 0.05$).

FIGURE 12. Condition index mean values for 0, 30, 60 and 90 days.

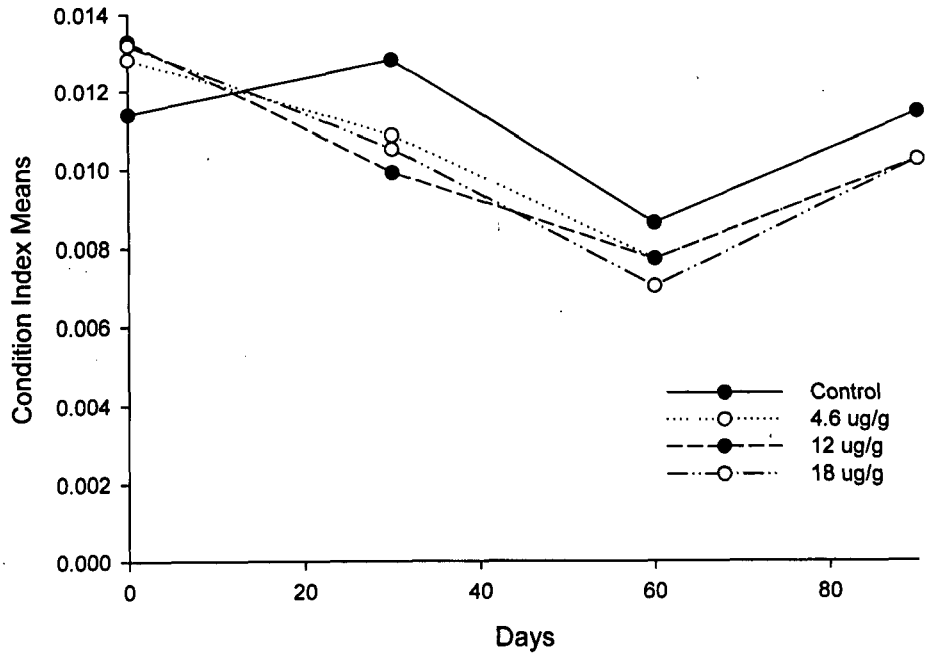
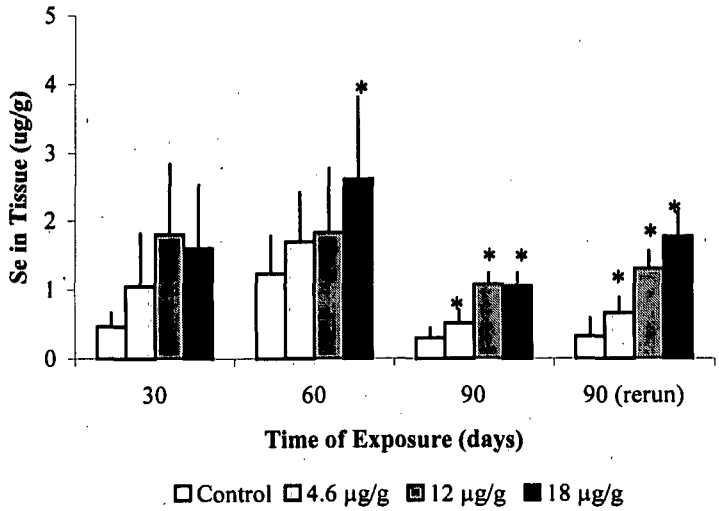
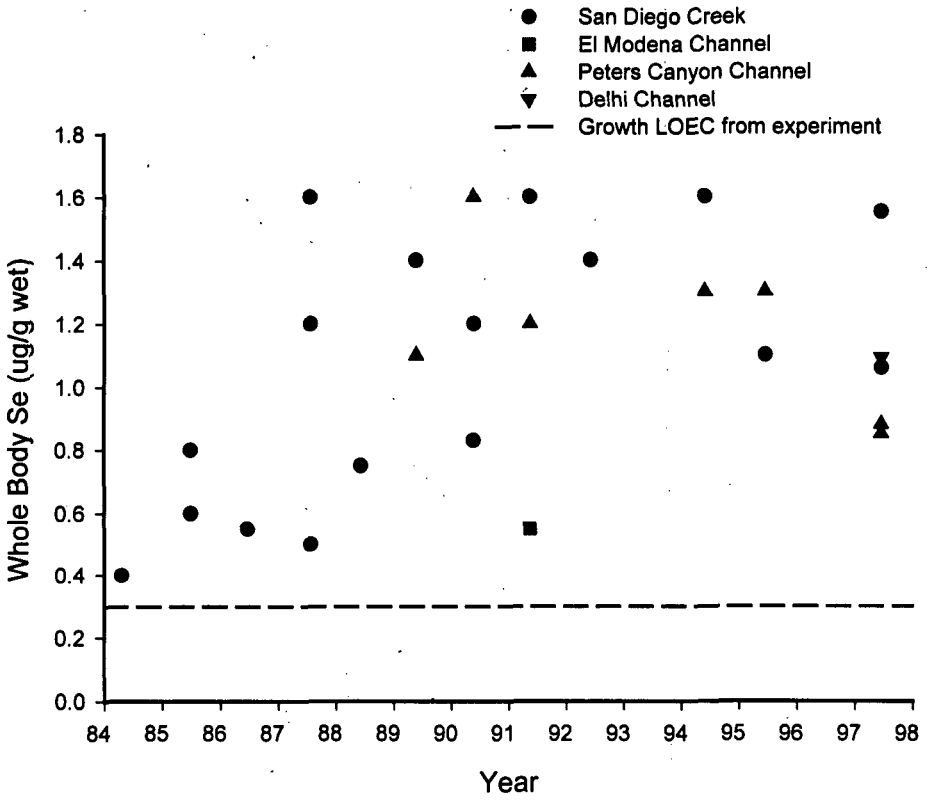


FIGURE 13. Whole body total Se concentration (μg/g tissue wet weight) in rainbow trout after 30, 60, and 90 day food SeMe exposure (error bars represent the standard deviation).



* Statistically significant difference from control ($p \leq 0.05$)

FIGURE 14. Whole body concentration of Selenium in red shiner collected from San Diego Creek and other channels in the Newport Bay watershed (data from the Toxic Substances Monitoring Program). The estimated growth LOEC from the experiment was calculated from the reported dry weight value assuming a water content of 40%.



APPENDIX

Fish Data

Individual fish values for weight, fork length and condition index after 0, 30, 60 and 90 days.

Treatment	0 Days			30 Days			60 Days			90 Days		
(µg/g)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)
	0.350	3.040	0.01186	0.900	4.10	0.01290	2.920	6.90	0.00825	5.215	7.90	0.01050
	0.384	3.280	0.01101	1.575	4.85	0.01384	4.260	7.25	0.01156	7.290	8.52	0.01162
Control	0.286	2.900	0.01083	1.400	4.80	0.01250	2.490	6.45	0.00885	5.534	8.10	0.01036
	0.343	3.070	0.01140	1.170	4.40	0.01310	1.800	6.30	0.00717	7.225	8.60	0.01300
	0.493	3.400	0.01180	1.620	5.15	0.01179	3.320	7.65	0.00738	—	—	—
	0.473	3.333	0.01230	1.090	4.55	0.01130	2.320	6.65	0.00745	3.400	6.67	0.01022
	0.393	3.170	0.01229	1.030	4.60	0.01039	1.650	5.90	0.00786	3.305	6.88	0.00989
4.6	0.327	3.000	0.01200	1.580	5.25	0.01084	1.775	6.20	0.00742	3.613	6.94	0.01014
	0.380	3.130	0.01265	1.250	4.80	0.01140	3.140	7.50	0.00723	3.968	7.22	0.01054
	0.463	3.233	0.01390	1.295	5.00	0.01030	2.750	7.20	0.00736	3.474	6.92	0.01023
	0.540	3.333	0.01390	1.070	4.80	0.00970	2.095	6.60	0.00714	2.882	6.10	0.01000
	0.560	3.700	0.01104	1.190	4.70	0.01095	2.730	7.05	0.00778	3.538	6.60	0.01036
12	0.560	3.433	0.01389	1.660	5.40	0.01055	2.625	6.80	0.00843	3.490	6.60	0.00991
	0.533	3.370	0.01395	1.660	5.75	0.00873	3.005	7.40	0.00728	3.464	7.90	0.01020
	0.517	3.370	0.01365	1.075	4.80	0.00968	2.165	6.55	0.00770	3.860	7.15	0.01033
	0.550	3.533	0.01240	1.950	5.75	0.01027	2.580	7.25	0.00675	4.553	8.20	0.00990
	0.600	3.633	0.01267	0.995	4.50	0.01024	2.515	6.75	0.00780	3.660	6.90	0.00985
18	0.437	3.570	0.01257	1.245	5.10	0.00938	2.550	6.90	0.00762	4.358	6.70	0.01071
	0.573	3.130	0.01393	1.175	4.90	0.01005	2.340	6.65	0.00791	3.140	7.40	0.01003
	0.620	3.150	0.01447	1.190	4.60	0.01230	2.990	7.05	0.00697	3.362	6.80	0.01036

Individual fish values of total selenium in tissue (wet weight).

Treatment µg/g	Replicate	Day 30	Day 60	Day 90
Control	A	0.642	1.360	0.530
	B	0.402	1.760	0.413
	C	0.503	0.520	0.140
	D	0.299	2.025	0.230
	E	0.286	1.378	0.210
	F	0.309	0.790	0.170
	G	0.797	0.874	0.370
4.6	A	1.790	4.600	0.520
	B	2.330	2.265	0.540
	C	1.200	0.719	0.540
	D	0.780	0.789	0.530
	E	0.208	2.365	0.530
	F	0.523	1.425	0.130
	G	0.534	2.288	0.770
	H		2.060	-------
12	A	0.802	2.045	1.070
	B	2.450	1.980	1.030
	C	3.335	3.712	1.460
	D	1.470	2.195	1.132
	E	2.251	1.697	1.193
	F	2.562	2.445	0.941
	G	0.597	1.440	1.000
	H	0.417	1.375	0.920
	I	2.960	1.456	0.920
	J	1.215	-------	1.120
18	A	4.000	2.678	0.770
	B	1.900	3.311	1.070
	C	1.370	2.909	1.030
	D	1.200	1.855	1.460
	E	0.730	2.835	1.132
	F	0.836	4.595	1.193
	G	1.132	2.735	0.941
	H	1.355	2.640	0.920
	I	1.727	-------	0.920
	J	1.773	-------	1.120