FINAL REPORT: Parts I-VII

Submitted to**:**
Meadowlands Environmental Research Institute

Evaluating the Effects of Contamination at Kearny Marsh

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I. Executive Summary

This document serves as Part I-VII of a final report for research conducted at Kearny Marsh and funded by MERI from June 2002 until January 2003. The overall purpose of the research was to evaluate the environmental health of the marsh with the goal of finding restoration strategies. Evaluation included measuring abiotic parameters of water (pH, Eh, temperature, salinity and dissolved oxygen), sediment (grain size, total organic carbon, acid-volatile sulfides and heavy metal content) and detritus (heavy metal content) at six sites around the marsh. It also included biomonitoring macroinvertebrates *in situ* as well as toxicity testing (96 h survival and whole body carbohydrate levels, 10 d survival and growth) and heavy metal bioaccumulation studies with sediment and detritus using a benthic macroinvertebrate, *Chironomus riparius*. Data for bioaccumulation and analyses involving integration of endpoints will be included in the final document.

Field site data indicated that low DO was the most obvious detrimental factor to marsh health. Extremely low benthic levels (0.58 to 3.44 mg/l) were found at all sites even into October. Shallowness of the marsh (8 to 29 in.) made it very sensitive to temperature fluctuations in surrounding air. Water temperatures in mid summer averaged 29-30 ºC, further driving down DO. This shallowness prohibited development of a strong thermocline: benthic and surface temperatures were similar. Redox potential indicated suboxic conditions at the water-sediment interface (-71.6 ± 44.2 mV in July). This may have kept the high levels of heavy metals found in sediments out of solution.

Seventeen taxa of benthic macroinvertebrates were identified, mostly to the Genus level. DO appeared to be the driving factor based on the types of taxa found and the increased numbers and diversity of organisms found at sites averaging the highest DO. Chironomid, *Gammarus* and nematode were the predominant organisms. Shannon-Weiner Index (H') showed low biodiversity. H' averages for Hester-Dendy ranged from 0.0 to 0.59, while H' averages for Dip Net ranged from 0.48 to 1.24. Healthy ecosystems usually have an H' value of 3.0.

Chemical analyses found high levels of heavy metals in sediments and detritus. Analyses included Cd, Cu, Cr, Fe, Hg, Mn, Ni, Pb and Zn. Heavy metal concentrations in sediments from all sites exceeded at least some of the severe effects limits (SEL) and all of the lowest effects limits (LEL) established by the Ontario Aquatic Sediment Criterion. Concentrations in detritus exceeded the LEL in many samples and SEL in some. By this criterion, both sediment and detritus were considered toxic. Correlations between heavy metals and %TOC, SEM−AVS and Fe concentrations indicated that metals were associated with all three fractions. SEM−AVS showed a seasonal trend where by free sulfides increased in October compared to June. Also, the relative concentrations of certain metals (Cd, Fe and Pb) in detritus versus sediment showed a seasonal trend. Data indicated that wetland plants were probably contributing to these seasonal trends in two ways: 1) plants provided oxygen that lead to the release of metals from sulfide precipitates during October and 2) detritus from plants provided labile pools of heavy metals that were more or less affected by changes in redox. Of all sediment parameters, Fe concentrations most consistently correlated with total heavy metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) in sediments.

Toxicity testing included acute (96 h) and subchronic (10 d) exposure to two types of substrates, whole sediment and detritus, with and without supplemental feeding. Endpoints for acute tests were survival and carbohydrate levels and for subchronic tests

were survival and growth. Findings indicated 1) that survival was not a sensitive endpoint for chironomids in acute or subchronic tests and 2) that physical contact with the substrates did not affect growth but ingestion of substrates did. Meaning the substrates were not toxic as long as the chironomids could eat fish food and were not depend on the substrates for sustenance. Most differences between substrates were found in Unfed treatments with subchronic exposure (10 d). However, sediment data for June and October were inconsistent. For June, sites 9 and 18 showed the best growth and site 7 the least; while for October, sites 3 and 22 had the best growth and sites 9, 10 and 18 the least. Detritus data was more consistent in that site 9 had the best growth and site 10 the least in both June and October. The inconsistencies could have reflected differences in substrate homogeneity, need for more replicates and/or seasonal sediment parameters that influenced toxicity. Substrates from site 10 consistently resulted in the least growth.

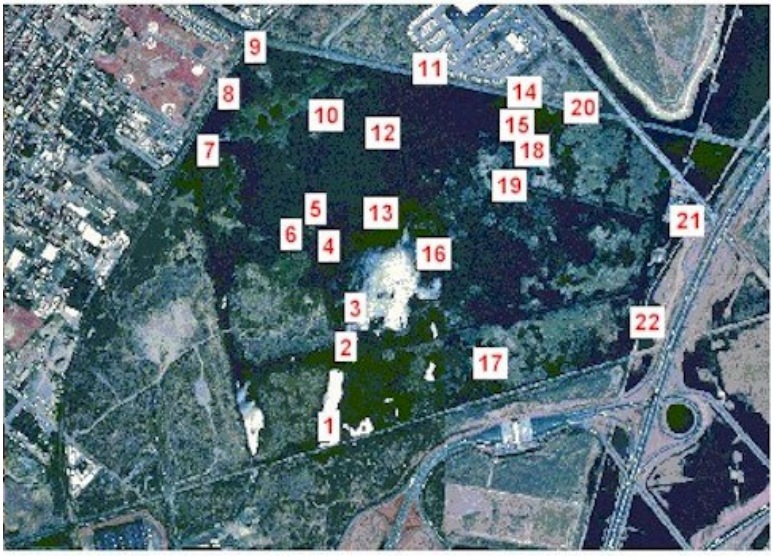
Comparison of initial to final weights in Unfed showed that larvae could use sediment and detritus alone for moderate grow. Growth in Unfed treatments was slightly better than growth in Cd spiked treatments with added fish food. It was therefore likely that poor growth in substrates was due to contamination. Sediment and detritus had similar levels of toxicity. Data did show significant differences between sediment and detritus for a few sites. However, in some cases sediment was more toxic (site 10 June) and in others detritus was more toxic (site 9 October). This endpoint indicated that, in general, neither substrate was more toxic than the other, meaning detritus was just as toxic as whole sediment.

Toxicants and stressors have been found to reduce carbohydrate levels by utilizing part of the organism's energy budget to maintain homeostasis. In this study, the fluorescence-assisted carbohydrate electrophoresis (FACE) assay was used as a sublethal biomarker for detection of sediment and detritus toxicity. Results showed differences between sites only by comparing Fed to Unfed treatments. When carbohydrates levels were similar in Fed and Unfed, the implication was that larvae were utilizing more energy than they could consume or that they had a reduced feeding rate in the presence of contaminants. In sediment experiments, sites 7 and 10 had similar carbohydrate levels in Fed and Unfed, indicating that these two sites were more toxic than the others. Data for detritus showed no significant differences between Fed and Unfed for any of the sites. Taken together results indicated that detritus was possibly more toxic than sediment using the FACE endpoint. Few studies have evaluated the contribution of detritus to sediment toxicity. These results indicated that decay of wetland plants has made a significant contribution to sediment toxicity in Kearny Marsh.

II. Introduction

This document is the final report on the research project undertaken at Kearny Marsh, NJ, 2002-2003. The purpose of this project was to obtain biotic and abiotic data on factors that might be affecting the environmental health of the marsh. Assessing biotic factors included biomonitoring macroinvertebrates *in situ*, testing sediment and detritus toxicity and measuring heavy metal bioaccumulation in laboratory exposed chironomids. The contribution of abiotic factors was assessed by measuring water parameters (pH, Eh, temperature, salinity and dissolved oxygen), sediment parameters (grain size, total organic carbon and acid-volatile sulfides) and heavy metal concentrations (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in field sediment and detritus. Data and analyses of completed work are presented in this report. Data for bioaccumulation studies has not yet been completed and will be supplied in an addendum at a later date.

Six sites at Kearny Marsh were evaluated over 1.5 years. They were chosen based on a report of contaminants in water and sediment by Langan Engineering and Environmental Services, 1999. The report indicated that these sites had varying levels of heavy metal concentrations ranging from moderate to severely contaminated. The monitored sites were W-3, W-7, W-9, W-10, W-18 and W-22 (Picture 2.01). Samples were collected on June 5, 2002, June 13, 2002, July 15, 2002, August 12, 2002, September 20, 2002, October 18, 2002, April 21, 2003 and May 21, 2003.



Picture 2.01. Kearny marsh sampling sites for this project included sites, 3, 7, 9, 10, 18 and 22.

The design of this study allowed biological and chemical data to be integrated so as to determine the current status of the marsh and what abiotic factors may be influencing its biota. Heavy metals were of special concern. The concentrations of Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn were analyzed in sediment and detritus separately in order to evaluate the contribution of detritus to overall sediment toxicity. Correlations of heavy metal concentrations and other sediment parameters with chironomid responses indicated which parameters were associated with sediment and detritus toxicity as well as heavy metal bioavailability. The different types of benthic macroinvertebrates in Kearny Marsh were identified to the lowest taxonomic level possible. These data were then used to provide information on the current level of biodiversity. Differences in diversity between sites were correlated with water, sediment and detritus parameters in order to determine which parameters were having the most influence. These data can be used to evaluate changes in the marsh ecosystem over time and to devise remediation strategies.

Specific activities for biotic factors:

1. Tests for lethality were conducted in triplicate at 96 h and 10 d for sediment and detritus collected June 5, 2002 and October 18, 2002 plus a negative and positive control. Each sediment and detritus sample was tested twice with supplemental feeding and twice without.

2. FACE testing (fluorescence assisted carbohydrate electrophoresis) was performed twice in triplicate at 96 h for sediment and detritus collected October 18, 2002 plus a negative control (sand and cerophyll) and positive control (sand and cerophyll plus 0.3 mM Cd). Treatments included fed and unfed groups.

3. Tests for growth were conducted at 10 d for sediment and detritus collected June 5, 2002 and October 18, 2002 plus a negative control (sand or cerophyll, respectively) and positive control (sand or cerophyll, respectively, plus 0.3 mM Cd). Each sediment and detritus sample was tested twice with supplemental feeding and twice without.

4. Benthic macroinvertebrates were collected four times during the study: June 13, 2002, August 12, 2002, October 18, 2002 and May 21, 2003. Collection techniques included Hester-Dendy set for one month and Dip Net. Most species were classified to the Genus level, but chironomid and nematode were classified to Family and Phylum, respectively. Site diversity was characterized by Taxa Richness and Shannon-Weiner Index.

Specific activities for abiotic factors:

1. Water quality parameters were collected eight times over the course of the study: June 5, 2002, June 13, 2002, July 15, 2002, August 12, 2002, September 20, 2002, October 18, 2002, April 21, 2003 and May 21, 2003. Measurements were taken at the surface and just above the sediment. Parameters included pH, redox, temperature, salinity, DO and depth.

2. Sediment and detritus were collected from each site on June 5, 2002 and October 18, 2002 and analyzed for Hg, Cd, Cu, Cr, Fe, Mn, Ni, Pb and Zn. MERI performed all metal analyses.

3. Sediment from each collection was analyzed for simultaneously extracted metals (SEM), acid volatile sulfides (AVS), grain size, moisture and total organic carbon (TOC). MERI performed grain size, moisture and TOC analyses. Dr. Bentivegna provided a student to carry out the SEM and AVS analyses at MERI. SEM and AVS analyses were carried out twice on each sample.

4. Chironomids, sediment and detritus from 10 d growth experiments were analyzed for Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn. MERI performed all metal analyses. To date, all chironomid analyses have been completed, but those for sediment and detritus have not been completed. Therefore, this data can not be provided in this report.

III. On Site Water Chemistry

A. Materials and Methods

Water chemistry parameters measured at Kearny Marsh were temperature (°C), pH, dissolved oxygen (DO, mg/l), redox potential (mV), salinity (mg/l) and depth (inches). Data were collected eight times: June 5, 2002, June 13, 2002, July 15, 2002, August 12, 2002, September 20, 2002, October 18, 2002, April 21, 2003 and May 21, 2003.

Site data were taken from a boat in the same area from which substrates and organisms were collected. These areas were located next to stands of wetland grasses, phragmites primarily. The exception was site 22, which was reached from shore. Data at this site were collected by walking out on a fallen log. No substantial stands of wetland grasses were present. Organisms were collected by Dip Net along the log and shoreline that had some aquatic vegetation.

Either an YSI 6600 Model 6600EDS-SV or Hydroloab Minisonde was used to collect data on temperature, pH, DO and redox. Data was collected from the surface and just above the sediments. Probes were allowed to rest on the sediment for benthic measurements. For surface measurement, probes were submerged just far enough to function properly, about 3 inches down. Depth was measured using a yard stick.

Data were analyzed using the statistical computer program, SPSS. Pearson correlations were generated by controlling for site and date using a two-tailed test of significance. The correlation between the two factors was considered statistically significant if $p \leq 0.05$

B. Results

1. Temperature

Temperature was measured at the surface and benthos of each site during each field trip. At the surface, it ranged from a low of 14 ºC to a high of 33.6 ºC (Table 3.01). At the benthos, it ranged from a low of 13.7 ºC to a high of 32.6 ºC. Surface and benthic temperatures for a particular site were usually similar, only varying by 1 degree or less (Figures 3.01 and 3.02). This was probably due to the marsh being shallow. A notable exception was during the mid summer months when surface exceeded benthic temperatures by about 3 ºC at sites 7, 9, and 18 for July and 9, 10, and 18 for August. Correlation between surface and benthos temperature was $r = 0.969$, $p \leq 0.001$. Site 3 had the coolest surface temperature on average (22.1 ± 6.1), site 22 had the warmest (24.3 ± 7.3). Comparing sampling dates, October 18, 2002 had the coolest surface temperature on average (14.9 ± 0.8) and August 12, 2002 had the warmest (30.5 ± 2.6).

2. pH

The pH was measured at the surface and benthos of each site during each field trip. At the surface, it ranged from a low of 7.2 to a high of 8.6 (Table 3.02). At the benthos, it ranged from a low of 6.6 to a high of 8.4. Benthic pH was generally lower than surface pH by 0 – 0.6 units. Correlation between surface and benthic pH was significant, $r = 0.742$, $p \leq 0.001$. However, the correlation was not as strong as for temperature. Of interest was the fluctuation in pH from higher in May-June 2002, lower in July, highest in August, lower through September and October and then higher again by April and May of 2003. This variation may have coincided with algal blooms. Site 9

showed a distinctly different pattern over the sampling period compared to the other sites (Figures 3.03 and 3.04). It began low in June 2002, increased through August and, as with the others, declined through October. The pH increased again by April 2003 but began declining in May. Site 9 had the lowest average pH (7.5 ± 0.2 surface and 7.3 ± 0.4 benthos), and site 10 had the highest (8.0 ± 0.4 surface and 7.8 ± 0.3 benthos). Comparing sampling dates, August 12, 2002 had the highest average pH (8.1 ± 0.3 surface and 7.8 ± 0.3 benthos) and October 18, 2002 had the lowest (7.3 ± 0.1 surface and 7.5 ± 0.1 benthos).

3. Dissolved oxygen (DO)

DO (mg/l)) was measured at the surface and benthos of each site during each field trip. At the surface, it ranged from a low of 1.33 to a high of 13.78 (Table 3.03). At the benthos, it ranged from a low of 0.07 to a high of 10.56. Benthic DO was occasionally much lower than surface DO despite the shallow water (Figures 3.05 and 3.06). For example, at site 10 on August 12, 2002, it was 0.91 and 10.30 mg/l for benthos and surface, respectively, and at site 7 on September 20, 2002, it was 0.57 and 9.17 mg/l for benthos and surface, respectively. Correlation between surface and benthos DO was statistically significant, $r = 0.677$, $p < 0.001$. The correlation coefficient was lower than for pH ($r = 0.742$) and temperature ($r = 0.969$). The lowest average DO occurred at sites 7 (5.66 ± 3.71 mg/l surface and 1.09 ± 1.60 mg/l benthos) and 9 (5.46 ± 4.88 mg/l surface and 1.61 ± 0.233.58 mg/l benthos), and the highest at site 22 (7.75 ± 4.03 mg/l surface and 4.35 ± 3.18 mg/l benthos). Comparing sampling dates, April 21, 2003 had the highest average surface and benthic DO (12.01 ± 1.86 mg/l surface and 8.11 ± 3.73 mg/l benthic). October 18, 2002 had the lowest average surface DO (3.35 ± 1.71 mg/l), while June 13, 2002 the lowest average benthic DO (1.11 ± 1.16 mg/l).

4. Redox potential

Redox potential (mV) was measured at the surface and benthos of each site during each field trip. At the surface, it ranged from a low of −73 mV to a high of 343 mV (Table 3.04). At the benthos, it ranged from a low of −110 mV to a high of 335 mV. Benthic redox was generally lower than that at the surface (Figures 3.07 and 3.08). Surface redox was significantly correlated with benthic redox, $r = 0.647$, $p < 0.001$. Average redox did not vary widely by site except during two sampling periods, 4-21-03 and 5-21-03, when it was unusually high and low, respectively, at the surface and benthos (this doubled standard deviations for averages). At the surface, site 10 had the lowest average redox (90.2 ± 89.1 mV) while site 9 had the highest (152.7 ± 103.8 mV). At the benthos, site 18 averaged the lowest redox (3.7 ± 55.8 mV) while site 22 had the highest (63.8 ± 103.2 mV). Average surface redox was lowest in May 21, 2003 (−44.1 ± 23.3 mV) and the highest in April 21, 2003 (263.2 ± 65.5 mV). Benthic redox was generally lower than surface redox dipping in July 2002, August 2002 and May 2003. The reason for the sharp decline in May is unknown; but July had the lowest average redox (−71.6 ± 44.2 mV) and April 21, 2003 had the highest (207.2 ± 128.8 mV).

5. Salinity

Salinity (ppt) was measured at each sampling date except June 13, 2002. It was measured at each site except sites 7 and 9 on July 15, 2002. Measurements were not taken due to the lack of meter availability on that date or technical problems. Only surface salinity was reported in Table 3.05 as surface and benthic salinities were similar. Salinities were fairly consistent over time and ranged from 0.20 ppt (freshwater) at site 9 to 2.25 ppt (oligohaline) at site 22 (Figure 3.09). Salinity levels increased at sampling sites closer to Sawmill Creek, which is northeast of the marsh. For example, sites 18 and 22 were closest to Sawmill Creek and had average salinities of 1.75 ± 0.19 and 1.84 ± 0.35 ppt, respectively. Sites 9 and 7 were furthest and their salinities averaged 0.49 ± 0.27 and 1.15 ± 0.18 ppt, respectively. This indicated that there was some flow between Sawmill Creek and Kearny Marsh at the northeast corner of the marsh. The month with the lowest average salinity was July (1.98 ± 0.12), indicating evaporative loss. April 21, 2003 had the lowest average salinity (1.24 ± 0.21).

6. Depth

Depth was measured during each field trip except June, 5, 2002. Measurements showed that the marsh was shallow, ranging from 8 to 34.5 in. (Table 3.05). Depth was measured close to phragmites stands where organism collection occurred. Therefore, data did not fully represent the marsh. Average depth was similar between sites with site 18 being the deepest, averaging 23.5 ± 8.7 in., and site 22 being the most shallow, averaging 13.5 ± 4.5 in. (Figure 3.10). The large variation for site 18 might have been due to taking measurements at slightly different locations around the site. Depth did change over time. It was most shallow on September 20, 2002, averaging 12.8 ± 4.5 in., and deepest on April 21, 2003, averaging 24.8 ± 6.8 in.

Table 3.01. Temperature (°C) at the surface and benthos of Kearny Marsh sites from 6-5-02 through 5-21-03.

		Site					
Date	Depth	3	7	9	10	18	22
6-5-02	surface	26.2	23.9	21.2	27.2	25.1	28.6
	benthic	25.7	23.3	20.9	26.7	25.1	27.4
6-13-02	surface	22.8	22.5	21	22.8	22.3	22.5
	benthic	21.8	22.1	21.4	23.2	22.1	21.7
7-15-02	surface	28.9	29.3	30	26.3	31.4	33.2
	benthic	28.8	26.7	28.2	25.9	26.3	32.2
8-12-02	surface	27.1	29.4	30.3	33.6	29.1	33.6
	benthic	26.8	28.8	27.4	29.4	26.4	32.6
9-20-02	surface	24.5	26.5	26.2	25.8	25.1	26.4
	benthic	22.9	26	24.2	26.1	24.2	24.6
10-18-02	surface	14.0	15.9	15.2	14	14.8	15.5
	benthic	13.7	16	15.8	13.7	14.8	15.5
4-21-03	surface	15.0	15.4	15.5	15.8	14.7	16
	benthic	14.3	14.7	14.5	15.2	12.3	15.8
5-21-03	surface	18.4	18	18	18.6	17.2	18.2
	benthic	18.4	18	17.9	18.5	17.2	18.2

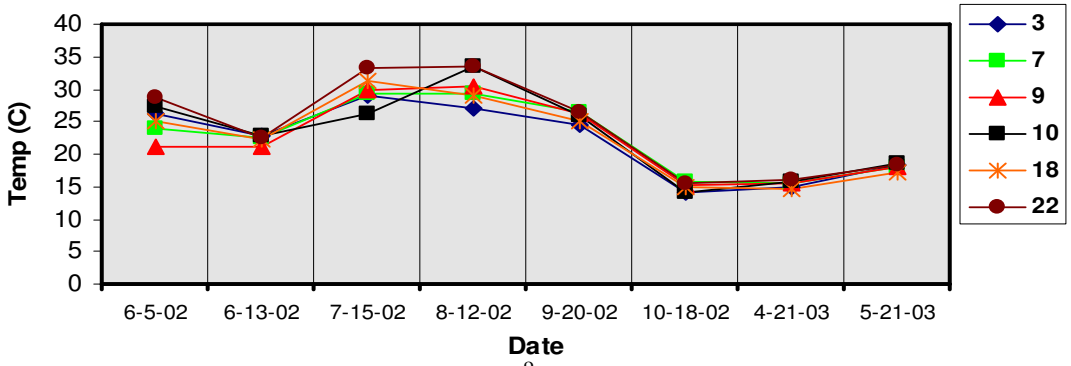


Figure 3.01. Surface temperatures (°C) at Kearny Marsh sites from June 2002 to May 2003.

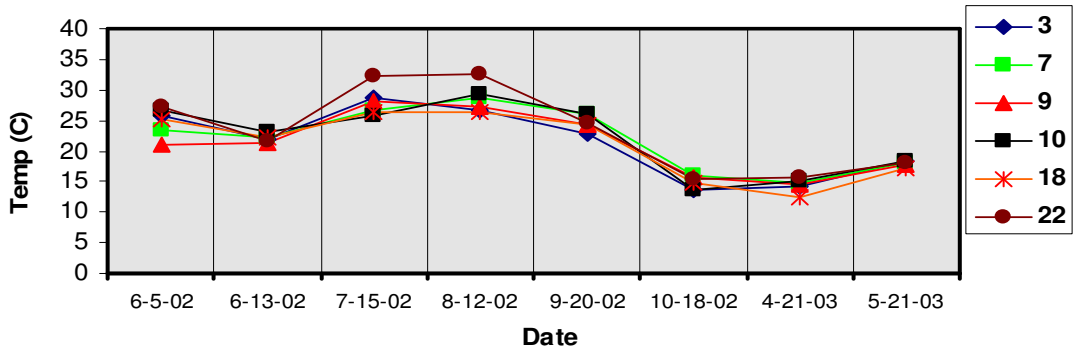


Figure 3.02. Benthic temperatures (°C) at Kearny Marsh sites from June 2002 to May 2003.

Table 3.02. The pH at the surface and benthos of Kearny Marsh sites from 6-5-02 through 5-21-03.

		Site					
Date	Depth	3	7	9	10	18	22
6-5-02	surface	7.8	7.7	7.2	8.2	7.9	8.2
	benthic	8.0	7.4	6.6	7.6	7.9	8.0
6-13-02	surface	7.6	7.5	7.4	7.8	7.8	8.1
	benthic	7.5	7.7	6.9	7.9	7.3	8.0
7-15-02	surface	7.5	7.5	7.5	7.6	7.6	7.7
	benthic	7.5	7.3	7.3	7.6	7.4	7.6
8-12-02	surface	7.9	7.8	7.8	8.3	8.1	8.4
	benthic	7.9	7.6	7.4	8.0	7.8	8.2
9-20-02	surface	7.7	7.6	7.4	7.9	7.7	7.8
	benthic	7.7	7.5	7.4	7.4	7.7	7.8
10-18-02	surface	7.3	7.2	7.2	7.4	7.4	7.4
	benthic	7.4	7.5	7.7	7.4	7.4	7.4
4-21-03	surface	7.7	7.7	7.6	7.9	7.8	8.0
	benthic	7.7	7.7	7.6	7.9	7.7	8.0
5-21-03	surface	8.2	7.9	7.6	8.6	7.4	7.7
	benthic	7.8	7.5	7.3	8.4	7.2	7.5

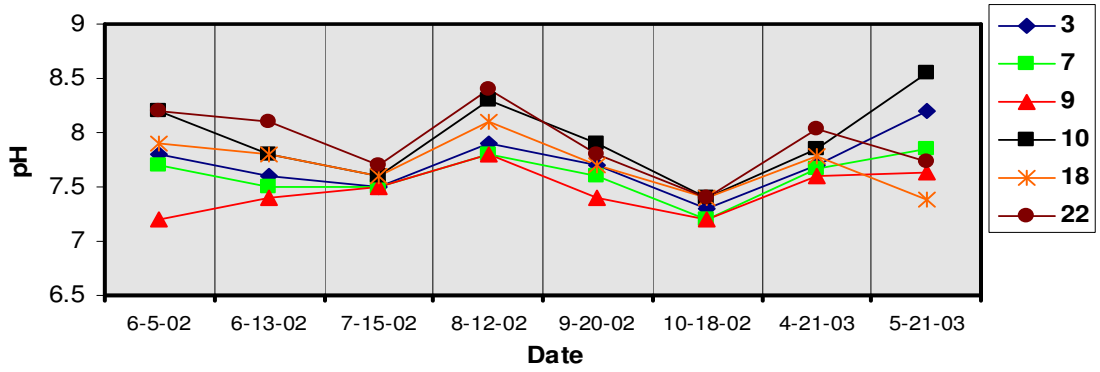


Figure 3.03. Surface pH at Kearny Marsh sites from June 2002 to May 2003.

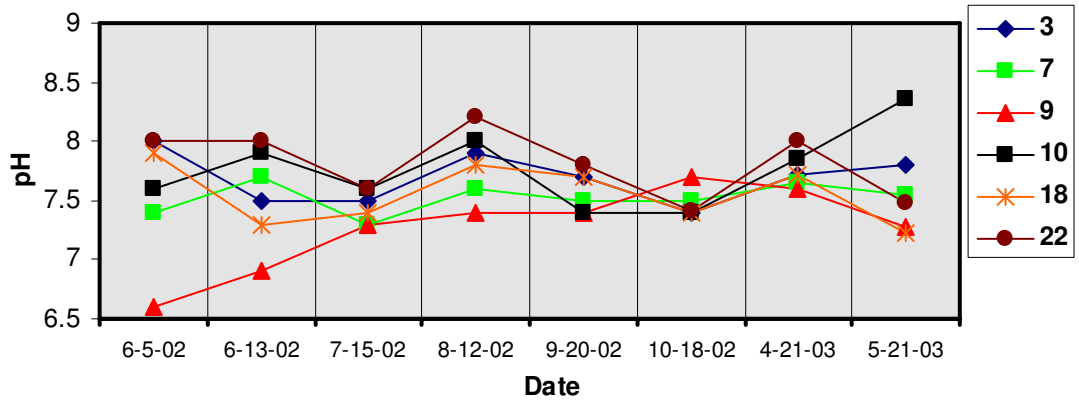


Figure 3.04. Benthic pH at Kearny Marsh sites from June 2002 to May 2003.

Table 3.03. Dissolved oxygen (mg/l) at the surface and benthos of Kearny Marsh sites from 6-5-02 through 5-21-03.

		Site					
Date	Depth	3	7	9	10	18	22
6-5-02	surface	5.04	3.75	3.85	6.08	5.83	8.41
	benthic	0.10	0.13	0.12	0.11	0.12	6.21
6-13-02	surface	3.16	2.04	3.21	5.18	3.42	4.08
	benthic	1.64	0.07	0.07	0.12	2.30	2.48
7-15-02	surface	2.56	3.05	3.59	4.31	3.20	4.08
	benthic	0.91	1.13	0.62	2.42	0.68	2.34
8-12-02	surface	7.40	10.46	11.7	10.30	7.35	13.16
	benthic	6.30	1.54	0.38	0.91	1.12	2.57
9-20-02	surface	5.92	9.17	1.33	9.11	10.55	8.40
	benthic	2.29	0.57	0.45	9.21	7.48	5.30
10-18-02	surface	2.98	1.80	1.50	3.64	3.46	3.42
	benthic	0.72	0.05	0.07	1.33	0.65	1.76
4-21-03	surface	9.94	10.31	14.5	12.33	11.22	13.78
	benthic	9.49	4.83	10.44	10.56	2.11	11.2
5-21-03	surface	6.85	4.72	4.00	6.05	3.40	6.63
	benthic	6.36	0.40	0.70	3.37	1.40	2.96

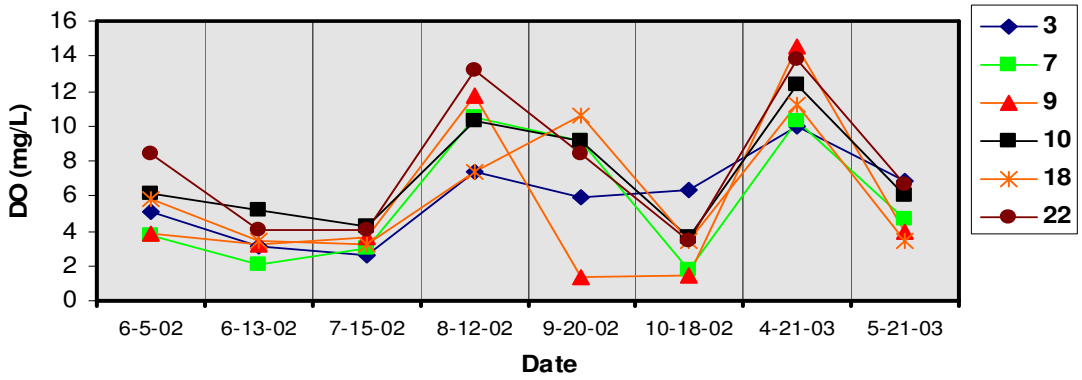


Figure 3.05. Surface DO (mg/l) at Kearny Marsh sites from June 2002 to May 2003.

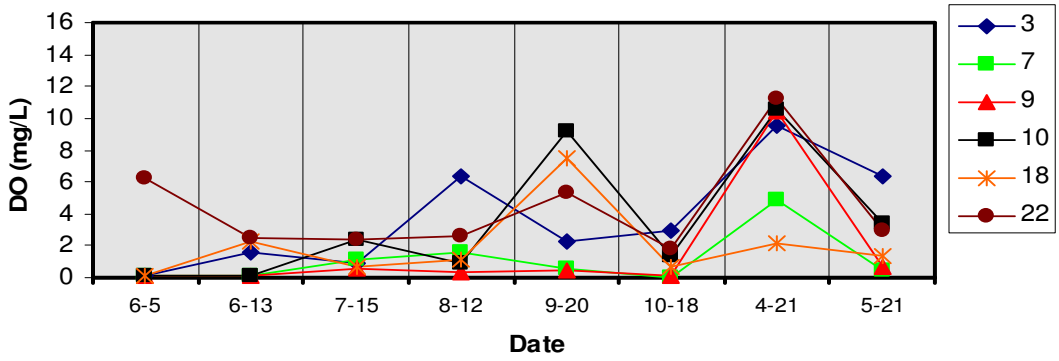


Figure 3.06. Benthic DO (mg/l) at Kearny Marsh sites from June 2002 to May 2003.

Table 3.04. Redox potential (mV) at the surface and benthos of Kearny Marsh sites from 6-5-02 through 5-21-03.

		Site					
Date	Depth	3	7	9	10	18	22
6-5-02	surface	163	187	141	108	89	22
	benthic	107	35	119	62	53	7
6-13-02	surface	86	90	196	90	59	121
	benthic	-44	-1	50	58	-53	118
7-15-02	surface	76	80	183	35	63	99
	benthic	-78	-110	-107	-71	12	-75
8-12-02	surface	190	129	134	109	149	140
	benthic	88	27	-24	-126	-64	20
9-20-02	surface	51	109	158	165	151	139
	benthic	-17	16	-3	71	56	129
10-18-02	surface	91	96	95	87	85	83
	benthic	84	68	60	81	84	83
4-21-03	surface	274	330	343	201	183	248
	benthic	274	217	335	204	-38	251
5-21-03	surface	-73	-45	-28	-69	-14	-36
	benthic	-51	-51	-16	-81	-19	-24

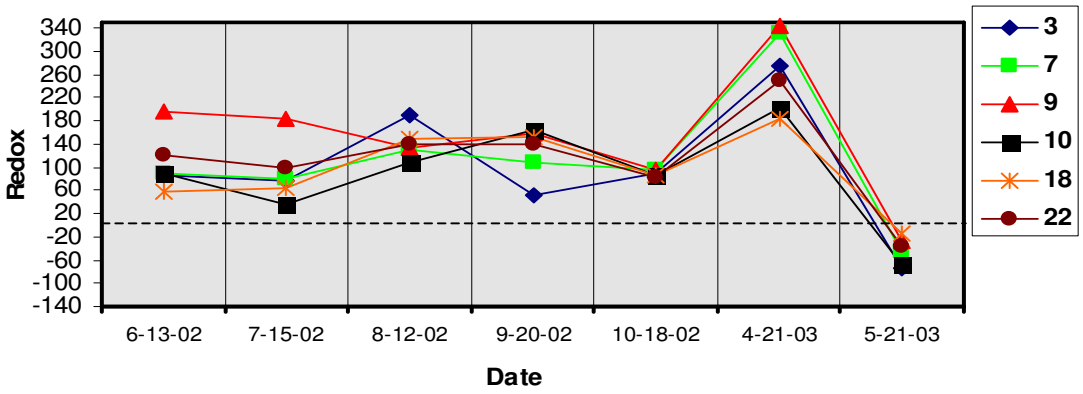


Figure 3.07. Surface redox (mV) at Kearny Marsh sites from June 2002 to May 2003.

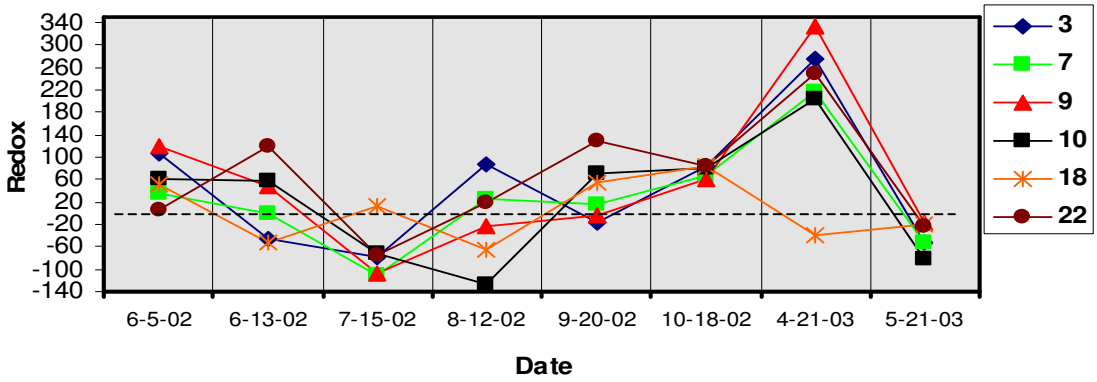


Figure 3.08. Benthic redox (mV) at Kearny Marsh sites from June 2002 to May 2003.

Table 3.05. Salinity (ppt) and Depth (in.) at the surface and benthos of Kearny Marsh sites from 6-5-02 through 5-21-03.

		Site					
Parameter	Date	3	7	9	10	18	22
Salinity	6-5-02	1.60	1.40	0.60	1.60	1.70	1.70
(ppt)	6-13-02	ND	ND	ND	ND	ND	ND
	7-15-02	1.92	ND	ND	1.88	1.97	2.15
	8-12-02	1.92	1.02	0.32	1.75	1.99	2.25
	9-20-02	0.88	0.88	0.26	1.81	1.74	2.13
	10-18-02	1.40	1.20	0.20	1.50	1.80	1.80
	4-21-03	1.23	1.2	0.85	1.32	1.48	1.35
	5-21-03	1.3	1.2	0.7	1.4	1.6	1.5
Depth	6-5-02	ND	ND	ND	ND	ND	ND
(in.)	6-13-02	18	15	28	14	14	14
	7-15-02	12	16	13	19	16	10
	8-12-02	17	15	12.5	20.5	29.5	9.5
	9-20-02	21	10	12	13	13	8
	10-18-02	21	19	15	19	32	19
	4-21-03	19.0	22	22	34.5	32	19
	5-21-03	17.0	18	13.5	32	28	15

ND = No data

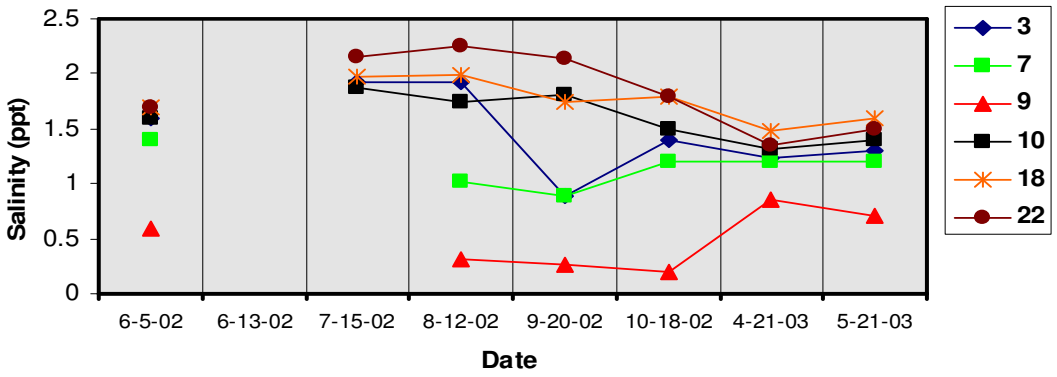


Figure 3.09. Salinity (ppt) at Kearny Marsh sites from June 2002 to May 2003.

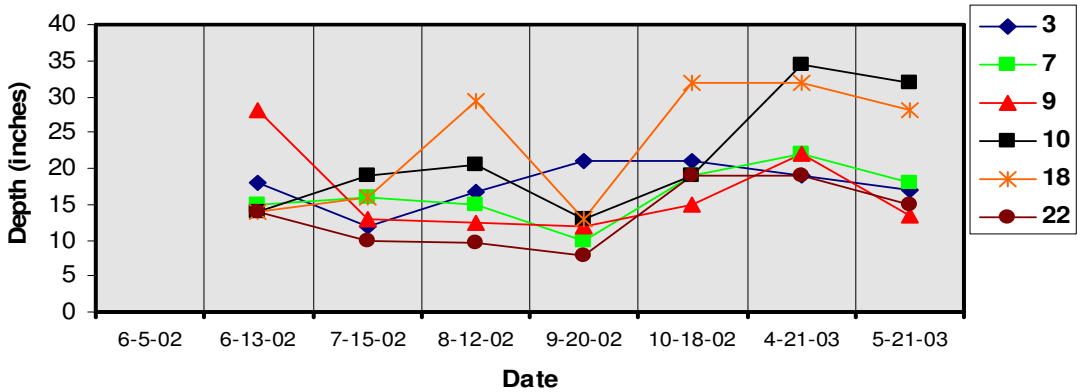


Figure 3.10. Depth (in.) at Kearny Marsh sites from June 2002 to May 2003.

C. Analysis and Discussion:

Field monitoring found Kearny Marsh to be shallow, oligohaline, and suboxic. Water temperatures fluctuated from 14 ºC in October to very warm temperatures, 29-33 ºC, in July and August (Table 3.01). Surface temperature and DO were correlated, $r = 0.409$, $p = 0.004$ (Pearson correlation coefficient controlling for date). This was expected as temperature influences how much oxygen can dissolve into water. However, the correlation between benthic temperature and benthic DO was not significant, $r = 0.162$, $p = 0.276$, indicating that microbial activity in the benthos was also influencing DO levels. This was further illustrated by the large difference in DO between the surface and benthos at several sites (Figures 3.05 and 3.06). Benthic DO was in general very low with sites 7 and 9 having the lowest levels, 1.09 ± 1.06 and 1.61 ± 3.58 mg/l, respectively, and site 22 having the highest, 4.35 ± 3.18. These levels of DO would definitely influence the type of organisms that could live in the marsh.

Photosynthesis appeared to influence water chemistry. This was shown by a strong correlation between surface DO and surface pH, $r = 0.542$, $p \leq 0.001$, and between surface temperature and pH, $r = 0.534$, $p \leq 0.001$. Theoretically, warm temperatures produced algal blooms, which in turn generated oxygen. This oxygen chemically converted hydrogen ions into water and hydroxide ions, thereby increasing pH. The relationship was most evident on August 12, 2002 when a spike occurred simultaneously in temperature, pH and DO (Figures 3.01, 3.03 and 3.05). Given this scenario, it was likely that pH dropped overnight as algal released carbon dioxide into the water. How low the pH dropped was not known as sampling was not done at night. Benthic pH levels were lower than surface levels but not low enough during the day to be environmental compromising, < 6.5.

Redox potential has been found to affect heavy metal chemistry thereby influencing bioavailability. Benthic levels were of primary interest due to the effect of redox on Fe and Mn oxides, which are known to sequester heavy metals. Benthic redox levels varied from oxic (207 ± 128 mV) in April 2003 to anoxic (-71.6 ± 44.2 mV) in July 2002. Levels were not as low as anticipated given the observed release of methane gas when sediments were disturbed. However, sampling periods were subject to weather changes: the sharp change in redox between April (207.2 ± 128.8) and May (-40.4 ± 25.3) 2003 could have reflected a change in rainy cool conditions to warmer drier weather. The temperature on average did increase 4º C between sampling periods. Sediments were probably more anoxic below than at the sediment-water interface, which was where measurements were taken. It has been suggested that anoxic sediments act as a "cap" on heavy metals by keeping them in insoluble forms. Cr could be an exception as it was found to be more soluble between −80 and −150 mV (Guo *et al*., 1997): conditions found in July at all but site 18. Cd and Zn have been found more soluble at mildly oxic conditions, > -60, such as those in June, September and October. Overall, the mildly oxic conditions found at sediment surfaces might allow leaching of heavy metals into the water column and therefore allow bioaccumulation by exposed organisms.

IV. Biomonitoring

A. Materials and Methods

Organisms from each site were collected using both Hester Dendy and dip net techniques. Biomonitoring was done on June 13, 2002, August 12, 2002, October 18, 2002 and May 21, 2003.

Hester-Dendy Samplers were EPA approved, 5 in. diameter, round (Wildlife Supply Company, Saginaw, MI). They were set by tying them to bricks and positioning them so that they rested on sediments in an upright position. Samples were collected 1 month later by placing each Hester Dendy in a separate plastic bag containing approximately 400 ml of 95 % ETOH. In the laboratory, the Hester Dendy was disassembled and the content of each surface was brushed into a dish containing 70% ETOH. The contents of the dish were stored in plastic bags until identification. Hester Dendy sections were placed in water until rehydrated and free of ETOH then reassembled for use.

The dip net used was an Explorer Tri-Dip Net, 40 in. (Wildlife Supply Company, Saginaw, MI). Organisms were collected by swiping the net through the water and into the base of the vegetation for 1 minute at each site. The contents of the net were put into a plastic bag containing 95 % ETOH. All of the vegetation in the net was included in the bag. The organisms were sorted and stored in 70 % ETOH.

All of the contents collected from Hester Dendy and dip net samplings were viewed under a dissecting scope, and the organisms were separated from vegetative matter. Organisms were identified, counted and stored in separate containers. They were identified using *Ecology and Classification of North American Freshwater Invertebrates*, edited by Jame H. Thorp and Alan P. Covich (2001). Organisms were identified to Genus level when possible.

B. Results for Biomonitoring June 13, 2002 and August 12, 2002

Benthic macroinvertebraes were monitored for four dates (6-13-02, 8-12-02, 10-18-03 and 5-21-03) using two sampling techniques, Hester-Dendy and Dip Net. Seventeen different types of organisms were identified: chironomids were put together in one taxon, the Family, *Chironomidae*; nematodes were put together in one taxon, the Phylum, *Nematoda*; the other 15 taxa were identified to the Genus level and included *Coptotomus* (water beetle), *Cordulegaster* (dragonfly), *Ephemerella* (Mayfly), *Gammarus* (amphipod), *Gerris* (water strider), *Haliplus* (water beetle), *Helobdella* (leech), *Hesperagrion* (damsel fly), *Idotea* (isopod), *Menetus* (snail), *Nehalennia* (damsel fly), *Palaemonetes* (shrimp), *Physella* (snail), *Somatochlora* (dragonfly), and *Tropisternus* (water beetle).

Tables 4.01 -4.04 show the number of individuals in each taxon collected on a particular date. Several Hester-Dendy samplers were lost most likely due to vandalism making this a less reliable technique than dip net even though it is quantitative while dip net is not. Three types of taxa dominated on Hester-Dendy, chironomid, *Gammarus* and nematode. Chironomid and nematode were usually found at all sites, while *Gammarus* was only consistently found at site 3. However, in June, *Gammarus* was found at all sites. Chironomid and nematode were probably found on Hester-Dendy because these taxa seek out submerged surface areas and can tolerate the low DO above sediments.

Dip net samples showed more diversity in keeping with the increased number of niches that can be sampled using this technique. Organisms were picked off of vegetation found in the net and also from within vegetation: the inner, hollow stems of *Phragmites* were colonized by some species, especially chironomids. Dominating taxa in Dip Net were the same as those on Hester-Dendy- chironomid, nematode and *Gammarus*. The presence of other types of taxa was more seasonal and site specific. For example, the snail, *Physella*, was found primarily in October. Larvae of the damsel fly, *Nehalenia*, were found primarily in August and October. The shrimp, *Palaemonetes*, was found primarily in June and August. Most of the mayflies, *Ephemerella*, were found only in August at site 9.

Comparisons of composite data for sampling sites showed that site 3 consistently had the greatest diversity in both Hester-Dendy and Dip Net (Figure 4.01 and 4.02). For Hester-Dendy, sites 7 and 9 had no organisms. This was in keeping with the low benthic DO found at these sites. On the other hand in Dip Net, sites 3, 9 and 22 showed the most diversity. Results, especially for site 9, suggested that several taxa could live on vegetation near the surface even when DO was low.

Comparison of composite data for sampling dates showed that August had the greatest number of individuals and taxa while May had the lowest (Figures 4.03 and 4.04). This was surprising at first, but does correspond to a spike in surface DO in August presumably due to algal blooms (Figure 3.05). The DO was higher at the surface in August than in October, June or May.

Benthic macroinvertebrate diversity was evaluated using the Shannon-Weiner (H') Index and Taxa Richness. Taxa Richness is the sum of taxa (not individuals) in a sample. The formula for H' was as follows.

$$H' = -^{G}\Sigma\, P_i \ln P_i$$

Where: G = sum of $P_i \ln P_i$ for each taxon in each sample
$P_i = n_i$/ total number of individuals in a sample
n_i = number of individuals in a taxon

H' was calculated using the taxons Genus, Family (Chironmidae) and Phylum (Nematoda). Data showed low diversity over all (Table 4.05). The range for Hester-Dendy was 0.0 to 1.07 H', with site 22 in May 2003 showing the greatest diversity. Site 3 had the highest average (0.59 ± 2.1 H') for Hester-Dendy, and sites 7 and 9 had zero organisms over the entire sampling period. H' diversity using Dip Net was better but still low in general. The range for Dip Net was 0.0 to 2.04 H', with site 9 in August 2002 showing the greatest diversity. Site 9 had the highest average (1.24 ± 0.58 H'), but this was primarily based on one sample. Site 3 more consistently showed high diversity (1.03 ± 0.54 H'). Sites 10 and 18 had the lowest diversity, 0.78 ± 0.36 and 0.48 ± 0.32, respectively. An H' value of 3 is considered a healthy level of diversity: all of these averages are well below that.

Data for Taxon Richness also showed low diversity (Table 4.06). For Hester-Dendy, site averages ranged from 0.5 ± 0.5 taxa (site 9) to 3.00 ± 1.22 taxa (site 3). The number of taxa found in Dip Net was somewhat higher, ranging from 2.5 ± 1.29 (site 18) to 5.75 ± 0.96 (site 22). As in H', sites 10 and 18 had the lowest diversity for Dip Net, while sites 7 and 9 had the lowest diversity for Hester-Dendy. All sampling periods had

similar levels of Taxon Richness with Hester-Dendy averaging from 1.17 to 2.75 taxa and Dip Net again higher averaging from 3.5 to 5.17 taxa.

Table 4.01. Biomonitoring data for samples collected June 13, 2002. Samples were collected using Hester Dendy and Dip Net techniques. Organisms were identified to Genus, Family (chironomid) or Phylum (nematode). ND = Hester-Dendy were lost.

Hester-Dendy	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	10	ND	2	11	56	ND
Coptotomus	0	ND	0	1	0	ND
Cordulegaster	0	ND	0	0	0	ND
Ephemerella	0	ND	0	0	0	ND
Gammarus	85	ND	0	49	10	ND
Gerris	0	ND	0	0	0	ND
Haliplus	0	ND	0	0	0	ND
Helobdella	0	ND	0	0	0	ND
Hesperagrion	0	ND	0	2	0	ND
Idotea	0	ND	0	0	0	ND
Menetus	0	ND	0	0	0	ND
Nehalennia	0	ND	0	0	0	ND
Nematode	0	ND	0	0	6	ND
Palaemonetes	0	ND	0	0	0	ND
Physella	6	ND	0	5	0	ND
Somatochlora	0	ND	0	0	0	ND
Tropisternus	0	ND	0	0	0	ND
Dip Net	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	8	18	19	5	1	16
Coptotomus	0	0	0	0	0	0
Cordulegaster	0	0	0	0	0	0
Ephemerella	0	0	0	0	0	0
Gammarus	19	0	0	27	22	58
Gerris	1	0	0	0	1	0
Haliplus	0	0	0	0	0	2
Helobdella	0	0	0	0	0	1
Hesperagrion	0	0	0	1	0	0
Idotea	0	0	0	0	0	3
Menetus	0	0	0	0	0	0
Nehalennia	0	0	1	0	0	0
Nematode*	0	13	14	2	0	3
Palaemonetes	8	0	0	8	2	1
Physella	2	4	0	1	0	2
Somatochlora	0	0	0	0	0	0
Tropisternus	0	0	0	0	0	0

Table 4.02. Biomonitoring data for samples collected August 12, 2002. Samples were collected using Hester Dendy and Dip Net techniques. Organisms were identified to Genus, Family (chironomid) or Phylum (nematode). ND = Hester-Dendy were lost.

Hester-Dendy	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	175	0	0	ND	0	1686
Coptotomus	0	0	0	ND	0	0
Cordulegaster	0	0	0	ND	0	0
Ephemerella	0	0	0	ND	0	0
Gammarus	28	0	0	ND	0	0
Gerris	0	0	0	ND	0	0
Haliplus	0	0	0	ND	0	0
Helobdella	0	0	0	ND	0	0
Hesperagrion	3	0	0	ND	0	0
Idotea	0	0	0	ND	0	0
Menetus	0	0	0	ND	0	0
Nehalennia	0	0	0	ND	0	0
Nematode	54	0	0	ND	0	0
Palaemonetes	0	0	0	ND	0	0
Physella	3	0	0	ND	0	0
Somatochlora	0	0	0	ND	0	0
Tropisternus	0	0	0	ND	0	0
Dip Net	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	20	5	21	5	3	33
Coptotomus	0	0	0	0	0	0
Cordulegaster	1	0	1	0	0	0
Ephemerella	3	0	29	0	0	0
Gammarus	0	0	0	0	0	0
Gerris	3	0	0	0	0	2
Haliplus	0	0	0	0	0	0
Helobdella	0	0	3	0	0	0
Hesperagrion	4	0	9	0	0	0
Idotea	0	0	9	0	0	0
Menetus	0	0	0	0	0	0
Nehalennia	0	0	12	1	0	1
Nematode	0	5	18	0	0	8
Palaemonetes	46	1	7	1	3	2
Physella	1	0	0	0	0	0
Somatochlora	0	0	3	0	0	0
Tropisternus	0	0	1	0	0	0

Table 4.03. Biomonitoring data for samples collected October 18, 2002. Samples were collected using Hester Dendy and Dip Net techniques. Organisms were identified to Genus, Family (chironomid) or Phylum (nematode). ND = Hester-Dendy were lost.

Hester-Dendy	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	160	7	0	124	1	65
Coptotomus	0	0	0	0	0	0
Cordulegaster	0	0	0	0	0	0
Ephemerella	0	0	0	0	0	0
Gammarus	27	0	0	0	0	0
Gerris	0	0	0	0	0	0
Haliplus	0	0	0	0	0	0
Helobdella	0	0	0	0	0	0
Hesperagrion	0	0	0	0	0	0
Idotea	0	0	0	0	0	0
Menetus	0	0	0	0	0	0
Nehalennia	2	0	0	0	0	0
Nematode	0	0	0	0	0	0
Palaemonetes	0	0	0	0	0	0
Physella	0	0	0	0	0	0
Somatochlora	0	0	0	0	0	0
Tropisternus	0	0	0	0	0	0
Dip Net	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	3	28	11	7	84	19
Coptotomus	0	0	0	0	0	0
Cordulegaster	0	0	0	0	0	0
Ephemerella	0	1	0	0	0	0
Gammarus	0	0	1	0	0	3
Gerris	0	0	0	0	0	0
Haliplus	0	0	0	0	0	1
Helobdella	0	1	0	0	0	0
Hesperagrion	4	0	2	0	0	0
Idotea	0	0	0	0	0	0
Menetus	6	0	0	0	0	0
Nehalennia	2	3	0	1	11	2
Nematode	0	0	0	0	0	0
Palaemonetes	1	0	1	3	7	0
Physella	15	3	0	0	1	16
Somatochlora	0	0	0	0	0	0
Tropisternus	0	0	0	0	0	0

Table 4.04. Biomonitoring data for samples collected May 21, 2002. Samples were collected using Hester Dendy and Dip Net techniques. Organisms were identified to Genus, Family (chironomid) or Phylum (nematode). ND = Hester-Dendy were lost.

Hester-Dendy	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	0	10	1	23	2	27
Coptotomus	0	0	0	0	0	0
Cordulegaster	0	0	0	0	0	0
Ephemerella	0	0	0	0	0	0
Gammarus	6	0	0	0	0	17
Gerris	0	0	0	0	0	0
Haliplus	0	0	0	0	0	0
Helobdella	0	0	0	0	0	0
Hesperagrion	0	0	0	0	0	1
Idotea	1	0	0	0	1	8
Menetus	0	0	0	0	0	0
Nehalennia	0	0	0	0	0	0
Nematode	0	0	0	0	0	0
Palaemonetes	0	0	0	0	0	0
Physella	0	0	0	0	0	0
Somatochlora	0	0	0	0	0	0
Tropisternus	0	0	0	0	0	0
Dip Net	Site 3	Site 7	Site 9	Site 10	Site 18	Site 22
Chironomid	31	6	4	27	0	161
Coptotomus	0	0	0	0	0	0
Cordulegaster	0	0	0	0	0	0
Ephemerella	0	1	2	0	0	0
Gammarus	2	0	0	0	2	40
Gerris	0	0	0	0	0	0
Haliplus	0	0	0	0	0	0
Helobdella	0	0	0	0	0	0
Hesperagrion	0	1	2	1	0	2
Idotea	0	0	0	0	0	0
Menetus	0	1	1	0	0	0
Nehalennia	0	0	0	0	0	2
Nematode	0	0	0	0	0	0
Palaemonetes	0	0	0	1	0	1
Physella	0	1	0	0	0	5
Planorbidae	0	1	1	0	0	0
Somatochlora	0	0	0	0	0	0
Tropisternus	0	0	0	0	0	0

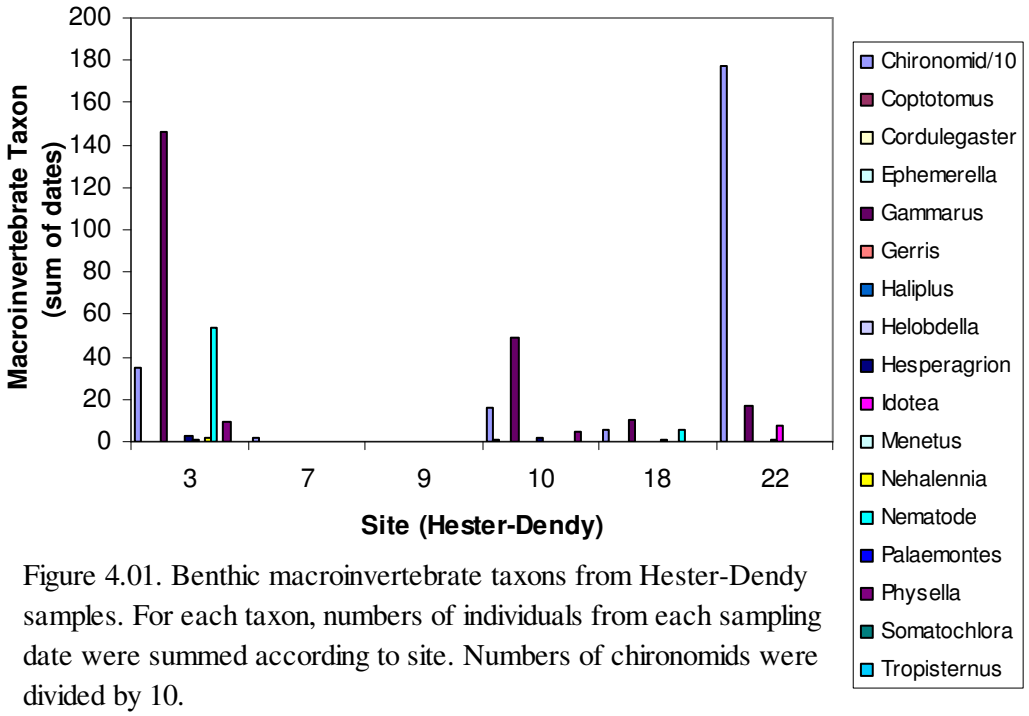


Figure 4.01. Benthic macroinvertebrate taxons from Hester-Dendy samples. For each taxon, numbers of individuals from each sampling date were summed according to site. Numbers of chironomids were divided by 10.

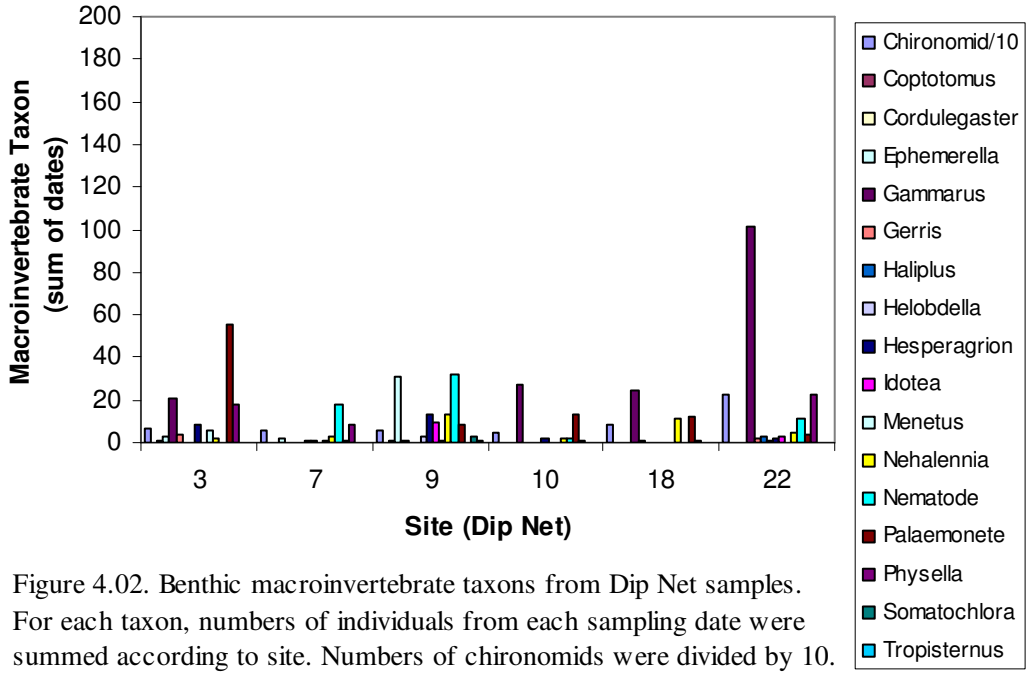


Figure 4.02. Benthic macroinvertebrate taxons from Dip Net samples. For each taxon, numbers of individuals from each sampling date were summed according to site. Numbers of chironomids were divided by 10.

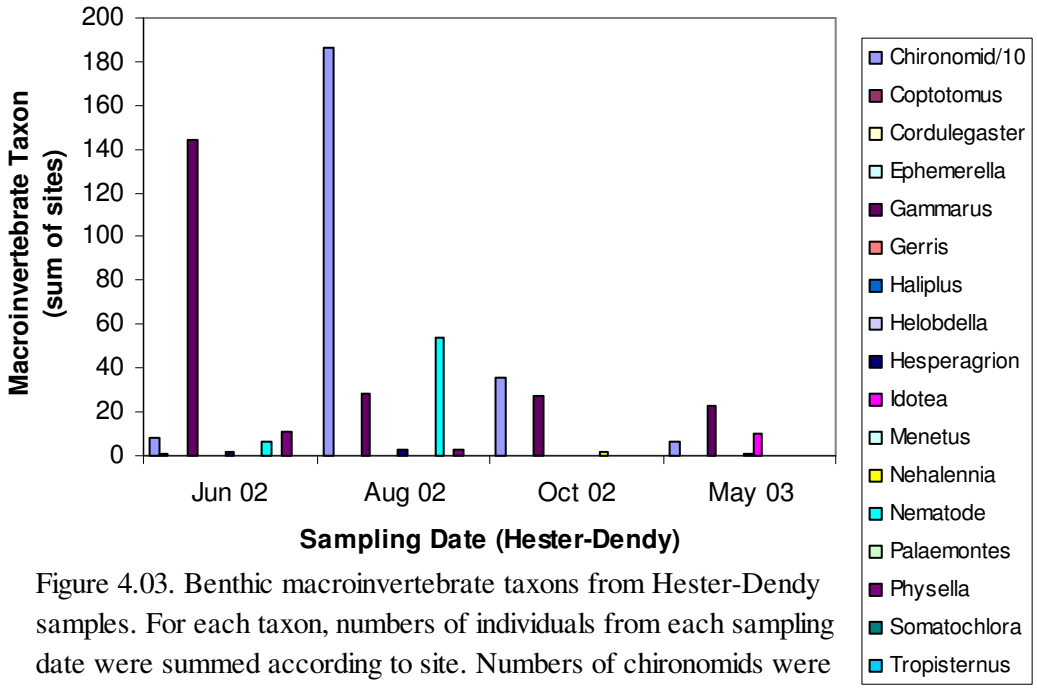


Figure 4.03. Benthic macroinvertebrate taxons from Hester-Dendy samples. For each taxon, numbers of individuals from each sampling date were summed according to site. Numbers of chironomids were divided by 10.

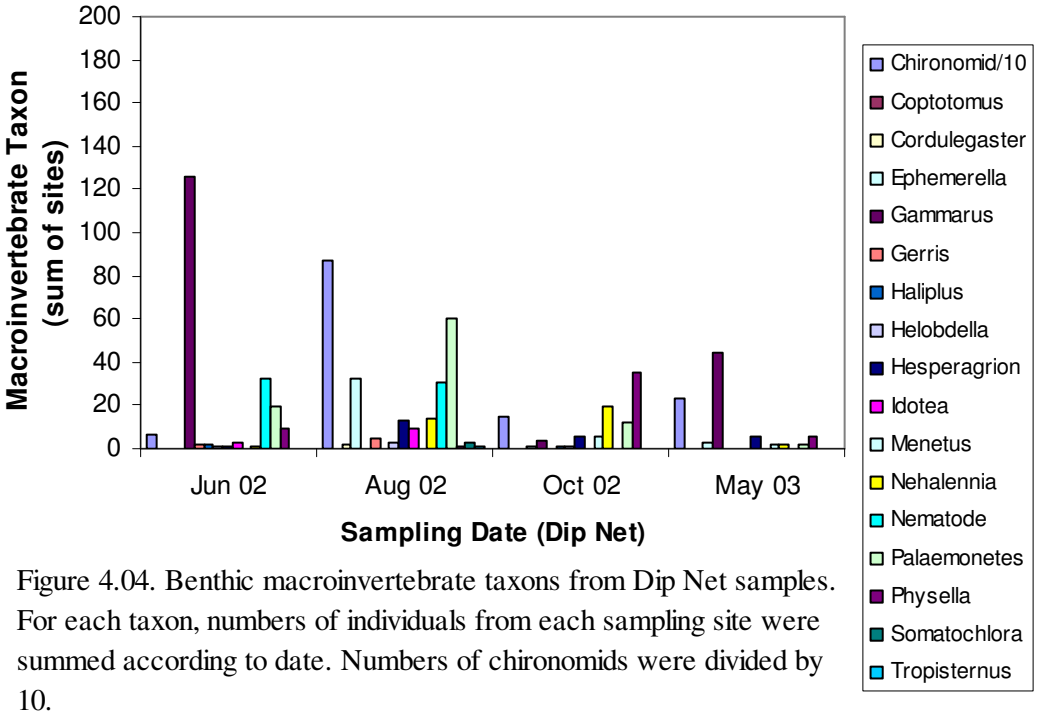


Figure 4.04. Benthic macroinvertebrate taxons from Dip Net samples. For each taxon, numbers of individuals from each sampling site were summed according to date. Numbers of chironomids were divided by 10.

Table 4.05. Shannon-Weiner Diversity Index* for Hester-Dendy (HD) and Dip Net (DN) samples.

		Sites						
Date	Method	3	7	9	10	18	22	Ave±SD
Jun-02	HD	0.54	ND	0.00	0.89	0.68	ND	0.53±0.38
	DN	1.25	0.96	0.79	1.17	0.59	1.09	1.25±0.25
Aug-02	HD	0.94	0.00	0.00	ND	0.00	0.00	0.19±0.42
	DN	1.18	0.93	2.04	0.80	0.69	0.90	1.09±0.49
Oct-02	HD	0.47	0.00	0.00	0.00	0.00	0.00	0.08±0.19
	DN	1.45	0.81	0.86	0.86	0.63	1.15	0.96±0.29
May-03	HD	0.41	0.00	0.00	0.00	0.64	1.07	0.35±0.44
	DN	0.23	1.23	1.27	0.30	0.00	0.72	0.63±0.54
Ave±SD	HD	0.59±0.21	0.00	0.00	0.30±0.42	0.33±0.33	0.36±0.50	
	DN	1.03±0.54	0.98±0.18	1.24±0.58	0.78±0.36	0.48±0.32	0.97±0.19	

*see text for formula
ND = no data

Table 4.06. Taxa Richness* for Hester-Dendy (HD) and Dip Net (DN) samples.

		Sites						
Date	Method	3	7	9	10	18	22	Ave±SD
Jun-02	HD	2	ND	1	5	3	ND	2.75±1.71
	DN	5	3	3	5	3	7	4.33±1.63
Aug-02	HD	5	0	0	ND	0	1	1.20±2.17
	DN	7	3	11	3	2	5	5.17±3.37
Oct-02	HD	3	1	0	1	1	1	1.17±0.98
	DN	6	5	4	2	4	5	4.33±1.37
May-03	HD	2	1	1	1	2	4	1.83±1.17
	DN	2	5	4	3	1	6	3.50±1.87
Ave±SD	HD	3.00±1.22	0.67±0.47	0.50±0.50	2.33±1.89	1.50±1.12	2.00±1.41	
	DN	5.00±2.16	4.00±1.15	5.50±3.70	3.25±1.26	2.50±1.29	5.75±0.96	

* Taxa Richness is the sum of different types of taxa found in samples: Genus (Coptotomus, Cordulegaster, Ephemerella, Gammarus, Gerris, Haliplus, Helobdella, Hesperagrion, Idotea, Menetus, Nehalennia, Palaemonetes, Physella, Somatochlora, Tropisternus), Family (chironomid) and Phylum (nematode).

C. Analysis and Discussion

Seventeen types of organisms were identified at six field sites in June, August, October and May samples. Sampling was fairly comprehensive covering all but the winter season, with locations all around the marsh. Hester-Dendy rested on top of the sediment and offered a substrate for colonization. They have been designed for use in streams where the movement of water selects for organisms that can cling to substrates. Kearny Marsh is a lentic system with little water movement, and by comparison to Dip Net, the number of taxa recovered on Hester-Dendy was low. Dip Net has not been considered a quantitative method, but it did include more niches. This proved important at Kearny Marsh as many of the organisms were found in vegetative matter floating at or near the surface. Alternative sampling methods need to be sought for wetland benthic macroinvertebrates.

Most organisms collected from Kearny Marsh are associated with organic pollution and known to be insensitive to low DO (Table 4.07). Either they get oxygen

from air as opposed to water (*Coptotomus, Gerris, Haliplus, Physella*, and *Tropisternus*) or have hemoglobin pigments (*Chironomidae* and *Menetus*). The predominant organisms in both Hester-Dendy and Dip Net samples were chironomid, *Gammarus* (amphipod) and nematode. The shrimp, *Palaemonetes*, was also prevalent in Dip Net. Data indicated a seasonal trend. While chironomid dominated throughout the sampling period, *Gammarus* was numerous in June and all but disappeared in August as the numbers of *Palaemonetes*, dragonfly and damsel fly increased. Finding a number of *Gammarus*, *Ephemerella* and *Idotea* (isopod) was something of a surprise. According to the literature, their Families have been associated with clean, oxygenated water (Thorp and Covich, 2001). One study did find that *G. pulex* could survive hypoxic conditions (1-2 mg/L DO) for at least 24 h (Maltby, 1995). Most *Gammarus* were found in June at sites 3 and 22. These two sites had the lowest sediment heavy metal contamination and highest benthic DO on average. This makes sense as *Gammarus* typically feed off of benthic substrates. *Ephemerella* (mayfly) is an herbivore, and some species have been found in organically enriched water (Thorp and Covich, 2001). At Kearny Marsh, the only time when more than 1-2 individuals were found was at site 9 in August. There appeared to be a major algae bloom at this time as surface DO rose significantly. Together these two factors, type of species and high DO, may explain the presence of *Ephemerella*. Available data on *Idotea* (isopod) have indicated that it is sensitive to low DO and heavy metals (Jones, 1975); conditions both present at Kearny Marsh. However, only 22 individuals were found during the entire sampling period and half of these were found at site 9 in August, the same place and time that *Ephemerella* was found.

Taxa diversity proved to be very low at Kearny Marsh. For Hester-Dendy, the average number of taxa during the sampling period ranged from 0.5 (site 9) to 3.0 (site 3). Average number of taxa increased somewhat in Dip Net, ranging from 2.5 (site 18) to 5.75 (site 22). Although the number of species in a healthy ecosystem varies, most ecosystems usually support dozens of different macroinvertebrate species (Thorp and Covich, 2001). Samples were carefully scoured so the list of macroinvertebrate taxa is fairly comprehensive. The Shannon-Weiner Index (H') also showed low diversity. H' averages for Hester-Dendy ranged from 0.0 (sites 7 and 9) to 0.59 (site 3). H' averages for Dip Net ranged from 0.48 (site 18) to 1.24 (site 9). These H' values were comparable to or lower than those found for stressed ecosystems. For example, in Gulf of Mexico near-coastal areas, benthic macroinvertebrate communities had H' values ranging from 3.8 to 1.0 (Butts *et al*., 2002). The low value corresponded with organically enriched sediments. Drainage of acid strip mines into streams caused a drop in H' from 3.10 to 1.95 (Tomkiewicz and Dunson, 1977). Sediment polycyclic aromatic hydrocarbon contamination (> NOEC) associated with the Exxon Valdez spill corresponded with an H' value of 0.92 at upper intertidal stations (Page *et al*., 2002). Clearly the ecosystem at Kearny Marsh has been compromised.

Table 4.07: Ecology of taxa found at Kearny Marsh

Taxon	Common name	Ecology	Ref
Chironomidae	chironomid	Survives under eutrophic conditions due to hemoglobin, heavy metal tolerant.	1
Coptotomus	diving beetle	Lives in stagnant water, preference for shallow, vegetative margins of ponds and marshes. Carries a bubble of oxygen allowing it to live in low DO and hunt prey.	2
Cordulegaster	dragon fly	Occurs in slow-water, silt-bottomed areas of streams, both large and small.	2
Ephemerella	mayfly	Larvae of most species inhabit clean streams. A few species may persist in organically enriched streams. Most larvae are herbivores.	1,2
Gammarus	scud	Typically found in slow-moving, lentic, freshwater. Feed off bottom substrates. Adults and juveniles can survive at relatively low DO, at least 1-2 mg/l for 24 h (Maltby, 1995), but Family is characterized by their ability to live in relatively clean, cool water.	1,3
Gerris	water strider	Insect predator found on the surface film of ponds and marshes. Hunt by skimming along the surface on long legs. Get oxygen from air not water.	1
Haliplus	crawling water beetle	Abundant in shallow lentic and lotic vegetation-choked habitats. Both larvae and adults are herbivores feeding on algae or macrophytes. Crawl about on the surface, so DO is not a factor.	1,2
Helobdella	leech	Most likely *H. stagnalis*, capable of living under anaerobic conditions, snails are its preferred prey.	1
Hesperagrion	damsel fly	Inhabits permanent ponds and marshes, on the wing throughout much of the summer.	1
Idotea	sow bug	Sensitive to low DO, toxic metals and organic enrichment. Data on brackish water isopods indicate that 1 ppm Hg in water can cause mortality at warm temperatures in low salinity.	1,4
Menetus	snail	Have gills but resist low DO using hemoglobin as a respiratory pigment. They are detrivores and bacterial feeders.	1,5
Nehalennia	damsel fly	Inhabits permanent ponds and marshes, on the wing throughout much of the summer.	1,2
Nematoda	nematode	Can inhabit organic enriched sediments.	1
Palaemonetes	grass shrimp	Inhabits vegetation in lentic systems, excellent scavengers that break down detritus, tolerate a wide range of salinities	1,6
Physella	sinistral pond snail	Widespread, abundant, and tolerant of pollution. Have a "lung" for taking in oxygen. Feed on tightly attached periphyton or detritus.	1,5
Somatochlora	dragonfly	Larvae are mostly lentic. Commonly occur in marshes, swamps, cool ponds and littoral areas of lakes.	1,2
Tropisternus	water scavenger beetle	Adults maintain an air supply on hydrofuge hairs but can crawl to the surface to obtain oxygen through plastron respiration.	1,2

1. Thorp and Covich, 2001
2. http://www.cedarcreek.umn.edu/insects/insects.html
3. Maltby, 1995
4. Jones, 1975
5. http://members.aol.com/mkohl2/Physidae.html
6. http://www.chesapeakebay.net/info/palaemonetes.cfm

V. Site Sediment and Detritus Analyses

A. Materials and Methods

Analyses of sediment and detrital characteristics were done at MERI. Grain size, total organic carbon (TOC), percent moisture, acid-volatile sulfides (AVS), simultaneously extracted metals (SEM) and heavy metals (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) were analyzed in June 5, 2002 and October 18, 2002 sediments. Detritus from the same sampling dates were analyzed only for heavy metals. Sampling sites included 3, 7, 9, 10, 18, and 22. Substrates were collected using an Ekman Grab. Detritus was prepared from whole sediment by sieving the sediment through a 1000 micron mesh on site using site water. Substrates were stored in polypropylene containers at −20 ºC until analysis, except those for AVS and SEM, which were stored at −70 ºC.

Sediment characterization was done as follows. TOC and grain size were analyzed by ASTM methods (American Society for Testing and Materials). For percent moisture, samples were weighed before and after oven drying. For TOC, organic matter from dry samples was burned off in a furnace for 16 hours at 550 ºC. % TOC was based on the change in sediment weight before and after ignition (ASTM-D2974). Grain size was determined by drying whole sediments, grinding them up and then sieving through different sized meshes that establish percent gravel, sand, silt and clay (ASTM-D422).

AVS and SEM were analyzed according to Allen and coworkers (1994). Briefly, the closed AVS apparatus consisted of an 8-16 vessel train linked together with Nalgene tubing. Nitrogen gas was used to volatilize and transport reactants through the train. Each station of the train consisted of: one reaction vessel containing oven-dried sediment (7-14 g), deionized water (200 ml) and 6 M HCl (10 ml) to acidify samples; one vessel containing pH buffer 4 (potassium phosphate 0.05 M, Fisher Scientific, Pittsburgh, PA) through which gas flowed to acidify the train; and two silver nitrate traps (200 ml 0.1M $AgNO_3$) into which sulfides flowed from the reaction vessel. At the end of the train was 1 M HCl (200 ml) for acidification of sediment samples. Before passing it through reaction vessels, nitrogen gas was deoxygenated and acidified by passing it through an oxygen scrubber (0 .02 M H_4NO_3V, 0.014 M $HgCl_2$) and pH 4 buffer. All solutions in the train were deoxygentated before use. Reactions ran for 2 hours after which vessel contents settled for 0.5 hours. Sediment sulfide content (AVS) was analyzed by filtering the combined contents of the two silver nitrate traps through 1.2 mm filter paper (Fisher Scientific, Pittsburgh, PA), drying (40 minutes, 104 ° C), desiccating the residue (20 minutes, room temperature), and then determining the change in filter paper weight. SEM was analyzed by collecting 100-160 ml of the acidified water from the reaction vessel and measuring Cd, Cu, Ni, Pb and Zn as described below. Silver nitrate traps were standardized by adding 3-6 ml of 0.1 M NaS to the $AgNO_3$ solution used in the train, then AVS was analyzed as described above. AVS from samples were based on the micromoles (µmol) of sulfides in traps, adjusted for the standard, and divided by quantity of dried sediment added to the reaction vessel, giving µmol/g. SEM was based on µmol of Cd, Cu, Ni, Pb and Zn summed and then divided by quantity of dried sediment added to the reaction vessel.

Heavy metal analyses for sediment and detritus included Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn. Analyses for SEM included Cd, Cu, Ni, Pb and Zn. Fe was also analyzed in SEM samples but was not used for calculating SEM. For metal analysis, sediment samples were oven-dried (yielding 1-2 g dry weight), weighed, and mineralized in 10 ml

trace metal grade HNO_3 in Teflon bombs in a microwave digester. The resultant mineralized solution was boiled off to near dryness and restored to 10 ml volume with 1% HNO_3 analysis. SEM samples were analyzed without further processing. Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analyzed by flame or by graphite furnace atomic absorption spectrophotometry (Varian Spectra AA-220FS) depending on metal concentration. Hg analyses were perform by cold-vapor generation (VGA-77) using a Bacharach MAS-50D mercury analyzer. Trace Metal Standard 1 (BAKER INSTRA-ANALYZED Reagent, Lot V47419') was used as a Quality Control Sample and run with each set of samples along with a blank. Results presented on heavy metals were generated following guidelines established in the New Jersey Department of Environmental Protection's Regulations Governing Laboratory Certification and Standards of Performance (N.J.A.C. 7: 18-1.1 et seq.) and certified to meet all analytical data quality objectives.

B. Results

Marsh sediments and detritus were characterized by measuring heavy metal concentrations and other parameters associated with heavy metal bioavailability and toxicity (Table 5.01). TOC levels in sediments were very high ranging from 7.11 to 87 %. This reflected the large detrital component of sediments and was not unexpected due to the annual died back of wetland grasses and poor microbial degradation found in hypoxic marshes. Sediments were primarily composed of sand, which ranged from 69.5 to 94 %. When combined, the smaller particles of silt and clay ranged from 4.5 to 31%. Taken together the % silt-clay was similar between June and October sediments. Notable exceptions were sites 18 and 22 in June sediments where % silt-clay was 2.5x and 6.3x higher in June than October sediments, respectively. This probably reflected variation in sediment composition at the sampling sites.

SEM−AVS values were all negative except for one replicate from site 10 collected in June (Table 5.02). This indicated that more sulfide was present then biologically available metals and that the sediments should not be toxic. There were apparent seasonal differences as values for SEM−AVS in October were considerably higher than for June in 4 of 6 samples. For example, SEM−AVS for site 3 was -367.2 µmol/g in October and -28.39 µmol/g in June. The seasonal difference was more closely associated with increases in AVS than changes in SEM; in the case of site 3 in October, the increase for AVS was 10-fold while that for SEM only doubled on average. Data for metals indicated that SEM was controlled by Zn concentrations. Zn was typically 10 times higher than Cu and Pb and 100 times higher than Cd and Ni. While Fe had the highest concentration in the acid-extracted sediment, it was not used to calculate SEM. Site differences in Fe plus Zn concentrations were not correlated to the seasonal increases in AVS, Pearson correlation coefficient was 0.102, $p = 0.636$. It therefore appeared that seasonal changes in AVS were not related to heavy metals. Sites 9 and 10 did not show notable seasonal differences in AVS.

Heavy metals were measured in both sediment and detritus and then compared to the Ontario Aquatic Sediment Criterions designated as the Lowest Effects Limit (LEL) and Severe Effects Limit (SEL). For most sites, sediments had concentrations of Cr, Cu and Pb above their respective SELs and would therefore be considered toxic (Table 4). Sites 7 and 9 had the most heavy metals exceeding SEL. Site 22 had no heavy metal concentrations exceeding SEL, but Cd, Cu, Ni and Pb exceeded LEL. Cd did not exceed

SEL in any of the sediments, but did exceed the LEL for all sites. Results for detritus showed that it was also highly contaminated (Table 5). Cu exceeded SEL in all samples. Cd exceeded SEL for all June samples and site 18 from October. Based on heavy metal concentrations, site 7 was the most contaminated while site 22 was the least. Substrate comparisons (% [detritus]/[sediment]) showed that detritus consistently had similar or greater concentrations of Cd, Cu, Ni and Zn than whole sediment (Figure 4). October detritus also had greater concentrations of Fe and Pb compared to sediment. June detritus from site 10 had 5 times more Cd than sediment. Whether the heavy metals in detritus were due to up take by the living plants or adsorption to outer surfaces, clearly detritus was an important source of heavy metal contamination in marsh sediments.

Table 5.01. Grain size, moisture (%) and TOC (%) in sediments from June and October collections.

Site	Mon	Moisture	TOC	Gravel	Coarse Sand	Medium Sand	Fine Sand	Silt	Clay
3	Jun	80.8	23.2	1.27	6.58	23.7	59.0	9.85	0.01
	Oct	89.9	44.9	0.07	11.7	37.8	39.6	1.76	9.15
7	Jun	85.9	32.0	1.67	5.50	25.5	56.2	11.2	0.00
	Oct	76.6	33.0	0.91	9.20	27.4	44.3	15.0	3.28
9	Jun	90.3	52.9	0.01	8.08	40.8	35.3	15.8	0.00
	Oct	89.5	43.8	1.90	17.2	34.4	28.8	11.0	6.81
10	Jun	93.8	77.0	0.93	20.7	34.6	31.4	12.9	0.00
	Oct	91.3	78.2	3.60	12.3	40.7	33.3	9.12	0.00
18	Jun	92.8	87.2	2.47	26.3	44.0	20.6	16.1	0.00
	Oct	95.8	83.2	0.82	30.3	51.2	16.8	6.40	0.00
22	Jun	52.7	11.8	0.48	3.15	11.7	54.7	23.2	6.88
	Oct	47.5	7.11	1.51	3.88	15.9	74.19	3.69	0.80

Table 5.02. SEM, AVS and SEM−AVS in sediments from June and October collections. Units were in µmol/g. SEM equaled combined concentrations of Zn, Ni, Cu, Cd and Pb in acid extracted samples (see Methods). S−A equaled SEM minus AVS concentration. For each month (Mn), samples were replicated (R) twice.

Site	Mn/R	Zn	Ni	Cu	Fe	Cd	Pb	SEM	AVS	S−A	AVE
3	Jun/1	3.33	0.24	0.17	57.0	0.01	0.43	4.20	37.67	-33.47	-28.39
	Jun/2	11.62	0.30	0.28	135.6	0.03	1.52	13.76	37.07	-23.31	
	Oct/1	12.86	0.81	2.76	214.5	0.06	1.94	18.43	381.4	-363.0	-367.2
	Oct/2	12.80	0.67	2.75	128.1	0.04	2.24	18.50	389.8	-371.3	
7	Jun/1	5.49	0.29	0.05	38.8	0.02	0.69	6.54	46.38	-39.84	-34.73
	Jun/2	12.35	0.43	0.35	110.1	0.08	3.67	16.87	46.49	-29.62	
	Oct/1	58.66	0.84	0.75	99.0	0.09	2.05	62.40	468.2	-405.8	-452.4
	Oct/2	21.77	1.26	1.15	135.9	0.12	6.57	30.88	529.8	-499.0	
9	Jun/1	24.38	0.62	0.07	282.8	0.03	0.92	26.02	105.0	-78.99	-54.50
	Jun/2	19.09	0.62	0.26	386.3	0.07	3.13	23.19	53.19	-30.01	
	Oct/1	19.72	0.81	3.88	288.2	0.10	5.71	30.23	141.8	-111.6	-83.08
	Oct/2	18.98	1.60	7.06	513.7	0.16	9.62	37.42	92.01	-54.59	
10	Jun/1	4.73	0.26	0.74	26.0	0.02	0.86	6.61	26.42	-19.81	-9.34
	Jun/2	61.92	0.60	0.91	68.7	0.05	3.57	67.06	65.92	1.14	
	Oct/1	6.69	0.44	1.41	35.6	0.02	1.30	9.85	39.74	-29.89	-36.23
	Oct/2	6.01	0.38	1.12	27.1	0.01	0.22	7.73	50.31	-42.58	
18	Jun/1	45.50	0.30	0.60	22.9	0.29	0.99	47.69	30.55	-17.14	-9.19
	Jun/2	43.54	0.19	0.20	13.1	0.01	0.84	44.79	80.30	-35.51	
	Oct/1	66.45	1.11	3.91	89.1	0.01	1.07	72.55	174.8	-102.3	-191.5
	Oct/2	40.91	2.64	8.11	331.4	0.02	2.63	54.30	335.1	-280.8	
22	Jun/1	1.40	0.11	0.20	59.4	0.00	0.37	2.08	8.19	-6.11	-3.95
	Jun/2	1.08	0.08	0.05	46.9	0.00	0.10	1.31	3.10	-1.79	
	Oct/1	10.51	1.30	4.57	474.0	0.02	2.61	19.01	98.15	-79.14	-55.03
	Oct/2	22.48	1.77	4.59	655.8	0.21	2.67	31.72	62.64	-30.92	

Table 5.03. Heavy metal concentrations (mg/kg) in Kearny Marsh sediments for June and October collections. Asterisks indicated concentrations above SEL.

Site	Mon	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Total[a]
3	Jun	3.13	120*	56	22822	0.55	168	31	153	172	435
	Oct	5.83	100	177*	35909	1.78	321	68	385*	646	1383
7	Jun	7.98	232*	210*	32206	8.32*	262	73	591*	671	1792
	Oct	5.75	101	159*	21885	6.97*	254	56	415*	447	1190
9	Jun	8.25	502*	243*	38983	2.23*	407	97*	661*	955*	2468
	Oct	9.75	512*	295*	53968	3.40*	542	93*	777*	1019*	2708
10	Jun	5.32	182*	153*	11701	1.61	183	57	435*	385	1218
	Oct	4.87	128*	137*	14819	1.29	386	61	458*	464	1255
18	Jun	4.27	66	148*	16227	1.22	382	66	526*	428	1240
	Oct	4.34	52	142*	14304	0.89	311	55	497*	410	1161
22	Jun	1.94	16	41	12032	0.21	183	23	61	79	222
	Oct	1.53	6	39	9386	0.16	148	22	70	78	218
LEL		0.60	26	16	NS	0.20	NS	16.0	31	120	
SEL		10.00	110	110	NS	2.00	NS	75.0	250	820	

[a]Total = Includes concentrations for Cd, Cr, Cu, Hg, Ni, Pb and Zn but not Fe and Mn.
LEL = Lowest Effects Limit based on Ontario Aquatic Sediment Criterion.
SEL = Severe Effects Limit based on Ontario Aquatic Sediment Criterion.
NS = No sediment criterion, Mon = Month collected.

Table 5.04. Heavy metal concentrations (mg/kg) in Kearny Marsh detritus for June and October collections. Asterisks indicated concentrations above SEL.

Site	Mon	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Total[a]
3	Jun	18.2*	27	270*	15816	0.97	446	40	256*	409	1021
	Oct	6.4	40	219*	26474	0.51	297	47	490*	314	1117
7	Jun	12.2*	66	240*	12636	3.56*	136	56	239	771	1389
	Oct	6.4	71	257*	41325	2.18*	149	57	546*	409	1349
9	Jun	22.2*	95	211*	19528	0.15	253	50	235	477	1090
	Oct	9.1	233*	205*	35481	2.23*	200	62	321*	428	1261
10	Jun	25.2*	37	187*	5677	0.27	194	43	108	567	968
	Oct	7.8	82	212*	19403	0.49	567	56	536*	628	1522
18	Jun	16.3*	9	223*	1896	0.56	89	27	71	394	741
	Oct	19.2*	50	278*	11209	0.55	220	47	496*	363	1253
22	Jun	8.8	18	145*	13220	0.84	212	28	49	249	499
	Oct	2.3	19	120*	22515	0.08	268	3	136	168	477
LEL		0.6	26	16	NS	0.20	NS	16	31	120	
SEL		10.0	110	110	NS	2.00	NS	75	250	820	

[a]Total = Includes concentrations for Cd, Cr, Cu, Hg, Ni, Pb and Zn but not Fe and Mn (mg/kg).
LEL = Lowest Effects Limit based on Ontario Aquatic Sediment Criterion.
SEL = Severe Effects Limit based on Ontario Aquatic Sediment Criterion.
NS = No sediment criterion, Mon = Month collected.

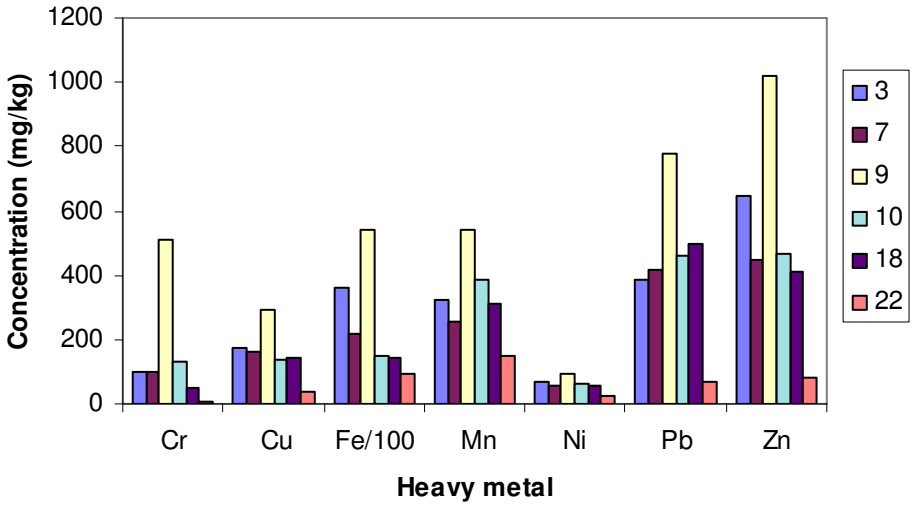


Figure 5.01. Heavy metal concentrations (mg/kg) in sediments from October collection. Data represent metals from each site. Fe concentrations were divided by 100. June data were similar to those in October.

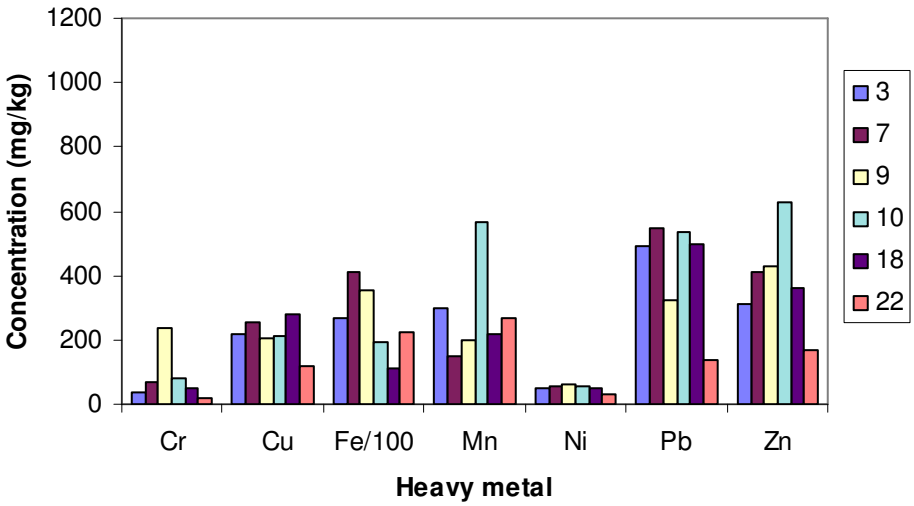


Figure 5.02. Heavy metal concentrations (mg/kg) in detritus from October collection. Data represent metals from each site. Fe concentrations were divided by 100. June data were similar to those in October, except for Pb which was higher in October.

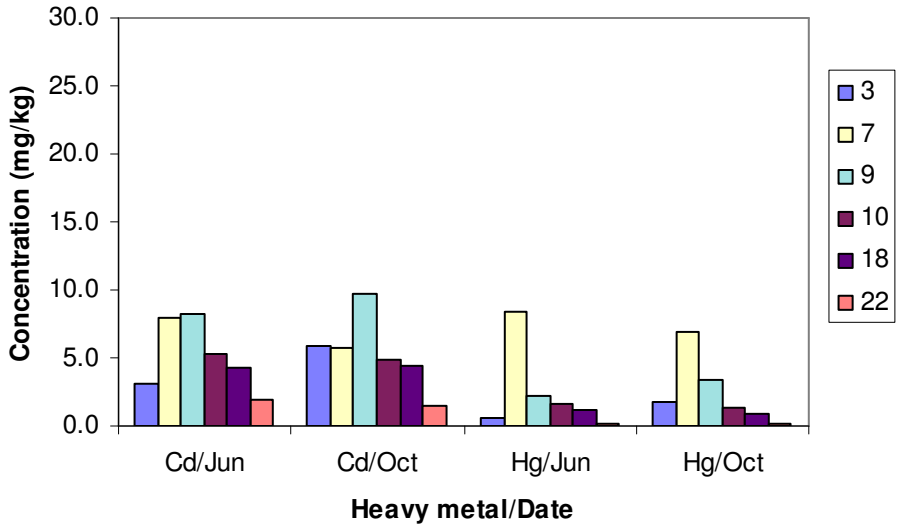


Figure 5.03. Cd and Hg (mg/kg) in sediments from June and October collections. Data represent metals from each site

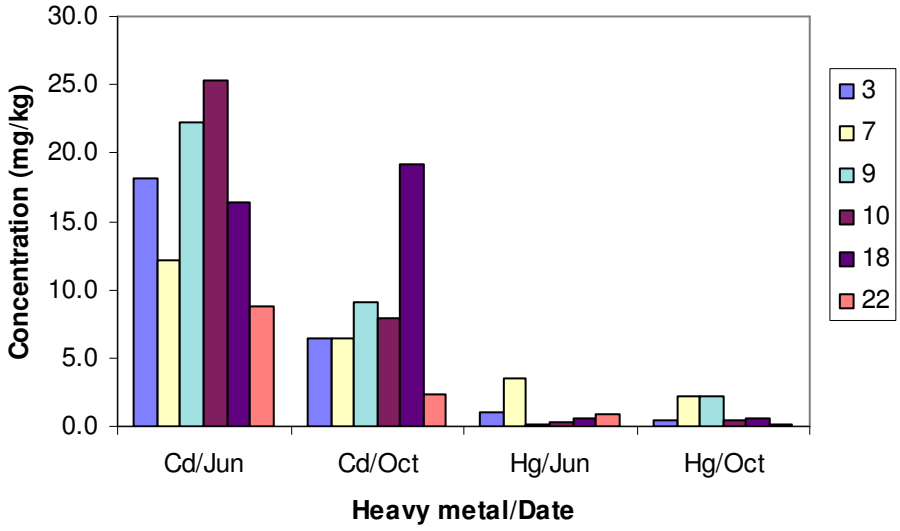


Figure 5.04. Cd and Hg (mg/kg) in detritus from June and October collections. Data represent metals from each site

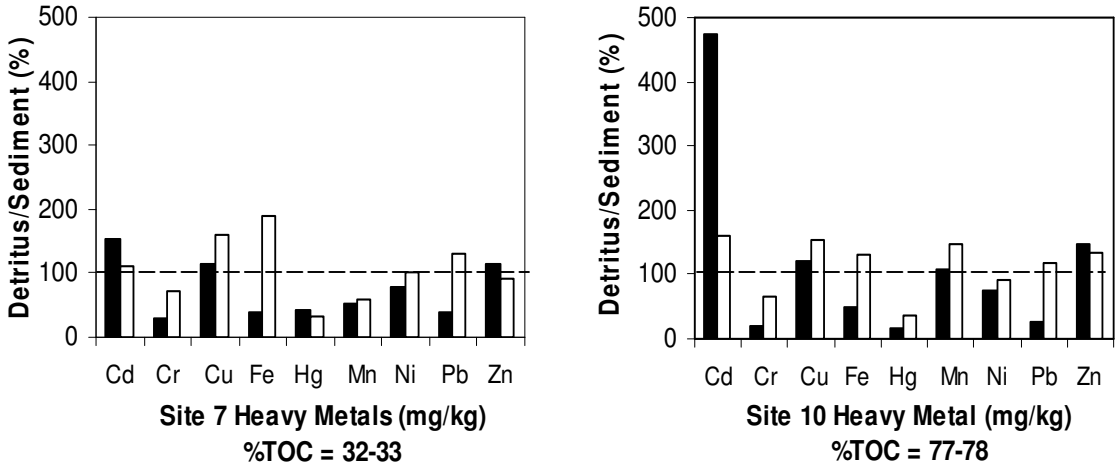


Figure 5.05. Comparison of heavy metals in detritus and sediment. Ratios of heavy metals in detritus versus whole sediment (%) are shown for sites 7 and 10 collected in June (closed column) and October (open column). A reference line for equivalent levels of metals in detritus and sediments is provided. The two sites had different levels of TOC, which did not appear to influence detritus to sediment ratios overall.

F. Analysis and Discussion

Kearny Marsh sediments were characterized in terms of common parameters such as grain size, % TOC, SEM−AVS and heavy metals. TOC in Kearny Marsh sediments ranged from 7.1-87.2 %, most samples had over 32 %. This large amount of organic matter was found primarily as poorly decomposed plant matter (detritus) probably due to suboxic conditions in the marsh. Similar TOC levels were found in Canadian mesohaline (approximately 0.5-2 ppt saline) wetlands, which ranged from 50 – 70 % (Bendell-Young *et al*., 2002). TOC has varied widely even for similar ecosystems. Foundry Cove is a mesohaline wetland that is part of the Hudson River watershed in New York, sediments there had lower TOC, 0.8-13 % (Hansen *et al*., 1996). Kearny Marsh grain size was dominated by sand, typically > 80 %. The level of sand was similar to that in Massachusetts salt marshes, which averaged 80 % (Hansen *et al*., 1996). This seemed reasonable as Kearny marsh was once connected to the Hackensack River estuary system.

The high concentrations of heavy metals found in sediments and detritus indicated that both substrates should be toxic for all sites. Many metal concentrations in sediment exceeded the Ontario SEL criterion for aquatic sediments. In addition, many detrital concentrations exceeded the LEL criterion. The highest heavy metal concentrations were found in sediment and detritus from sites 7 and 9. Their close proximity to one of the landfills might account for this. Sediment heavy metal concentrations were generally lower for the Kearny Marsh data reported here than reported in the Langan report (Langan Engineers and Environmental Services, 1999). For example, total concentration of Cd, Cu, Cr, Hg, Ni, Pb and Zn in 0-1 inches of sediment from site 7 was 2453 mg/kg for our sediment and 6380 mg/kg in Langan, 1999. Site 9 concentrations were 2593 for our sediment and 12275 in Langan, 1999. On the other hand, concentrations at sites 3 and 22 were higher here (536 and 1428, respectively) than in Langan (100 and 245,

respectively). The differences might be due to the depth at which the sediment samples were collected. Metal concentrations in Kearny Marsh were similar to or greater than those found in the Hackensack River and Newark Bay which were 10 ± 6 mg/kg Cd, 237 ± 222 mg/kg Cu, 2.1 ± 2.6 mg/kg Hg, 39 ± 49 mg/kg Ni, 421 ± 571 mg/kg Pb and 395 ± 403 mg/kg Zn (Bonnevie *et al*., 1994).

The relationship between sediment and detrital concentrations was interesting. Fe, Pb and Zn had the highest concentrations in both sediment and detritus at all sites (Figures 5.01 and 5.02). However, site 9 had the highest metal concentrations in sediment compared to other sites but not in detritus. This suggested that something was limiting the availability of sediment metals to plants at site 9. Cd was actually higher in detritus than in sediment at all sites while Hg levels were lower (Figures 5.03 and 5.04). Ratios of detritus to sediment concentrations showed that some heavy metals, particularly Cd, Cu and Zn were higher in detritus than sediment (Figure 5.05). Seasonal trends were also apparent. In June, ratios of Cd were much higher than in October, while Fe and Pb ratios were usually higher in October than June (Tables 5.03 and 5.04). The detritus may have been contributing to a labile pool of metals that were more or less available during the year. These results were consistent with release of Cd from metal precipitates under oxic conditions (early June) and of Pb under suboxic ones (early fall) (Reddy and Patrick, 1977).

Data did not discriminate between heavy metals adsorbed on to detritus (Windham *et al*., 2004) and those accumulated by plants during their lifetime (Scholes *et al*., 1998, Peltier *et al*., 2003). Results from Windham and coworkers indicated that adsorption from surroundings is important. They found that submerged litter from wetland plants accumulated heavy metals in excess of sediment concentrations. Cu, Pb and Zn adsorption was greater than that of Cr and Hg as in this study. They did not measure Cd. On the other hand, live Phragmites plants have been found to bioaccumulate heavy metals in leaves and roots (Scholes *et al*., 1998, Peltier *et al*., 2003, Weis *et al*., 2002). Relative proportions of metals in plant tissue were similar to those in Kearny marsh detritus, Zn>Cu>Pb>Cr=Cd. Ratios of plant to sediment concentrations for Zn and Cu were also similar to those found at Kearny Marsh (Scholes *et al*., 1998). The ratio for Cd, however, could not be deduced from the data provided. Overall, Kearny Marsh data indicated that detritus was an important source of heavy metals whether or not the original source was bioaccumulated metals or adsorption from surroundings.

Negative values for SEM−AVS (Table 5.02) indicated that sediments should not be toxic even though metal concentrations exceeded LELs and SELs. Interpretation of the negative values would be that there was sufficient sulfide available for binding up heavy metals and making them unavailable to biota (Hansen *et al*., 1996). Formation of metal sulfides has been used to explain the apparent lack of toxicity for anaerobic sediments that are highly contaminated with heavy metals (Lau and Chu, 2000). However, the metals might have been associated with other more bioavailable fractions such as detritus. Metals in detritus contributed to concentrations in whole sediment but were not necessarily available for sulfide binding. Correlation between % TOC and heavy metals in sediment showed that heavy metals were tied up in the organic carbon fraction of sediments (Figure 5.06). Correlations between %TOC and total heavy metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) in sediment from all sites was not significant (R^2 =

0.361). However, if data from sites 10 and 18, which had sediments with the highest %TOC were not included, then sediment heavy metals were significantly correlated with % TOC ($R^2 = 0.886$, $p = 0.003$). Apparently sites 10 and 18 had a source of organic matter less associated with metals.

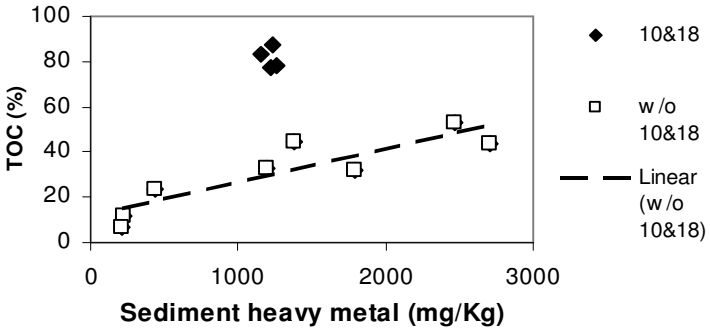


Figure 5.06. Total heavy metals (mg/kg) in sediment versus TOC (%). Total heavy metals included Cd, Cr, Cu, Hg, Ni, Pb and Zn but not Fe and Mn.

Correlations between SEM−AVS and heavy metals in June and October sediment showed seasonal differences. The correlation in June was nearly significant ($R^2 = 0.739$, $p = 0.091$), while that in October was not ($R^2 = 0.018$, $p = 0.973$). Seasonal differences have been observed by other investigators (Azzoni *et al.*, 2001). They found that the return of oxic conditions after summer released Fe and other heavy metals into the water column. This was attributed to oxidation of the sulfide in the heavy metal complex to sulfate. Wetland plants have complicated this scenario by releasing oxygen from their roots into water and generating oxic microenvironments. Under these conditions, labile metal sulfides were dissolved releasing heavy metals into the water. The seasonal variation and toxicity of Kearny Marsh sediments might be explained in part by this effect of wetland plants. In June, plants provided enough oxygen to convert sulfides to sulfate so the pool of AVS was lower. In October, free sulfides increased as plant activity declined and temperatures were still warm enough to sustain microbial sulfate reduction. Therefore, the amount of sulfides was no longer correlated with metal concentrations.

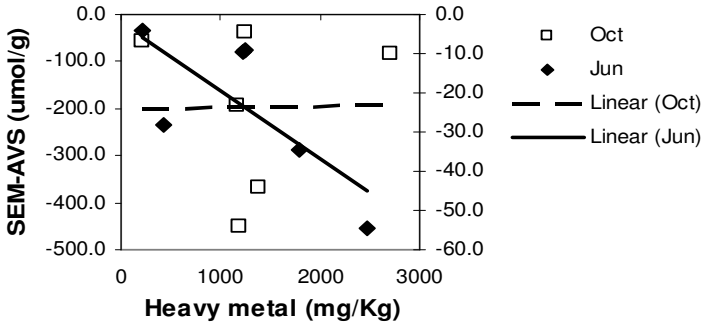


Figure 5.07. Total heavy metals in sediment versus SEM-AVS. Total heavy metals included Cd, Cr,Cu, Hg, Ni, Pb and Zn.

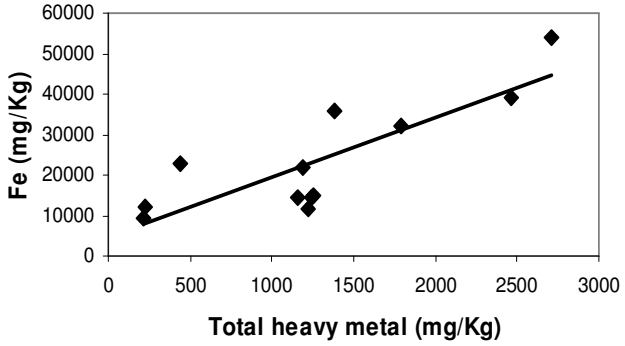


Figure 5.08. Fe (mg/kg) versus total heavy metal (mg/Kg) in sediment. Total heavy metal include Cd, Cr, Cu, Hg, Ni Pb and Zn.

Fe compounds such as organic-Fe complexes and Fe oxides have also been found to adsorb heavy metals (MartInez and McBride, 2001). Correlation between Fe and total heavy metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) was significant using data from all sites (R^2 = 0.822, p = 0.001). Overall, data suggested that heavy metals were associated with various sediment fractions, with sulfides playing a less important role than organic matter and Fe oxides.

VI. Toxicity Testing

A. Materials and Methods

Toxicity tests were performed in triplicate with 10 larvae per container. Acute toxicity tests used 4th instars and ran for 96 h. Subchronic toxicity tests used 2nd-3rd instars and ran for 10 d. Initial weights were taken for subchronic tests. Each toxicity test included fed and unfed larvae for each sample and control. Two separate acute and subchronic tests were performed for June 5, 2002 sediment. For detritus, one acute and two subchronic tests were done due to insufficient sample quantities. Endpoints for acute tests were percent survival and whole-body carbohydrate levels (µg monosaccharide/mg larvae). Endpoints for subchronic tests were percent survival and weight (mg/larvae).

Conditions for toxicity tests were as follows. Containers were 1 L polypropylene with either 3 g detritus or 50 ml sediment and 250 ml test water [particle and carbon filtered water using filters CDPRM1206 and CDFC012 04, respectively (Millipore Corp., MA)]. Substrate and water were combined and allowed to sit overnight; larvae were added the next day. The light cycle was 12 h light/12 h dark. Temperature ranged from 23-26 ºC. The pH was taken at the beginning and end of each experiment (Sentron Model 2001 pH System, Sentron Inc., WA). In acute toxicity tests of sediment, pH ranged from 7.4-7.9 in controls and 7.1-7.8 in samples. Sediment subchronic tests had pH ranging from 7.4-7.9 in controls and 7.1-7.7 in samples. In acute toxicity tests of detritus, pH ranged from 6.7-7.0 in controls and 7.5-7.8 in samples. Detritus subchronic tests had pH ranging from 6.5-7.4 in controls and 7.3-7.8 in samples. Fed larvae received 3 drops of fish food (1 g/10 ml, ground TetraDoro Green® Floating Food Sticks, Tetra, Germany) on the first day of the experiment in acute tests and twice a week in subchronic tests.

Controls for toxicity tests were as follows. For sediment experiments, negative control consisted of 40 ml (60 g) acid-washed sand (American Stone Mix, Inc., MD) and 250 ml test water. Positive control consisted of 40 ml acid-washed sand, 250 ml test water and 0.3 mM Cd. A positive control was not run for the first acute sediment toxicity test. For June detritus experiments, negative control consisted of 3 g hydrated cerophyll (Ward's Natural Science Establishment, Inc., NY) added to 250 ml test water. Positive control consisted of 3 g rehydrated cerophyll, 250 ml test water and 0.3 mM Cd. Cd was mixed into the containers after the substrate and water were added. High levels of bacterial growth with cerophyll caused poor survival in June controls; therefore, controls for October detritus experiments were changed. For October detritus, negative control for the first experiment consisted of 3 g June detritus from site 22 added to 250 ml test water. Positive control consisted of 3 g of the same detritus, 250 ml test water and 0.3 mM Cd. For the second experiment, positive control consisted of 3 g of October detritus from site 3, 250 ml test water and 0.3 mM Cd. There was no negative control in the second October experiment; detritus from site 3 with and without Cd were compared.

Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey posthoc test. This determined whether or not samples were significantly different from one another and from controls. Responses of Fed versus Unfed larvae were also evaluated by student T-test. Groups were considered statistically different if $p \leq 0.05$.

B. Results for Acute Toxicity Tests

1. Sediment

With June sediment, neither the first nor second acute toxicity tests (96 h) showed significant differences between treatment groups for survival (%) in Fed chironomids. One way ANOVA found $p = 0.564$ and 0.632, respectively. Unfed chironomids also showed no acute sediment lethality in the first, $p = 0.738$, or second, $p = 0.840$, experiment. As seen in Tables 6.01 and 6.02, there were low values (< 80 % survival) for Unfed groups in some of the triplicates: site 3- 1st acute, site 22- 1st acute, site 7- 2nd acute and site 9- 2nd acute. However, the lack of consistency within triplicates and between toxicity tests suggested the responses were irrelevant. Similar data was found with October sediment (Tables 6.03 and 6.04). One way ANOVA found $p = 0.594$ and 0.236 for Fed in the first and second experiments, respectively. For Unfed, $p = 0.798$ and 0.096 in the first and second experiments, respectively. T-tests of data combined from the two experiments showed no significant differences in survival between Fed and Unfed chironomids in June or October sediment, $p > 0.05$ (Figures 6.01 and 6.02). Therefore, adding food supplements did not affect survival at 96 h.

2. Detritus

There was only sufficient June detritus for one acute toxicity test (96 h). Both the negative and positive controls had significantly reduced survival, averaging 0 and 20 % in Fed and 0 and 10 % in Unfed, respectively (Table 6.05). The control substrate consisted of hydrated cerophyll. This is a formula of dried grasses sold for the purpose of culturing certain protists and crustaceans. Lethality in controls was attributed to high levels of bacteria, which were observed as unusually cloudy water. Statistical analysis of data, therefore, did not include the controls. One way ANOVA found no statistically significant treatment effects between sites in Fed, $p = 0.092$, and Unfed, $p = 0.333$, groups. In Fed, detritus from site 10 did reduce survival compared to site 3, but it was not significant, $p = 0.068$ in Tukey posthoc test. Adding food supplements to June detritus did not have an affect on survival at 96 h, $p > 0.05$ (Figure 6.03). Two separate experiments were run with October detritus (Tables 6.06 and 6.07). There were no apparent effects on survival for Fed or Unfed. For Fed, one way ANOVA for the first experiment was $p = 0.878$, for the second, it was $p = 0.724$. For Unfed, values for the first and second experiment were $p = 0.859$ and 0.177, respectively. T-tests of data combined from the two experiments showed no significant differences in survival between Fed and Unfed chironomids in October detritus, $p > 0.05$ (Figure 6.04). October results supported the lack of affects on survival found for June data and that adding ground fish food did not improve survival at 96 h.

3. Sediment versus detritus

Survival in sediment and detritus from June and October were compared. T-tests for each treatment showed no statistically significant differences for Fed and Unfed

chironomids for either month, $p > 0.05$. Only figures for October are shown (Figures 6.05 and 6.06). Overall, the endpoint of survival at 96 h showed no toxicity in any of the sediments and detritus tested.

Table 6.01. First acute toxicity test (96 h) for sediment from 6-5-02 collection. No significant differences were found between treatments for Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	100	86.7±15.3	–C-1	80	86.7±5.8
–C-2	70		–C-2	90	
–C-3	90		–C-3	90	
+C-1	ND		+C-1	ND	
+C-2	ND		+C-2	ND	
+C-3	ND		+C-3	ND	
3-1	90	86.7±5.8	3-1	60	73.3±23.1
3-2	80		3-2	60	
3-3	90		3-3	100	
7-1	80	83.3±5.8	7-1	100	90.0±10.0
7-2	80		7-2	80	
7-3	90		7-3	90	
9-1	90	86.7±5.8	9-1	90	90.0±0.0
9-2	90		9-2	90	
9-3	80		9-3	90	
10-1	90	90.0±10.0	10-1	80	86.7±11.5
10-2	100		10-2	80	
10-3	80		10-3	100	
18-1	90	93.3±5.8	18-1	90	83.3±11.5
18-2	100		18-2	90	
18-3	90		18-3	70	
22-1	80	80.0±0.0	22-1	100	76.7±25.2
22-2	80		22-2	80	
22-3	80		22-3	50	

–C = Control = acid-washed sand and 250 ml test water.
+C = Control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.

Table 6.02. Second acute toxicity test (96 h) for sediment from 6-5-02 collection. No significant differences were found between treatments for Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	70	86.7±15.3	–C-1	70	80.0±17.3
–C-2	90		–C-2	100	
–C-3	100		–C-3	70	
+C-1	90	86.7±5.8	+C-1	90	76.7±32.1
+C-2	90		+C-2	100	
+C-3	80		+C-3	40	
3-1	80	96.7±15.3	3-1	90	86.7±5.8
3-2	110		3-2	80	
3-3	100		3-3	90	
7-1	ND	ND	7-1	90	70.0±26.5
7-2	ND		7-2	80	
7-3	ND		7-3	40	
9-1	90	80.0±17.3	9-1	100	70.7±42.3
9-2	90		9-2	90	
9-3	60		9-3	22.2	
10-1	100	96.3±6.4	10-1	100	93.3±5.8
10-2	100		10-2	90	
10-3	88.9		10-3	90	
18-1	80	90.0±10.0	18-1	90	93.3±5.8
18-2	90		18-2	90	
18-3	100		18-3	100	
22-1	90	93.3±5.8	22-1	60	80.0±17.3
22-2	90		22-2	90	
22-3	100		22-3	90	

–C = Control = acid-washed sand and 250 ml test water.

+C = Control = acid-washed sand, 250 ml test water and Cd (0.3 mM).

ND = No data.

Table 6.03. First acute toxicity test (96 h) for sediment from 10-18-02 collection. No significant differences were found between treatments for Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	90	90.0±0.0	–C-1	80	90.0±10.0
–C-2	90		–C-2	90	
–C-3	90		–C-3	100	
+C-1	90	83.3±11.5	+C-1	70	80.0±17.3
+C-2	70		+C-2	100	
+C-3	90		+C-3	70	
3-1	100	96.7±5.8	3-1	80	90.0±10.0
3-2	100		3-2	90	
3-3	90		3-3	100	
7-1	100	90.0±10.0	7-1	90	90.0±10.0
7-2	90		7-2	100	
7-3	80		7-3	80	
9-1	100	93.3±5.8	9-1	90	90.0±0.0
9-2	90		9-2	90	
9-3	90		9-3	90	
10-1	80	93.3±11.5	10-1	70	86.7±15.3
10-2	100		10-2	100	
10-3	100		10-3	90	
18-1	90	96.7±5.8	18-1	80	90.0±10.0
18-2	100		18-2	90	
18-3	100		18-3	100	
22-1	100	90.0±10.0	22-1	100	96.7±5.8
22-2	80		22-2	100	
22-3	90		22-3	90	

–C = Control = acid-washed sand and 250 ml test water.

+C = Control = acid-washed sand, 250 ml test water and Cd (0.3 mM).

ND = No data.

Table 6.04. Second acute toxicity test (96 h) for sediment from 10-18-02 collection. No significant differences were found between treatments for Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	90	93.3±5.8	–C-1	100	90.0±17.3
–C-2	100		–C-2	100	
–C-3	90		–C-3	70	
+C-1	60	60.0±0.0	+C-1	60	66.7±11.5
+C-2	60		+C-2	80	
+C-3	60		+C-3	60	
3-1	90	56.7±35.1	3-1	80	60.0±20.0
3-2	60		3-2	40	
3-3	20		3-3	60	
7-1	80	80.0±10.0	7-1	60	66.7±11.5
7-2	90		7-2	80	
7-3	70		7-3	60	
9-1	60	70.0±17.3	9-1	80	80.0±10.0
9-2	90		9-2	90	
9-3	60		9-3	70	
10-1	80	80.0±10.0	10-1	90	96.7±5.8
10-2	70		10-2	100	
10-3	90		10-3	100	
18-1	60	56.7±35.1	18-1	90	83.3±20.8
18-2	90		18-2	100	
18-3	20		18-3	60	
22-1	80	83.3±5.8	22-1	60	70.0±17.3
22-2	80		22-2	90	
22-3	90		22-3	60	
+22-1	80	83.3±5.8			
+22-2	80				
+22-3	90				

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
+22 = Site 22 sediment with cadmium (0.3 mM)
ND = No data.

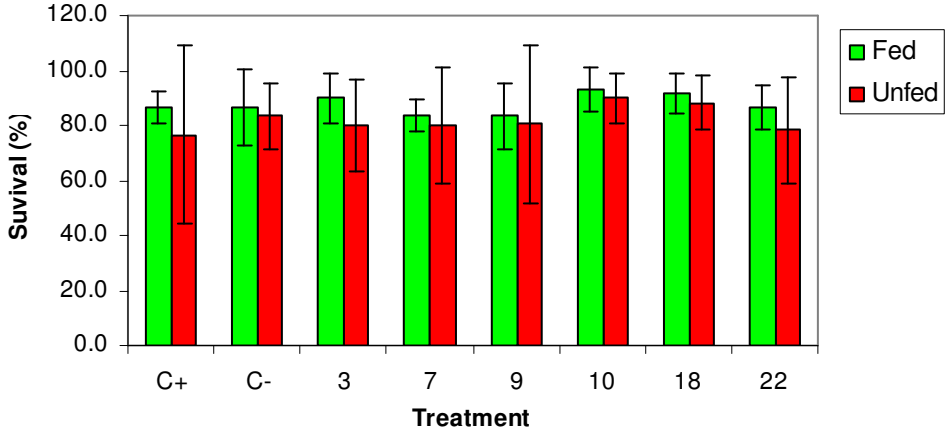


Figure 6.01. Percent survival in June sediment at 96 h. Data for the 1st and 2nd experiments were combined, n=3-6. Error bars represent 1 SD. There were no significant differences between or within (Fed versus Unfed) treatment groups, p>0.05.

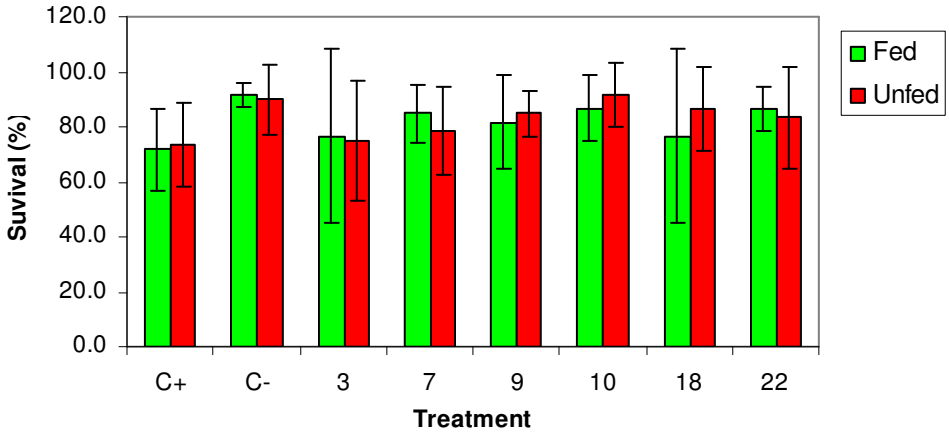


Figure 6.02. Percent survival in October sediment at 96 h. Data for the 1st and 2nd experiments were combined, n=6. Error bars represent 1 SD. There were no significant differences between or within (Fed versus Unfed) treatment groups, p>0.05.

Table 6.05. Acute toxicity test (96 h) of detritus from 6-5-02 collection. Cerophyll was used in negative and positive controls, which developed high levels of bacteria and compromised survival. No statistically significant differences were found between sites in Fed and Unfed treatment groups.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	0	0.0±0.0	–C-1	0	0.0±0.0
–C-2	0		–C-2	0	
–C-3	ND		–C-3	0	
+C-1	0	20.0±20.0	+C-1	0	10.0±10.0
+C-2	40		+C-2	20	
+C-3	20		+C-3	10	
3-1	100	100.0±0.0	3-1	100	100.0±0.0
3-2	100		3-2	100	
3-3	100		3-3	100	
7-1	90	90.0±0.0	7-1	100	100.0±0.0
7-2	90		7-2	100	
7-3	90		7-3	100	
9-1	100	96.7±5.8	9-1	100	96.7±5.8
9-2	100		9-2	100	
9-3	90		9-3	90.0	
10-1	80	83.0±5.1	10-1	80	90.8±10.1
10-2	80		10-2	92	
10-3	89		10-3	100	
18-1	80	90.0±10.0	18-1	80	90.0±10.0
18-2	90		18-2	90	
18-3	100		18-3	100	
22-1	100	90.0±10.0	22-1	90	96.7±5.8
22-2	90		22-2	100	
22-3	80		22-3	100	

–C = Negative control = hydrated cerophyll and 250 ml test water.
+C = Positive control = hydrated cerophyll, 250 ml test water and Cd (0.3 mM).
ND = No data.

Table 6.06. First acute toxicity test (96 h) of detritus from 10-18-02 collection. No statistically significant differences were found between treatment groups in Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	100	95.0±7.1	–C-1	100	100.0±0.0
–C-2	90		–C-2	100	
–C-3	ND		–C-3	ND	
+C-1	100	100.0±0.0	+C-1	100	100±0.0
+C-2	100		+C-2	100	
+C-3	ND		+C-3	ND	
3-1	90	93.3±5.8	3-1	100	90.0±17.3
3-2	100		3-2	70	
3-3	90		3-3	100	
7-1	100	96.7±5.8	7-1	100	86.7±11.5
7-2	90		7-2	80	
7-3	100		7-3	80	
9-1	100	96.7±5.8	9-1	100	93.3±11.5
9-2	90		9-2	100	
9-3	100		9-3	80.0	
10-1	100	93.3±11.5	10-1	90	93.3±5.8
10-2	80		10-2	100	
10-3	100.0		10-3	90	
18-1	90	90.0±10.0	18-1	100	90.0±10.0
18-2	100		18-2	90	
18-3	80		18-3	80	
22-1	90	93.3±5.8	22-1	100	93.3±11.5
22-2	90		22-2	100	
22-3	100		22-3	80	

–C = Negative control = detritus from June, site 22, and 250 ml test water.
+C = Positive control = detritus from June, site 22, 250 ml test water and Cd (0.3 mM).
ND = No data.

Table 6.07. Second acute toxicity test (96 h) for detritus from 10-18-02 collection. No statistically significant differences were found between treatment groups in Fed or Unfed.

FED			UNFED		
Sample	% survival	Ave. ± SD	Sample	% survival	Ave. ± SD
–C-1	ND	ND	–C-1	ND	ND
–C-2	ND		–C-2	ND	
–C-3	ND		–C-3	ND	
+C-1	90	90.0±0.0	+C-1	80	83.3±5.8
+C-2	90		+C-2	90	
+C-3	90		+C-3	80	
3-1	70	86.7±15.3	3-1	60	80.0±17.3
3-2	90		3-2	90	
3-3	100		3-3	90	
7-1	60	83.3±20.8	7-1	60	50.0±36.1
7-2	90		7-2	80	
7-3	100		7-3	10	
9-1	90	93.3±5.8	9-1	90	93.3±5.8
9-2	100		9-2	100	
9-3	90		9-3	90	
10-1	90	86.7±5.8	10-1	60	80.0±20.0
10-2	90		10-2	80	
10-3	80		10-3	100	
18-1	80	80.0±10.0	18-1	70	80.0±10.0
18-2	70		18-2	80	
18-3	90		18-3	90	
22-1	100	93.3±5.8	22-1	80	66.7±11.5
22-2	90		22-2	60	
22-3	90		22-3	60	

–C = Negative control = Not done

+C = Positive control = detritus from October, site 3, 250 ml test water and Cd (0.3 mM).

ND = No data.

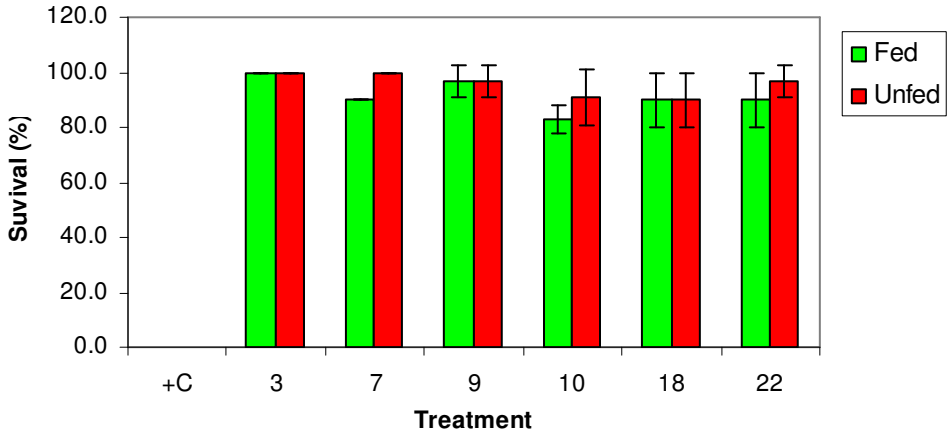


Figure 6.03. Percent survival in June detritus at 96 h. Data for the 1st and 2nd experiments were combined, n=3. Error bars represent 1 SD. There were no significant differences between or within (Fed versus Unfed) treatment groups, p>0.05.

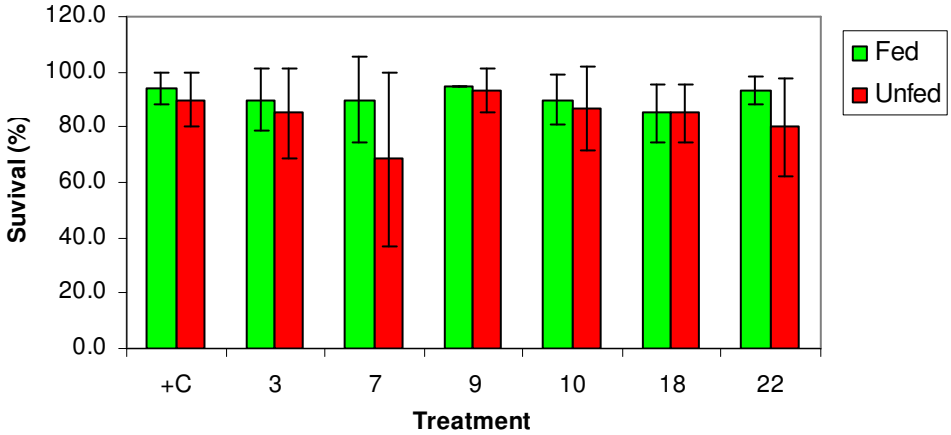


Figure 6.04. Percent survival in October detritus at 96 h. Data for the 1st and 2nd experiments were combined, n=5-6. Error bars represent 1 SD. There were no significant differences between or within (Fed versus Unfed) treatment groups, p>0.05.

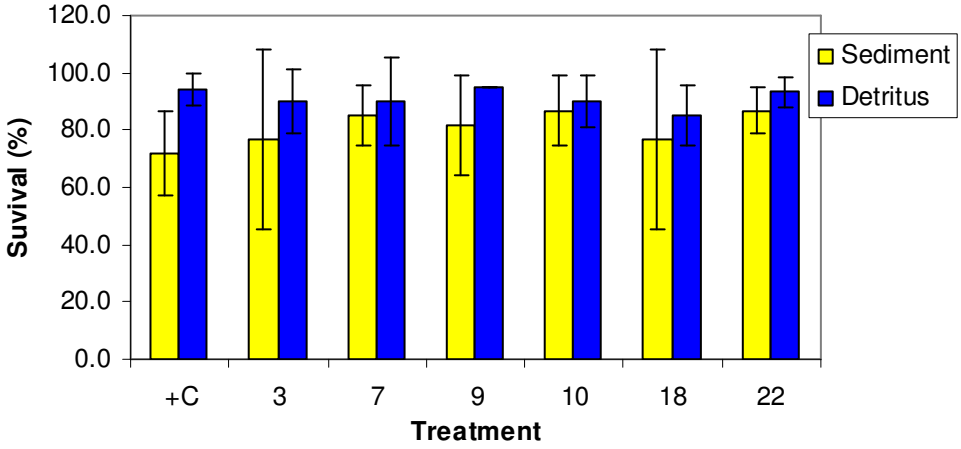


Figure 6.05 Comparison of survival (%) in October sediment and detritus in Fed at 96 h. Data for the 1st and 2nd experiments were combined, n=6. Error bars represent 1 SD. There were no significant differences between control or sites, p>0.05.

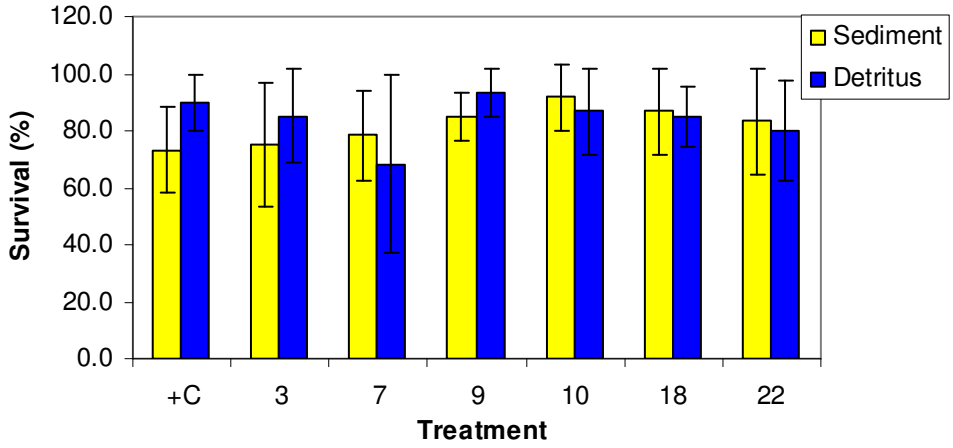


Figure 6.06 Comparison of survival (%) in October sediment and detritus in Unfed at 96 h.Data for the 1st and 2nd experiments were combined, n=6. Error bars represent 1 SD. There were no significant differences between control or sites, p>0.05.

C. Results for Subchronic Toxicity Tests

1. Sediment

Sediments collected June 5, 2002 and October 18, 2002 were used in subchronic toxicity tests with 10 d growth (mg/larvae) and survival (%) as endpoints. Two separate experiments were conducted in triplicate for each sediment, n = 6. Results for June sediment in experiments 1 and 2 are shown in Tables 6.08-6.09 and 6.10-6.11, respectively. Results for October sediment in experiments 1 and 2 are shown in Tables 6.12-6.13 and 6.14-6.15, respectively.

For June experiments, Fed larvae showed no statistically significant differences in growth for negative control and sediments, $p > 0.05$. Average values ranged from 4.337 to 6.368 mg/larvae. Growth in positive control was reduced compared to all other treatments, $p \leq 0.001$. Average values for positive controls were 0.688 and 0.538 in experiments 1 and 2, respectively. Unfed larvae showed statistically significant differences in growth for controls and sediments. Growth was reduced in negative and positive controls. Growth in negative control was undoubtly suppressed due to the absence of food and lack of nutritional value from acid-washed sand. For sediments, growth in site 7 (0.761±0.001 and 1.127±0.471 mg/larvae) was reduced in the 1st and 2nd experiments, respectively, compared to sites 9 (1.644±0.152 and 1.433±0.521 mg/larvae) and 18 (1.569±0.148 and 1.394±0.057). The difference was statistically significant when data from the two experiments were combined, n = 5-6, $p \leq 0.05$ (Figure 6.08).

Results for Fed larvae in October experiments were similar to those in June. One exception was that growth in negative control (2.349±0.525 and 1.155±0.629) was reduced compared to growth in sediments (range = 3.207±0.404 to 4.519±0.343). Less growth in the October negative control might have been due to addition of less fish food than in June experiments. It also suggested that sediments were providing some nutrition, themselves, in addition to the supplemental fish food. In Unfed, both positive and negative controls had little growth again. Sediment from site 10 (0.757±0.188 and 0.636±0.099) was significantly reduced in the first and second experiment, respectively, compared to sites 3 (1.447±0.108 and 1.277±0.140) and 22 (1.749±0.133 and 2.034±0.189). In addition, sediments from sites 9 and 18 had reduced compared to site 22 in both experiments. Combined data from the two experiments, n = 5-6, showed that growth in site 10 sediment was reduced compared to all other sediments, while that in sites 9 and 18 were reduced compared to sites 3 and 22, $p \leq 0.05$ (Figure 6.10).

It was notable that June and October results were not similar in terms of which sediments caused the worst or best growth. In June, site 7 sediment caused the worst growth and sites 9 and 18 the best. In October, site 10 sediment caused the worst growth and sites 3 and 22 the best. Comparison of Fed and Unfed showed that adding food significantly increased growth for all sites (Figures 6.08 and 6.10). However, sediment alone did provide enough nutritional value for some growth. For example, larvae in Unfed groups grew 2.17x to 3.49x their initial weights in October sediments compared to 1.39x in sand (Unfed negative control).

Survival (%) was not affected in controls or any of the sites in Fed larvae from June experiments (Tables 6.10 and 6.11). It was relatively consistent, ranging from 70 to 100 % with an occasional replicate of 30-40 %. October results were similar except that positive control had significantly reduced survival compared to other treatments (Tables 6.14 and 6.15). In Unfed, survival was significantly reduced in both controls for June

and October results. This was likely due to the absence of food as stated above. In June experiments, survival was significantly reduced in site 7 sediments compared to sites 3 and 22; however, the difference was only statistically significant when experimental data were combined, n =5-6 (Figure 6.09). October sediments for Unfed had high replicate variation with none of the sites showing significant reductions in survival (Figure 6.11). Comparisons between Fed and Unfed for each site showed that feeding larvae did not improve survival.

Overall, data indicated good growth and survival in June and October sediments as long as larvae were fed. Results for Unfed larvae showed that compared to sand, sediments provided enough nutrition to support survival but only with very modest growth. This lack of growth over 10 d indicated sediment toxicity at all sites. Growth in Unfed larvae showed some significant differences between sites. However, results were inconsistent for the different sites in June versus October sediments. This suggested seasonal differences in factors associated with toxicity.

2. Detritus

Two separate toxicity tests were performed for each detritus sample collected June 05, 2002 and October 18, 2002. Endpoints were growth (mg/larvae) and survival (%) at 10 d in Fed and Unfed larvae. Detritus was prepared by sieving whole sediment through a 100 micron screen using on site water. The material left on the screen after sieving was considered detritus and largely consisted of plant matter in various states of decay. In experiments using June detritus, cerophyll was used for controls. It caused an unusual amount of mortality especially in the second experiment. Observations indicated that the cerophyll supported heavy bacterial growth which compromised the chironomids. Because of this problem, data from June controls were not used in statistical analyses. For experiments with October detritus, detritus samples collected from the marsh were used for positive control. Cd was mixed into either site 22 detritus from June (1^{st} experiment) or site 3 detritus from October (2^{nd} experiment). There was no negative control per se, except that chironomids in a particular experiment demonstrated the ability to survive and grow in Fed test groups.

Results for growth in June detritus showed no significant differences between sites in Fed for either the first (Table 6.16) or second (Table 6.17) experiment, $p > 0.359$ and 0.829, respectively. In Unfed larvae from the first experiment, growth was significantly reduced in site 10 detritus (0.671 ± 0.070) compared to sites 3 (1.442 ± 0.206), 9 (1.685 ± 0.069) and 22 (1.227 ± 0.300), $p \leq 0.023$. Growth was also reduced in site 18 compared to site 9 ($p = 0.046$). In the second experiment, site 10 (0.815 ± 0.196) reduced growth compared to site 18 (2.026 ± 0.282), $p = 0.008$. Combining data from June experiments, n = 5-6, showed that Fed larvae grew approximately 6x better than Unfed, that site 10 detritus significantly reduced growth, and that site 9 detritus consistently provided the best growth (Figure 6.12).

Results for growth in October detritus were similar to those for June in that there were no significant differences between sites in Fed larvae for the first (Table 6.20) or second experiment (Table 6.21). In Unfed, detritus from sites 3 (0.809 ± 0.052), 10 (0.830 ± 0.430), 18 (0.673 ± 0.129) and 22 (0.619 ± 0.142) significantly reduced growth compared to sites 7 (1.177 ± 0.123) and 9 (1.507 ± 0.160) in the first experiment, $p \leq 0.05$. In the second experiment, sites 10 (0.754 ± 0.070) and 18 (1.023 ± 0.206) were

again reduced compared to sites 7 (1.668 ± 0.395) and 9 (1.638 ± 0.166). Combining data from October experiments, n = 5-6, showed that Fed larvae grew approximately 5x more than Unfed and that sites 3, 10 and 18 had significantly reduced growth compared to sites 7 and 9 (Figure 6.14). Data from site 22 was inconsistent showing reduced growth in the first experiment and increased growth in the second. Due to its large SD, data from site 22 in Unfed was not used in statistical analysis of combined data. The inconsistency might have been due to hot spots in the detritus sample.

Survival was also determined in detritus experiments. It was based on the number of larvae found at 10 d. Results were complicated by the difficulty of recovering small larvae from the substrate, particularly in Unfed experiments. In the first experiment for June detritus (Table 6.18), Fed larvae showed significantly reduced survival in detritus from sites 9 (43.3 ± 5.8) and 10 (50.0 ± 34.6) compared to sites 7 (93.3 ± 5.8), 18 (96.7 ± 5.8) and 22 (100 ± 0), $p \leq 0.049$. Fed larvae also had reduced survival in detritus 9 and 10 in the second experiment (Table 6.19), but the differences were not statistically significant due to triplicate variability. In Unfed larvae of the first June experiment, survival was significantly lower in site 10 (53.3 ± 20.8) compared to sites 18 (93.3 ± 5.8) and 22 (86.7 ± 11.5), $p = 0.023$ and 0.053, respectively. These differences were again not apparent in the second experiment due to high triplicate variability. Observations indicated that predatory nematodes might have caused some of the mortality associated with triplicate variability in the second June experiment. Combining data, n = 5-6, from June experiments showed that site 10 detritus significantly reduced survival compared to other sites in Fed and Unfed test groups (Figure 6.13).

For October detritus, there were no significant differences in survival between sites for Fed in the first or second experiment, $p > 0.05$. Survival in Unfed larvae was not statistically different between sites in the first experiment, but survival in site 10 detritus was significantly reduced compared to other sites in the second experiment. Combined data, n = 5-6, showed that site 10 detritus significantly reduced survival compared to other sites in Unfed test groups (Figure 6.15).

Spiking detritus with Cd significantly reduced growth without affecting survival (Figure 6.14 and 6.15). This showed that growth was the more sensitive endpoint. Cd exposed chironomids grew only 1.5x compared to initial weights whether or not they were fed. Cd spiked detritus inhibited growth to the same extent as detritus from sites 10 and 18 in both the first and second October experiments.

Overall, responses to detritus were similar to those for sediments. Detritus provided enough nutrition for modest growth as Unfed larvae grew approximately 3x their initial weights (0.527 ± 0.00 and 0.501 ± 0.040 mg/larvae for June and October, respectively). Comparison of Fed and Unfed for each site showed that feeding significantly increased growth without affecting survival. Differences between sites were best seen in Unfed. However, unlike sediment, results were more consistent in that detritus from site 10 reduced growth in both June and October experiments.

3. Sediment versus detritus

Subchronic toxicity in sediment was compared to that in detritus for each particular site to ascertain whether one substrate or the other was more toxic. Since there were few statistical differences between sites in Fed experiments, only Unfed groups

were compared. Comparisons were done for survival in June (Figure 6.16) and October (Figure 6.17) and for growth in June (Figure 6.18) and October (Figure 6.19).

Results showed minor differences between whole sediment and detritus for growth and survival. Exceptions included June samples from site 10 in which growth and survival were reduced in detritus compared to sediment, indicating that the detritus was more toxic, $p \leq 0.001$ and 0.004, respectively. In October, there were significant differences between site 3 and site 9 substrates for growth. Growth was reduced in site 3 detritus compared to sediment, $p \leq 0.001$; however, growth was increased in site 9 detritus compared to sediment, $p \leq 0.001$. The reason why one substrate was not consistently more toxic than the other is unknown. The inconsistency may have reflected real differences between site 3 and site 9 sediments or the need for more repetition. Overall, there was growth in detritus was similar to that in sediment.

Table 6.08. Effects on growth in first subchronic toxicity test (10 d) for sediment from 6-5-02 collection. In Fed, site 3 sediment provided better growth than negative control, and positive control was reduced compared to all other treatments. In Unfed, growth was significantly reduced in both controls and site 7.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	4.665	4.468±0.295[a]	–C-1	ND	0.560±0.042[a]
–C-2	4.610		–C-2	0.530	
–C-3	4.128		–C-3	0.590	
+C-1	0.658	0.688±0.209[b]	+C-1	0.650	0.566±0.167[a]
+C-2	0.496		+C-2	0.675	
+C-3	0.910		+C-3	0.373	
3-1	6.909	6.464±0.650[c]	3-1	1.529	1.449±0.118[b]
3-2	6.764		3-2	1.313	
3-3	5.718		3-3	1.504	
7-1	5.294	5.273±0.066[ac]	7-1	0.760	0.761±0.001[ac]
7-2	5.326		7-2	ND	
7-3	5.199		7-3	0.761	
9-1	6.355	6.368±0.218[ac]	9-1	1.536	1.644±0.152[b]
9-2	6.156		9-2	1.751	
9-3	6.592		9-3	ND	
10-1	3.514	5.124±1.642[ac]	10-1	1.180	1.127±0.075[bc]
10-2	6.796		10-2	1.073	
10-3	5.061		10-3	ND	
18-1	6.247	5.993±0.366[ac]	18-1	1.400	1.569±0.148[b]
18-2	6.159		18-2	1.634	
18-3	5.573		18-3	1.674	
22-1	6.583	6.115±0.534[ac]	22-1	1.157	1.361±0.228[b]
22-2	6.228		22-2	1.608	
22-3	5.534		22-3	1.319	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

Table 6.09. Effects on growth in second subchronic toxicity test (10 d) for sediment from 6-5-02 collection. Positive control in Fed and positive and negative controls in Unfed were significantly reduced. There were no significant differences between sites.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	4.688	4.337±0.524[a]	–C-1	0.570	0.634±0.183[a]
–C-2	3.735		–C-2	0.840	
–C-3	4.588		–C-3	0.492	
+C-1	0.451	0.538±0.121[b]	+C-1	ND	0.580±0.113[ac]
+C-2	0.488		+C-2	0.500	
+C-3	0.676		+C-3	0.660	
3-1	5.250	5.188±0.120[a]	3-1	1.439	1.290±0.180[ab]
3-2	5.264		3-2	1.342	
3-3	5.050		3-3	1.090	
7-1	4.084	4.470±0.508[a]	7-1	1.460	1.127±0.471[ab]
7-2	4.282		7-2	0.793	
7-3	5.046		7-3	ND	
9-1	4.573	5.181±0.789[a]	9-1	1.221	1.433±0.521[b]
9-2	6.072		9-2	2.026	
9-3	4.896		9-3	1.051	
10-1	5.140	5.241±0.295[a]	10-1	1.327	1.205±0.105[ab]
10-2	5.010		10-2	1.149	
10-3	5.573		10-3	1.140	
18-1	4.017	5.149±1.095[a]	18-1	1.348	1.394±0.057[bc]
18-2	6.202		18-2	1.458	
18-3	5.229		18-3	1.375	
22-1	4.910	5.346±0.399[a]	22-1	1.010	1.006±0.036[ab]
22-2	5.693		22-2	1.039	
22-3	5.435		22-3	0.968	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

Table 6.10. Effects on survival (%) in first subchronic toxicity test (10 d) for sediment from 6-5-02 collection. No significant differences were found for Fed treatments. Controls in Unfed treatments had significantly reduced survival compared to sites 10 and 22.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	80	90.0±10.0[a]	–C-1	10	16.5±11.5[a]
–C-2	90		–C-2	10	
–C-3	100		–C-3	30	
+C-1	90	86.7±15.3[a]	+C-1	20	23.3±5.8[a]
+C-2	100		+C-2	20	
+C-3	70		+C-3	30	
3-1	70	90.0±17.3[a]	3-1	80	83.3±15.3[ab]
3-2	100		3-2	70	
3-3	100		3-3	100	
7-1	90	96.7±5.8[a]	7-1	10	26.7±37.9[ab]
7-2	100		7-2	0	
7-3	100		7-3	70	
9-1	100	96.7±5.8[a]	9-1	80	60.0±26.5[ab]
9-2	100		9-2	70	
9-3	90		9-3	30	
10-1	100	96.7±5.8[a]	10-1	100	86.7±15.3[b]
10-2	90		10-2	90	
10-3	100		10-3	70	
18-1	90	96.7±5.8[a]	18-1	10	60.0±43.6[ab]
18-2	100		18-2	80	
18-3	100		18-3	90	
22-1	30	73.3±37.9[a]	22-1	100	93.3±11.5[b]
22-2	90		22-2	100	
22-3	100		22-3	80	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

Table 6.11. Effects on survival (%) in second subchronic toxicity test (10 d) for sediment from 6-5-02 collection. No significant differences were found for Fed treatments. Controls in Unfed treatments had significantly reduced survival compared to sites 3, 18 and 22.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	80	93.3±11.6[a]	–C-1	20	30.0±26.5[a]
–C-2	100		–C-2	10	
–C-3	100		–C-3	60	
+C-1	80	66.7±23.1[a]	+C-1	0	10.0±0.0[a]
+C-2	40		+C-2	10	
+C-3	80		+C-3	10	
3-1	90	96.7±5.8[a]	3-1	90	93.3±5.8[b]
3-2	100		3-2	100	
3-3	100		3-3	90	
7-1	80	76.7±15.3[a]	7-1	80	66.7±32.2[ab]
7-2	60		7-2	90	
7-3	90		7-3	30	
9-1	90	93.3±5.8[a]	9-1	80	76.7±25.2[ab]
9-2	90		9-2	50	
9-3	100		9-3	100	
10-1	100	80.0±34.6[a]	10-1	90	86.7±5.8[a]
10-2	40		10-2	90	
10-3	100		10-3	80	
18-1	100	100.0±0.0[a]	18-1	90	93.3±5.8[b]
18-2	100		18-2	90	
18-3	100		18-3	100	
22-1	100	96.7±5.8[a]	22-1	90	96.7±5.8[b]
22-2	90		22-2	100	
22-3	100		22-3	100	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

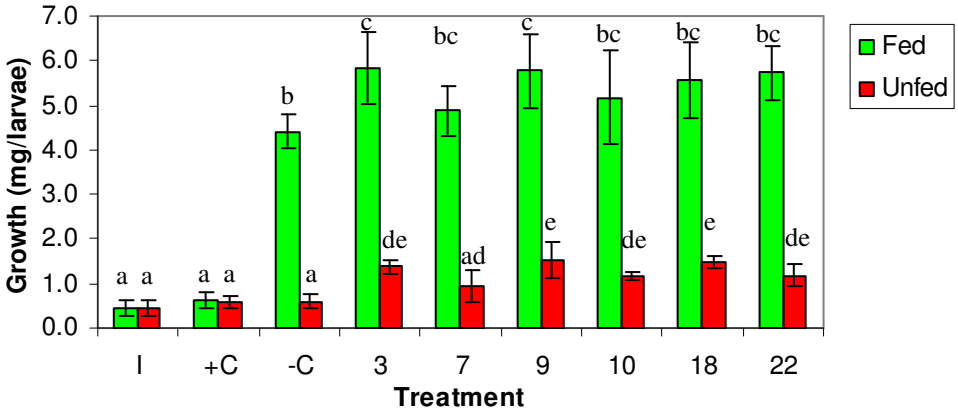


Figure 6.08: Effect of June sediment on growth (mg/larvae) in Fed and Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values that share a common letter were not significantly different, p > 0.05.
I = Initial weight, +C = positive control, -C = negative control

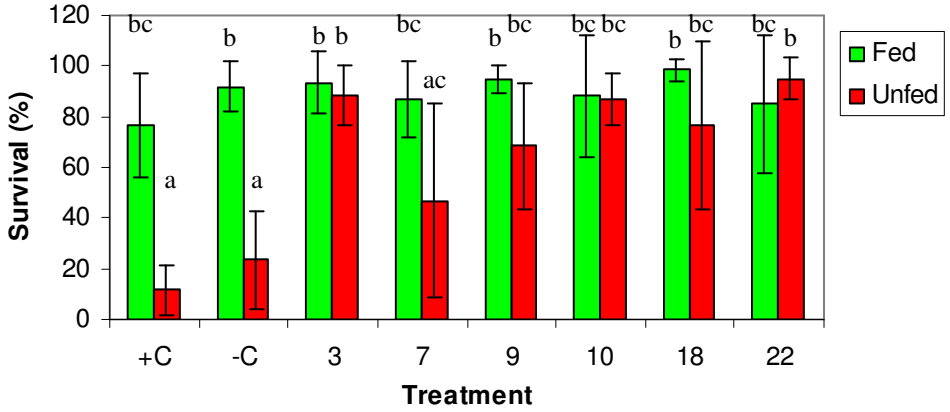


Fig. 6.09: Effect of June sediment on subchronic survival (%) in Fed and Unfed larvae at 10 d. Data represent averages +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values with a common letter are not significantly different, p > 0.05.
+C = positive control, -C = negative control

Table 6.12. Effects on growth in first subchronic toxicity test (10 d) for sediment from 10-18-02 collection. In Fed, controls had significantly reduced growth compared to sediments. In Unfed, positive control and site 10 was significantly reduced compared to sites 3, 7, and 22.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	1.976	2.349±0.525[a]	–C-1	0.430	0.430±0.0
–C-2	2.950		–C-2	ND	
–C-3	2.122		–C-3	ND	
+C-1	0.470	0.392±0.110[b]	+C-1	0.430	0.432±0.004[a]
+C-2	ND		+C-2	0.430	
+C-3	0.314		+C-3	0.435	
3-1	5.553	4.289±1.104[c]	3-1	1.142	1.277±0.140[b]
3-2	3.798		3-2	1.423	
3-3	3.515		3-3	1.266	
7-1	4.339	4.177±0.412[c]	7-1	1.245	1.246±0.172[b]
7-2	3.708		7-2	1.074	
7-3	4.483		7-3	1.418	
9-1	4.264	4.450±0.175[c]	9-1	1.110	1.010±0.098[bc]
9-2	4.474		9-2	1.005	
9-3	4.613		9-3	0.914	
10-1	3.896	4.264±0.408[c]	10-1	0.581	0.636±0.099[ac]
10-2	4.194		10-2	0.576	
10-3	4.703		10-3	0.751	
18-1	4.800	4.519±0.343[c]	18-1	0.775	0.925±0.230[bc]
18-2	4.621		18-2	1.190	
18-3	4.137		18-3	0.809	
22-1	4.521	4.358±0.203[c]	22-1	1.900	2.034±0.189[d]
22-2	4.131		22-2	2.168	
22-3	4.422		22-3	ND	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

Table 6.13. Effects on growth in second subchronic toxicity test (10 d) for sediment from 10-18-02 collection. In Fed, controls had significantly reduced growth compared to sediment treatments. Sites 3 and 22 had significantly increased growth compared to sites 7, 9, and 18 in Unfed.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	1.846	1.155±0.629[a]	–C-1	ND	ND
–C-2	0.613		–C-2	ND	
–C-3	1.007		–C-3	ND	
+C-1	0.650	0.613±0.053[a]	+C-1	ND	ND
+C-2	0.575		+C-2	ND	
+C-3	ND		+C-3	ND	
3-1	3.171	3.529±0.361[b]	3-1	1.561	1.447±0.108[ac]
3-2	3.524		3-2	1.434	
3-3	3.892		3-3	1.347	
7-1	3.051	3.544±1.020[b]	7-1	ND	1.125±0.138[ab]
7-2	2.864		7-2	1.027	
7-3	4.716		7-3	1.223	
9-1	3.194	3.458±0.602[b]	9-1	1.084	0.973±0.098[b]
9-2	3.034		9-2	0.938	
9-3	4.148		9-3	0.897	
10-1	3.422	3.431±0.367[b]	10-1	0.892	0.757±0.188[b]
10-2	3.069		10-2	0.542	
10-3	3.802		10-3	0.837	
18-1	2.897	3.207±0.404[b]	18-1	1.052	1.035±0.066[b]
18-2	3.664		18-2	0.962	
18-3	3.059		18-3	1.090	
22-1	3.717	4.107±0.340[b]	22-1	1.622	1.749±0.133[c]
22-2	4.260		22-2	1.887	
22-3	4.343		22-3	1.740	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.
Data that share a common letter were not statistically different, $p \geq 0.05$.

Table 6.14. Effects on survival (%) in first subchronic toxicity test (10 d) for sediment from 10-18-02 collection. In Fed, survival was significantly reduced in positive control only. In Unfed, survival was reduced in both controls as well as site 22.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	70	83.3±11.6[a]	–C-1	0	3.3±5.8[a]
–C-2	90		–C-2	0	
–C-3	90		–C-3	10	
+C-1	20	23.3±25.2[b]	+C-1	10	13.3±5.8[a]
+C-2	0		+C-2	10	
+C-3	50		+C-3	20	
3-1	90	93.3±5.8[a]	3-1	90	86.7±5.8[b]
3-2	90		3-2	80	
3-3	100		3-3	90	
7-1	90	96.7±5.8[a]	7-1	80	73.3±20.8[b]
7-2	100		7-2	90	
7-3	100		7-3	50	
9-1	90	86.7±5.8[a]	9-1	80	76.7±5.8[b]
9-2	90		9-2	80	
9-3	80		9-3	70	
10-1	100	83.3±15.3[a]	10-1	70	80.0±10.0[b]
10-2	70		10-2	80	
10-3	80		10-3	90	
18-1	30	63.3±35.1[ab]	18-1	20	63.3±37.9[ab]
18-2	100		18-2	90	
18-3	60		18-3	80	
22-1	100	96.7±5.8[a]	22-1	10	20.0±17.3[a]
22-2	90		22-2	40	
22-3	100		22-3	10	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.

Table 6.15. Effects on survival (%) in second subchronic toxicity test (10 d) for sediment from 10-18-02 collection. In Fed, survival in the positive control was significantly reduced compared to all other treatments. In Unfed, both controls were reduced compared to the sites; none of the sites were significantly different from one another.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	70	83.3±11.6[a]	–C-1	0	0.0±0.0[a]
–C-2	90		–C-2	0	
–C-3	90		–C-3	0	
+C-1	40	20.0±20.0[b]	+C-1	0	0.0±0.0[a]
+C-2	20		+C-2	0	
+C-3	0		+C-3	0	
3-1	90	90.0±0.0[a]	3-1	90	90.0±0.0[b]
3-2	90		3-2	90	
3-3	90		3-3	90	
7-1	90	80.0±26.5[a]	7-1	0	36.7±40.4[ab]
7-2	100		7-2	30	
7-3	50		7-3	80	
9-1	80	83.3±5.8[a]	9-1	80	60.0±20.0[b]
9-2	80		9-2	40	
9-3	90		9-3	60	
10-1	100	93.3±5.8[a]	10-1	50	70.0±20.0[b]
10-2	90		10-2	90	
10-3	90		10-3	70	
18-1	100	100.0±0.0[a]	18-1	90	66.7±20.8[b]
18-2	100		18-2	60	
18-3	100		18-3	50	
22-1	90	96.7±5.8[a]	22-1	60	80.0±17.3[b]
22-2	100		22-2	90	
22-3	100		22-3	90	

–C = Negative control = acid-washed sand and 250 ml test water.
+C = Positive control = acid-washed sand, 250 ml test water and Cd (0.3 mM).
ND = No data.

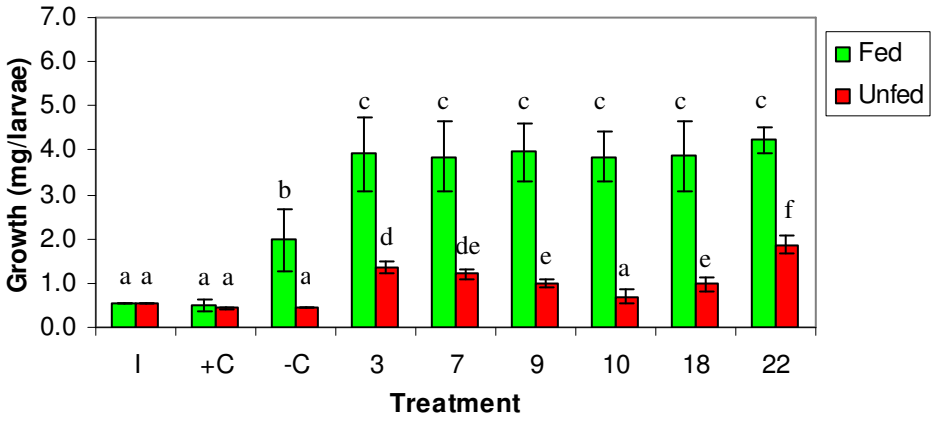


Figure 6.10: Effect of October sediment on growth (mg/larvae) in Fed and Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values that share a common letter were not significantly different, $p > 0.05$.
I = Initial weight, +C = positive control, -C = negative control

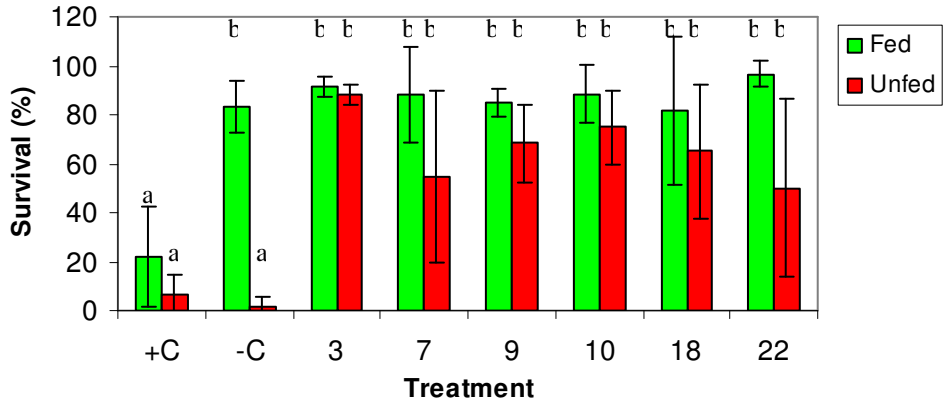


Fig. 6.11: Effect of October sediment on subchronic survival (%) in Fed and Unfed larvae at 10 d. Data represent averages +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values with a common letter are not significantly different, $p > 0.05$
+C = positive control, -C = negative control

Table 6.16. Effects on growth (mg/larvae) in first subchronic toxicity test (10 d) for detritus from 6-5-02 collection. Controls using cerophyll did not work properly. For sites, there was no significant difference in Fed. Growth in Unfed was significantly reduced in site 10 and increased in site 9 compared to some sites.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	2.671	2.292±0.537[a]	–C-1	0.653*	1.848±1.044[a]
–C-2	1.912		–C-2	2.584	
–C-3	ND		–C-3	2.306	
+C-1	ND	1.570±0.000	+C-1	0.470	0.491±0.030[b]
+C-2	1.570*		+C-2	ND	
+C-3	ND		+C-3	0.513	
3-1	ND	5.815±0.777[b]	3-1	1.282	1.442±0.206[cd]
3-2	6.364		3-2	1.674	
3-3	5.266		3-3	1.370	
7-1	6.192	5.930±0.612[b]	7-1	1.217	1.146±0.066[cde]
7-2	6.367		7-2	1.131	
7-3	5.230		7-3	1.089	
9-1	6.603	7.045±0.719[b]	9-1	1.734	1.685±0.069[d]
9-2	7.875		9-2	1.636	
9-3	6.657		9-3	ND	
10-1	5.776	7.162±1.440[b]	10-1	0.749	0.671±0.070[be]
10-2	7.060		10-2	0.650	
10-3	8.650		10-3	0.613	
18-1	6.057	5.868±0.635[b]	18-1	1.296	1.125±0.151[ce]
18-2	6.386		18-2	1.010	
18-3	5.160		18-3	1.070	
22-1	5.178	5.682±0.488[b]	22-1	0.955	1.227±0.300[cd]
22-2	6.153		22-2	1.549	
22-3	5.715		22-3	1.176	

–C = 3 g hydrated cerophyll and 250 ml test water.
+C = 3 g hydrated cerophyll, 250 ml test water plus 0.3 mM Cd.
ND = No data.
*Value not included in one way ANOVA.

Table 6.17. Effects on growth (mg/larvae) in second subchronic toxicity test (10 d) for detritus from 6-5-02 collection. Controls using cerophyll did not work properly. For sites, there was no significant difference in Fed. Growth in Unfed was significantly reduced in site 10 compared to site 18.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	1.260*	1.260±0.000	–C-1	ND	4.512±0.000
–C-2	ND		–C-2	ND	
–C-3	ND		–C-3	4.512*	
+C-1	ND	0.740±0.000	+C-1	ND	0.900±0.000
+C-2	0.740*		+C-2	ND	
+C-3	ND		+C-3	0.900*	
3-1	6.498	5.836±0.618[a]	3-1	1.320	1.370±0.048[ab]
3-2	5.733		3-2	1.376	
3-3	5.276		3-3	1.415	
7-1	5.264	5.333±0.643[a]	7-1	1.237	1.345±0.154[ab]
7-2	4.728		7-2	1.454	
7-3	6.009		7-3	ND	
9-1	5.233	5.580±0.409[a]	9-1	1.364	1.424±0.085[ab]
9-2	5.475		9-2	1.484	
9-3	6.031		9-3	ND	
10-1	4.950	5.317±0.451[a]	10-1	0.663	0.815±0.196[b]
10-2	5.820		10-2	1.037	
10-3	5.180		10-3	0.747	
18-1	5.709	5.383±0.574[a]	18-1	1.713	2.026±0.282[a]
18-2	5.720		18-2	2.105	
18-3	4.720		18-3	2.260	
22-1	6.041	5.538±0.443[a]	22-1	1.784	1.274±0.602[ab]
22-2	5.362		22-2	0.610	
22-3	5.209		22-3	1.427	

–C = 3 g hydrated cerophyll and 250 ml test water.
+C = 3 g hydrated cerophyll, 250 ml test water plus 0.3 mM Cd.
ND = No data.
*Value not included in one way ANOVA.

Table 6.18. Effects on survival (%) in first subchronic toxicity test (10 d) for detritus from 6-5-02 collection. Controls using cerophyll did not work properly. In Fed, survival was significantly reduced in sites 9 and 10. Survival in site 10 was also significantly reduced in Unfed.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	70*	60.0±14.1	–C-1	30*	70.0±36.1
–C-2	50*		–C-2	80*	
–C-3	ND		–C-3	100*	
+C-1	0	3.3±5.8	+C-1	20*	30.0±14.1
+C-2	10*		+C-2	0	
+C-3	0		+C-3	40*	
3-1	ND	85.0±7.1[ab]	3-1	50	70.0±20.0[ab]
3-2	80		3-2	70	
3-3	90		3-3	90	
7-1	90	93.3±5.8[b]	7-1	80	76.7±5.8[ab]
7-2	100		7-2	80	
7-3	90		7-3	70	
9-1	40	43.3±5.8[a]	9-1	50	65.0±21.2[ab]
9-2	50		9-2	80	
9-3	40		9-3	ND	
10-1	90	50.0±34.6[a]	10-1	70	43.3±23.1[b]
10-2	30		10-2	30	
10-3	30		10-3	30	
18-1	100	96.7±5.8[b]	18-1	90	93.3±5.8[a]
18-2	90		18-2	100	
18-3	100		18-3	90	
22-1	100	100±0[b]	22-1	100	86.7±11.5[a]
22-2	100		22-2	80	
22-3	100		22-3	80	

–C = 3 g hydrated cerophyll and 250 ml test water.
+C = 3 g hydrated cerophyll, 250 ml test water plus 0.3 mM Cd.
ND = No data.
*Value not included in one way ANOVA.

Table 6.19. Effects on survival (%) in second subchronic toxicity test (10 d) for detritus from 6-5-02 collection. Controls using cerophyll did not work properly. For sites, there were no significant differences in Fed or Unfed.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	10*	3.3±5.8	–C-1	0	16.7±28.9
–C-2	0		–C-2	0	
–C-3	0		–C-3	50*	
+C-1	0	3.3±5.8	+C-1	0	6.7±11.5
+C-2	10*		+C-2	0	
+C-3	0		+C-3	20*	
3-1	60	66.7±5.8[a]	3-1	80	83.3±5.8[a]
3-2	70		3-2	90	
3-3	70		3-3	80	
7-1	80	80.0±0.0[a]	7-1	60	43.3±37.9[a]
7-2	80		7-2	70	
7-3	80		7-3	0	
9-1	70	60.0±17.3[a]	9-1	80	85.0±7.1[a]
9-2	40		9-2	90	
9-3	70		9-3	ND	
10-1	20	53.3±35.1[a]	10-1	70	53.3±20.8[a]
10-2	50		10-2	60	
10-3	90		10-3	30	
18-1	90	56.7±35.1[a]	18-1	70	33.3±32.1[a]
18-2	60		18-2	20	
18-3	20		18-3	10	
22-1	70	80.0±10.0[a]	22-1	50	40.0±26.5[a]
22-2	80		22-2	10	
22-3	90		22-3	60	

–C = 3 g hydrated cerophyll and 250 ml test water.
+C = 3 g hydrated cerophyll, 250 ml test water plus 0.3 mM Cd.
ND = No data.
*Value not included in one way ANOVA.

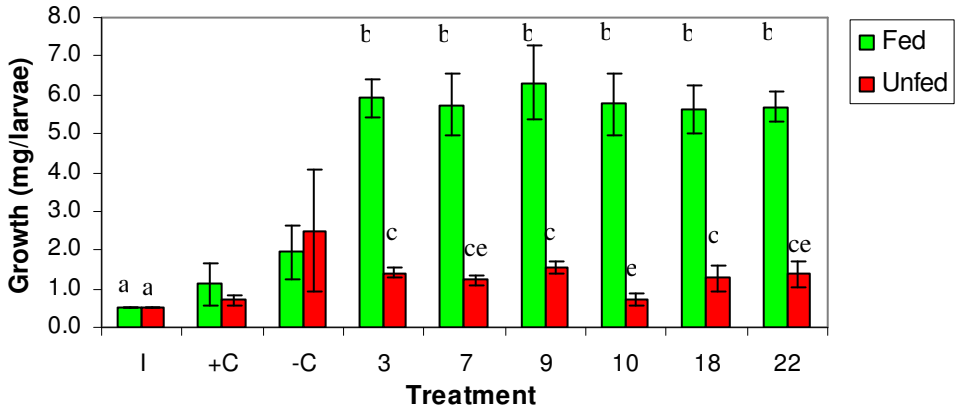


Figure 6.12: Effect of June detritus on growth (mg/larvae) in Fed and Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 3-6. Values that share a common letter were not significantly different, p > 0.05.
I = Initial weight, +C = positive control, -C = negative control

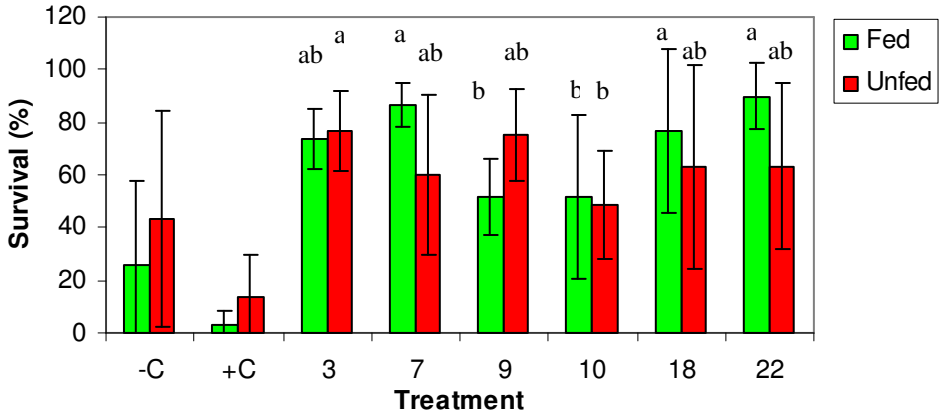


Fig. 6.13: Effect of June detritus on subchronic survival (%) in Fed and Unfed larvae at 10 d. Data represent averages +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values with a common letter are not significantly different, p > 0.05.
I = Initial weight, +C = positive control, -C = negative control

Table 6.20. Effects on growth (mg/larvae) in first subchronic toxicity test (10 d) for detritus from 10-18-02 collection.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	5.539	5.287±0.356[a]	–C-1	1.684	1.738±0.076[a]
–C-2	5.034		–C-2	1.792	
–C-3	ND		–C-3	ND	
+C-1	0.709	0.740±0.043[b]	+C-1	0.766	0.692±0.105[b]
+C-2	0.770		+C-2	0.618	
+C-3	ND		+C-3	ND	
3-1	4.608	4.791±0.183[a]	3-1	0.766	0.809±0.052[b]
3-2	4.973		3-2	0.866	
3-3	4.792		3-3	0.794	
7-1	5.853	4.958±0.828[a]	7-1	1.072	1.177±0.123[a]
7-2	4.218		7-2	1.147	
7-3	4.803		7-3	1.313	
9-1	5.447	4.934±0.444[a]	9-1	1.692	1.507±0.160[a]
9-2	4.678		9-2	1.420	
9-3	4.678		9-3	1.410	
10-1	4.021	4.397±0.347[a]	10-1	0.733	0.830±0.430[b]
10-2	4.705		10-2	0.458	
10-3	4.465		10-3	1.300	
18-1	4.628	4.730±0.118[a]	18-1	0.717	0.673±0.129[b]
18-2	4.701		18-2	0.775	
18-3	4.860		18-3	0.528	
22-1	3.784	4.432±0.619[a]	22-1	0.693	0.619±0.142[b]
22-2	5.017		22-2	0.708	
22-3	4.496		22-3	0.456	

–C = 3 g detritus from 6-5-02, site 22, and 250 ml test water.
+C = 3 g detritus from 6-5-02, site 22, 250 ml test water and 0.3 mM Cd.
ND = No data.

Table 6.21. Effects on growth (mg/larvae) in second subchronic toxicity test (10 d) for detritus from 10-18-02 collection.

FED			UNFED		
Sample	mg/larvae	Ave. ± SD	Sample	mg/larvae	Ave. ± SD
–C-1	ND	ND	–C-1	ND	ND
–C-2	ND		–C-2	ND	
–C-3	ND		–C-3	ND	
+C-1	0.578	0.521±0.049[a]	+C-1	0.879	0.779±0.089[b]
+C-2	0.487		+C-2	0.710	
+C-3	0.499		+C-3	0.748	
3-1	4.714	4.605±0.412[b]	3-1	0.946	1.048±0.088[bc]
3-2	4.150		3-2	1.099	
3-3	4.951		3-3	1.098	
7-1	4.884	4.964±0.114[b]	7-1	1.389	1.668±0.395[ac]
7-2	5.094		7-2	ND	
7-3	4.913		7-3	1.947	
9-1	5.511	5.073±0.388[b]	9-1	1.477	1.638±0.166[a]
9-2	4.936		9-2	1.631	
9-3	4.772		9-3	1.808	
10-1	4.410	4.511±0.422[b]	10-1	0.758	0.754±0.070[b]
10-2	4.148		10-2	0.682	
10-3	4.974		10-3	0.822	
18-1	4.519	4.739±0.234[b]	18-1	0.906	1.023±0.206[b]
18-2	4.714		18-2	0.902	
18-3	4.985		18-3	1.260	
22-1	4.619	5.012±0.445[b]	22-1	2.754	2.513±0.325[d]
22-2	5.495		22-2	2.143	
22-3	4.921		22-3	2.643	

+C = 3 g detritus from 10-18-02, site 3, 250 ml test water and 0.3 mM Cd.
ND = No data.

Table 6.22. Effects on survival (%) in first subchronic toxicity test (10 d) for detritus from 10-18-02 collection. There were no significant differences between treatments in Fed or Unfed.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	80	90.0±14.1[a]	–C-1	90	75.0±21.2[a]
–C-2	100		–C-2	60	
–C-3	ND		–C-3	ND	
+C-1	100	95.0±7.1[a]	+C-1	80	90.00±14.1[a]
+C-2	90		+C-2	100	
+C-3	ND		+C-3	ND	
3-1	90	90.0±10.0[a]	3-1	70	70.0±0.0[a]
3-2	80		3-2	70	
3-3	100		3-3	70	
7-1	100	93.3±5.8[a]	7-1	90	93.3±5.8[a]
7-2	90		7-2	90	
7-3	90		7-3	100	
9-1	40	76.7±32.5[a]	9-1	90	63.3±46.2[a]
9-2	100		9-2	90	
9-3	90		9-3	10	
10-1	90	80.0±17.3[a]	10-1	60	53.3±30.6[a]
10-2	60		10-2	80	
10-3	90		10-3	20	
18-1	80	83.3±5.8[a]	18-1	60	50.0±10.0[a]
18-2	80		18-2	40	
18-3	90		18-3	50	
22-1	100	100.0±0.0[a]	22-1	60	60.0±10.0[a]
22-2	100		22-2	50	
22-3	100		22-3	70	

–C = 3 g detritus from 6-5-02, site 22, and 250 ml test water.
+C = 3 g detritus from 6-5-02, site 22, 250 ml test water and 0.3 mM Cd.
ND = No data.

Table 6.23. Effects on survival (%) in second subchronic toxicity test (10 d) for detritus from 10-18-02 collection.

FED			UNFED		
Sample	Survival (%)	Ave. ± SD	Sample	Survival (%)	Ave. ± SD
–C-1	ND	ND	–C-1	ND	ND
–C-2	ND		–C-2	ND	
–C-3	ND		–C-3	ND	
+C-1	40	76.7±32.2[a]	+C-1	100	90.0±10.0[a]
+C-2	100		+C-2	90	
+C-3	90		+C-3	80	
3-1	90	93.3±5.8[a]	3-1	80	83.3±5.8[a]
3-2	100		3-2	80	
3-3	90		3-3	90	
7-1	90	93.3±5.8[a]	7-1	80	85.0±7.1[a]
7-2	90		7-2	ND	
7-3	100		7-3	90	
9-1	80	90.0±10.0[a]	9-1	90	83.3±5.8[a]
9-2	90		9-2	80	
9-3	100		9-3	80	
10-1	100	96.7±5.8[a]	10-1	60	60.0±0.0[b]
10-2	100		10-2	60	
10-3	90		10-3	60	
18-1	90	86.7±5.8[a]	18-1	90	90.0±10.0[a]
18-2	90		18-2	100	
18-3	80		18-3	80	
22-1	80	90.0±10.0[a]	22-1	100	90.0±10.0[a]
22-2	100		22-2	90	
22-3	90		22-3	80	

+C = 3 g detritus from 10-18-02, site 3, 250 ml test water and 0.3 mM Cd.
ND = No data.

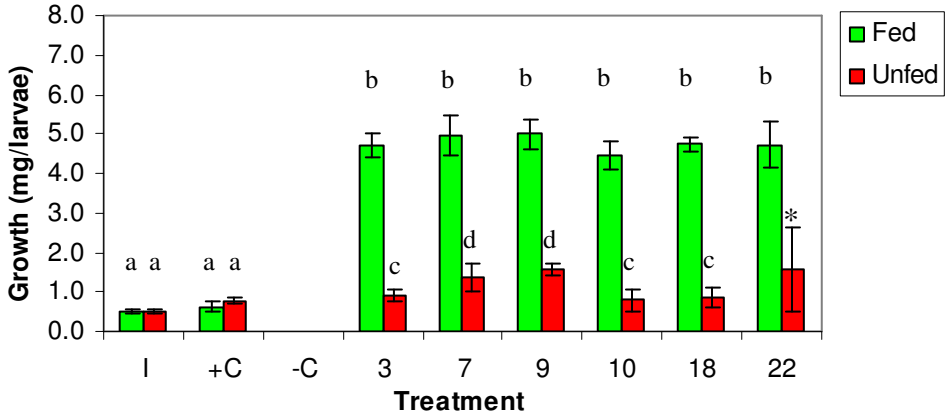


Figure 6.14: Effect of October detritus on growth (mg/larvae) in Fed and Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values that share a common letter were not significantly different, $p > 0.05$.
I = Initial weight, +C = positive control, -C = negative control
* = data not included in ANOVA

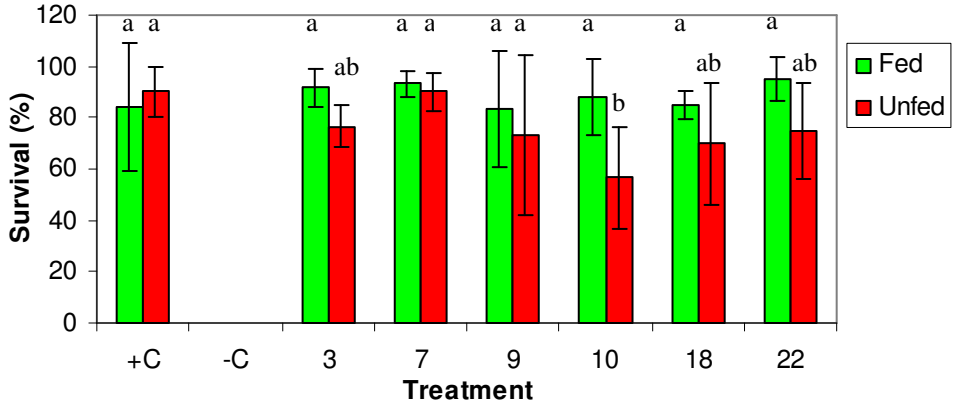


Fig. 6.15: Effect of October detritus on subchronic survival (%) in Fed and Unfed larvae at 10 d. Data represent averages +/- SD of the 1st and 2nd experiments combined, n = 5-6. Values with a common letter are not significantly different, $p > 0.05$.
I = Initial weight, +C = positive control, -C = negative control

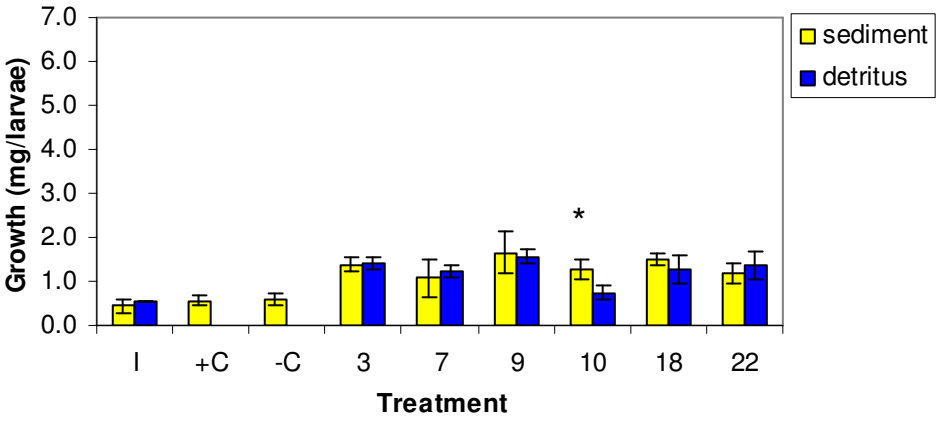


Figure 6.16. Comparison of June sediment and detritus growth (mg/larvae) in Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Asterisks (*) indicate a difference between sediment and detritus, $p > 0.05$.
I = Initial weight, +C = positive control, -C = negative control

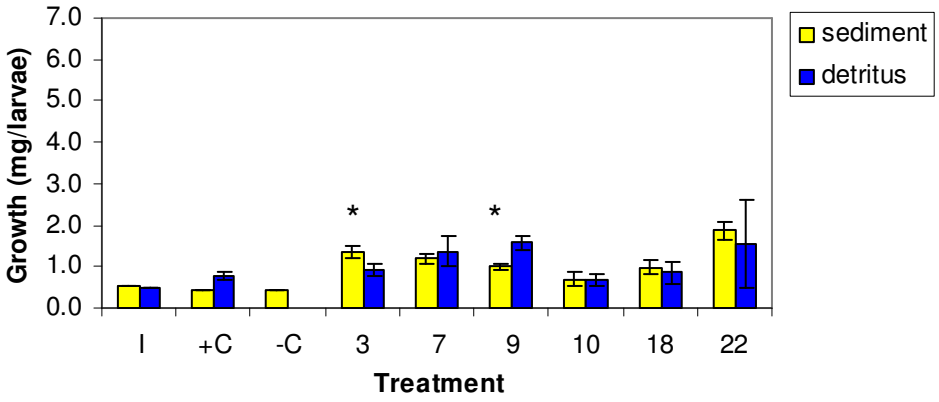


Figure 6.17. Comparison of October sediment and detritus growth (mg/larvae) in Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Asterisks (*) indicate a difference between sediment and detritus, $p > 0.05$.
I = Initial weight, +C = positive control, -C = negative control

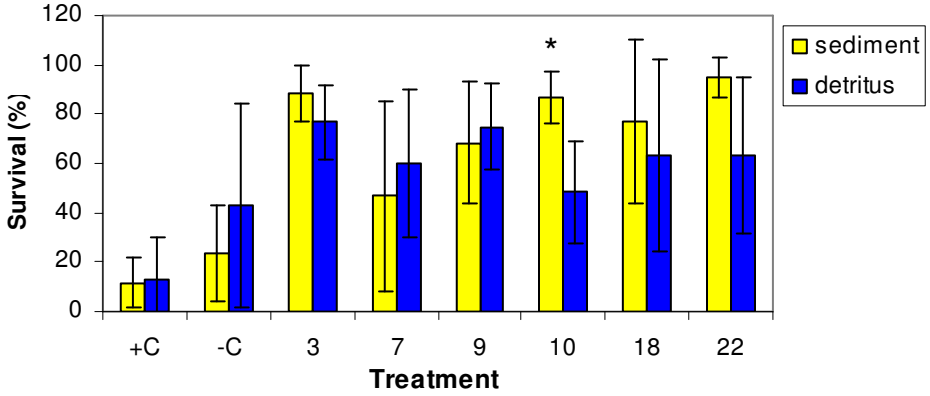


Figure 6.18. Comparison of June sediment and detritus survival (%) in Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Asterisks (*) indicate a difference between sediment and detritus, p > 0.05..

I = Initial weight, +C = positive control, -C = negative control

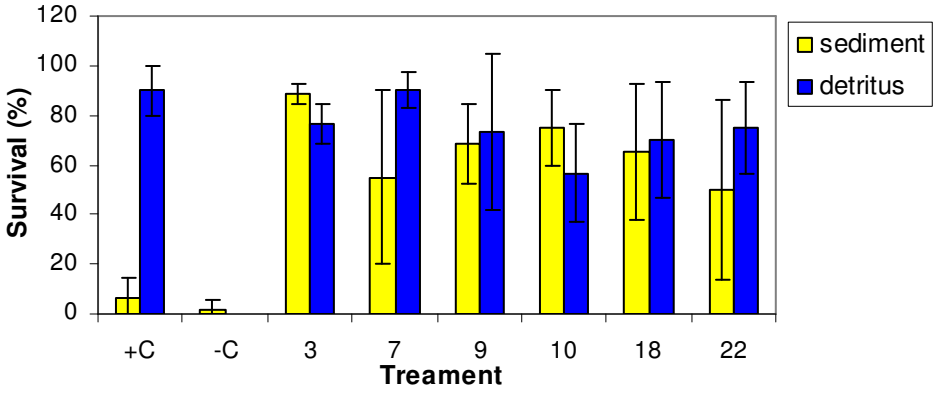


Figure 6.19. Comparison of October sediment and detritus survival (%) in Unfed at 10 d. Data represent average +/- SD of the 1st and 2nd experiments combined, n = 5-6. Asterisks (*) indicate a difference between sediment and detritus, p > 0.05.

I = Initial weight, +C = positive control, -C = negative control

D. Analysis and Discussion of Sediment and Detritus Toxicity Tests

Toxicity testing included acute (96 h) and subchronic (10 d) exposure to two types of substrates, whole sediment and detritus, with and without supplemental feeding. Endpoints for acute tests were survival and carbohydrate levels (see Section VII) and for subchronic tests were survival and growth. Acute tests started with 3rd instar larvae which were larger than the 2nd instars used in subchronic tests. This made them easier to find at the end of the experiment, and replicates for survival in acute tests were more consistent than in subchronics. Comparison of results showed that none of the sediment or detritus samples was acutely toxic (96 h exposure) as measured by survival. Differences between sites were most consistently found with subchronic tests using growth as an endpoint in Unfed larvae.

Although some sites showed significant differences in subchronic survival for Fed larvae (Figure 6.13), treatments with supplemental feeding typically showed no differences between sites for sediment (Figures 6.08-6.11) or detritus (6.12, 6.14-6.15) for either endpoint. These findings indicated 1) that survival was not a sensitive endpoint for chironomids in acute or subchronic tests and 2) that physical contact with the substrates did not affect growth but ingestion of substrates did. Meaning the substrates were not toxic as long as the chironomids could eat fish food and were not depend on the substrates for sustenance. Importance of ingestion was further demonstrated by effects seen in positive control. In Fed treatments, Cd in sand significantly reduced growth compared to June (Figure 6.08) and October sediment (Figure 6.10) as well as October detritus (Figure 6.14). June detritus lacked a valid positive control. Results indicated that Cd spiked into water adsorbed on to food particles and was consumed. Other research has shown similar results with greater Cd toxicity in fed versus starved chironomids, *C. riparius* (Pascoe *et al*., 1990, Bentivegna, 2002). Results for Fed treatments also indicated that metals in sediment and detritus did not dissolve into the water and contaminate the supplemental food to a toxic level.

Most differences between substrates were found in Unfed treatments with subchronic exposure (10 d). However, sediment data for June and October were inconsistent. For June, sites 9 and 18 showed the best growth and site 7 the least; while for October, sites 3 and 22 had the best growth and sites 9, 10 and 18 the least. Detritus data was more consistent in that site 9 had the best growth and site 10 the least in both June and October. The inconsistencies between months could have reflected differences in substrate homogeneity, need for more replicates and/or seasonal sediment parameters that influenced toxicity. Overall, site 10 provided for the least growth.

One question was whether reduced growth resulted from substrate toxicity or poor substrate nutritional value. Comparison of initial weights to average final weights in Unfed showed that larvae could use sediment and detritus alone for moderate grow. For sediment, initial weights were 0.43 and 0.54 mg/larvae while average final weights were 1.28 and 1.18 mg/larvae for June and October, respectively. Therefore larval growth was 2.94 and 2.19x initial for June and October sediment, respectively. Similar results were found for detritus. Initial weights were 0.53 and 0.50 mg/larvae while average final weights were 1.26 and 1.18 mg/larvae for June and October, respectively. Therefore larval growth was 2.40 and 2.36x initial for June and October detritus, respectively. Growth in Fed treatments was greater, 12.63 and 7.30x initial for June and October sediment, respectively, and 11.09 and 9.05x initial for June and October detritus,

respectively. Changes in Fed positive control showed effects of spiked Cd in the presence of food. In sediment, final average weights with Cd were 0.61 and 0.50 mg/larvae representing an increase of 1.41 and 0.93x initial weight in June and October, respectively. In detritus, final average weights with Cd were 1.12 and 0.61 mg/larvae representing an increase of 2.13 and 1.21x initial weights in June and October, respectively. Taken together, data showed that growth in substrates of Unfed treatments was slightly better than growth in Cd spiked treatments in the presence of fish food (Figure 6.20). It was therefore likely that poor growth in substrates was due to contamination. This data did not exclude the influence of substrate nutritional value.

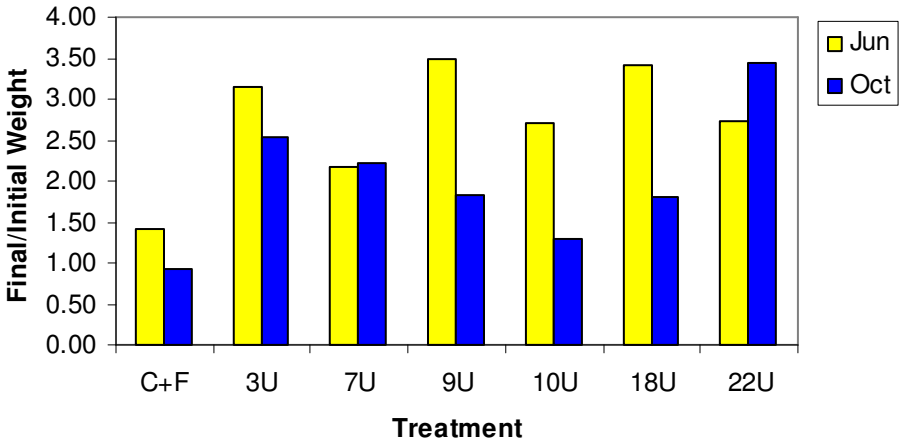


Figure 6.20. Ratio of final to initial weights for subchronic exposures to June and October sediments and positive control. Data for treatments were derived from averages of combined experiments, n = 5-6. C+F = Fed positive control. U= Unfed treatment.

Sediment and detritus had similar levels of toxicity (Figures 6.16-6.19). Data did show significant differences between sediment and detritus for a few sites. However, in some cases sediment was more toxic (site 10 June) and in others detritus was more toxic (site 9 October). This indicated that, in general, neither substrate was more toxic than the other. Therefore, detritus was just as toxic as whole sediment. Other researchers have found detritus to be toxic and that metals can be bioaccumulated from detritus (Weis *et al*., 2002). No literature was found where detritus and sediment toxicity were compared. So detritus appeared to make an important contribution to Kearny Marsh sediment, but no comparison can be made with other locations.

VII. Carbohydrate Analyses for Toxicity Testing

A. Materials and Methods

Carbohydrate analyses were performed on larvae following 96 h exposures to sediment or detritus. Date was gathered from two separate experiments with each treatment done in triplicate. As described in the acute toxicity tests above, sediment controls consisted of sand and water (negative control) or sand and water plus 0.3 mM Cd (positive control). Control for detritus consisted by 0.3 mM Cd added to W22 for the first experiment and 0.3 mM Cd added to W3 for the second experiment. For each treatment group, larvae were either Fed or Unfed. Feeding consisted of adding 0.1 g/ml of ground fish food on day 1 of the experiment. Larvae were starved 3 d prior to initiating experiments.

Carbohydrates were isolated from 4 larvae per replicate. The larvae were first weighed together and then homogenized in 200 µl water containing 400 µg/ml allose (internal standard). This solution was immediately frozen at −70 ºC until further processing. To this solution, 200 µl phenol/choloform (3:1) was added, followed by mixing and centrifugation (12,000 rpm for 10 min at 4 ºC). The upper layer was then transferred to a clean tube. To this layer, 200µl chloroform was added, followed by mixing and centrifugation. The upper layer was again transfer to a clean tube and 50 µl of 50 % trifluoroacetic acid (TFA) was added. Samples were allowed to incubate at 4 ºC for one hour. Residual protein was pelleted by centrifuging. The supernatant was transferred to a clean tube and lyophilized. The dried carbohydrate samples were stored at −70 ºC.

Carbohydrates were detected by attaching the fluorophore, 2-amino acridine, and separating them on polyacrylamide gels by electrophoresis. This involved dissolving the isolated carbohydrates in 200 µl ddH2O. Then 50 µl of the sample was combined with 50 µl of 4 N TFA and digested at 95 ºC for 5 h: this process digested polysaccharides to monosaccharides and opened their ring structure. After digestion samples were cooled for 30 min at −20 ºC and lyophilized. Samples were then dissolved in 3µl glacial acetic acid. To this, 2.5 µl of 1.0 M 2-amino-acridine (in DMSO) and 2.5 µl of 1.0 M sodium cyanotrihydroborate (in DMSO) were added. Samples were incubated overnight at 37 ºC and then lyophilized. To this 5 µl of glacial acetic acid and 15 µl ddH2O was added. Samples were mixed and 3 µl was loaded on to BioRad Ready gels containing 12 % polyacrylamide. A monosaccharide ladder (400 µg/ml each of allose, mannose, glucose, galactose and fructose) and an external standard (400 µg/ml allose) were loaded on each gel. Samples were run at 30 mA per gel for about 30 min. Results were visualized on an UV transilluminator (Foto/Prep I, Fotodyne, Hartland, WI) and documented with a digital documentation system (Alpa Imager 2002, Alpha Innotech, Co. San Leandro, CA).

Data from gels was quantified be determining the area for the glucose band using ImageQuant (Molecular Dynamics, Inc., Sunnyvale, CA). Individual bands from larval samples were normalized for sample loss and differences in photography. This was done by converting area to µg/ml carbohydrate using a standard curve (Bentivegna, 2002). Values were adjusted for differences between the external standard and standard curve and differences between the internal and external standards. Quotients were multiplied by 5 to account for total carbohydrates in 200 µl aliquots of homogenate, as only 50 µl aliquots were used in carbohydrate analyses. Finally, products were divided by milligram of larval wet weight resulting in µg carbohydrate/mg tissue. Treatment differences were

determined by one-way ANOVA followed by Tukey's posthoc test, $p \leq 0.05$ (SSPS, Inc., 2001). Differences between Fed and Unfed or Sediment and Detritus samples were determined by Independent Samples T-test, $p \leq 0.05$.

B. Results

1. Sediment

Results for each carbohydrate experiment are shown separately in Tables 7.01 and 7.02. In the first experiment, there were no statistical differences between treatments for Fed or Unfed larvae (Table 7.01). However, carbohydrate levels were reduced by sites 7 and 10 in Fed and sites 7 and 9 in Unfed. The second experiment did show significant treatment effects (Table 7.02). Carbohydrates from sites 7 and 10 in Fed were again reduced and statistically different from site 18. Unfed larvae showed no statistical differences. Neither the positive nor negative control showed significant differences between sediments in Fed and Unfed. Positive control larvae were smaller than those in negative control and sediments (data not shown) but in terms of carbohydrate levels per milligram were not different.

Combining data from the first and second experiments (n =5-6) did show treatment affects between sites in Fed larvae and between Fed and Unfed larvae from the same site. Fed larvae exposed to sediment from sites 9, 18 and 22 showed significantly higher levels of carbohydrates compared to those from sites 7 and 10 (Figure 7.01). Sediment from site 3 was also significantly higher than that from site 7. Comparison of Fed and Unfed groups showed significant increases in carbohydrate levels for Fed larvae in negative control and sites 3, 9, 18 and 22 (Fig. 7.02). For sites 7 and 10, carbohydrate levels for Fed and Unfed were similar. Data indicated that in the presence of supplemental food those sediments reduced feeding rate and/or enhanced metabolic stress thereby reducing µg carbohydrate/mg larvae. No statistical differences occurred between Unfed treatments.

2. Detritus

Results for each carbohydrate experiment are shown in Tables 7.03 and 7.04. Data from the first experiment (n = 2-3) showed no statistical differences between treatments for either Fed or Unfed. In the second Fed experiment, carbohydrate levels were increased by site 3 detritus compared to positive control, site 3 detritus plus 0.3 mM Cd. This indicated that Cd could significantly reduce carbohydrate levels at 96 h and that the spiked Cd was bioavailable. No differences were found in Unfed.

Combined data (n = 5-6) showed no statistical differences between sites for Fed or Unfed treatments (Fig. 7.03). When Fed and Unfed were compared, supplemental feeding only increased carbohydrates in larvae exposed to detritus from site 3 (Fig. 7.04). This data indicated that larvae did no better in detritus when fed than when unfed, except for site 3. Also, none of the detrital samples themselves provided more nutrition or less stress relative to one another.

3. Sediment versus detritus

Data for sediment and detrital samples were compared in order to evaluate their relative contribution to site toxicity. Statistical differences were found in Fed (Fig. 7.05) but not Unfed treatments (Fig. 7.06). This indicated that neither media enhanced

carbohydrate levels by itself but rather suppressed the benefit of an external food source at some sites. Feeding increased larval carbohydrate levels in sediment versus detritus for sites 9, 18, and 22 (Fig. 7.05). This indicated that sediment was less toxic than detritus at these sites. Carbohydrate levels were low for sites 7 and 10 in both sediment and detritus even with supplemental food. This indicated that both media were toxic at these sites. Overall, data indicated that detritus alone reduced feeding and/or increased stress than whole sediment.

Table 7.01. Effects on carbohydrates (µg/mg) in first acute toxicity test (96h) for sediment from 10-18-02 collection. Treatments showed no statistical differences for Fed or Unfed groups

FED			UNFED		
Sample	carbs µg/mg	Ave. ± SD	Sample	carbs µg/mg	Ave. ± SD
–C-1	14.26	15.12±1.43	–C-1	3.48	6.0±2.17
–C-2	16.77		–C-2	7.41	
–C-3	14.32		–C-3	7.10	
+C-1	13.07	15.79±5.11	+C-1	12.76	9.17±5.74
+C-2	21.68		+C-2	12.20	
+C-3	12.61		+C-3	2.55	
3-1	15.00	14.11±2.30	3-1	11.94	11.21±0.72
3-2	15.83		3-2	10.49	
3-3	11.50		3-3	11.20	
7-1	12.99	10.63±2.06	7-1	8.67	7.74±1.80
7-2	9.16		7-2	8.89	
7-3	9.76		7-3	5.67	
9-1	15.99	16.52±2.93	9-1	6.28	6.53±0.35
9-2	19.68		9-2	6.77	
9-3	13.90		9-3	ND	
10-1	13.89	12.41±1.47	10-1	14.11	11.32±3.94
10-2	12.37		10-2	8.53	
10-3	10.96		10-3	ND	
18-1	18.77	18.32±3.71	18-1	12.47	10.52±2.42
18-2	21.78		18-2	7.81	
18-3	14.40		18-3	11.26	
22-1	15.69	17.26±2.29	22-1	11.21	9.38±2.69
22-2	16.21		22-2	ND	
22-3	19.89		22-3	7.54	

–C = Control without cadmium
+C = Sand with cadmium (0.3 mM).
ND = No data.

Table 7.02. Effects on carbohydrates (µg/mg) in second acute toxicity test (96 h) for sediment from 10-18-02 collection. Treatments showed statistical differences for Fed but not Unfed groups

FED			UNFED		
Sample	carbs µg/mg	Ave. ± SD	Sample	carbs µg/mg	Ave. ± SD
−C-1	13.14	14.18±1.40[ab]	−C-1	13.24	9.76±4.58
−C-2	15.78		−C-2	11.47	
−C-3	13.64		−C-3	4.57	
+C-1	10.30	12.36±1.81[ab]	+C-1	9.33	8.78±6.94
+C-2	13.67		+C-2	1.58	
+C-3	13.11		+C-3	15.43	
3-1	20.06	18.26±5.42[ac]	3-1	ND	16.69±8.35
3-2	12.18		3-2	13.86	
3-3	22.55		3-3	10.11	
7-1	8.68	10.86±3.34[b]	7-1	9.47	8.87±0.53
7-2	14.70		7-2	8.70	
7-3	9.20		7-3	8.45	
9-1	18.25	17.76±2.55[ab]	9-1	ND	11.61±1.54
9-2	15.00		9-2	12.70	
9-3	20.03		9-3	10.53	
10-1	11.88	11.59±0.85[bc]	10-1	10.72	9.66±1.22
10-2	12.25		10-2	9.94	
10-3	10.63		10-3	8.33	
18-1	19.84	19.39±0.98[a]	18-1	13.62	11.70±2.94
18-2	18.27		18-2	8.31	
18-3	20.07		18-3	13.18	
22-1	16.96	17.99±0.89[ab]	22-1	11.13	9.88±1.21
22-2	18.60		22-2	8.70	
22-3	18.41		22-3	9.82	

−C = Control without cadmium ; +C = Sand with cadmium (0.3 mM); ND = No data.
Averages that share a common letter are not statistically different, ANOVA, Tukey's posthoc, $p>0.05$.

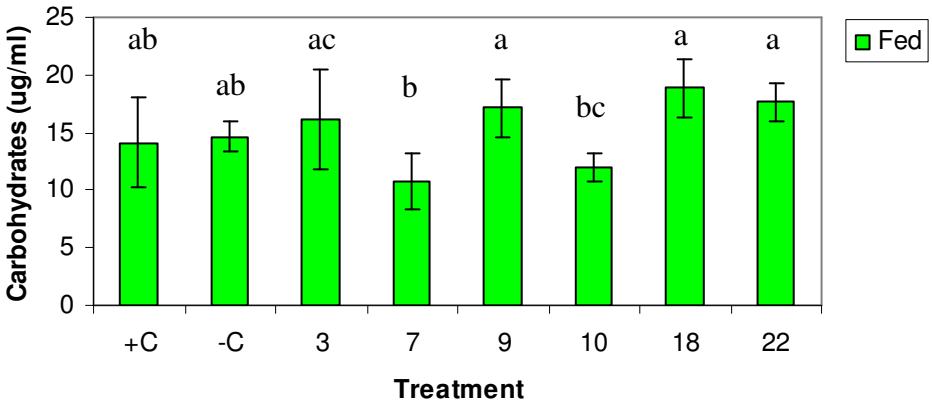


Figure 7.01: Effect of October sediment on carbohydrate levels at 96 h in Fed. Data from acute toxicity tests were combined, n =5-6. Values that share letters were not significantly different.

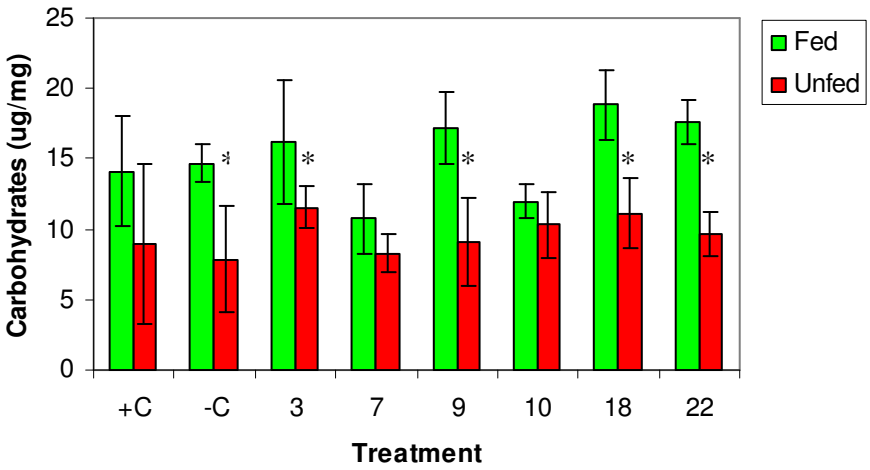


Figure 7.02: Effect of October sediment on carbohydrate levels at 96 h. Data from acute toxicity tests were combined, n =5-6. * = significant differences between Fed and Unfed.

Table 7.03. Effects on carbohydrates (µg/mg) in first acute toxicity test (96 h) for detritus from 10-18-02 collection. Treatments showed no statistical differences for Fed or Unfed groups

FED			UNFED		
Sample	carbs µg/mg	Ave. ± SD	Sample	carbs µg/mg	Ave. ± SD
+C-1	9.12	10.30±1.67	+C-1	10.21	9.03±1.67
+C-2	11.48		+C-2	7.84	
+C-3	ND		+C-3	ND	
3-1	12.53	13.14±2.78	3-1	10.93	8.92±1.85
3-2	10.71		3-2	8.55	
3-3	16.17		3-3	7.28	
7-1	9.93	10.69±2.49	7-1	9.29	9.18±2.44
7-2	8.67		7-2	6.68	
7-3	13.48		7-3	11.56	
9-1	6.54	7.95±1.99	9-1	5.93	6.51±1.37
9-2	7.09		9-2	8.07	
9-3	10.23		9-3	5.53	
10-1	6.48	7.85±1.21	10-1	5.58	5.59±0.50
10-2	8.76		10-2	6.10	
10-3	8.32		10-3	5.10	
18-1	11.48	11.70±0.33	18-1	7.14	7.39±0.35
18-2	11.53		18-2		
18-3	12.08		18-3	7.64	
22-1	6.93	8.34±1.35	22-1	11.40	9.83±1.42
22-2	9.63		22-2	8.61	
22-3	8.46		22-3	9.49	

+C = Detritus from June samples, site 22 with cadmium (0.3 mM).
ND = No data.

Table 7.04. Effects on carbohydrates (µg/mg) in second acute toxicity test (96 h) for detritus from 10-18-02 collection. Treatments showed statistical differences for Fed but not Unfed groups.

FED			UNFED		
Sample	carbs µg/mg	Ave. ± SD	Sample	carbs µg/mg	Ave. ± SD
+C-1	9.23	9.34±1.83[a]	+C-1	9.06	7.92±1.61
+C-2	11.21		+C-2	6.08	
+C-3	7.56		+C-3	8.63	
3-1	18.97	16.29±2.34[b]	3-1	10.87	11.91±1.47
3-2	14.66		3-2	12.94	
3-3	15.24		3-3		
7-1	16.04	14.24±1.80[ab]	7-1	9.41	12.65±2.87
7-2	16.22		7-2	13.64	
7-3	12.69		7-3	14.89	
9-1	14.50	13.27±1.30[ab]	9-1	11.39	12.47±0.95
9-2	11.91		9-2	12.83	
9-3	13.39		9-3	13.18	
10-1	12.86	14.39±1.61[ab]	10-1	11.63	10.28±1.57
10-2	16.08		10-2	10.67	
10-3	14.24		10-3	8.55	
18-1	17.48	14.52±2.58[ab]	18-1		13.44±2.23
18-2	13.33		18-2	11.86	
18-3	12.74		18-3	15.02	
22-1	15.71	12.65±2.73[ab]	22-1	14.81	14.57±0.34
22-2	11.78		22-2		
22-3	13.54		22-3	12.02	

+C = Detritus from October samples, site 3 with cadmium (0.3 mM); ND = No data.
Averages that share a common letter are not statistically different, ANOVA, Tukey's posthoc, $p>0.05$.

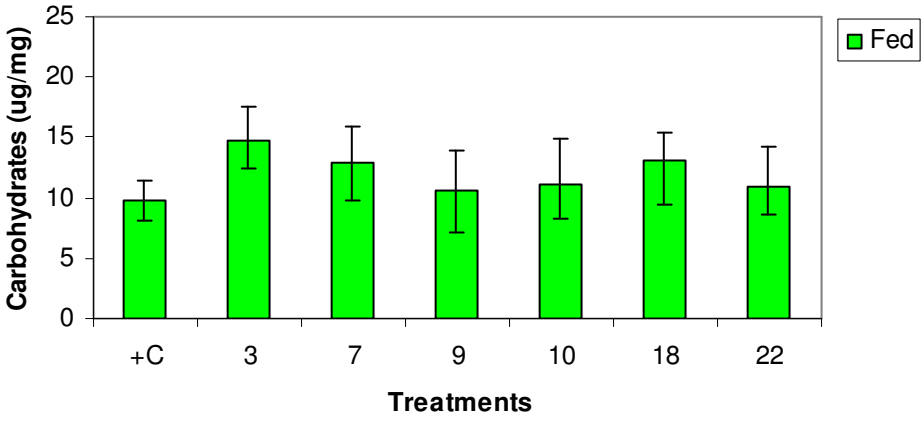


Figure 7.03: Effect of October detritus on carbohydrate levels at 96 h in Fed. Data from acute toxicity tests were combined, n =5-6. Treatments showed no signficant differences.

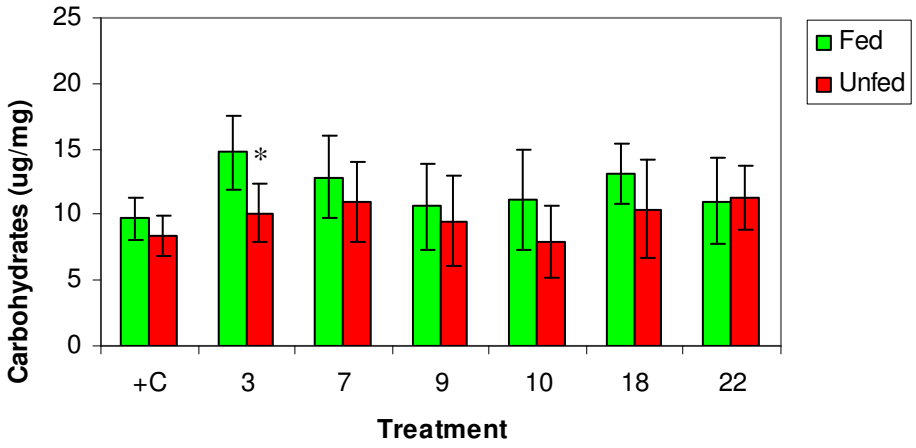


Figure 7.04: Effect of October detritus on carbohydrate levels at 96 h. Data from acute toxicity tests were combined, n =5-6. * = significant differences between Fed and Unfed.

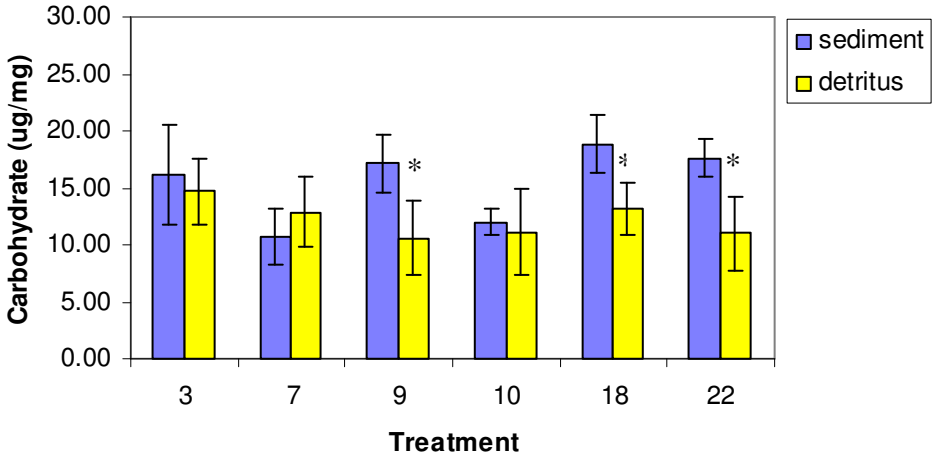


Figure 7.05: Effect of October sediment and detritus on carbohydrates in Fed larvae at 96 h. *= significant different between sediment and detritus.

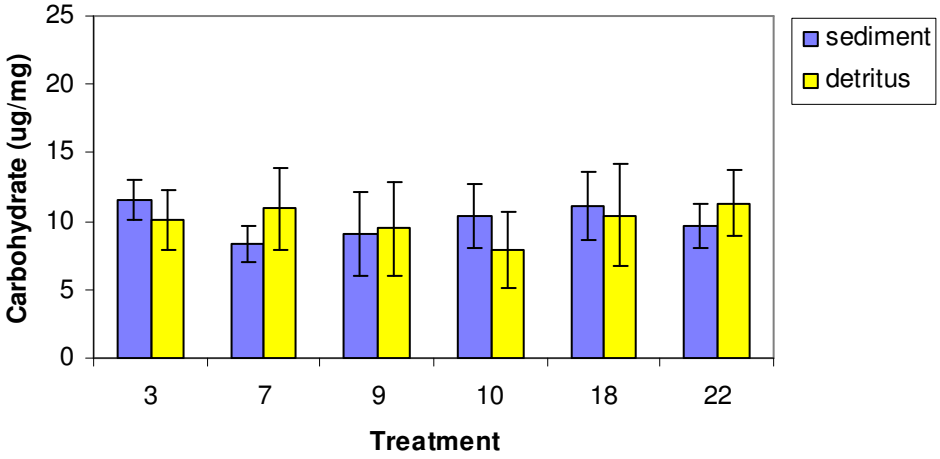


Figure 7.06: Effect of sediment and detritus on carbohydrates in Unfed larvae at 96 h. There were no significant differences between sediment and detritus.

C. Analysis and Discussion

Carbohydrates have an important structural (chitin exoskeleton) and metabolic role in insects. Metabolic energy is primarily derived from glucose (monosaccharide), trehalose (disaccharide of glucose) and glycogen (polysaccharide of glucose). Many types of stressors have been found to affect carbohydrate levels in organisms including parasitism (Bischof, 1995), diet (Ortel, 1996) and toxic chemicals (Bentivegna, 2002b, Bodar *et al*., 1988, Rajalekshmi and Mohandas, 1993, Martin and Black, 1996). Toxicants and stressors are believed to reduce carbohydrate levels by utilizing part of the organism's energy budget to maintain homeostasis.

In this study, the FACE carbohydrate assay was used as a sublethal biomarker for detection of sediment and detritus toxicity. The objective was to determine if there were any differences in toxicity between collection sites or between the sediment and detritus at a particular site. While reduced carbohydrate levels indicated a reduction in energy resources, they could not discriminate between stress-induced toxicity or poor nutritional value of substrate. Other researchers have found low caloric value to be coincident with toxic insult (Martinez *et al*., 1994). The FACE assay converts all carbohydrates to monosaccharides, and results in this study are based on glucose concentrations.

Results showed differences between sites only in Fed larvae. Sediment from sites 7 and 10 significantly suppressed carbohydrate levels compared to other sites, indicating that these two sites were more toxic than the others (Fig. 7.01). Unfed treatments showed no statistical differences. The importance of feeding for this bioassay was previously demonstrated (Bentivegna, 2002b). Bentivegna showed that affects from 24-72 h starvation were similar to those for fed and unfed larvae exposed to Cd. The implication was that larvae were stressed from the Cd added to their diet or that they had a reduced feeding rate in the presence of Cd, which simulated starvation. In this study, larvae increased their carbohydrate levels when offered an uncontaminated, external food source (Fed) compared to when they were depend on substrate only (Unfed). Data for Fed and Unfed were similar for sites 7 and 10, which suggested that even in the presence of food, feeding rate was suppressed or toxic insult was leading to a decline in metabolic resources (Fig. 7.02). Results for detritus showed no significant differences between sites for Fed or Unfed treatments (Fig. 7.03). Comparison of Fed to Unfed showed a significant difference only for site 3 (Fig. 7.04). This indicated that detritus from site 3 was less toxic than those from other sites.

Comparison of detritus and sediment showed that carbohydrates levels in Fed and Unfed detritus were similar to those in Unfed sediment (Figs. 7.05 and 7.06). Also, comparing Fed treatments showed carbohydrates were significantly lower in detritus than sediment for sites 9, 18 and 22 (Fig. 7.05). Taken together results indicated that detritus was more toxic than sediment at these sites. Bioaccumulation data should be able to discriminate between whether Fed larvae were accumulating metals through their diet (metal concentration higher in Fed than Unfed) or simply not feeding (metal concentration similar in Fed and Unfed).

Positive controls for sediment did not show reduced carbohydrate levels for either Fed or Unfed treatments. There was no significant differences between Cd spiked sand and sediments with or without supplemental food (Figure 7.02). However, results for detritus did show modulation of carbohydrate levels by Cd in Fed treatments. Carbohydrates levels were significantly reduce in detritus from site 3 that was spiked

with Cd compared to the same detritus unspiked (Table 7.04). One explanation for the lack of effect in sediment studies could be that too much food was added and the concentration of Cd diluted out. Another possibility is the biomarker was not sensitive or consistent enough to statistically differentiate between some groups. Analyses were done on the major glucose band in this study instead of the more sensitive, glycogen-based bands found in other studies (Bentivegna, 2002a). The manufacturer of the kit used in those studies discontinued production and the adapted protocol for this study did not produce the glycogen-based bands. In any case, differences found between sites for Fed larvae were consistent with effects on subchronic growth and demonstrated the assay's ability to detect changes in metabolic resources.

VIII. Bioaccumulation

IX. Integration of Endpoints

X. References

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