SEDIMENT TOXICITY TESTS

Pickle Pond Sediments

Tested September 2013

By the Wisconsin State Laboratory of Hygiene

Environmental Toxicology Section

Laboratory Report Number: FY000113 – FY000118

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Compiled By: Mallory Berrey

Reviewed By: Dawn Perkins Date: December 9, 2013

INTRODUCTION

Craig Roesler and Joe Graham of the Wisconsin DNR collected six sediment samples for the Pickle Pond sediment project on August 27, 2013 and the samples were delivered to the Wisconsin State Laboratory of Hygiene (WSLH). WSLH tested the sediments for toxicity along with formulated sediment as a laboratory control. Solid phase sediment toxicity tests were performed using the amphipod, *Hyalella azteca*, and the larval stage of the midge, *Chironomus tentans*. These two organisms, which burrow and come into direct contact with the sediments, are recommended for use in sediment toxicity testing (USEPA, 2000).

TEST METHODS

Sediments were received in 2 gallon high density polyethylene buckets on August 29, 2013 and stored in the dark at 4°C. On September 6, each sediment sample and the synthetic laboratory control sediment were thoroughly homogenized by mixing the sample using a large stainless steel spoon. Homogenized sediment was placed in test beakers and stored at 4°C until September 8. On September 8, dechlorinated municipal tap water was added to each test beaker at a ratio of 1:1.75, sediment to overlying water, and the test beakers were randomly placed into a walk-in environmental chamber at 23±1°C with a 16 hour: 8 hour light:dark cycle. After allowing the sediments to settle overnight, organisms were randomly added to the test beakers on September 9, 2013. Test conditions are summarized in Table 1 (USEPA, 2000).

Chironomus tentans

Chironomus tentans egg masses were purchased from Aquatic Biosystems, Fort Collins, CO and hatched in the Environmental Toxicology section of WSLH. Larval *C. tentans* were 14 days old on the day the test was initiated. Ten individuals were randomly placed in each test beaker with eight replicates per sediment site and Lab Control. Overlying water was replaced twice daily and organisms were fed 1.5 ml Tetramin® flake fish food mixture daily (1.5 ml contained 6.0 mg of dry solids). Dissolved oxygen, pH, and temperature of the overlying water were recorded daily. Hardness, alkalinity, ammonia and conductivity were measured at the beginning and at the end of the test (day 0 and day 10, respectively). On day 10, the organisms were recovered from the sediment to determine the number of survivors. Surviving organisms were subsequently

dried overnight at 100°C and weighed to determine dry weight. The organisms were then ashed at 550°C for a minimum of 2 hours and weighed to determine ash-free dry weight (USEPA, 2000).

Hyalella azteca

Juvenile *H. azteca*, cultured in the Environmental Toxicology section of WSLH, were 13-14 days old on the day of test initiation. Ten individuals were randomly placed in each test beaker with eight replicates per sediment site and Lab Control. Overlying water was replaced twice daily and organisms were fed 1.0 ml YFC (yeast/fish food/cereal leaves). Dissolved oxygen, pH, and temperature of the overlying water were recorded daily. Hardness, alkalinity, ammonia and conductivity were measured at the beginning and at the end of the test (day 0 and day 10, respectively). On day 10, the organisms were recovered from the sediment to determine the number of survivors in each replicate. Survivors were subsequently dried overnight at 100°C and weighed to determine dry weight (USEPA, 2000).

Statistical analyses

Statistical analyses were conducted using a PC-version of SAS® (SAS Institute, Cary, NC). One-way analysis of variance (ANOVA) followed by a multiple comparison test (Student-Newman-Keuls) was used to identify differences among treatments in survival and weight of survivors of *Chironomus tentans* and *Hyalella azteca.* Results with $p < 0.05$ were considered significant.

SUMMARY OF RESULTS

Tests for both species met the minimum requirements for test acceptability (see Table 1). *C. tentans* survival was 70% in the control sediment and the average weight per individual was greater than 0.48 mg (at 1.49 mg). *H. azteca* survival in the control sediment was 97.5% and the average weight increased from 0.055 mg/individual to 0.087 mg/individual.

Overlying water chemical parameters

Dissolved oxygen (DO) and temperature values in overlying water were within acceptable limits for both tests according to USEPA, 2000 (see Table 1 and Figures 3, 5, 9, and 11). The only exception to this was that some temperatures on

Day 1 in both the *C. tentans* and *H. azteca* test chambers were slightly above the 23±1°C acceptable limit. The incubator's temperature was adjusted and the subsequent temperatures were within the limits. DO should remain above 2.5 mg/L, which was the case throughout the test as the lowest DO measurement observed was 2.58 mg/L. There are no criteria set for pH values but results are summarized in Figures 4 and 10. Results of conductivity, hardness, alkalinity, and ammonia analyses from samples collected on the first and last days of the tests are summarized in Figures 6 and 12. According to USEPA (2000), values for hardness, alkalinity and ammonia should not vary by more than 50% during the test. This was the case for hardness in both species, and alkalinity in the *C. tentans* test. Alkalinity did vary by more than 50% in the *H. azteca* Lab Control (210 to 330 mg/L). However, the rest of the *H. azteca* sites were within the 50% acceptable range. Ammonia values also varied by greater than 50% for both species in most of the Pickle Pond test sites. Overall, the levels of ammonia in the overlying water were low ($\leq$ 1.78 mg/L) and not at levels associated with toxicity in previous sediment tests (20-310 mg/L, USEPA, 2000). There are no criteria set for conductivity measurements.

Survival and Growth

Statistical analyses indicated significant differences among sites in growth of *C. tentans* and of *H. azteca*; however, there was no significant difference in survival ($p < 0.05$).

Chironomus tentans

Survival of *C. tentans* was not significantly different among any of the sites and the Lab Control (Figure 1).

The ash-free dry weight (AFDW) of *Chironomus tentans* was significantly higher in sites PP01 and PP02 A/B compared to the Lab Control. The AFDW of sites LF-10, PP04, PP07, and PP09 were not significantly different than the Lab Control, PP01 and PP02 A/B (Figure 2).

At the initiation of the test, an average dry weight of 20 organisms was taken to confirm the organisms were in their 2nd to 3rd instar. The average dry weight is supposed to be between 0.08 – 0.23 mg/individual. The *C. tentan* organisms used to initiate this test averaged 0.3355 mg/individual. This may explain why at the termination of the test (on Day 10) the laboratory observed multiple replicates containing pupated larvae and a few containing emerged adults. These individuals

were included in the survival data, however were excluded when calculating ash-free dry weight.

Hyalella azteca
Survival of *H. azteca* was not significantly different among the sites and the Lab Control (Figure 7).

Dry weight of *H. azteca* was not significantly different between site LF-10 and the Lab Control (Figure 8). However, both were significantly lower than all other sites. The dry weights of sites PP01, PP04, PP07, and PP09 were not significantly different from each another, but all four were significantly higher than site PP02 A/B.

CONCLUSIONS

None of the Pickle Pond sediment samples tested in September 2013 had significant negative effects on the survival of *Hyalella azteca* or *Chironomus tentans*. In contrast, the AFDW (*C. tentans*) and the dry weight (*H. azteca*) across all ambient sites were significantly higher compared to the Lab Control. This indicates there is no evidence of toxicity from the Pickle Pond sediment samples.

Interestingly, ammonia levels decreased in all *H. azteca* treatments from the beginning to the end of the test. In contrast, ammonia increased in all *C. tentans* treatments. It is not known whether this is significant or not.

It is also worth noting that in several of the ambient sites tested, *C. tentans* survival within a given site varied greatly – with some replicates showing much lower survival compared to the other replicates within the same site. At site PP02 A/B an indigenous leech (genus and species unknown) was found in the replicate cup where only four *C. tentans* survived. A few other indigenous leeches were encountered (in the ambient sites only), unfortunately it was not noted as to what specific treatments and/or replicates they were found in. A brief peer-review literature search found there is evidence demonstrating some species of freshwater leeches are known to eat invertebrates, including *Chironomids* (Ingersoll 1990, Kutschera 2003). Whether the presence of leeches was an attributing factor to the observed *C. tentans* inter-replicate survival variability is not known.

REFERENCES

United States Environmental Protection Agency (USEPA). Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second Edition. 2000. EPA/600/R-99/064. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

Ingersoll CG, Nelson MK. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). ASTM STP, WG Landis and WH van der Schalie, eds, American Society for Testing and Materials, Philadelphia, PA.

Kutschera U. 2003. The Feeding Strategies of the Leech *Erpobdella octoculata* (L.): A Laboratory Study. Internat Rev Hydrobiol, 88:1:94-101.

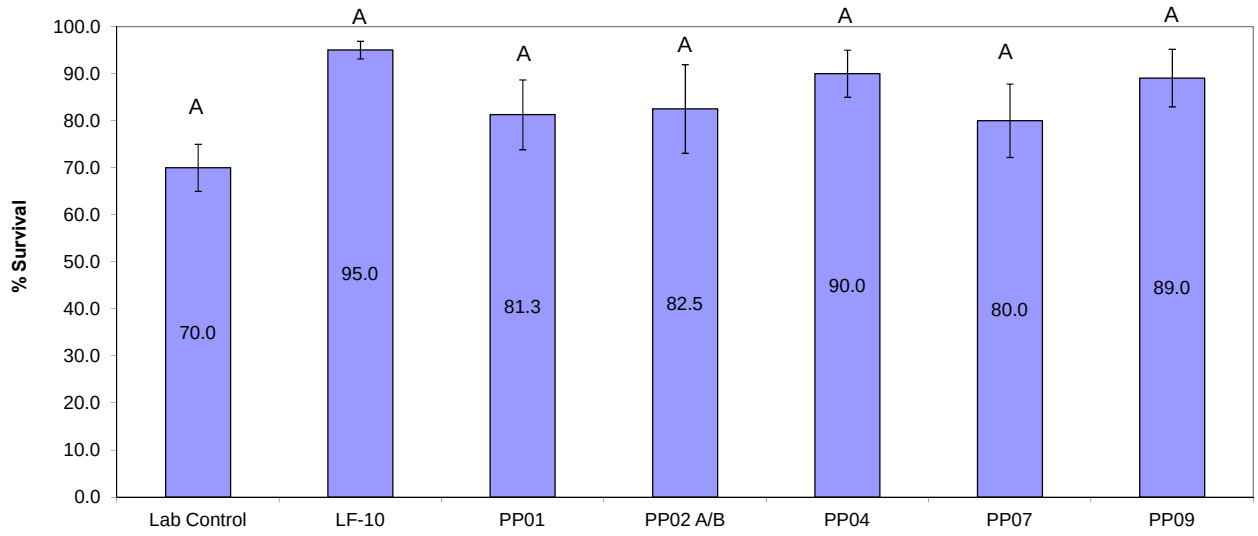
Table 1. Summary of Test Conditions for Conducting Sediment Toxicity Tests

	Parameter	Conditions
1.	Test Type	Whole sediment toxicity test with renewal of overlying water
2.	Temperature	$23 \pm 1^{o}C$
3.	Light Quality	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod	16L:8D
6.	Test Chamber	470 ml polypropylene Beaker (*C. tentans* and *H. azteca*)
7.	Sediment Volume	100 ml (*C. tentans* and *H. azteca)*
8.	Overlying Water Volume	175 ml (*C. tentans* and *H. azteca)*
9.	Renewal of Overlying Water	2 volume additions/d (*C. tentans* and *H. azteca*)
10.	Age of Organisms	Second to third instar larvae (*C. tentans*) 7- to 14-d old, within a 1 to 2 day range (*H. azteca)*
11.	Number of organisms/chamber	10 (*C. tentans* and *H. azteca)*
12.	Number of replicates/treatment	8 (*C. tentans* and *H. azteca)*
13.	Feeding	1.0 ml YFC (1800 mg/l stock) daily to each test chamber (*H. azteca*) 1.5 ml Tetramin flake fish food mixture (1.5ml contains 6.0 mg of dry solids) to each test chamber (*C. tentans*)
14.	Aeration	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L
15.	Overlying water	dechlorinated tap water
16.	Test chamber cleaning	If screens become clogged during a test; gently brush the outside of the screen
17.	Overlying water quality	Hardness, alkalinity, ammonia, DO, pH, and conductivity at the beginning and end of a test. Temperature, pH and dissolved oxygen daily.
18.	Test duration	10 d (*H. azteca* and *C. tentans*)
19.	Endpoints	Survival and growth (dry weight) (*H. azteca*) Survival and growth (ash free dry weight) (*C. tentans*)
20.	Test acceptability	Minimum mean control survival of 70%, minimum mean weight per surviving control organism of 0.48 mg ash free dry weight (*C. tentans)* Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment (*H. azteca)*

Figure 1
Pickle Pond, September 2013
***Chironomus tentans* Survival**
Sediment collected: August 27, 2013
Test Date: September 9, 2013

			Percent survival by replicate									
Lab Number	Site Name	Description	Rep 1	2	3	4	5	6	7	8	Mean	SE
LC	Lab Control	synthetic sediment	70	60	70	50	100	70	70	70	70.0	5.0
FY000113	LF-10	Loons Foot Landing Area	100	100	100	90	90	100	90	90	95.0	1.9
FY000114	PP01	Pickle Pond site 01	80	100	70	90	100	40	100	70	81.3	7.4
FY000115	PP02 A/B	Pickle Pond site 02 A/B	90	40	40	100	90	100	100	100	82.5	9.4
FY000116	PP04	Pickle Pond site 04	60	80	100	90	100	100	100	90	90.0	5.0
FY000117	PP07	Pickle Pond site 07	90	80	90	70	90	90	30	100	80.0	7.8
FY000118	PP09	Pickle Pond site 09	80	90	100	50	100	100	100	92.3	89.0	6.1

Pickle Pond Sediments
***C. tentans* Survival**

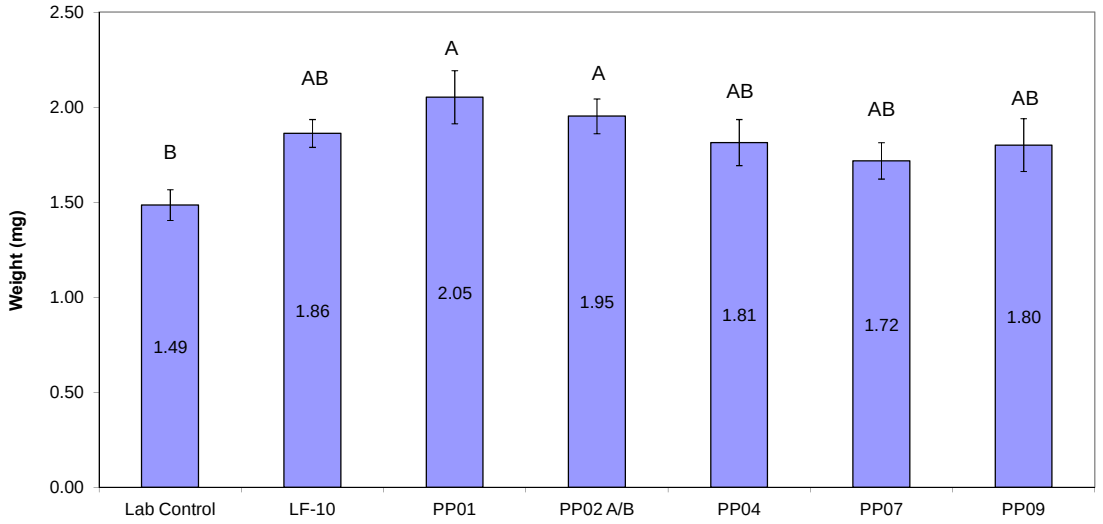


Bars with the same letter are not statistically different

Figure 2
Pickle Pond, September 2013
***Chironomus tentans* Ash Free Dry Weight (AFDW)**
Sediment collected: August 27, 2013
Test Date: September 9, 2013

			mg/surviving larva									
Lab Number	Site Name	Description	Rep 1	2	3	4	5	6	7	8	Mean	SE
LC	Lab Control	synthetic sediment	1.36	1.56	1.33	1.90	1.62	1.61	1.18	1.32	1.49	0.08
FY000113	LF-10	Loons Foot Landing Area	1.80	1.65	1.78	2.29	2.05	1.74	1.78	1.81	1.86	0.07
FY000114	PP01	Pickle Pond site 01	2.19	2.30	2.31	2.01	1.52	2.64	1.49	1.95	2.05	0.14
FY000115	PP02 A/B	Pickle Pond site 02 A/B	2.11	1.79	2.31	1.97	2.12	2.03	1.82	1.46	1.95	0.09
FY000116	PP04	Pickle Pond site 04	2.41	2.10	1.78	2.05	1.53	1.43	1.56	1.64	1.81	0.12
FY000117	PP07	Pickle Pond site 07	1.63	2.08	1.64	1.64	1.69	1.50	2.17	1.39	1.72	0.10
FY000118	PP09	Pickle Pond site 09	2.46	1.82	1.71	2.33	1.37	1.60	1.44	1.67	1.80	0.14

Pickle Pond Sediments
***C. tentans* Mean Ash Free Dry Weight**



Bars with the same letter are not statistically different

Figure 3
Pickle Pond, September 2013
Chironomus tentans
Dissolved Oxygen (mg/L)

Lab Number	Site Name	Description	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	6.67	4.86	4.88	5.33	4.93	5.90	5.82	4.85	4.72	5.29	4.14	5.07	0.69
FY000113	LF-10	Loons Foot Landing Area	5.85	4.59	4.39	4.19	3.46	4.48	3.99	3.77	3.40	3.94	3.16	3.94	0.74
FY000114	PP01	Pickle Pond site 01	5.59	4.21	3.77	3.64	3.54	4.69	4.33	3.33	3.45	3.69	3.39	3.80	0.69
FY000115	PP02 A/B	Pickle Pond site 02 A/B	5.47	3.97	4.22	3.64	3.76	4.34	3.85	3.96	3.13	3.44	2.58	3.69	0.73
FY000116	PP04	Pickle Pond site 04	5.69	3.75	3.85	3.26	3.51	4.64	4.12	3.18	3.12	3.73	3.19	3.64	0.77
FY000117	PP07	Pickle Pond site 07	5.73	4.21	4.58	4.42	4.60	5.32	5.45	4.59	4.11	4.03	3.53	4.48	0.66
FY000118	PP09	Pickle Pond site 09	5.63	4.56	4.58	4.42	4.18	4.73	5.57	3.67	3.25	3.95	3.19	4.21	0.81

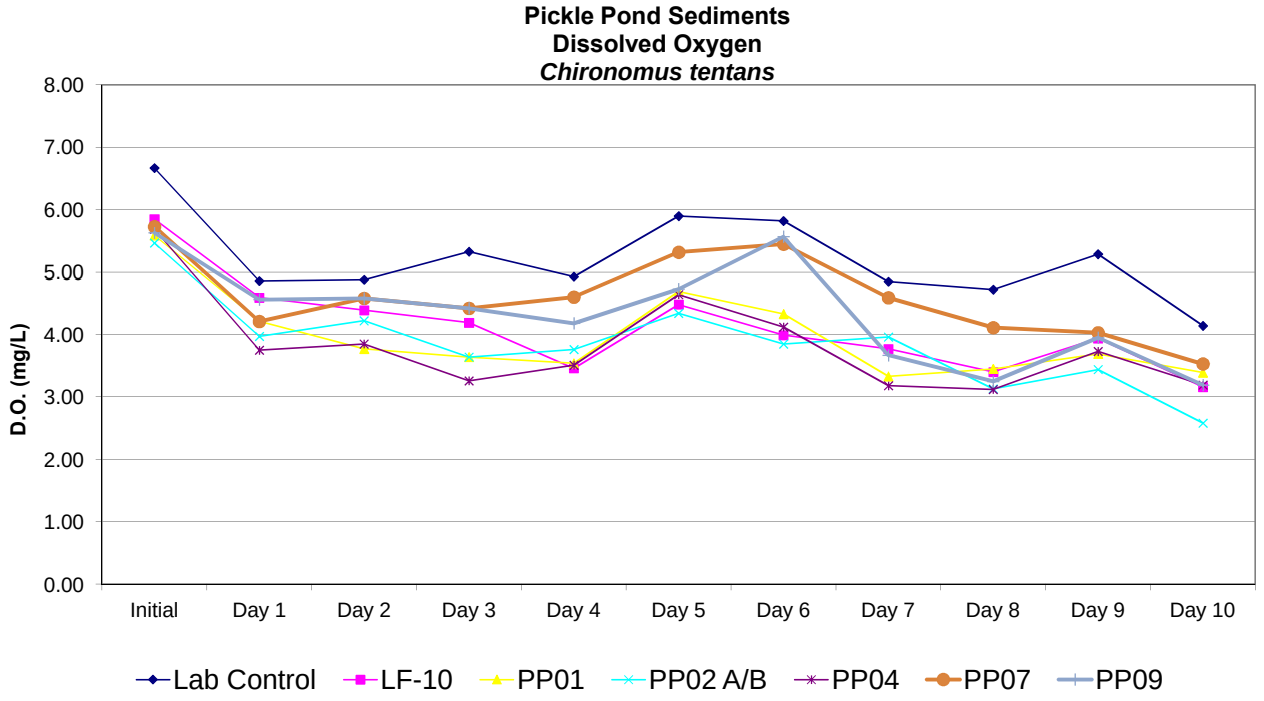


Figure 4
Pickle Pond, September 2013
Chironomus tentans
pH

Lab Number	Site Name	Description	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	8.53	8.11	8.12	8.23	8.25	8.33	8.43	8.20	8.24	8.28	8.22	8.24	0.12
FY000113	LF-10	Loons Foot Landing Area	7.82	7.73	7.82	7.87	7.88	7.97	8.00	7.98	7.92	7.97	7.94	7.91	0.08
FY000114	PP01	Pickle Pond site 01	7.79	7.67	7.70	7.75	7.85	7.93	8.01	7.88	7.84	7.86	7.87	7.84	0.10
FY000115	PP02 A/B	Pickle Pond site 02 A/B	7.95	7.78	7.87	7.90	7.97	8.02	8.07	8.09	7.95	7.94	7.90	7.95	0.09
FY000116	PP04	Pickle Pond site 04	7.88	7.70	7.77	7.78	7.88	8.00	8.00	7.91	7.86	7.89	7.90	7.87	0.09
FY000117	PP07	Pickle Pond site 07	7.78	7.62	7.76	7.79	7.85	7.96	8.17	8.01	7.89	7.90	7.93	7.89	0.15
FY000118	PP09	Pickle Pond site 09	7.91	7.86	7.94	7.99	8.01	8.05	8.20	8.03	7.95	8.00	7.99	8.00	0.09

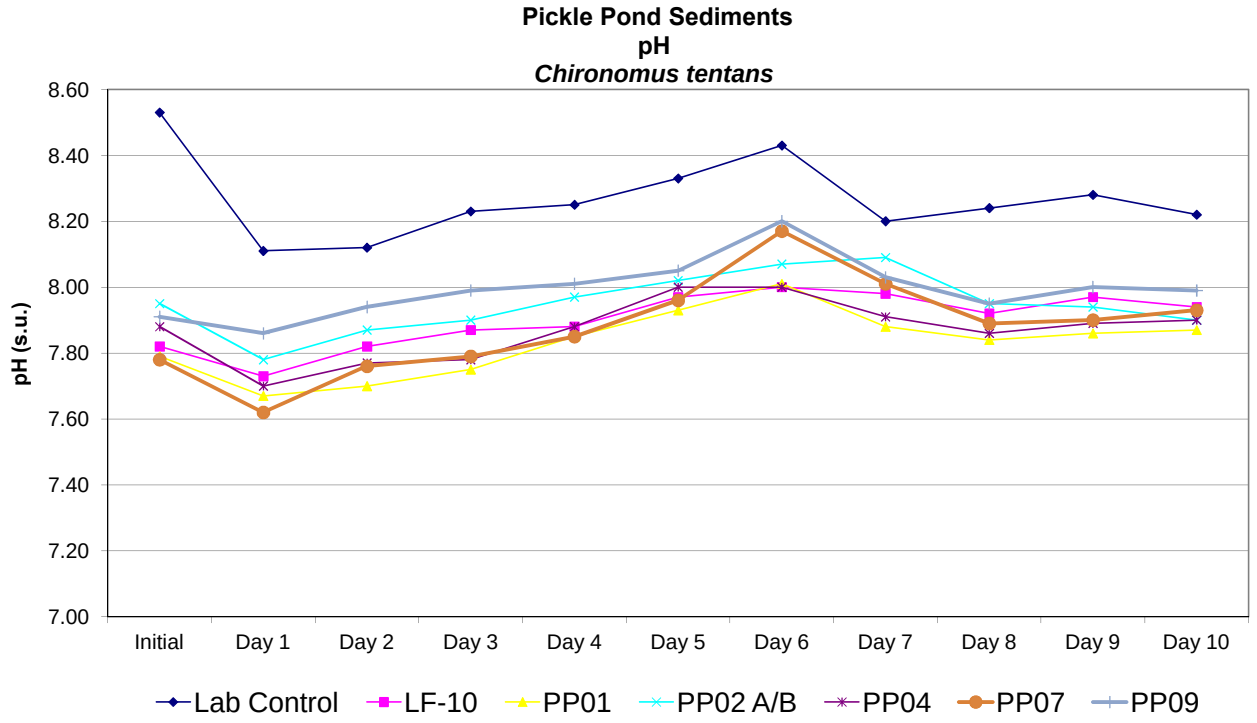


Figure 5
Pickle Pond, September 2013
Chironomus tentans
Temperatures (ºC)

Lab Number	Site Name	Description	Initial *	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	24.0	22.6	22.8	22.9	23.1	22.8	22.9	22.6	22.5	22.8	22.5	22.8	0.4
FY000113	LF-10	Loons Foot Landing Area	23.8	22.2	22.5	22.9	23.0	22.5	22.8	22.4	22.3	22.6	22.4	22.6	0.4
FY000114	PP01	Pickle Pond site 01	24.0	22.5	22.8	23.1	22.8	22.8	23.0	22.8	22.8	22.8	22.7	22.8	0.4
FY000115	PP02 A/B	Pickle Pond site 02 A/B	23.6	22.2	22.4	22.9	22.5	22.4	22.7	22.6	22.4	22.7	22.5	22.5	0.4
FY000116	PP04	Pickle Pond site 04	24.1	22.7	22.9	23.1	23.2	23.1	22.9	23.0	22.8	23.0	22.8	23.0	0.4
FY000117	PP07	Pickle Pond site 07	23.7	22.4	22.6	22.9	22.9	22.6	22.7	22.6	22.5	22.8	22.5	22.7	0.4
FY000118	PP09	Pickle Pond site 09	23.7	22.4	22.5	22.9	23.0	22.5	22.7	22.5	22.4	22.7	22.5	22.6	0.4

* The incubator temperature was turned down after the initial temperatures were at or above 23+1°C

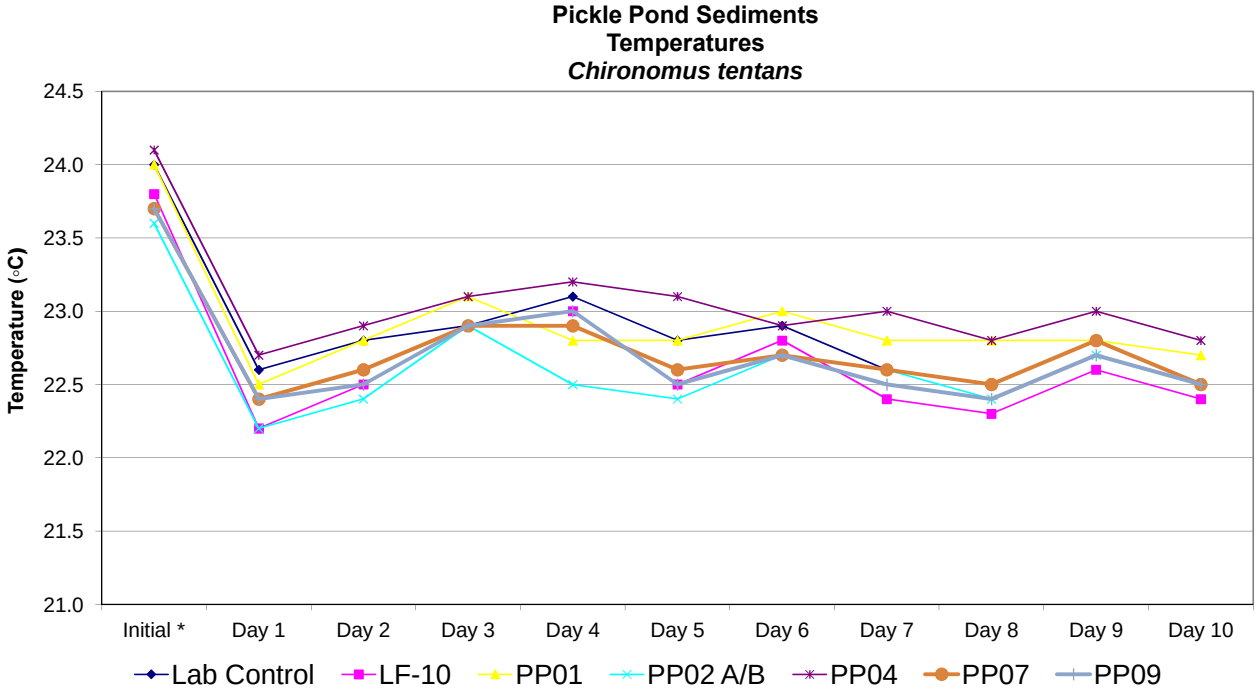


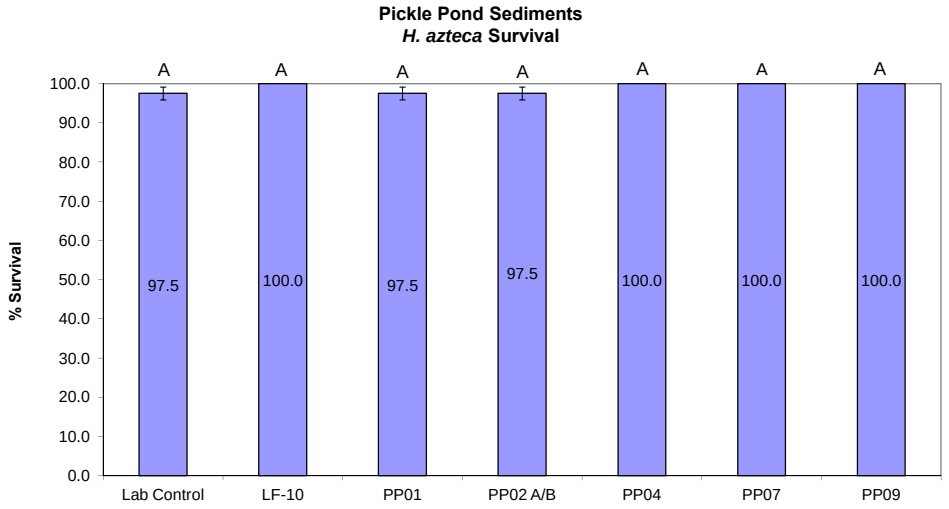
Figure 6
Pickle Pond, September 2013
Chironomus tentans
Conductivity, Hardness, Alkalinity and Ammonia

			Conductivity (µS)		Hardness (mg/L)		Alkalinity (mg/L)		Ammonia (mg/L)	
Lab Number	Site Name	Description	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)
LC	Lab Control	synthetic sediment	695	619	160	152	210	315	0.243	0.545
FY000113	LF-10	Loons Foot Landing Area	525	575	152	156	215	295	0.451	1.170
FY000114	PP01	Pickle Pond site 01	700	612	140	148	225	305	0.836	1.780
FY000115	PP02 A/B	Pickle Pond site 02 A/B	732	620	148	140	245	285	0.744	1.670
FY000116	PP04	Pickle Pond site 04	721	604	144	152	240	295	0.640	1.300
FY000117	PP07	Pickle Pond site 07	589	614	152	152	250	310	0.190	1.200
FY000118	PP09	Pickle Pond site 09	564	636	144	148	250	315	0.880	0.944

Figure 7
Pickle Pond, September 2013
***Hyallela azteca* Survival**
Sediment collected: August 27, 2013
Test Date: September 9, 2013

			Percent survival by replicate									
Lab Number	Site Name	Description	Rep 1	2	3	4	5	6	7	8	Mean	SE
LC	Lab Control	synthetic sediment	90	100	90	100	100	100	100	100	97.5	1.6
FY000113	LF-10	Loons Foot Landing Area	100	100	100	100	100	100	100	100	100.0	0.0
FY000114	PP01	Pickle Pond site 01	100	100	100	90	90	100	100	100	97.5	1.6
FY000115	PP02 A/B	Pickle Pond site 02 A/B	100	100	100	100	90	100	100	90	97.5	1.6
FY000116	PP04	Pickle Pond site 04	100	100	100	100	100	100	100	100	100.0	0.0
FY000117	PP07	Pickle Pond site 07	100	100	100	100	100	100	100	*LA	100.0	0.0
FY000118	PP09	Pickle Pond site 09	100	100	100	100	100	100	100	100	100.0	0.0

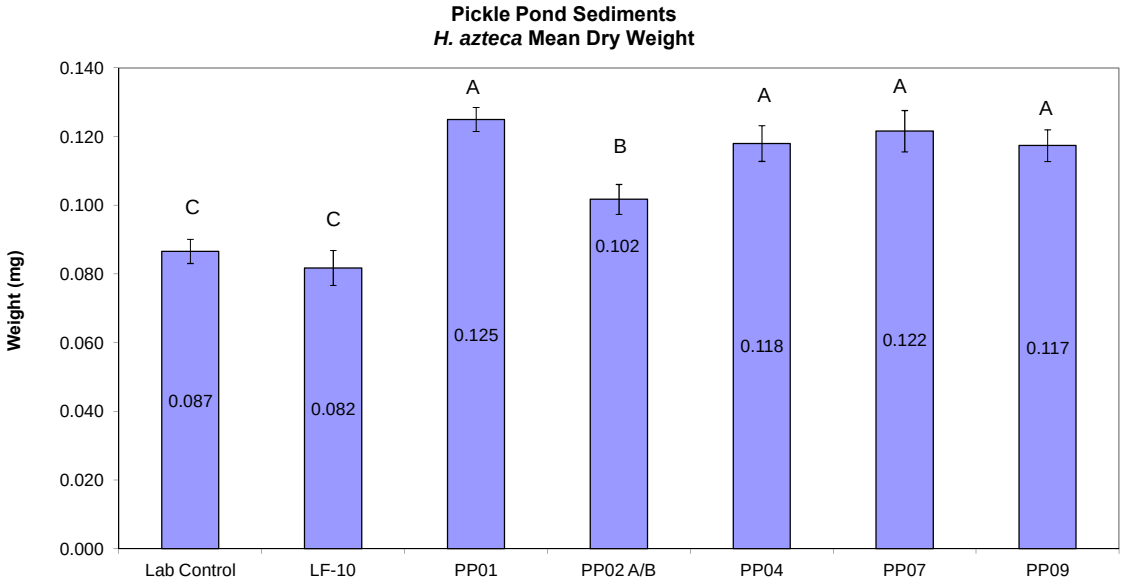
*Lab Accident with Replicate 8 of PP07 site (cup spilled during test termination on Day 10)



Bars with the same letter are not statistically different

Figure 8
Pickle Pond, September 2013
***Hyalella azteca* Dry Weight/Surviving Individual (mg)**
Sediment collected: August 27, 2013
Test Date: September 9, 2013

			mg/survivng individual									
Lab Number	Site Name	Description	Rep 1	2	3	4	5	6	7	8	Mean	SE
LC	Lab Control	synthetic sediment	0.081	0.098	0.075	0.077	0.082	0.102	0.094	0.085	0.087	0.00
FY000113	LF-10	Loons Foot Landing Area	0.115	0.076	0.076	0.075	0.084	0.068	0.077	0.085	0.082	0.01
FY000114	PP01	Pickle Pond site 01	0.131	0.117	0.114	0.116	0.124	0.142	0.122	0.135	0.125	0.00
FY000115	PP02 A/B	Pickle Pond site 02 A/B	0.108	0.093	0.094	0.090	0.089	0.120	0.103	0.118	0.102	0.00
FY000116	PP04	Pickle Pond site 04	0.119	0.091	0.126	0.128	0.110	0.114	0.117	0.141	0.118	0.01
FY000117	PP07	Pickle Pond site 07	0.153	0.136	0.129	0.113	0.103	0.120	0.118	0.102	0.122	0.01
FY000118	PP09	Pickle Pond site 09	0.129	0.131	0.129	0.105	0.093	0.118	0.118	0.118	0.117	0.00



Bars with the same letter are not statistically different

Figure 9
Pickle Pond, September 2013
Hyalella azteca
Dissolved Oxygen (mg/L)

Lab Number	Site Name	Description	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	5.63	6.73	6.88	6.80	6.90	7.26	7.21	6.59	6.82	6.72	6.60	6.85	0.43
FY000113	LF-10	Loons Foot Landing Area	5.87	6.12	6.27	6.20	6.33	7.30	6.43	6.04	6.22	6.77	6.16	6.38	0.39
FY000114	PP01	Pickle Pond site 01	5.17	5.60	5.94	6.06	6.18	6.55	6.53	6.03	6.23	6.44	5.99	6.16	0.41
FY000115	PP02 A/B	Pickle Pond site 02 A/B	5.32	5.38	5.70	5.79	6.12	6.27	6.56	5.94	6.08	6.26	5.81	5.99	0.38
FY000116	PP04	Pickle Pond site 04	5.34	5.52	5.77	5.65	5.78	6.30	6.10	5.48	5.73	6.00	5.49	5.78	0.29
FY000117	PP07	Pickle Pond site 07	5.58	5.68	5.91	5.85	6.02	6.77	6.67	5.85	5.93	5.87	5.55	6.01	0.40
FY000118	PP09	Pickle Pond site 09	5.45	5.75	5.78	5.65	5.82	6.56	6.56	5.66	5.85	6.09	5.81	5.95	0.36

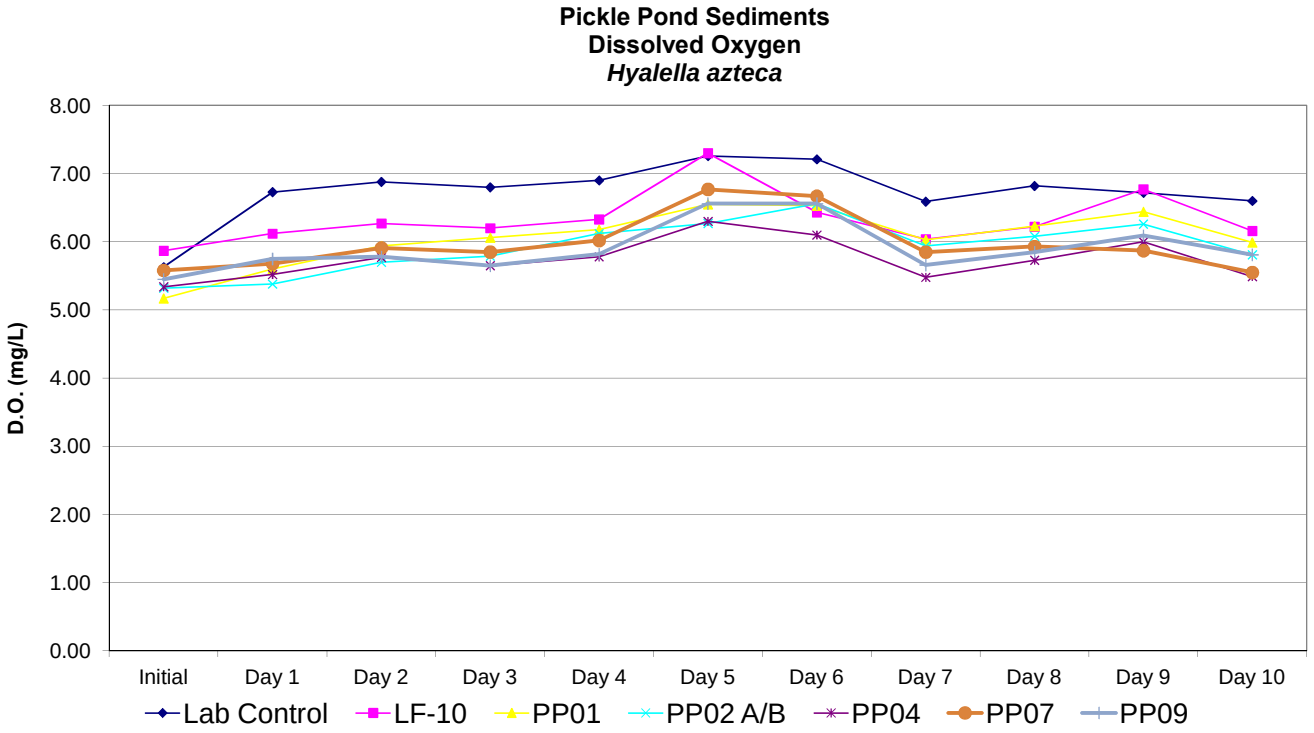


Figure 10
Pickle Pond, September 2013
Hyalella azteca
pH

Lab Number	Site Name	Description	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	8.40	8.37	8.41	8.41	8.45	8.51	8.56	8.49	8.46	8.49	8.49	8.46	0.06
FY000113	LF-10	Loons Foot Landing Area	7.85	7.95	8.06	8.11	8.16	8.21	8.38	8.24	8.24	8.49	8.35	8.22	0.19
FY000114	PP01	Pickle Pond site 01	7.68	7.82	7.99	8.07	8.14	8.21	8.29	8.24	8.24	8.32	8.27	8.16	0.21
FY000115	PP02 A/B	Pickle Pond site 02 A/B	7.90	7.95	8.04	8.14	8.21	8.22	8.39	8.27	8.27	8.26	8.27	8.20	0.15
FY000116	PP04	Pickle Pond site 04	7.81	7.89	7.99	8.04	8.11	8.20	8.28	8.19	8.20	8.26	8.35	8.15	0.17
FY000117	PP07	Pickle Pond site 07	7.78	7.88	8.02	8.07	8.16	8.23	8.31	8.15	8.19	8.26	8.23	8.15	0.17
FY000118	PP09	Pickle Pond site 09	7.93	7.99	8.08	8.13	8.19	8.22	8.33	8.29	8.28	8.23	8.41	8.22	0.15

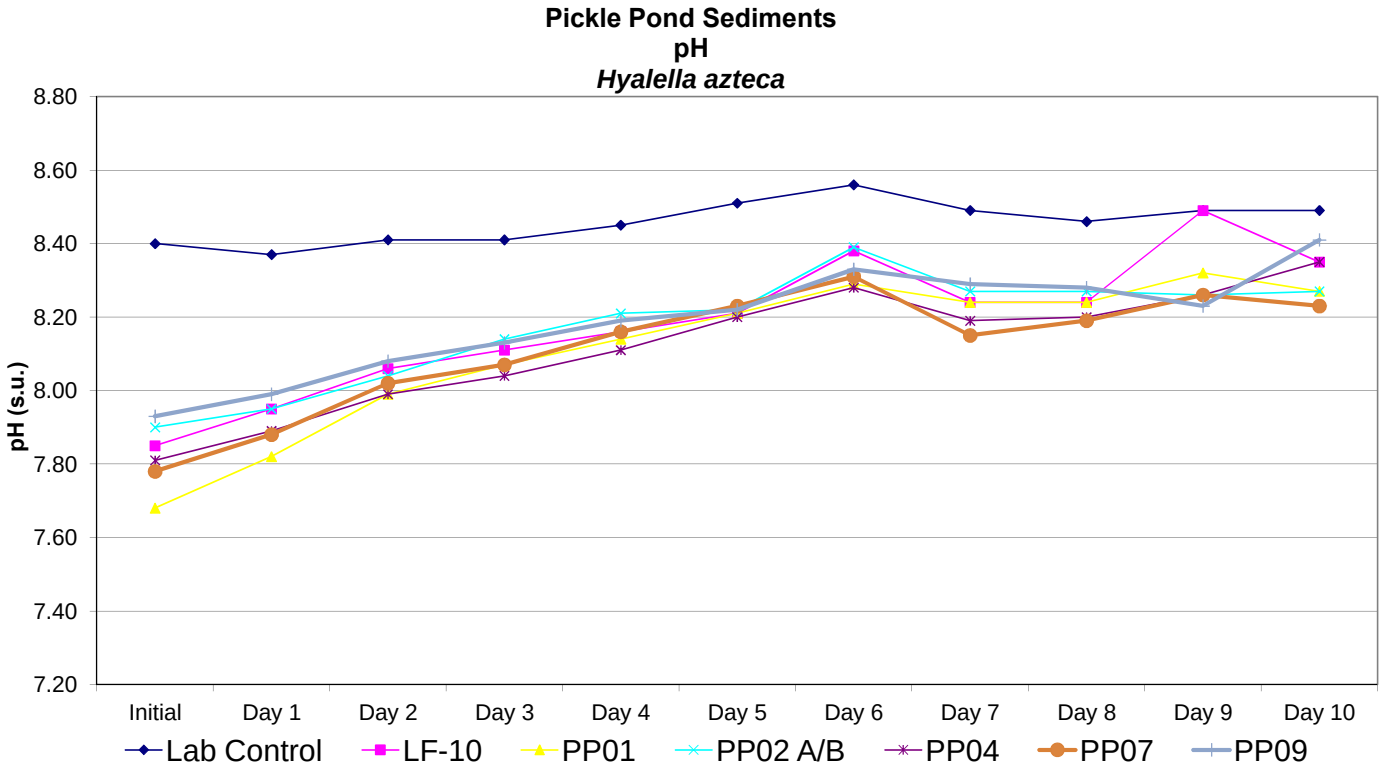


Figure 11
Pickle Pond, September 2013
Hyalella azteca
Temperatures (°C)

Lab Number	Site Name	Description	Initial *	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	sd
LC	Lab Control	synthetic sediment	24.4	22.6	22.7	23.9	23.3	22.8	22.8	22.6	22.6	22.6	22.8	22.9	0.6
FY000113	LF-10	Loons Foot Landing Area	24.0	22.4	22.5	22.9	23.0	22.5	23.0	22.5	22.3	22.5	22.6	22.6	0.5
FY000114	PP01	Pickle Pond site 01	24.4	22.6	22.8	23.0	23.2	22.8	23.0	22.7	22.6	22.8	22.6	22.8	0.5
FY000115	PP02 A/B	Pickle Pond site 02 A/B	23.8	22.2	22.3	22.9	23.1	22.3	22.8	22.4	22.3	22.6	22.7	22.6	0.5
FY000116	PP04	Pickle Pond site 04	24.4	22.6	22.7	23.0	23.1	22.8	23.0	22.6	22.6	22.8	22.6	22.8	0.5
FY000117	PP07	Pickle Pond site 07	24.1	22.4	22.6	22.9	23.2	22.7	22.8	22.5	22.4	22.7	22.4	22.7	0.5
FY000118	PP09	Pickle Pond site 09	23.9	22.4	22.6	22.9	23.1	22.6	23.0	22.5	22.4	22.6	22.4	22.7	0.5

* The incubator temperature was turned down after the initial temperatures were at or above 23+1°C

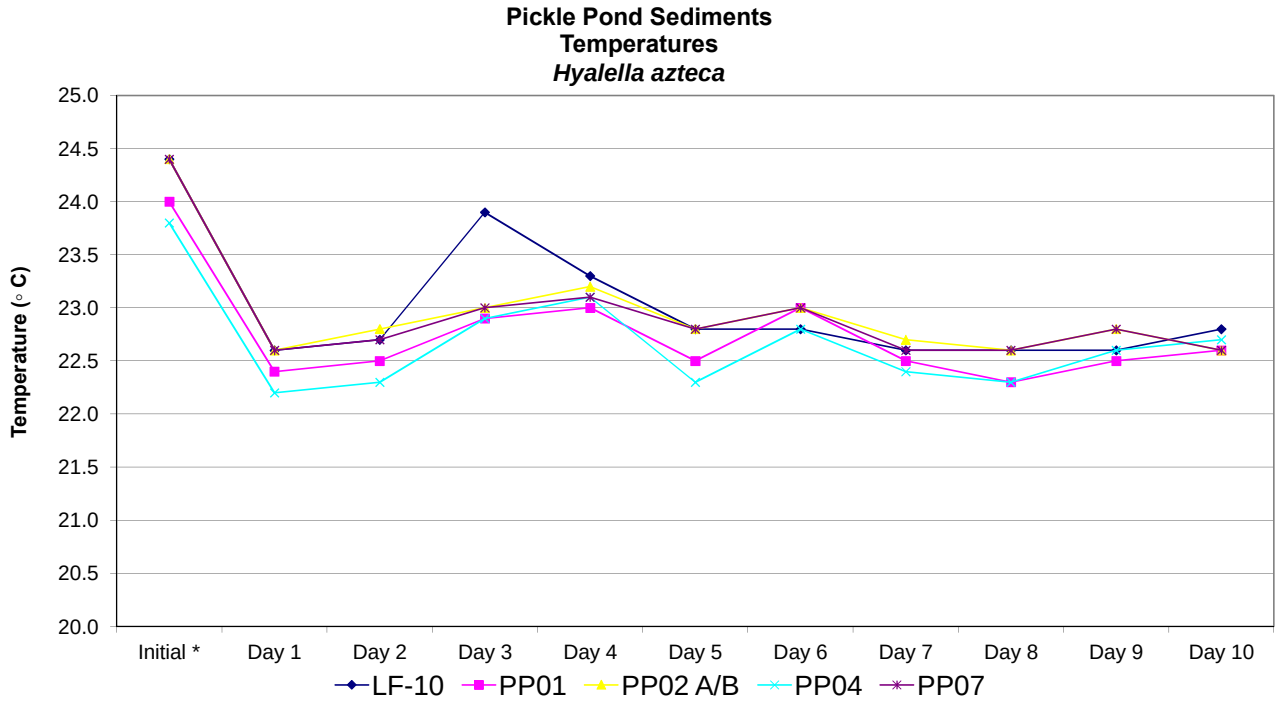


Figure 12
Pickle Pond, September 2013
Hyalella azteca
Conductivity, Hardness, Alkalinity and Ammonia

			Conductivity (µS)		Hardness (mg/L)		Alkalinity (mg/L)		Ammonia (mg/L)	
Lab Number	Site Name	Description	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)
LC	Lab Control	synthetic sediment	717	608	160	168	210	330	0.243	0.080
FY000113	LF-10	Loons Foot Landing Area	519	534	152	164	215	310	0.451	0.082
FY000114	PP01	Pickle Pond site 01	685	626	140	144	225	285	0.836	0.312
FY000115	PP02 A/B	Pickle Pond site 02 A/B	739	607	148	140	245	285	0.744	0.299
FY000116	PP04	Pickle Pond site 04	745	640	144	148	240	290	0.640	0.258
FY000117	PP07	Pickle Pond site 07	591	629	152	168	250	285	0.190	0.128
FY000118	PP09	Pickle Pond site 09	566	631	144	144	250	300	0.880	0.076