Appendix 1.
CICEET Macroinvertebrate Sampling Status Report, 8/22/09
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Methods

Experimental Design and Field Sampling

In July 2007, we collected 15 replicate samples (90 total) from four habitat types (i.e., high and low marsh, scrub-shrub, and forested tidal wetlands) at six locations at six separate wetlands. Benthic invertebrate sampling modules (10 X 10 m) were established adjacent to each of the project's permanent plots. Our sampling procedures followed the Estuarine Habitat Assessment Protocol (Simenstad et al. 1991) for benthic invertebrates. Benthic invertebrates were sampled from dewatered channel sediments using a 6.35-cm diameter plastic corer. Cores were taken to a depth of 5 cm for a total volume of 160.8 cm^3. Exact sampling location was randomized within the module boundaries using a random number table and grid, and location of the grid was recorded using a Trimble ProXR GPS. We retained samples in labeled sample jars, and fixed them in the field with a 10% solution of buffered formalin. Then, we analyzed the number of taxonomic groups represented in our replicated samples following methods described in Hurtubia (1973) to determine the optimum number of samples for each site. In July 2008, we collected 12 replicate samples (72 total) from 5 of the 6 sites sampled in 2007, using the same methods as in 2007. The 2008 sampling omitted Blind Slough scrub-shrub wetland due to the difficulty of locating an appropriate substrate for the benthic cores.

Table 1 explains site abbreviations used in figures and tables in the Results section, and shows the corresponding permanent plot numbers.

Table 1. Site abbreviations and corresponding permanent plots

Abbreviation	Macroinvertebrate sample area description	Corresponding permanent plot
BSFW	Blind Slough forested wetland	Blind Slough Plot 1
BSSW	Blind Slough scrub-shrub wetland	Blind Slough Plot 2
CCFW	Coal Creek forested wetland	Coal Creek Plots 1 and 2
HCHM	Hidden Creek high marsh	Hidden Marsh Plots 3 and 4
HCLM	Hidden Creek low marsh	Hidden Marsh Plots 1 and 2
MSHM	Millport Slough high marsh	Millport Slough Plots 1 and 2
SKLM	Siletz Keys low marsh	Siletz Keys Plots 1 and 2

Laboratory Setup and Processing

In September 2007, two experienced laboratory technicians were given a 1 ½ day refresher course conducted by Ayesha Gray (Cramer Fish Sciences) at the South Slough NERR Estuarine and Coastal Lab in Charleston, OR. Training included proper handling of samples, identification of invertebrates, microscope photography and graphic file exchange (to check IDs), and database development. In the laboratory, sample contents were washed through a 0.5 mm sieve to remove fine particulates and retain macrofauna.

Samples were then be transferred to water or isopropanol (depending on length of time until organism identification), and stained with Rose Bengal (a biological dye). After 24 hours in dye, using a light dissecting scope, all organisms were counted and identified to the finest taxonomic resolution possible without dissection, generally family or species identification for most common estuarine invertebrates. Unknown organisms were photographed and identified by Cramer Fish Sciences personnel or Jeff Cordell (University of Washington) from images and voucher specimens.

Statistical Analysis

In total 162 samples were processed; 90 from 2007 and 72 from 2008. Data were collated and stored in a Microsoft Access database and analyzed as described below. Statistical analysis focused on characterizing total abundance, taxonomic richness, percent composition, assemblage structure and presence of indicator taxonomic groups. Abundance results were determined as the number of invertebrates per sample, and taxonomic richness was measured as the total number of taxonomic groups (separating life stages) per sample.

Invertebrate assemblage characteristics were explored using multivariate statistics: nonmetric multidimensional scaling (NMDS), analysis of similarity (ANOSIM), and similarity percentage analysis (SIMPER) using PRIMER 6.0 (Clarke and Gorley 2006) (Note, SIMPER analysis will be completed for the final report). Among multivariate statistics, NMDS is an especially powerful technique for determining assemblage differences among ecological data. Data are log transformed and taxonomic groups accounting for less than 3% of any sample are discounted. NMDS graphically plots differences in invertebrate assemblages in ordination space (axes with no scale) based on the Bray-Curtis similarity matrix.

ANOSIM ("Analysis of similarities") looks for differences between groups of community samples (defined a priori), using permutation/randomization methods. It is a statistical test to determine significant differences among groupings delivered by NMDS and returns a p-value (similar to ANOVA) ($p < 0.10$ represents significance at the 90% confidence level). An R value, scaled between -1 and +1, is also reported with 0 representing no difference and a value of 1 representing biological difference among samples. A value of 0.4 is commonly used as a cutoff with values <0.4 having no difference, and those >0.4 being considered statistically different. Indicator analysis (INDVAL) developed by Dufrene and Legendre (1997) is used to identify “indicator species” among the reference wetland types using the software PC-Ord (McCune et al. 2002). Indicators can be compared among sites and with indicators of ecosystem state as identified in Gray (2005). Specific indicators may provide information on site characteristics in terms of invertebrates.

Results

Based on data obtained from 162 processed samples, we have evaluated total abundance, taxonomic richness, percent composition, invertebrate assemblage patterns, and indicator

species. Total abundance, taxonomic richness, and percent composition were summarized by year and site (Figures 1 and 2). Total abundance was highest at Hidden Creek Low Marsh in both sampling years, and lowest in the forested wetlands and scrub marsh. Taxonomic richness was also highest in the Hidden Creek Low Marsh, but comparable with all other sites except Coal Creek Forested Wetland where taxonomic richness was lower. An increasing proportion of New Zealand mudsnails (*Potamopyrgus antipodarum;* non-native, invasive) were found in the Coal Creek Forested Wetland. Over 50% of the sample was mudsnails in 2007, and the proportion increased to over 90% in 2008 (Figure 2).

A statistical comparison of the percent composition is represented by the multivariate statistics and NMDS plots for both years (Figure 3). ANOSIM analysis showed that assemblage structure of high and low marshes is relatively similar, while the forested wetland and scrub marsh are significantly different from each other and the high/low marshes (Table 1). All sites had significant differences in invertebrate assemblage, except for the Hidden Creek High Marsh compared with the Millport Slough High Marsh and Siletz Keys Low Marsh. High and low marshes were distinguished at Hidden Creek, but significant differences were less reliable between Millport Slough and Siletz Keys.

The indicator analysis provided repeatable indicators at all sampling sites, except for Siletz Keys Low Marsh (Table 2). New Zealand mudsnails were identified as the only indicator at the Coal Creek site in both years. Indicators at Blind Slough Plot 1 (forested wetland) included the common bivalve, *Macoma* spp., and the brackish-water isopod, *Caecidotea* spp. Indicators at the two high marsh sites were different with Hidden Creek having isopods and the non-native amphipod *Grandidierella japonica*, and Millport Slough having flatworms and polychaetes. Several indicators were identified in the Hidden Creek Low Marsh sites, including nematodes, the free-living estuarine anemone, and dipteran larvae. Only the ostracod was identified as an indicator (2008) at the Siletz Keys Low Marsh sites. No indicators were found in 2007 at the scrub-shrub Blind Slough Plot 2 (scrub-shrub wetland), and this wetland was not sampled in 2008.

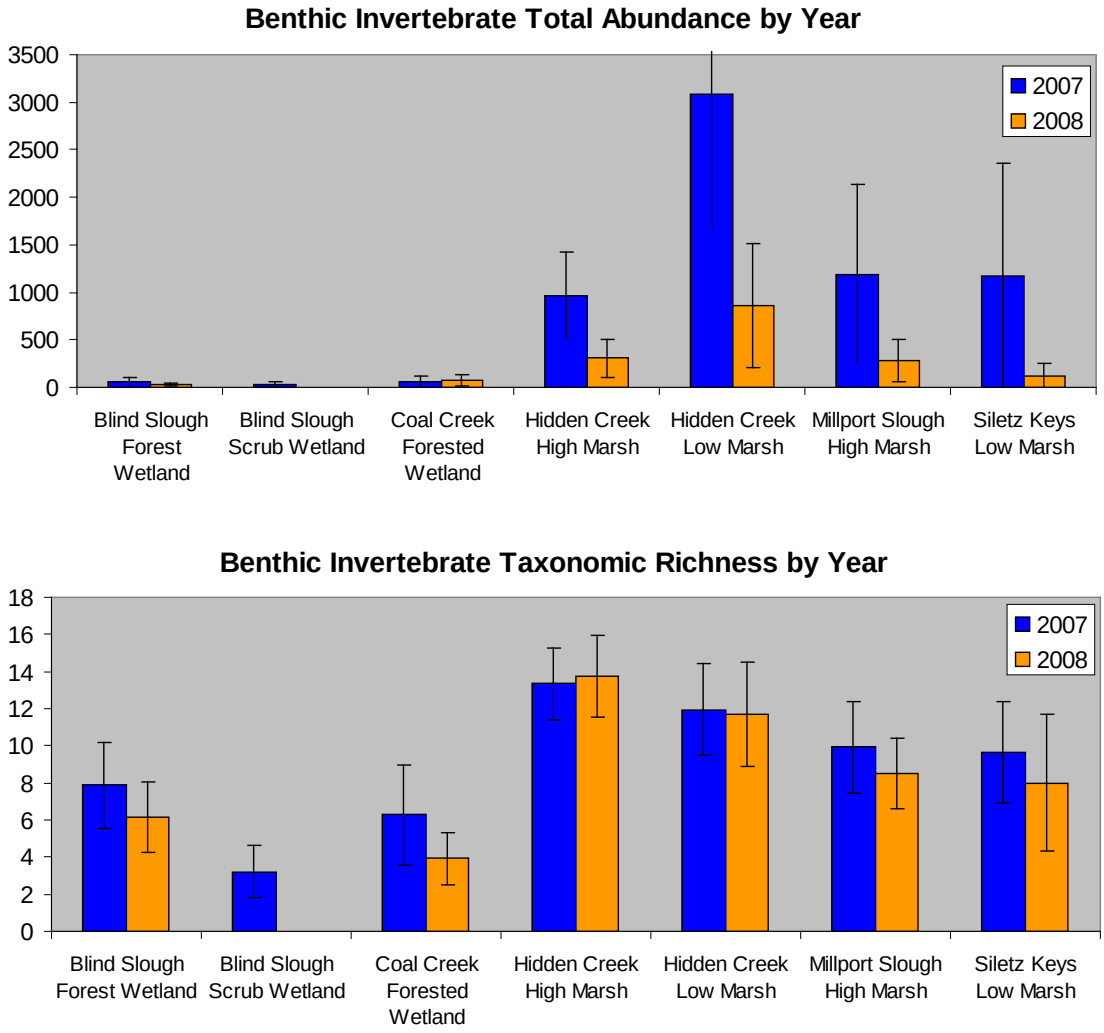

Figure 1. Total abundance (above) and taxonomic richness (below) in each sampling year. Note: Blind Slough Plot 2 (scrub-shrub wetland) was not sampled in 2008.

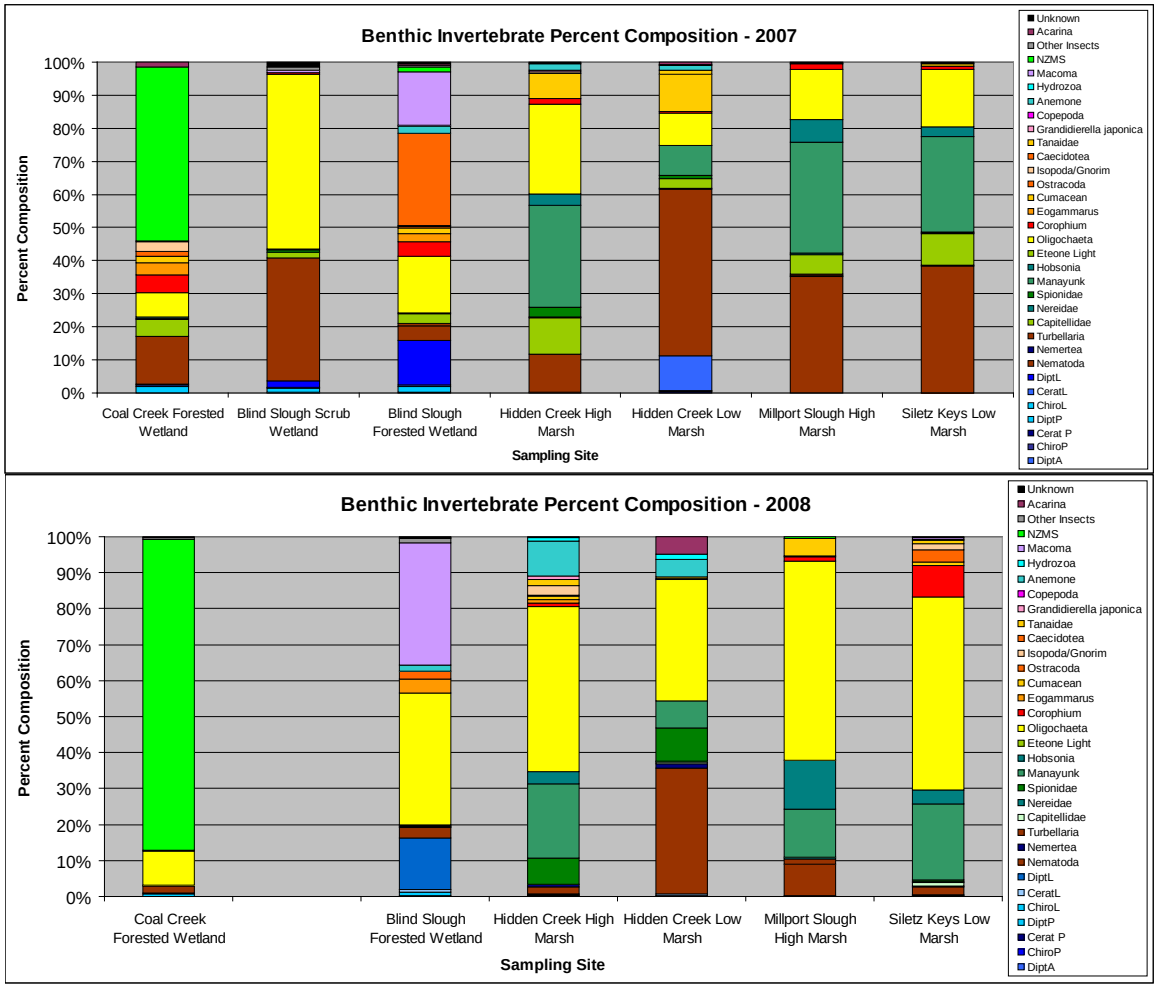

Figure 2. Average percent composition of benthic invertebrate samples from 2007 and 2008. Note: Blind Slough Plot 2 (scrub-shrub wetland) was not sampled in 2008.

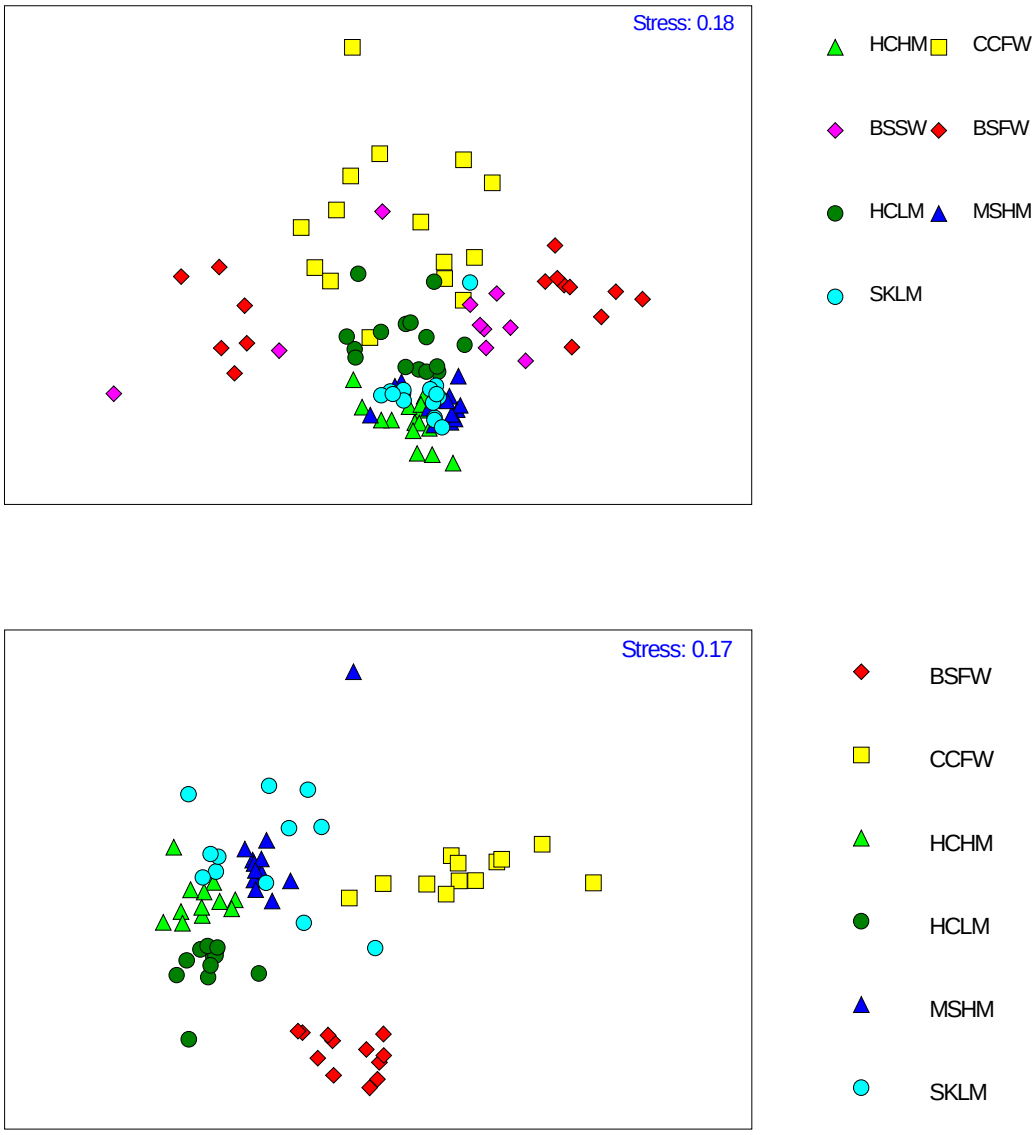

Figure 3. NMDS multivariate analysis plots the relative position (in terms of abundance and composition of taxonomic groups) of each sample in 2007 (top) and 2008 (bottom).

Table 2. ANOSIM results for NDMS analysis (bold statistics in shaded boxes indicate groups that are not significantly different).

	2007		2008	
Groups	R value	P value	R value	P value
HCHM, CCFW	0.819	0.1	0.994	0.1
HCHM, BSSW	0.814	0.1		
HCHM, BSFW	0.555	0.1	0.997	0.1
HCHM, HCLM	0.699	0.1	0.769	0.1
HCHM, MSHM	0.291	0.1	0.731	0.1
HCHM, SKLM	0.225	0.1	0.477	0.1
CCFW, BSSW	0.526	0.1		
CCFW, BSFW	0.507	0.1	0.996	0.1
CCFW, HCLM	0.763	0.1	0.981	0.1
CCFW, MSHM	0.812	0.1	0.925	0.1
CCFW, SKLM	0.746	0.1	0.939	0.1
BSSW, BSFW	0.319	0.6		
BSSW, HCLM	0.651	0.1		
BSSW, MSHM	0.756	0.1		
BSSW, SKLM	0.631	0.1		
BSFW, HCLM	0.536	0.1	0.996	0.1
BSFW, MSHM	0.559	0.1	0.941	0.1
BSFW, SKLM	0.530	0.1	0.888	0.1
HCLM, MSHM	0.775	0.1	0.841	0.1
HCLM, SKLM	0.618	0.1	0.766	0.1
MSHM, SKLM	0.069	7.7	0.330	0.1

Table 2. INDVAL indicators identified at each sampling site for sites sampled in both 2007 and 2008. Highlighted names were consistent indicators between years.

2007 INDVAL Indicators (value >40%)

Coal Creek Forested Wetland	Blind Slough Forested Wetland	Hidden Creek High Marsh	Hidden Creek Low Marsh	Millport Slough High Marsh	Siletz Keys Low Marsh
NZMS	*Macoma* spp.	Polychaeta: Spionidae	Nematoda	Turbellaria	
	Isopoda: *Caecidotea* spp.	Isopoda	Anemone	Polychaeta: Nereidae	
		Amphipoda: *Grandidierella japonica*	Tanaidae	Polychaeta: *Hobsonia florida*	
			Diptera: Ceratopogonidae Pupae		
			Diptera: Ceratopogonidae Larvae		
			Acarina		
			Hydrozoa		

2008 INDVAL Indicators (value >40%)

Coal Creek Forested Wetland	Blind Slough Forested Wetland	Hidden Creek High Marsh	Hidden Creek Low Marsh	Millport Slough High Marsh	Siletz Keys Low Marsh
NZMS	*Macoma* spp.	Amphipoda: *Eogammarus* spp.	Nematoda	Turbellaria	Ostracoda
	Isopoda: Caecidotea	Isopoda	Polychaeta: Capitellidae	Polychaeta: Nereidae	
	Diptera: Ptychopteridae	Amphipoda: *Grandidierella japonica*	Polychaeta: Spionidae	Polychaeta: *Hobsonia florida*	
	Diptera: *Bittacomorphella* spp.		Anemone	Cumacea	
			Diptera: Ceratopogonidae Larvae		
			Acarina		
			Nemertea		
			Hydrozoa		

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