Chronic Toxicity Methods Comparison: Preliminary Summary of Results

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Introduction

The California State Water Resources Control Board is in the process of developing sediment quality objectives. Acute and chronic sediment test methods are expected to be part of the implementation guidance for these objectives. Acute testing has been routinely conducted in California and elsewhere in the past. Chronic testing has been conducted on a much more limited basis. Because of this, there are questions regarding whether chronic test methods are practical, reproducible, and more sensitive than the acute methods.

This document describes the initial results of the first phase of a project to identify methods appropriate for use in California. The first phase of the project consists of a comparison of candidate methods, in which laboratories that have expertise in various chronic methods conducted tests on split sediment samples from stations in southern California and San Francisco Bay. Based on the results of this first set of testing, a subset of methods that provide the best combination of sensitivity and practicality will be chosen for further examination. The next step will be an interlaboratory comparison of the chosen methods using a set of split samples. This will help to determine the feasibility of turning these procedures into routinely used test methods.

For the current round of testing, the sediment samples were collected as part of two regional monitoring surveys, one in the Southern California Bight (Bight 03) and the other in San Francisco Bay (RMP). The stations represented a wide range of expected contamination levels and habitat types (Table 1). Since the Bight03 stations were chosen randomly, there were no historical data available for any of the locations except BRI-02 which was a fixed station. The RMP stations have been sampled for about 10 years and were chosen for this project based on a large geographic distribution and a range of acute toxicity to amphipods. Ten day *Eohaustorius* acute survival tests were performed on sediment from each station (Figure 1). Samples were also taken for organic and metals chemistry, TOC, grain size and benthic infauna. To date, we have received data only for the metals, TOC and grain size from the southern California stations (Tables 2 and 3).

Below are shortened versions of the methods documents that each group submitted with their data. Following the methods is a series of tables and figures showing all the endpoints for each test method. The data have been converted to percent of control to facilitate comparisons between methods and sampling periods. Data that is indicated as different from control values was both reported by the testing laboratory as significantly different and was also greater than the “biologically meaningful” value provided by the laboratory for each specific endpoint.

Methods

Leptocheirus and *Neanthes* (ERDC-EL-MS, Daniel Farrar and Guilherme Lotufo)

Field-collected sediments provided by SCCWRP were evaluated using 10-d acute and 28-d chronic exposures to the estuarine amphipod *Leptocheirus plumulosus* and 28-d chronic exposure to the marine polychaete *Neanthes arenaceodentata*. Testing was conducted in two phases as determined by the collection and subsequent arrival of two sediment shipments at the ERDC. Six sediments were evaluated in phase I and nine sediments in phase II. A control sediment, collected from Sequim Bay, Washington, was provided by the ERDC for evaluating test performance and for conducting statistical comparisons.

10-d L. plumulosus experiments. Approximately 175 mL of each sediment was added to each of 5 replicate 1-L beakers to obtain a 2 cm depth. Sediment was then overlain with 725 mL of 20 ppt synthetic seawater. The beaker was placed into a temperature and light cycle controlled exposure module under gentle aeration. Temperature was maintained at 25 °C and light cycle was set for 24-hour illumination. At day 0, *L. plumulosus* (500-750 µm sieve size class) were obtained from in-house cultures and placed into counting chambers (10 animals per chamber). After verifying the correct number of animals in each counting chamber the contents of two counting chambers were then gently transferred to a replicate beaker. Water quality measurements (Dissolved oxygen, pH, salinity and overlying water ammonia) were determined at day 0 and prior to test termination. Pore water ammonia was also measured at day 0. Exposure module temperature (min/max) was monitored and recorded daily. Observations of each beaker were conducted daily. Animals were not fed during the test. On day 10, the sediment in each beaker was gently sieved (500 µm sieve) and surviving animals recovered. Surviving organism were counted and recorded.

28-d L. plumulosus experiments. The day prior to test initiation, sediments were removed from cold storage and mixed for 10 minutes with a laboratory impeller mixer. Approximately 175 mL of each sediment was added to 5 replicate 1-L beakers to obtain the required depth of 2 centimeters. Sediment was then overlain with 725 mL of 20 ppt synthetic seawater. Beakers were placed in an exposure module under gentle aeration. Temperature was maintained at 25°C and light cycle was set at 16:8 hours light:darkness. At day 0, *L. plumulosus* (250-600 µm sieve size class) were obtained from in-house cultures and placed into counting chambers (10 animals per chamber). After verifying the correct number of animals in each counting chamber the contents of two counting chambers were then gently transferred to a replicate beaker. Water quality measurements (Dissolved oxygen, pH, salinity and overlying water ammonia) were determined at day 0, prior to test termination and in one replicate per sediment on Monday, Wednesday and Friday (M,W,F). Pore water ammonia was also determined at day 0. Exposure module temperature (min/max) was monitored and recorded daily. Observations of each beaker were conducted daily. Water was exchanged (~ 400 mL) from each beaker on M,W,F after water quality parameters were measured. Each beaker was provided with 20 mg of Tetramin® the first two weeks and 40 mg per beaker the final two weeks of testing. On

day 28, the sediment in each beaker was gently sieved (300 µm sieve) and surviving animals recovered. Surviving adults and neonates were counted and recorded. The surviving adults from each replicate were placed on a pre-weighed pan and placed in a drying oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans were removed, allowed to cool in a dessicator then weighed to obtain total dry-weight for each replicate.

For the first set of sample delivered, the control reproduction did not meet the acceptance criteria for the method. These samples were retested at a later date.

28-d N. arenaceodentata experiments. The day prior to test initiation, sediments were removed from cold storage and mixed for 10 minutes with a laboratory impeller mixer. Approximately 75 mL of each sediment was added to 10 replicate 300 mL tall-form beakers to obtain the required depth of 2 centimeters. Sediment was then overlain with 125 mL of 30 ppt synthetic seawater Beakers were placed in an exposure module under gentle aeration. Temperature was maintained at 20°C and light cycle was set at 12:12 hours light:darkness. At day 0, *N. arenaceodentata* (≤7 days old) were placed into counting chambers (1 animal per chamber). Organisms were obtained from Dr. Don Reish (California State University, Long Beach, CA). After verifying the correct number of worms in each counting chamber the contents of a single counting chambers were then gently transferred to a replicate beaker. Water quality measurements (Dissolved oxygen, pH, salinity and overlying water ammonia) were determined at day 0, prior to test termination and in three replicates per sediment once weekly. In addition, pore water ammonia was determined at day 0. Exposure module temperature (min/max) was monitored and recorded daily. Observations of each beaker were conducted daily. Water was exchanged (~ 60 mL) from each beaker once per week after water quality parameters were measured. Each beaker was provided with 2 mg of Tetramarin® on Tuesdays and 2 mg of Tetramarin® and 2 mg of Alfalfa on Fridays. On day 28, the sediment contained in each beaker was gently sieved (425 µm sieve) and surviving worms recovered. Surviving worms were counted and recorded. Surviving animals in each replicate were placed on a pre-weighed pan and placed in a drying oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans were removed, allowed to cool in a dessicator then weighed to obtain the individual dry weight for each replicate/animal.

Survival data for *L. plumulosus* and growth and reproduction data for all organisms were analyzed using Fisher's LSD test for comparing sediment mean values with control mean values. Survival data were arc-sine square root transformed prior to analysis. Survival data for *N. arenaceodentata* were analyzed using Fisher's exact test. Significance level for all tests was set at $\alpha = 0.05$. All data were analyzed using SigmaStat Statistical Software.

Copepod Lifecycle (University of South Carolina, Tom Chandler)

Fresh sediment samples were received from the Southern California Coastal Water Research Project and kept at 4ºC for no more than 4 days. A sediment reference sample from Oyster Landing at North Inlet (Baruch Lab, Georgetown, SC) was collected prior to

Day 0 and kept at 4ºC. Sediments were press-sieved and collected into acetone-rinsed glass beakers containing an acetone-rinsed Teflon-coated stir-bar. BA41, BC11, BRI2, 4085, 4202, 4262, NI-Reference, and NI-Sterile were sieved through a 125 μm sieve. Sediment samples 4008 and 4695 were sieved through a 250 μm sieve while 4209 and 4130 were sieved through a 212 and 180 μm sieve, respectively. Sediment samples 4066 and 4142 were too sandy to pass a 250 μm sieve, and could not be run with our bioassay. Over each beaker, sieved sediment was covered with parafilm and allowed to re-settle for a minimum of 1 hour at 4°C. Teflon 50 ml Erlenmeyer flasks with mesh-covered outflow holes (our standard test chamber; per original proposed protocol) were filled with 0.45 μm filtered, aerated seawater. Press-sieved sediment samples were then packed into Teflon syringes and slowly extruded onto the bottoms of their respective chambers (4 replicates per sediment sample). For samples containing a lot of water, the water percentage was estimated visually with the added sample amount adjusted to achieve a final volume of 10 ml settled sediment slurry. Chambers were lightly tapped on the benchtop to settle/spread out the sediment. Chambers were inspected to insure that at least 1 cm of overlying water was present in each. Approximately 10-ml of 0.45 μm filtered seawater was gently added along the side of the chamber so as not to disturb the sediment. Adult non-gravid female and adult male copepods (*Amphiascus tenuiremus*) were then counted into each quadruplicate test chamber (25/sex). Chambers were placed in an incubator at 20°C under continuous dripping flow for 14 days with a 12:12 light:dark cycle. Replicate chambers were fed every third day a mixture of frozen algal stock (10^7 cells of 1:1:1 *Isochrysis galbana, Phaeodactylum tricornutum* and *Dunaliella tertiolecta*). Measured water quality parameters were consistently of high quality.

At the end of 14 days of exposure, copepods were collected on a 63 μm sieve and rinsed into polypropylene jars. Samples were checked/counted for dead bodies. Copepods were stained with Rose Bengal and preserved by doubling the volume of the contents of the jar with 10% borate-buffered formalin (yielding a ~ 5% final concentration). Non-gravid adult females, gravid adult females, adult males, copepodites, nauplii, and clutch sizes were enumerated under a Nikon SMZ-U stereo dissection microscope. A general linear model was performed on the data set using SAS software. Tukey's multiple comparison tests were performed in order to delineate where significant differences occurred among site means ($p \geq 0.95$).

Juvenile Clam and Oyster Lysosomal Destabilization (Marine Resources Research Institute, Amy Ringwood and Chuck Keppler)

Clam studies. Juvenile clams (*Mercenaria mercenaria*) used for all experiments were obtained from Atlantic Littleneck Clam Farm, Charleston, SC. Clams were acclimated in 25 ‰ seawater at 23 to 25°C for at least 24 hours, then sieved through two mesh sizes (1.0 mm and 1.2 mm) to ensure that the clams were of a similar size range. Twenty-five clams were used for each replicate. Pre-assay wet weights of each clam group were taken for growth rate estimates, and to ensure that all replicate groups had similar initial weights. Replicate subsets of clams were also counted, wet weighed, dried overnight and reweighed to verify the wet:dry weight ratio used to estimate initial dry weights.

Sediment samples were press sieved through a 500 μm sieve, homogenized, and 50 ml aliquots were placed into four replicate 250 ml beakers. Seawater (150 ml, adjusted to 25 ‰ with distilled water) collected from a clean site at Folly Beach, SC was carefully added to each beaker. The replicates were gently aerated for the duration of the experiment, and the assays were conducted at room temperature (22 to 25 °C) for 7 days. Each replicate was fed on the first, third and sixth days of the assay (50:50 mix of *Isochrysis galbana* and *Chaetoceros gracilis*: 20×10^6 cells / replicate, counted using a hemacytometer). The phytoplankton was dialyzed in dialysis tubing (12,000 to 14,000 molecular weight pore size) against seawater for approximately 5 hours, with one water change. This step was included to remove excess nutrients and metals associated with the phytoplankton culture media.
At the end of the 7 day exposures, clams were sieved from the sediments and placed in fresh 25 ‰ seawater for approximately 2 hours to depurate excess sediment. The clams were then resieved and dead clams were counted and removed, and percent mortalities were calculated. The surviving clams were counted using a bench-top magnifier and rinsed with distilled water to remove excess salts. Post-assay wet weights were determined, and clams were then dried for 48 hours (at 70°C). Each clam replicate was recounted and final dry weight per clam was determined. A microbalance was used for all weight determinations. Initial dry weights were subtracted from the final dry weights, and the results were expressed as growth rates (μg/clam/day).

Control charts based on laboratory growth in seawater controls and control sediments (mean $\pm$ 95% confidence limits, based on 9 or more recent test sets) are maintained in order to insure the reliability of the assay results. A site is considered toxic if the seed clam growth rate is both significantly less than control growth and < 80% of control growth. The 15 sites were run as two separate assays (6 in the first, 9 in the second), as sediment collection was nearly a month apart.

Oyster studies. Oysters (*Crassostrea virginica*, 5.3 $\pm$ 0.7 cm) used for laboratory sediment exposures were collected from control sites and bubbled in site water for at least 24 hours prior to the start of the experiment. Sediment samples homogenized and 100 ml aliquots were placed into three replicate 1000 ml beakers. Seawater (800 ml, adjusted to 25 ‰ with distilled water) collected from a clean site at Folly Beach, SC was carefully added to each beaker. The beakers were allowed to settle for 2 hours, then 3 clean-scrubbed oysters were gently added to each replicate. The replicates were gently aerated for the duration of the experiment, and the assays were conducted at room temperature (22 to 25 °C) for 4 days. Each replicate was fed on the first and third days of the assay (*Isochrysis* paste mixed into filtered sea water, 70×10^6 cells / replicate).

Digestive gland tissue was diced and incubated on a shaker in calcium/magnesium free saline (CMFS) for 20 minutes. Trypsin (Type XIII) was added to each sample to complete cellular disaggregation, then cell suspensions were filtered (100um mesh) and centrifuged. The pellet was washed twice and resuspended in CMFS. A cell suspension aliquot was mixed with an equal aliquot of neutral red (NR) solution, placed on a microscope slide, covered with a cover slip and placed in a humidity chamber at room temperature. Digestive gland cells containing lysosomes were examined under a light

microscope to evaluate NR retention. A minimum of 50 cells were scored as stable (NR retention in the lysosomes) or destabilized (NR leaking into the cytoplasm), and the data was expressed as the percentage of cells with destabilized lysosomes per oyster.

Sediment-Water Interface (Marine Pollution Studies Laboratory, Brian Anderson and Bryn Phillips)

Sediment samples were shipped to the Marine Pollution Studies Laboratory (MPSL) in coolers with ice. Tests were initiated as soon as possible after receipt of samples. Samples were generally shipped with no overlying water in the core tubes, but four samples were received with clean seawater overlying the sediment (Stations 4130, 4008, 4085, and BR1-02). Water was replaced in Stations 4130 and 4008 prior to testing, but was allowed to remain in Stations 4085 and BR1-02 because of time constraints. The water volume in these stations was adjusted accordingly.

After receipt of samples, the bottom caps on the core tubes were sealed with Parafilm. One day prior to the start of the test 300 mL clean overlying seawater (1 μm filtered) was added to each core tube, and the tubes were placed in one-liter beakers containing 500 mL seawater. This procedure equalizes the pressure in core tubes that might otherwise be prone to leakage. Samples are then aerated overnight and allowed to equilibrate. On test day 0 water quality samples were collected from the core tubes and 25 μm screen tubes were placed on the sediment surface. Embryos of the mussel, *Mytilus galloprovincialis* or the sea urchin, *Strongylocentrotus purpuratus* were prepared and added to the screen tubes. Mussels were exposed for 48 hours and sea urchins were exposed for 96 hours. The exposures were terminated by removing the screen tube, rinsing developed larvae into a vial, and adding formalin to fix and preserve larvae.

Positive control reference toxicant tests were conducted for all species using cadmium or copper chloride dilutions to bracket the EC50 values for each species. The negative controls consisted of five laboratory replicates of reference sand that was placed in core tubes. Water quality parameters of dissolved oxygen, pH, and salinity were measured using a Hach SensIon selective ion meter with appropriate electrodes. Total ammonia was measured spectrophotometrically. Temperature was measured using a continuously recording thermograph and thermometer.

Toxicity testing data from each sample were compared to laboratory controls using separate-variance t-tests. The separate-variance t-test is similar to a simple Student's t-test, with degrees of freedom adjusted to account for any heterogeneity of variance between samples. The t-test was employed to simply indicate whether differences between test samples and controls were statistically significant at the given alpha level (0.05).

Additional statistical methods were applied to determine sample toxicity, because a t-test can often detect small differences between samples when there is low variance among laboratory replicates. Such small differences may not be biologically significant. Our analysis indicates that the *Strongylocentrotus* test is capable of identifying statistically

significant differences in 90% of cases where the difference between the treatment and the control is 22%. In this set of samples with the control response of 100%, samples with survival less than 78% would be considered toxic. The MSD value for mussels was also used in data analysis. The mussel 90th percentile MSD is 80% of the control. These criteria, in addition to t-tests, were applied to the present data.

Table 1. Descriptive information for stations tested for the chronic sediment toxicity methods evaluation project.

Station ID	Strata	Location/Comments
4066	Estuary	Mouth of San Gabriel River, surf zone area
4130	Estuary	Mouth of Cerritos Channel, low tidal energy
4142	Estuary	LA River, upstream, tidally influenced
4202	Mainland shelf	Offshore of PV peninsula, near wastewater outfall
4262	Bays and Marinas	King Harbor, Redondo Beach, turning basin
BRI-02	Special	Marina Del Rey, area with known benthic response
4085	Bays and Marinas	Entrance channel to Marina Del Rey
4008	Estuary	Santa Margarita River mouth, rural area, coarse grain size
4209	Estuary	Santa Margarita River mouth, rural area, coarse grain size
4695	Estuary	Tijuana River mouth
BA10	SF Bay	Coyote Creek mouth, southern most SF Bay
BA41	SF Bay	Redwood Creek, southwestern SF Bay
BC11	SF Bay	Yerba Buena Island east-central SF Bay
BD31	SF Bay	Pinole Point, southern shore, northern SF Bay
BF21	SF Bay	Grizzly Bay, northeastern most SF Bay

Table 2. Chronic sediment toxicity methods evaluation whole sediment metals concentrations. Mean ERMq are the concentration of the metals for which there are ERM values divided each ERM, summed for all the available elements and divided by the number of elements.

Metal (mg/ dry kg)	4066	4130	4142	4202	4262	BRI-02	4085	4008	4209	4695
Aluminum	4400	18550	3745	25000	13900	35700	24800	16300	5410	2895
Antimony	0.2	0.82	0.155	<2.98	<3.00	0.61	1.07	0.21	0.105	0.08
Arsenic	1.04	7.04	0.975	8.51	4.03	13.0	11.6	2.5	1.5	1.12
Barium	22.6	163	25.6	274	115	154	165	102	29.8	13.2
Beryllium	0.09	0.54	0.08	2.82	1.56	0.91	0.62	0.31	0.065	ND
Cadmium	0.05	0.845	0.225	5.15	0.57	0.335	1.74	0.14	ND	ND
Chromium	7.44	48.9	5.40	136	45.5	94.5	78.4	33.6	9.9	4.58
Cobalt	2.18	11.2	2.10	NA	NA	14.0	9.9	6.98	2.00	0.9
Copper	7.46	87.3	5.74	5.14	3.18	362	101	14.2	2.84	0.98
Iron	5810	33050	5860	32200	19600	51300	35500	23800	6615	3935
Lead	4.73	61.6	4.32	30.0	38.9	113	130	4.71	1.35	1.24
Manganese	84	380	74.8	NA	NA	354	294	267	85.4	43.0
Mercury	0.103	0.400	0.0645	0.464	0.226	0.983	0.41	0.084	0.024	0.0185
Molybdenum	0.19	1.75	0.28	NA	NA	2.00	2.08	0.66	0.145	0.105
Nickel	4.05	25.8	4.06	29.0	21.2	41.6	33.1	10.2	3.04	0.935
Selenium	0.26	1.28	0.25	1.08	<0.99	2.14	1.29	0.63	0.21	0.215
Silver	0.654	0.766	0.277	1.9	0.67	1.98	2.94	0.665	0.241	0.216
Strontium	41.6	84.8	25.2	NA	NA	93.4	89.7	45.1	17.2	22.5
Thallium	0.06	0.28	ND	NA	NA	0.335	0.34	0.23	0.07	ND
Tin	0.41	3.8	0.46	NA	NA	6.26	6.26	1.56	0.48	0.3
Titanium	531	2325	304	NA	NA	2440	1970	2110	753	409
Vanadium	13.1	74.6	12.4	NA	NA	141	93.3	68.4	21.5	13.2
Zinc	22	248	32.9	180	92.2	382	315	47.9	14.0	6.44
Metals mean ERMq	0.06	0.312	0.0464	0.372	0.174	0.666	0.479	0.092	0.0286	0.0173

Table 3. Organic and physical properties of sediment from stations analyzed for chronic sediment toxicity methods project.

Constituent	4066	4130	4142	4202	4262	BRI-02	4085	4008	4209	4695
TOC (%)	0.016	2.04	0.27	2.06	1.50	1.99	2.93	0.67	0.038	ND
TON (%)	ND	0.116	0.007	0.152	0.119	0.196	0.165	0.146	ND	ND
Gravel (%)	0	0	35.4	0	0	0	0	0	0	0
Sand (%)	100	44.2	62.5	39.3	56.5	7.9	30.4	53.8	97.5	100
Silt (%)	0	46.3	*	50.1	35.7	74.2	56.9	40.4	2.5	0
Clay (%)	0	9.5	*	10.7	7.8	17.9	12.6	5.9	0	0

* Due to large grain size, sample could not be analyzed with the laser method. Data is from sieve analysis, which found fines content (combination of silt and clay) to be 2.0%.

Table 4. Summary of results for all endpoints of tests performed for the chronic sediment toxicity methods evaluation project. Data is expressed as mean percentage of control response ± standard deviation. Values in bold are significantly different from control response.

	***Amphiascus* Endpoints**									**SWI**	
Station	Male Survival	Female Survival	Combined Survival	Percent Gravid Females	Mean Clutch Size	No. of Nauplii	No. of Copepodites	Naupliar/ Copepodite Ratio	Realized Offspring Production	Mussel Dev.	Sea Urchin Dev.
4066	NT	NT	NT	NT	NT	NT	NT	NT	NT	93 ± 10	NT
4130	78 ± 13.6	79 ± 9.5	79 ± 8.1	83 ± 11.1	81 ± 3.4	**21 ± 8.4**	**12 ± 5.4**	172 ± 48.5	**22 ± 7.7**	100 ± 19	NT
4142	NT	NT	NT	NT	NT	NT	NT	NT	NT	100 ± 8	NT
4202	95 ±28.4	**66 ± 12.9**	79 ± 19.8	**75 ± 1.9**	**58 ± 9.3**	**31 ± 10.2**	**13 ± 8.1**	281 ± 93.4	**37 ± 16.6**	95 ± 11	NT
4262	68 ± 22.3	102 ± 4.2	87 ± 12.0	92 ± 17.0	**66 ± 6.4**	47 ± 14.4	**13 ± 10.5**	**619 ± 527.2**	**30 ± 12.1**	93 ± 9	NT
BRI-02	105 ± 20.5	101 ± 10.2	105 ± 8.0	84 ± 5.6	77 ± 5.3	**12 ± 4.2**	**3 ± 2.2**	413 ± 195.6	**8 ± 2.8**	101 ± 6	NT
4085	88 ± 12.0	97 ± 12.9	97 ± 10.2	95 ± 15.7	**65 ± 7.2**	**21 ± 5.7**	**8 ± 31**	290 ± 143.7	**15 ± 4.2**	103 ± 5	NT
4008	76 ± 15.9	71 ± 26.9	74 ± 17.3	73 ± 11.2	**70 ± 5.5**	**33 ± 5.7**	**10 ± 5.0**	387 ± 161.4	**35 ± 17.8**	NT	87 ± 16
4209	105 ± 21.8	82 ± 18.8	93 ± 19.2	67 ± 20.2	**66 ± 6.5**	84 ± 18.3	**14 ± 5.5**	**634 ± 218.7**	64 ± 18.1	NT	94 ± 7
4695	99 ± 20.4	80 ± 11.6	89 ± 14.2	75 ± 25.4	**63 ± 6.9**	73 ± 12.2	25 ± 13.4	331 ± 116.4	65 ± 17.3	NT	105 ± 2
BA10	NT	NT	NT	NT	NT	NT	NT	NT	NT	92 ± 5	NT
BA41	91 ± 27.7	75 ± 3.6	82 ± 13.1	84 ± 9.6	**71 ± 6.4**	**37 ± 14.6**	**3 ± 1.4**	**1193 ± 99.3**	**28 ± 12.6**	116 ± 9	NT
BC11	77 ± 20.9	73 ± 11.6	75 ± 13.3	90 ± 7.6	**69 ± 10.4**	**30 ± 13.9**	**6 ± 7.8**	**1742 ± 2251**	**29 ± 22.1**	**27 ± 23**	NT
BD31	NT	NT	NT	NT	NT	NT	NT	NT	NT	75 ± 20	NT
BF21	NT	NT	NT	NT	NT	NT	NT	NT	NT	85 ± 7	NT

NT= Sample not tested by that method.

Table 4. Continued.

	Juvenile *Mercenaria*		***Crassostrea***	***Leptocheirus***				***Neanthes***	
Station	Survival	Growth	Lysosomal Destable.	10 Day Survival	28 day Survival	28 day Growth	28 day Repro	28 day survival	28 day growth
4066	95 ± 5.2	125 ± 6.3	**138 ± 25.2**	**46 ± 11.4**	**72 ± 41.2**	82 ± 15.4	27 ± 21.9	100 ± 0.0	105 ± 59.6
4130	101 ± 0.0	**67 ± 0.9**	111 ± 28.8	**61 ± 20.4**	84 ± 13.9	81 ± 28.8	76 ± 75.6	**50 ± 52.7**	**58 ± 20.2**
4142	96 ± 6.1	110 ± 4.4	85 ± 17.4	**77 ± 4.6**	80 ± 10.1	71 ± 28.4	78 ± 54.8	80 ± 42.2	104 ± 30.4
4202	99 ± 2.0	113 ± 5.8	109 ± 16.0	**58 ± 8.4**	**66 ± 39.8**	69 ± 24.5	**31 ± 37.4***	100 ± 0.0	87 ± 40.0
4262	97 ± 6.0	85 ± 2.9	104 ± 43.1	94 ± 8.1	82 ± 32.6	110± 25.5	**27 ± 27.6***	100 ± 0.0	**72 ± 33.1**
BRI-02	100 ± 0.0	91 ± 6.9	92 ± 20.1	**66 ± 13.4**	**63 ± 28.3**	**25 ± 16.6**	**2 ± 2.2***	100 ± 0.0	**57 ± 25.1**
4085	99 ± 2.0	133 ± 5.6	102 ± 24.0	**65 ± 13.8**	77 ± 22.5	59 ± 44.9	**7 ± 9.2***	100 ± 0.0	98 ± 42.2
4008	94 ± 3.9	**62 ± 1.7**	97 ± 18.4	90 ± 15.9	100 ± 5.3	130 ± 14.2	121 ± 96	100 ± 0.0	**54 ± 27.2**
4209	101 ± 0.0	120 ± 4.8	101 ± 15.9	103 ± 6.3	101 ± 6.9	90 ± 22.8	59 ± 35.6	100 ± 0.0	105 ± 28.9
4695	101 ± 0.0	138 ± 2.1	122 ± 17.9	83 ± 14.2	**46 ± 29.2**	**37 ± 17.0**	**0 ± 0**	90 ± 31.6	113 ± 39.2
BA10	98 ± 2.3	103 ± 8.5	120 ± 27.0	84 ± 8.7	95 ± 11.3	96 ± 32.0	**22 ± 15.6***	80 ± 42.2	**60 ± 16.5**
BA41	96 ± 3.3	112 ± 3.3	97 ± 23.4	88 ± 13.9	84 ± 17.4	98 ± 55.9	**35 ± 16.8***	90 ± 31.6	**58 ± 33.7**
BC11	94 ± 7.7	66 ± 13.5	**140 ± 24.7**	**60 ± 26.7**	81 ± 21.5	73 ± 33.3	**27 ± 17.7***	100 ± 0.0	**76 ± 45.0**
BD31	97 ± 3.8	92 ± 8.1	102 ± 18.3	**63 ± 13.2**	84 ± 10.0	71 ± 16.4	**31 ± 8.9***	100 ± 0.0	88 ± 26.1
BF21	99 ± 2.0	103 ± 10.0	126 ± 16.2	**64 ± 13.6**	99 ± 6.4	70 ± 19.8	**21 ±2 0.8***	90 ± 31.6	**50 ± 17.9**

*All stations tested in this batch had reproduction more than 3 X lower than the control. The lab has never experienced this before and considers the data suspect, but has no solid basis for exclusion.

Table 5. Sample holding time for chronic sediment toxicity methods stations. Whole sediment samples for each station were held at SCCWRP for varying times before being homogenized at SCCWRP and distributed in two batches. Therefore, for all tests except the SWI, the number of days represents the time between the individual lab's receipt of the sample and test initiation. Since the core samples for the SWI test were not held at SCCWRP like the whole sediment, the SWI times represent the time between sample collection and test initiation.

					Leptocheirus		
Station	*Amphiascus*	*Mercenaria*	*Crassostrea*	SWI	10 Day	28 Day	*Neanthes*
4066	na	5	14	6	18	108*	24
4130	5	5	14	6	18	108*	24
4142	na	5	17	6	18	108*	24
4202	4	6	15	6	26	75	43
4262	4	6	18	5	26	75	43
BRI-02	4	6	18	1	26	75	43
4085	4	6	18	1	26	75	43
4008	5	5	17	10	18	108*	24
4209	5	5	14	10	18	108*	24
4695	5	5	17	10	18	108*	24
BA10	na	6	18	9	26	75	43
BA41	4	6	18	12	26	75	43
BC11	4	6	15	14	26	75	43
BD31	na	6	15	14	26	75	43
BF21	na	6	15	16	26	75	43

*Calculated from the start date of re-test, which are the data presented in this of summary.

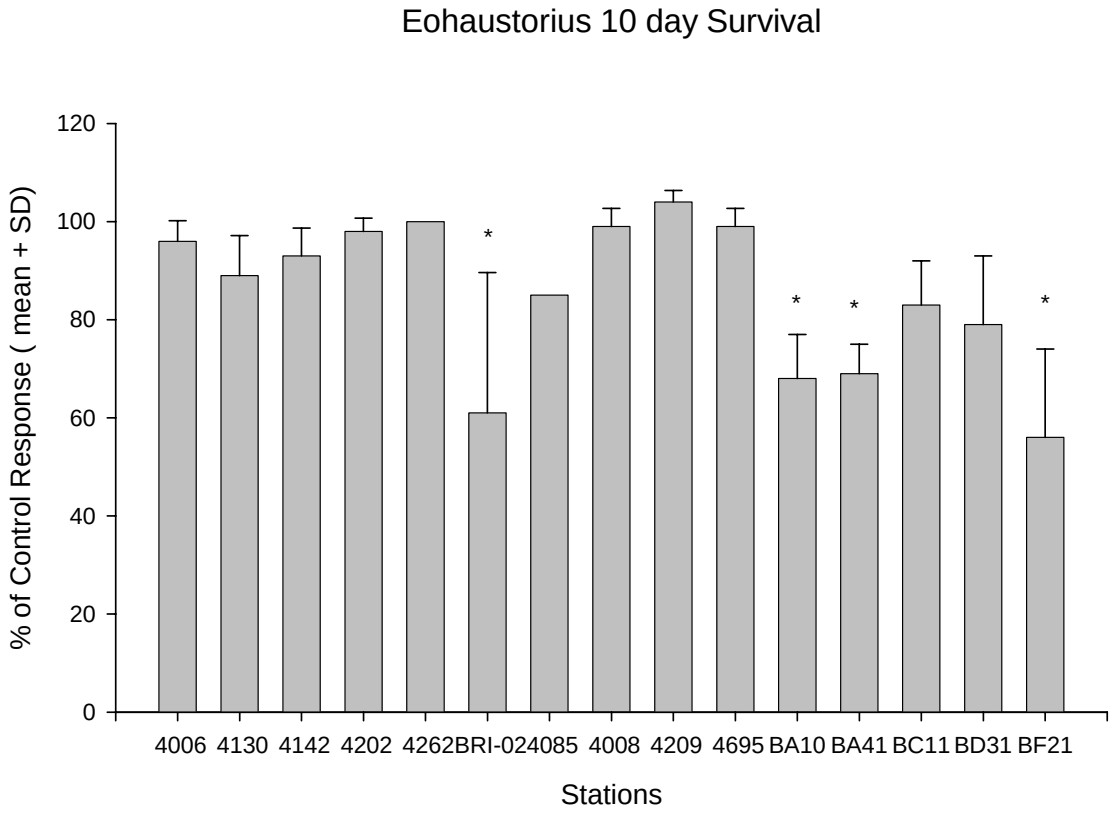


Figure 1. Results of *Eohaustorius* 10 day survival tests conducted as part of the Bight 03 and RMP regional monitoring programs. Stations marked with * are significantly different from control values ($p<0.05$) and less than 80% of the control response.

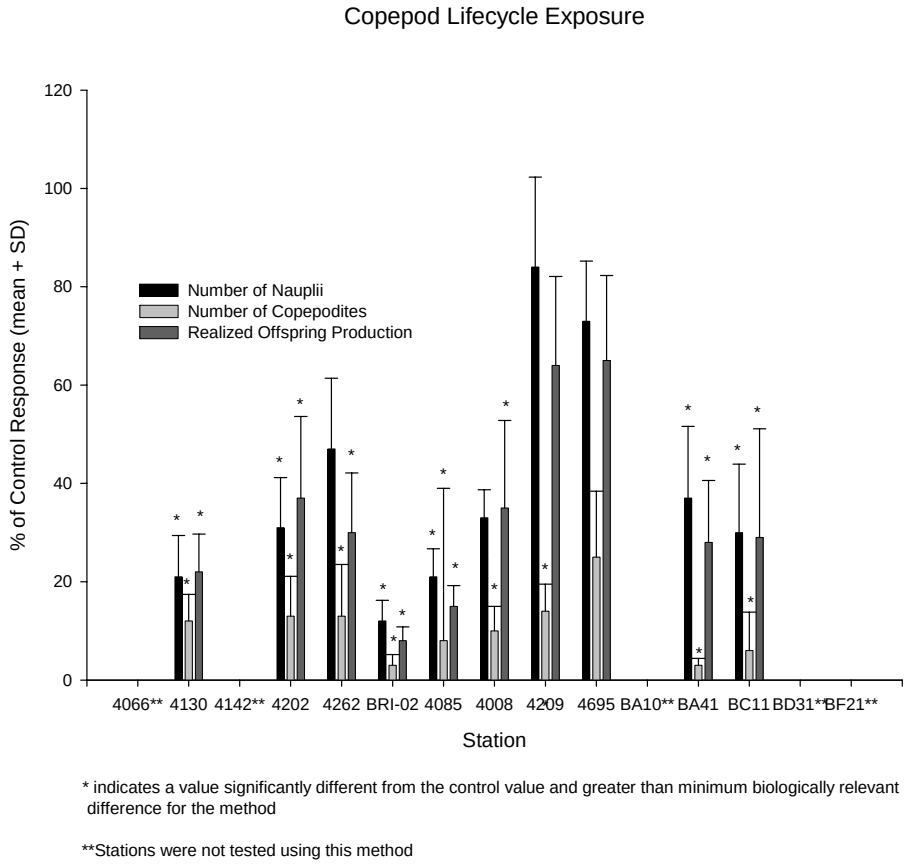
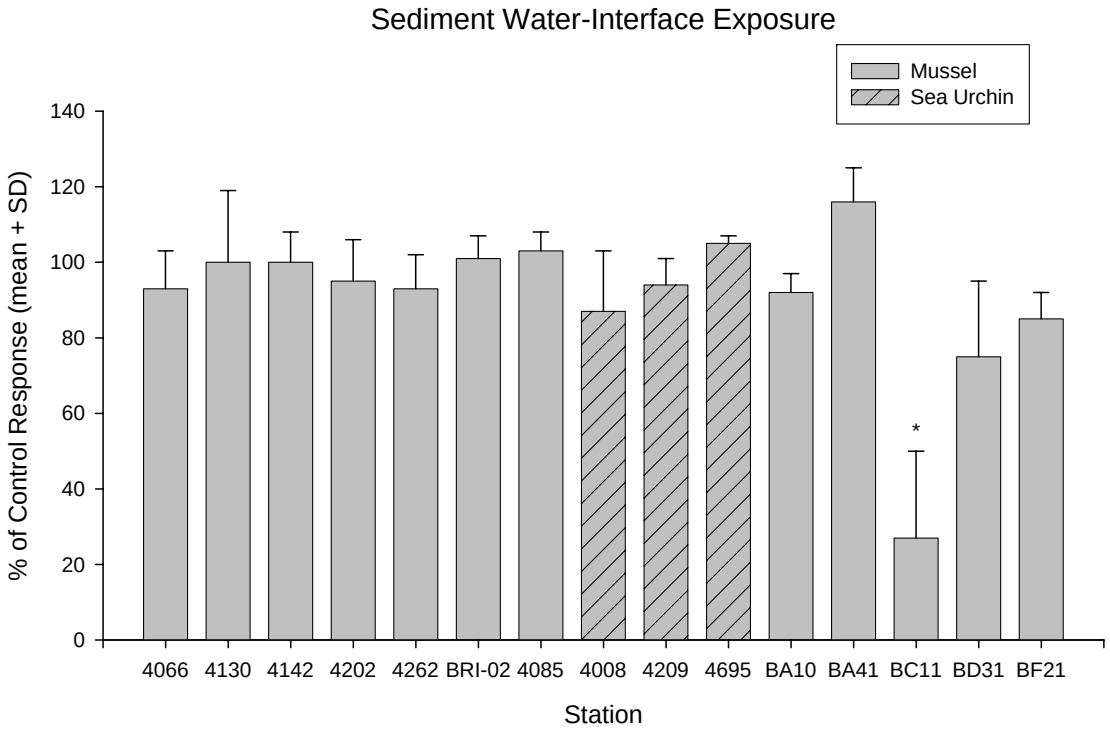


Figure 2. Results from selected endpoints of the copepod (*Amphiascus*) lifecycle exposures. Note that stations 4066, 4142, BA10, BD31 and BF21 were not tested using this method.



* indicates a value significantly different from the control value and greater than minimum significant difference for the exposure.

Figure 3. Results from sediment-water interface tests using developing mussel or sea urchin embryos.

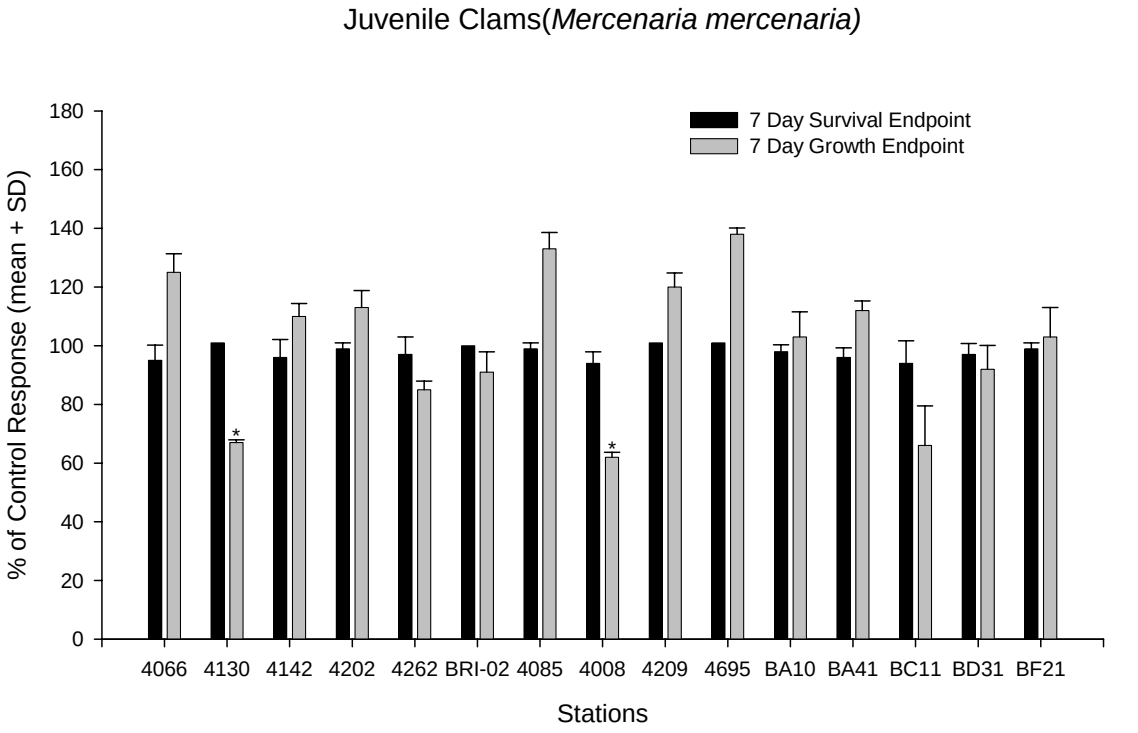
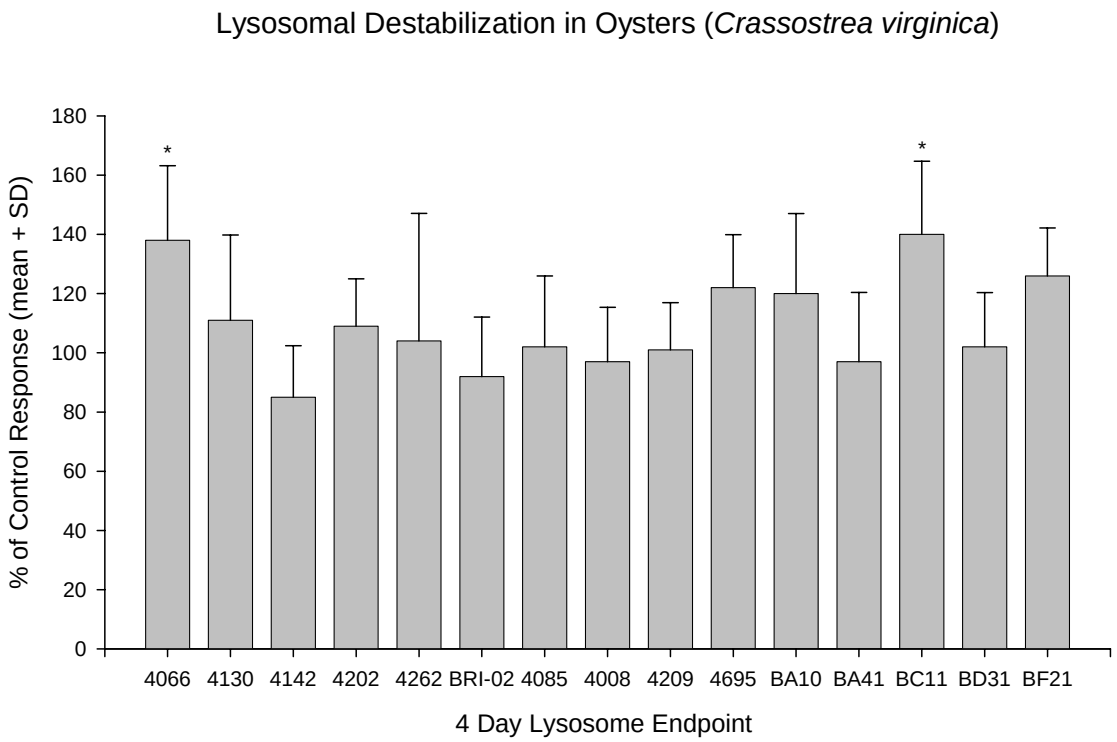


Figure 4. Results of juvenile clam 7 day exposures to whole sediments.



* indicates a value significantly different from the control value and greater than minimum biologically relevant difference for the method

Figure 5. Results of lysosomal destabilization tests performed on juvenile oysters.

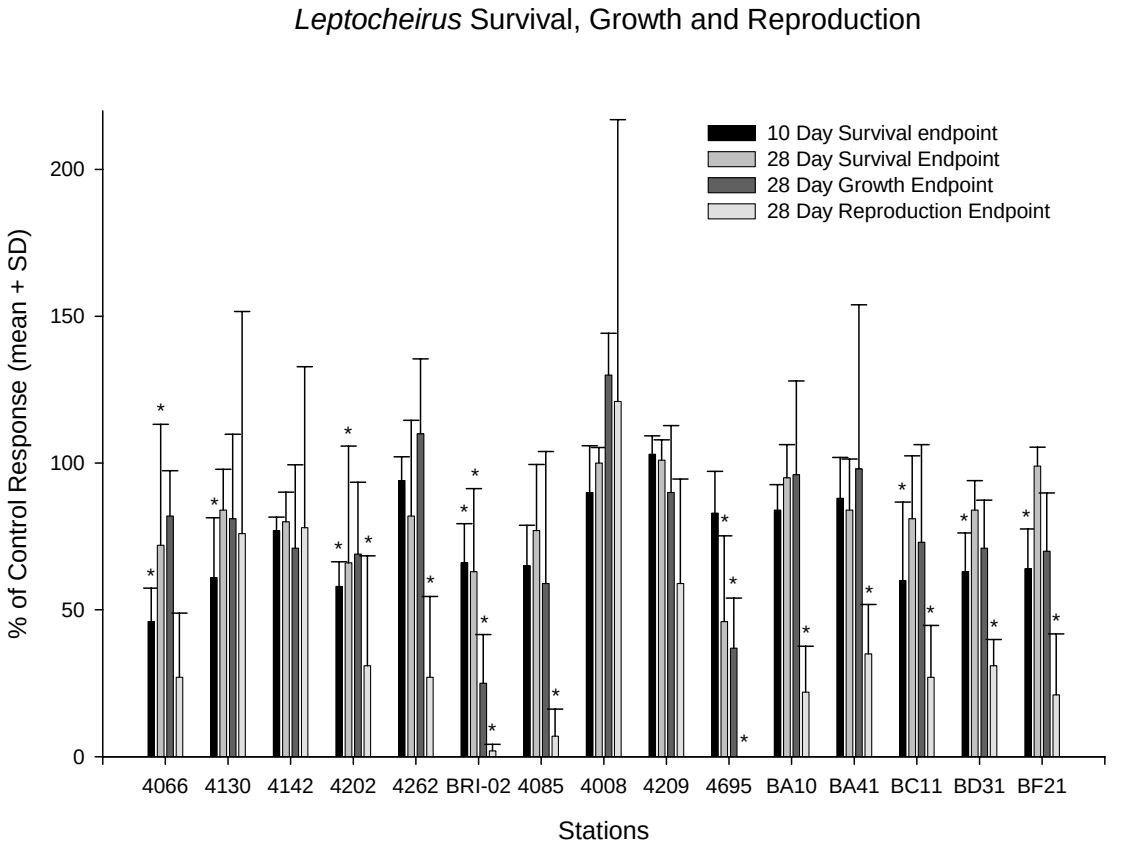


Figure 6. Results of acute and chronic exposures of *Leptocheirus* to whole sediment samples.

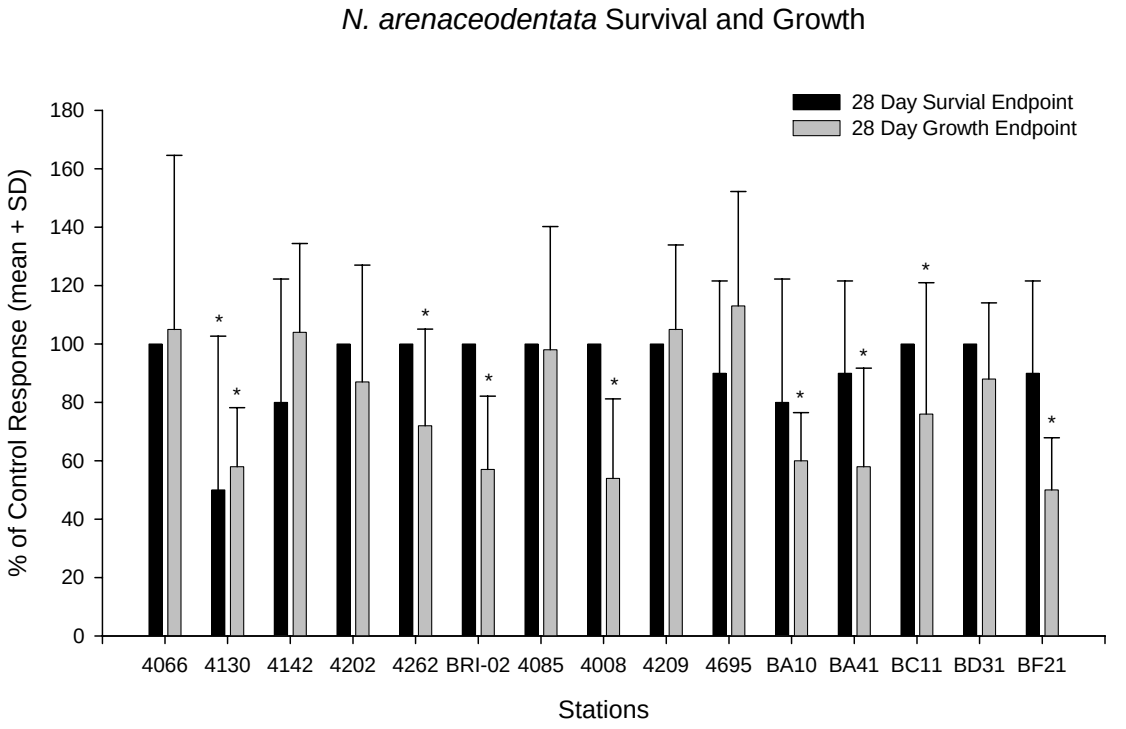


Figure 7. Results of 28 day *Neanthes* exposures to whole sediment samples.