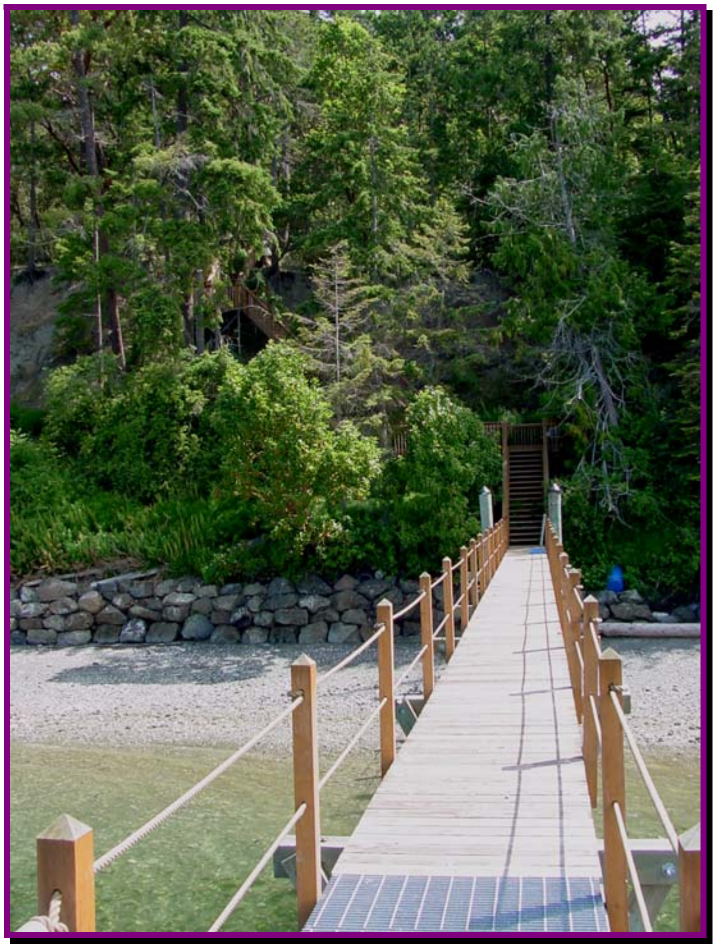
Environmental response to ACZA treated wood structures in a Pacific Northwest marine environment



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Background. The environmental industry and a few regulators within state and federal governments in the United States have raised concerns regarding the use of ACZA treated piling in marine environments. These concerns are based on perceptions that copper, arsenic and zinc, leached from the preserved wood will increase water and sediment concentrations to levels where adverse effects, particularly associated with copper, would be observed in marine biota. Brooks (1997) described the losses of preservative from ACZA treated wood in freshwater and marine environments and provided a computer model for predicting increases of both water and sediment concentrations of ACZA metals as a function of a number of environmental physicochemical parameters and the amount of immersed treated wood. The loss rate algorithm predicts a copper loss rate of $32.5 * \exp^{-0.1.114 * \text{time (in days)}}$ (μg Cu/cm^2-day) for marine environments were the salinity is ca. 30 parts per thousand. On the first day of immersion the loss is 32.5 μg Cu/cm^2-day. This value declines exponentially to 0.013 μg Cu/cm^2-day at the end of the first week. Concerns have also be raised that arsenic, lost at ca. 0.10 μg As/cm^2-day from ACZA treated wood at all times post construction, would bioconcentrate in shellfish tissues to levels posing a health risk to humans. The greatest risk in this regard would be from consumption of mussels (*Mytilus edulis trossulus*) growing directly on the piling or from clams living in sediments at the base of the piling. There is no empirical evidence describing the actual concentration of copper, arsenic or zinc in proximity to ACZA treated wood structures in marine environments. Concentrations of metal in wetland soils under and adjacent to the ACZA treated portions of the Wildwood Boardwalk in Oregon were described by and Lebow *et al.* (1999) and Brooks (1999) described metals and invertebrate communities under and around inundated portions of the boardwalk. Increases in copper, arsenic and zinc were observed in soils, water and sediments adjacent to the boardwalk, but the invertebrate community was unaffected by the small increases.

The purpose of this study is to determine water, sediment and shellfish concentrations of copper, arsenic and zinc in the immediate vicinity of ACZA treated structures. The risk assessment relies on a direct assessment of macrobenthic communities in the vicinity of ACZA treated structures and on regulatory criteria for marine sediments and water. Human health risks associated with the consumption of mussels and clams from the vicinity of ACZA treated structures are based on recommendations made by the U.S. Food and Drug Administration (FDA 1993).

Site Description. The primary structure evaluated in this assessment is a personal use pier, ramp and float constructed during 1999 in Sequim Bay, Washington (cover and Figure 1). Excepting the handrails, which were constructed of CCA-C preserved wood, the entire structure was treated with ACZA, with nominal retentions of 2.5 pounds ACZA per cubic foot (*pcf* in the treated zone) for piling and sawn lumber used below the high tide line (AWPA 2001, C2 and C18) and 0.6 *pcf* for sawn lumber used out of water but subject to salt water splash (AWPA 2001, C18). Figure 2 is a plan drawing of the facility with the location of the sample stations annotated. Sediment samples were also collected and analyzed for copper, arsenic and zinc under the Port Townsend City Pier and under the Fort Worden wharf. Both of these structures sit on creosote treated wood piling, but have expansive decks constructed of 3” by 8” ACZA treated hem-fir. Table 1 summarizes information for the three locations examined in this study.

Table 1. Characteristics of the four sites at which the environmental response to ACZA preserved piling and decking were evaluated in Puget Sound.

Site	Number of piling	Footprint (m^2)	Notes
Sequim Bay pier and float	23	102.6	Rural area
Fort Ward wharf and pier	292	1,635.8	Rural area
Port Townsend City pier	85	Not measured	Urban area

Figure 1. ACZA treated float in Sequim Bay, Washington that was subject of a risk assessment conducted on October 18, 2002. Triplicate water samples were collected at a local reference location and at the indicated locations adjacent to the structure during a rainstorm on December 13, 2002. Water collection depth was 0.1 m along side the float and 0.5 m in the center of the dolphin.

Water and sediment quality criteria. Table 2. Provides a summary of Washington State's marine sediment quality criteria (WAC 173-204) and the U.S. EPA acute and chronic marine water quality criteria for copper, arsenic and zinc.

Table 2. Regulatory sediment and water quality criteria for copper, arsenic and zinc.

Metal	Washington Sediment Quality Criteria (µg/g dry sediment)
Copper	390
Arsenic	57
Zinc	410

Marine water quality criteria

Metal	USEPA acute (µg/L)	US EPA chronic (µg/L)
Copper	4.8	3.1
Arsenic	69.0	36.0
Zinc	84.6	76.6

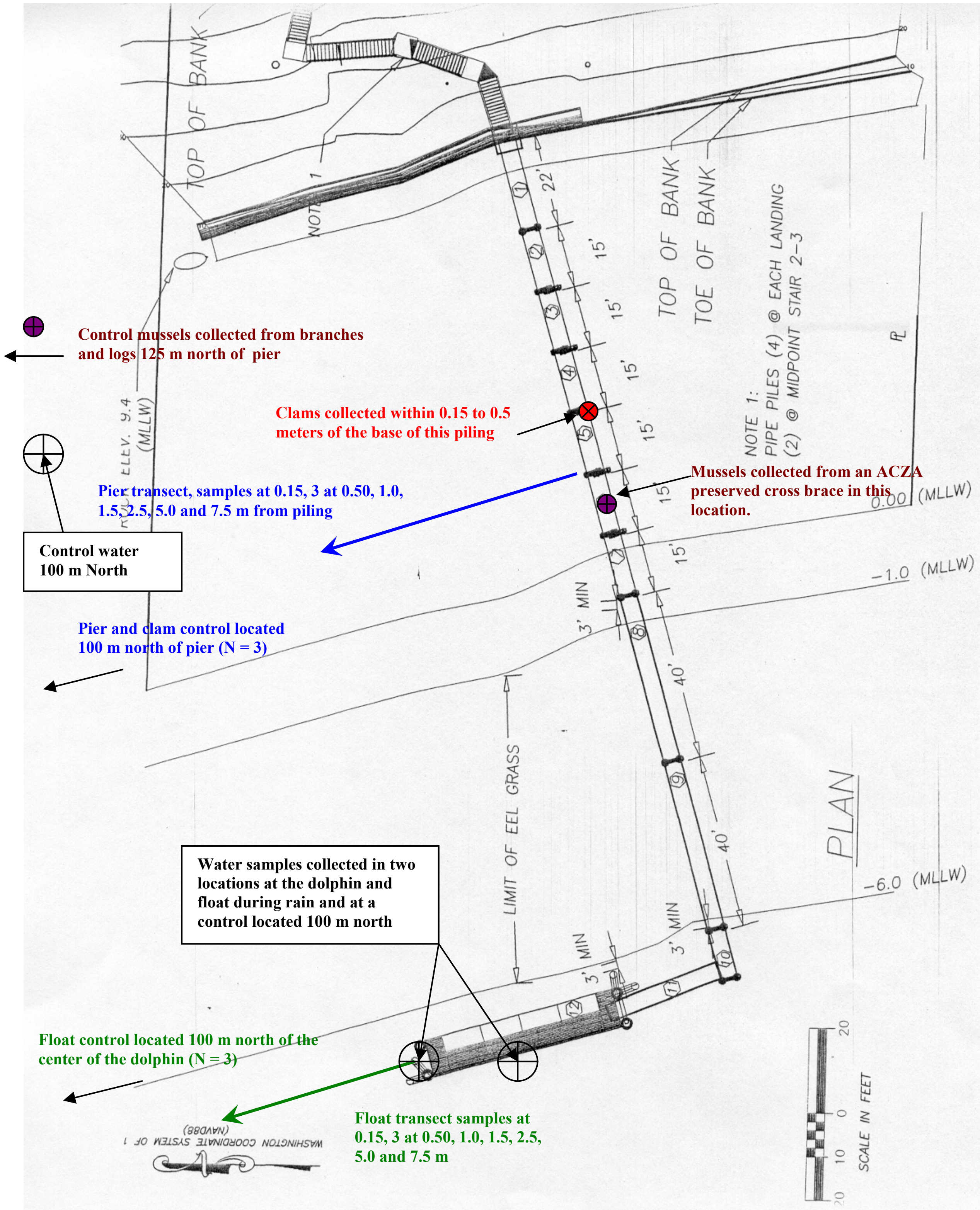

Figure 2. Plan of the pier and float located in Sequim Bay, Washington. The location of all sample stations is provided.

Arsenic in shellfish. The U.S. Food and Drug Administration (FDA 1993) reviews arsenic surveys in shellfish. The National Marine Fisheries Service (1978) reported arsenic concentrations of 3.0 to 4.0 μg As/g wet hardshell clam or oyster tissue with a mean concentration of 2.8 to 3.8 μg/g for all molluscan bivalves. Higher mean concentrations (8.6 to 10.6 μg As/g) were found for crustaceans and the highest concentrations were recorded in Pacific spiny lobster tails (20.0 to 30.0 μg As/g). The FDA concluded that ten percent of the total arsenic in shellfish is inorganic and that the tolerable daily intake for inorganic arsenic is 130 μg As for a 60 kg person. This would be equivalent to 1,300 μg total arsenic in shellfish tissues. Using survey results for mean molluscan shellfish consumption in the United States, FDA calculated an arsenic level of concern of 130 μg Total As/g wet tissue weight. This value will be used in this analysis as a benchmark.

Methods. Samples were collected by Mr. Lynn Goodwin of Strait and Sound Consulting using scuba equipment and the stainless steel fixture described in Figure 3. The fixture has a footprint of 0.032 m^2. Transect lines were laid out using a 100 m fiberglass tape and two samples were collected adjacent to the required distance on the left and right side of the tape. The first sample was used for physicochemical and the second for biological analyses. The lid of this sampler was inserted into the sediments and pulled toward the diver to create a shelf. The sampler was then pushed horizontally against the shelf to enclose samples that were ca. 10 cm deep. The lid was closed while the sampler was in the substrate, enclosing the sample for retrieval. At the surface, physicochemical samples were taken from the top two cm of the sampler and placed in precleaned glass containers for metal analysis and into 125 ml LDPE urine specimen bottles for other physicochemical analyses. The second sample, collected at the same time using a separate sampler, was washed into a five-gallon bucket using 125 μm filtered seawater for sieving and fixing on the day of collection. Samples for physicochemical analyses were placed on ice in a cooler while in the field. Samples were held at 4 ^{o}C until analyzed. Sulfide and redox analyses were conducted in the field within 15 minutes of collection. Mussels and clams were placed in Ziploc™ bags upon collection and held on ice in a cooler until shucked and shipped via an overnight delivery service on phase change ice packs to the analyzing laboratory.

Figure 3. Benthic sampler used by scuba divers to collect fully enclosed sediment samples

Sample dates. Samples were collected in Sequim Bay on October 18, 2002. Additional sediment samples were collected on January 6, 2002 at the Port Townsend City dock and at Fort Ward in Rich Passage, Washington on January 8, 2002. Water samples in Sequim Bay were collected on December 16, 2002 during a light rain.

Sample documentation and shipping. Samples were shipped in coolers on phase change ice packs by overnight delivery service to the appropriate analytical laboratory using chain of custody procedures that comply with the requirements of ASTM D4840-88.

Equipment and sample bottle cleaning. Multiple sampling fixtures, identical to that shown in Figure 3, were washed in hot water and detergent followed by soaking in 10% reagent grade nitric acid and a final rinse in distilled water. Fixtures were stored, along with similarly cleaned stainless steel sampling utensils constructed with a 2 cm high square shoulder, in new one gallon Ziploc™ bags until required in the field. Two freshly cleaned samplers and utensils were used for each transect. Sampling began at the local reference station and proceeded from the furthest to the closest station along the designated transects. Laboratory cleaned 250 ml wide-mouth bottles were supplied by the laboratories conducting the sediment and tissue metal analyses. New 125 ml urine specimen jars were used for storage of sediments for other physicochemical analyses (TVS, $S^{=}$, redox, SGS).

List of measured endpoints. The following endpoints were assessed at the Sequim Bay pier and float. Only SGS, TVS and sediment Cu, As and Zn concentrations were assessed at the Port Townsend City and Fort Ward piers. Physicochemical endpoints were measured only in the surficial sediment (0 - 2.0 cm) layer (PSEP, 1996).

Metals. All mussel and clam tissues, sediment and water samples were analyzed for copper, arsenic and zinc by Washington State Department of Ecology accredited laboratories.

Biological Endpoints. The entire contents of the 0.032 m^2 grab samples were sieved on 1.0 mm sieves and identified to the lowest level possible – generally to species.

Additional Tests

Sediment free sulfide concentrations
Sediment redox potential
Sediment Grain Size Distribution
Sediment Total Volatile solids

Sediment copper, arsenic and zinc concentrations were determined by Analytical Resources Incorporated in Seattle, Washington using EPA 6010B for copper and zinc and EPA 7060A for arsenic following a strong acid digestion using method 3050B. Method reporting limits were 0.3 mg As/kg dry sediment; 0.2 mg Cu/kg; and 0.7 mg Zn/kg. Quality assurance tests were all within data qualification limits (PSEP 1996) and included a standard reference material (Lot 247); a method blank (analyte not detected); and a spike (reported under tissues).

Seawater concentrations of copper, arsenic and zinc were collected at slack tide during a light rain and analyzed at the Battelle Marine Science Laboratory in Sequim, Washington using ICP/MS with detection limits of 0.1 µg As/L, 0.023 µg Cu/L, and 0.062 µg Zn/L. Total metal concentrations are reported (the samples were not filtered). Quality assurance tests included a laboratory blank in which copper was detected at 0.136 µg/L and zinc at 0.123 µg Zn/L. Analysis of a standard reference material (SRM) gave results within the data qualification limits (6% RPD for As; 4% for Cu and 20% for Zn).

Mussel (*Mytilus edulis trossulus*) and clam (*Protothaca staminea*) tissue concentrations of copper, arsenic and zinc were determined by Analytical Resources Incorporated in Seattle, Washington using EPA 6010B for copper and zinc and EPA 7060A for arsenic with a detection limit of 10 µg/kg following a strong acid digestion using method EPA 3050B. Quality assurance included recovery of a standard reference material (Lot 247) with results for all three metals in their advisory range, matrix spikes (100% recovery for As; 95% for Cu; and 94% for Zn); and one duplicate run (RPD = 6.5% for As; 0.0% for Cu; and 1.4% for Zn). A method blank revealed small amounts of all three metals. However, because the blank concentrations of metal were at the reporting limits of 0.1 mg As/kg; 0.2 mg Cu/kg; and 0.6 mg Zn/kg, and were at least an order of magnitude less than the concentrations reported in tissues, no corrective action was taken. All results were reported on a wet tissue weight basis.

Total volatile solids (TVS) analyses were accomplished on 50-gram surficial sediment samples (PSEP 1996). Samples were dried at 103 $\pm$ 2^{o} C in aluminum boats that had been tared following combustion at 550 ^{o}C for 30 minutes. Drying was continued until no further weight reduction was observed (generally overnight). The samples were than combusted at 550 ^{o}C for 2 hours or until no further weight loss was recorded. TVS were calculated as the difference between the dried and combusted weights. Triplicate analyses were completed on 5% of the samples or on a minimum of one per batch (PSEP 1996).

Sediment Grain Size Distribution (SGS) analyses were accomplished using 50 $\pm$ 15 grams of surficial sediment taken from the top two cm of the sediment column. The sample was wet sieved on a 0.64 µm sieve. The fraction retained on the 0.64 µm sieve was dried in an oven at 92 ^{o}C and processed using the dry sieve and pipette method of Plumb (1981). The sieves used for the analysis had mesh openings of 2.0, 0.89, 0.25 and 0.064 µm. Particles passing the 0.064 µm sieve during wet sieving were analyzed by sinking rates in a column of water (pipette analysis). Triplicate analyses were conducted on one, or a minimum of 5 percent, of the samples. The Root Squared Deviation (RSD) was $\leq$ 20% for these triplicate samples.

Benthic infaunal analysis. The entire contents of infaunal grab samples (including the overlying water) were washed from the grab into a five-gallon bucket using 125 µm filtered seawater. Samples were then sieved on 1.0 mm stainless steel screens. The retained material was placed in 1.0 or 2.0 liter HDPE bottles and fixed using 15% buffered formalin in seawater on the day of collection. Each sample jar had matching inside, outside and cap labels. Fixed samples were washed with filtered fresh water and preserved in 70% isopropyl alcohol after four days. Infaunal organisms were sorted from the background matrix under 10 x magnifications. A different technician repicked twenty percent of each sample. Quality assurance guidelines required a picking efficiency of >95%. Any sample failing this QA benchmark was completely repicked. Infauna were identified to the lowest level practicable – generally to species. All taxa were compared with verified specimens in a reference collection (Aquatic Environmental Sciences), or will be verified by an outside expert.

Redox potential (Eh) was measured in the field using an Orion™ advanced portable ISE/pH/mV/ORP/temperature meter Model 290A equipped with a Model 9678BN Epoxy Sure-Flow Combination Redox/ORP probe. The stated accuracy of the meter in the ORP mode is $\pm$ 0.2 mV or $\pm$ 0.05% of the reading, whichever is greater. Detailed protocols for preparation of standards and conducting the analyses are provided in Wildish (1999). Quality assurance required checking of the meter against two standards at the beginning and end of each batch of samples and triplicate analyses completed on 5% of the samples with a minimum of one per batch.

Free sediment sulfides ($S^{=}$) were evaluated using an Orion™ advanced portable ISE/pH/mV/ORP/temperature meter Model 290A equipped with a Model 9616 BNC Ionplus

Silver/Sulfide electrode. The meter has a concentration range of 0.000 to 19,900 μM and a relative accuracy of ± 0.5% of the reading. The probe and meter were three point calibrated using 100, 1,000 and 10,000 μM standards every three hours. Detailed protocols are provided in Wildish *et al.* (1999). Quality assurance required frequent checking of the meter against standards and triplicate analyses completed on 5% of the samples with a minimum of one per batch.

Statistical analyses. The experimental design and analyses relied on correlation, principle components and linear and non-linear regression analyses for single samples collected along transects and on analysis of variance for triplicate samples collected at treatment and reference stations. Raw data was entered into a Microsoft Excel™ spreadsheet and imported into Statistica™ software for analysis. Proportional data (TVS and SGS) were arcsine(square-root) transformed prior to inferential analyses (Zar 1984). Biological count data were transformed (Ln(N+1)) prior to determination of Pearson correlation coefficients. Biological responses were summarized in three-dimensional contour graphs using Statistica's distance weighted least squares subroutine. Statistical significance throughout this report is associated with the probability of making a Type I error of 0.05.

Shannon's Index (Shannon and Weaver, 1949). This index provides the average uncertainty per species in an infinite community of taxa. The form of the index used in this analysis is:

$$\text{Shannon's Index} = H' = -\Sigma_{over\ species}\,(n_i/n)*\ln(n_i/n)$$

The value of the index is zero when a single species is present. It is maximized when there are a large number of equally represented species and it is reduced in communities dominated by a few highly abundant species. The value of Shannon's Index in a sample containing 20 taxa equally represented in a total abundance of 600 animals would be 3.0.

Pielou's Index (Pielou 1977) is a commonly used measure of community evenness. It expresses the observed value of Shannon's Index relative to the maximum possible value (ln(S)). Where (S) is the number of taxa present. Pielou's index varies between 0 and 1.0 and generally co-varies with Shannon's Index.

$$\text{Pielou's Index} = J' = H'/\ln(S).$$

Temperature, salinity, dissolved oxygen and pH. Temperature was measured in the field using the temperature feature on a Yellow Springs Instrument Company (YSI) Model 33 SCT meter, which was also used to determine salinity. A YSI Model 57 dissolved oxygen meter was used to measure dissolved oxygen *in-situ.*

Results and discussion. Physicochemical endpoints used to characterize sediments in Sequim Bay, Fort Ward and Port Townsend are detailed in Table 3. Sediments at all of the sample locations were dominated by sand. The proportions TVS in Sequim Bay were high because the pier transect ran through an eelgrass meadow and the float transect was offshore by only about two meters from the outer edge of the meadow. As expected, sulfide concentrations were higher, and redox generally lower in the eelgrass meadow (pier transect) than in the offshore area. Concentrations of organic carbon and free sediment sulfides were relatively high near clusters of piling and lower near single piling or at reference locations. Eelgrass meadows are noted as valuable habitats in the Pacific northwest. However, note that the sulfide concentrations in the meadow were as high as 637 μM $S^{=}$ and the redox potential correspondingly low (-342 mV). These sulfide concentrations are within the range where Brooks and Mahnken (2003a) demonstrated the exclusion of 30 to 50% of macrobenthic taxa (Figure 4).

Table 3. Physicochemical endpoints characterizing sediments at Sequim Bay, Fort Ward and Port Townsend.

Site	Date	Distance (m)	Code	Gravel (%)	Sand (%)	Fines (%)	TVS (proportion)	Redox (mV)	Sulfides (μM)
Sequim Bay Float	10/18/02	0.15	BF(1)	3.36	89.34	7.30	0.093	-47.8	73.7
Sequim Bay Float	10/18/02	0.50	BF(2)	4.14	85.28	10.57	0.131	-24.9	55.5
Sequim Bay Float	10/18/02	0.50	BF(3)	6.09	83.02	10.89	0.131	-25.3	84.7
Sequim Bay Float	10/18/02	0.50	BF(4)	7.99	81.18	10.83	0.121	57.5	62.8
Sequim Bay Float	10/18/02	1.00	BF(5)	1.77	86.38	11.85	0.116	92.2	67.8
Sequim Bay Float	10/18/02	1.5	BF(6)	0.73	90.76	8.51	0.099	62.6	38.7
Sequim Bay Float	10/18/02	2.5	BF(7)	0.56	92.63	6.81	0.089	79.7	42.8
Sequim Bay Float	10/18/02	5.0	BF(8)	3.99	88.26	7.75	0.105	-106.2	50.3
Sequim Bay Float	10/18/02	7.5	BF(9)	0.53	89.79	9.68	0.102	-113.8	36.8
Sequim Bay Float	10/18/02	100	BF(10)	0.47	90.22	9.31	0.118	-268.1	70.7
Sequim Bay Float	10/18/02	100	BF(11)	0.47	90.22	9.31	0.116	-77.2	118.0
Sequim Bay Float	10/18/02	100	BF(12)	0.47	90.22	9.31	0.127	55.6	43.2
Sequim Bay Pier	10/18/02	0.15	BP(1)	2.66	91.67	5.67	0.086	-245.0	493.0
Sequim Bay Pier	10/18/02	0.50	BP(2)	0.87	93.53	5.60	0.094	-321.6	260.0
Sequim Bay Pier	10/18/02	0.50	BP(3)	0.87	93.53	5.60	0.084	-342.9	637.0
Sequim Bay Pier	10/18/02	0.50	BP(4)	0.87	93.53	5.60	0.111	-322.6	409.0
Sequim Bay Pier	10/18/02	1.00	BP(5)	3.51	90.81	5.68	0.099	-309.6	406.0
Sequim Bay Pier	10/18/02	1.5	BP(6)	2.61	91.89	5.50	0.091	-311.0	297.0
Sequim Bay Pier	10/18/02	2.5	BP(7)	12.77	82.58	4.65	0.088	63.4	106.0
Sequim Bay Pier	10/18/02	5.0	BP(8)	21.14	74.18	4.68	0.081	-233.8	246.0
Sequim Bay Pier	10/18/02	7.5	BP(9)	36.38	58.93	4.69	0.091	27.4	325.0
Sequim Bay Pier	10/18/02	100	BP(10)	11.81	82.83	5.36	0.087	75.5	316.0
Sequim Bay Pier	10/18/02	100	BP(11)	9.28	83.57	7.15	0.094	59.2	245.0
Sequim Bay Pier	10/18/02	100	BP(12)	9.66	85.35	5.00	0.092	-227.8	162.0
Port Townsend	4/19/2002	0.15	PT-S3(1)	24.40	56.38	19.22	0.030	-120.2	191.4
Port Townsend	4/19/2002	0.15	PT-S3(2)	22.78	57.01	20.21	0.030	-175.7	128.4
Port Townsend	4/19/2002	0.15	PT-S3(3)	25.17	55.50	19.34	0.030	-163.9	116.1
Fort Ward Pier	4/26/2002	0.3	FWP-S1					-102.4	119.4
Fort Ward Pier	4/26/2002	0.3	FWP-S2					-90.8	134.3
Fort Ward Pier	4/26/2002	0.3	FWP-S3					-96.3	158.2

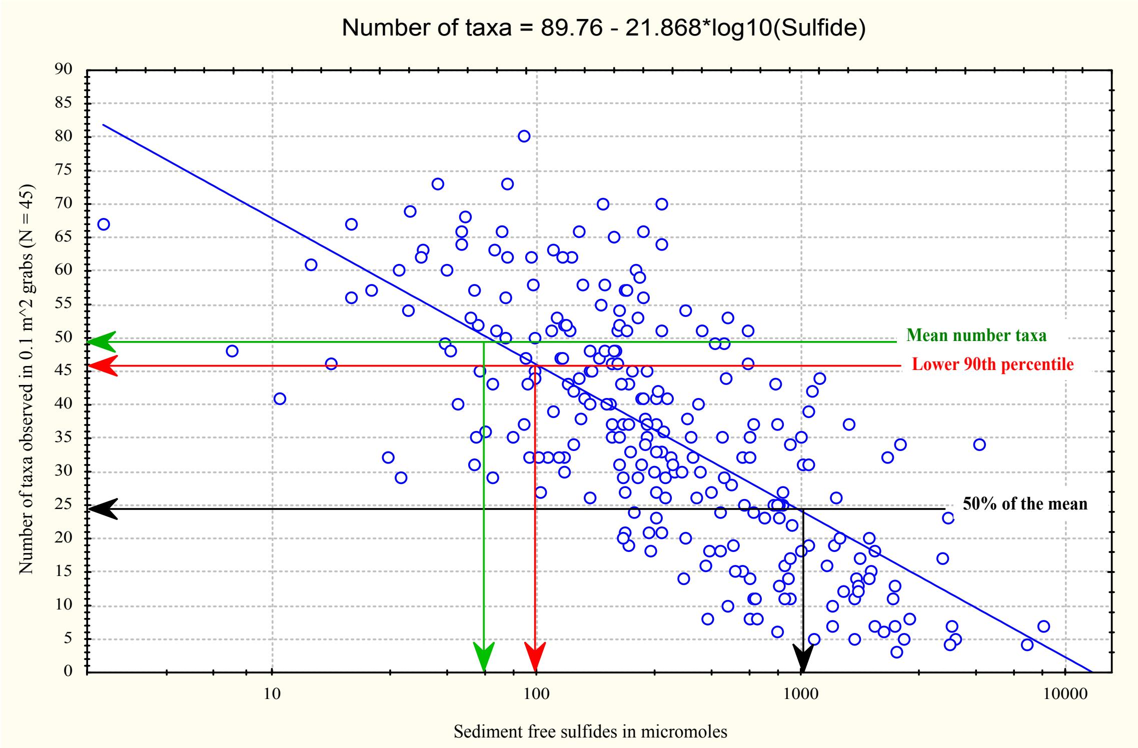

Figure 4. Number of taxa observed in British Columbia sediments as a function of the concentration of Free Sediment Sulfides ($S^{=}$). Graph from Brooks and Mahnken (2003a).

Figure 5. Piling supporting the Fort Ward wharf in Rich Passage, Washington. The wharf's deck is constructed of ACZA treated 3" x 8" lumber.

Free sediment sulfide concentrations adjacent to the Fort Ward wharf (2,706 µM $S^{=}$) were nearly as high as those observed by Goyette and Brooks (1998) at the Sooke Basin dolphins. Figure 5 depicts support piling on the north face of the Fort Ward wharf. Biodeposits from the fouling community were likely exceeding the assimilative capacity of the sediments creating localized anaerobic conditions leading to high sulfide concentrations. The point is that these high sulfide concentrations would confound macrobenthic affects associated with sediment metal concentrations. Sediment samples at Fort Ward were collected from under the ACZA treated pier deck within 30 cm of support piling at about 1.0' Mean Lower Low Water (MLLW). The Fort Ward pier pilings were fouled at this higher intertidal elevation, but not nearly to the degree seen in Figure 5 and as seen in Table 3, free sediment sulfides were <160 µM.

Metal concentrations in sediments. Table 4 summarizes all sediment concentrations of arsenic, copper and zinc observed in this assessment. Sediment concentrations of arsenic varied between 1.56 and 6.7 µg As/g dry sediment at all stations located ≤ 7.5 m from ACZA treated structures and between 0.49 and 6.34 µg/g at the reference stations. Highest arsenic concentrations were found at the Fort Ward pier and control stations. To put these concentrations

Table 4. Sediment concentrations of arsenic (As), copper (Cu) and zinc (Zn) observed at ACZA treated structures and reference locations in Sequim Bay, Port Townsend and Fort Ward in Washington State.

Location	Date	Structure	Distance (m)	Replicate	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Sequim Bay	10/18/2002	Pier piling	0.2	1	2.60	13.20	23.80
Sequim Bay	10/18/2002	Pier piling	0.5	1	2.20	7.30	22.30
Sequim Bay	10/18/2002	Pier piling	0.5	2	2.00	7.00	22.30
Sequim Bay	10/18/2002	Pier piling	0.5	3	2.00	6.60	20.80
Sequim Bay	10/18/2002	Pier piling	1.0	1	1.80	7.20	23.50
Sequim Bay	10/18/2002	Pier piling	1.5	1	1.90	6.90	22.40
Sequim Bay	10/18/2002	Pier piling	2.5	1	1.90	7.00	20.40
Sequim Bay	10/18/2002	Pier piling	5.0	1	1.70	6.60	20.00
Sequim Bay	10/18/2002	Pier piling	7.5	1	1.56	6.80	20.60
Sequim Bay	10/18/2002	Pier piling	100.0	1	2.00	7.30	24.00
Sequim Bay	10/18/2002	Pier piling	100.0	2	1.90	8.50	22.70
Sequim Bay	10/18/2002	Pier piling	100.0	3	2.00	7.10	23.90
Sequim Bay	10/18/2002	4 piling dolphin at the float	0.2	1	3.00	19.80	22.80
Sequim Bay	10/18/2002	4 piling dolphin at the float	0.5	1	3.50	16.30	32.40
Sequim Bay	10/18/2002	4 piling dolphin at the float	0.5	2	3.70	12.90	26.80
Sequim Bay	10/18/2002	4 piling dolphin at the float	0.5	3	3.30	12.50	25.70
Sequim Bay	10/18/2002	4 piling dolphin at the float	1.0	1	3.20	9.60	23.70
Sequim Bay	10/18/2002	4 piling dolphin at the float	1.5	1	2.90	6.60	19.00
Sequim Bay	10/18/2002	4 piling dolphin at the float	2.5	1	2.60	5.50	19.80
Sequim Bay	10/18/2002	4 piling dolphin at the float	5.0	1	2.90	6.30	21.60
Sequim Bay	10/18/2002	4 piling dolphin at the float	7.5	1	3.10	6.10	20.40
Sequim Bay	10/18/2002	4 piling dolphin at the float	100.0	1	3.40	7.00	23.90
Sequim Bay	10/18/2002	4 piling dolphin at the float	100.0	2	3.50	6.20	22.20
Sequim Bay	10/18/2002	4 piling dolphin at the float	100.0	3	3.20	6.00	21.50
Port Townsend	4/26/2002	3 piling dolphin at the pier	0.3	1	2.86	10.00	44.20
Port Townsend	4/26/2002	3 piling dolphin at the pier	0.3	2	3.06	15.30	39.00
Port Townsend	4/26/2002	3 piling dolphin at the pier	0.3	3	7.44	77.90	53.50
Port Townsend	4/26/2002	3 piling dolphin at the pier	100.0	1	0.49	6.02	24.70
Port Townsend	4/26/2002	3 piling dolphin at the pier	100.0	2	0.49	3.91	18.80
Port Townsend	4/26/2002	3 piling dolphin at the pier	100.0	3	0.49	5.83	24.90
Fort Ward	4/19/2002	Pier piling	0.3	1	6.70	9.31	71.00
Fort Ward	4/19/2002	Pier piling	0.3	2	6.25	6.81	76.40
Fort Ward	4/19/2002	Pier piling	0.3	3	5.91	9.78	57.60
Fort Ward	4/19/2002	Pier piling	100.0	1	3.85	11.20	32.10
Fort Ward	4/19/2002	Pier piling	100.0	2	4.34	7.50	31.80
Fort Ward	4/19/2002	Pier piling	100.0	3	6.34	8.71	34.30

into perspective, recall from Table 2 that the Washington State marine sediment quality criterion (SQC) for arsenic is 57 μg As/g. Thus all of the observed concentrations are less than the SQC by an order of magnitude.

More copper was lost from ACZA treated structures than arsenic and sediment copper concentrations at the treated structures generally ranged between 5.5 and 19.8 μg Cu/g dry sediment with the exception of one sample collected from the center of a three piling dolphin at the Port Townsend City pier which was 77.9 μg Cu/g. The SQC for copper is 390 μg Cu/g and in general the observed copper concentrations within even 15 cm of ACZA treated piling were an order of magnitude less than the SQC. The single sample at Port Townsend was 20% of the SQC. Copper concentrations at the four reference locations were uniformly low varying between 5.8 and 11.2 μg/g.

Except for the Fort Ward pier, zinc concentrations near ACZA treated structures were in the range 20 to 53.5 μg Zn/g and they were 22.7 to 34.3 μg/g at the four reference stations. The Fort Ward pier and wharf complex serves a local fish farm. Brooks and Mahnken (2003b) described increases in sediment zinc associated with aquaculture. These usually small increases are associated with uneaten feed and fish feces, because Zn is added to fish feeds as a micronutrient. Fish feed is delivered by truck and stored on the Fort Ward wharf prior to being transferred to lighters for delivery to the fish. This study was not designed to determine cause and effect. However, the higher zinc concentrations observed at Fort Ward's reference station may result from the dispersal of finely divided food particles abraded from the fed pellets (hypothesis). Under any circumstances, all of the measured concentrations are well below Washington State's marine zinc SQC of 410 μg Zn/g.

Figure 6, taken from the South side of the pier looking North, shows the location of the treatment and reference transects and piling and bracing where clams and mussels were collected for tissue analyses of metals. Sediment concentrations of Cu, As and Zn at the Sequim Bay pier, where macrobenthic samples were collected are summarized in Figure 7.

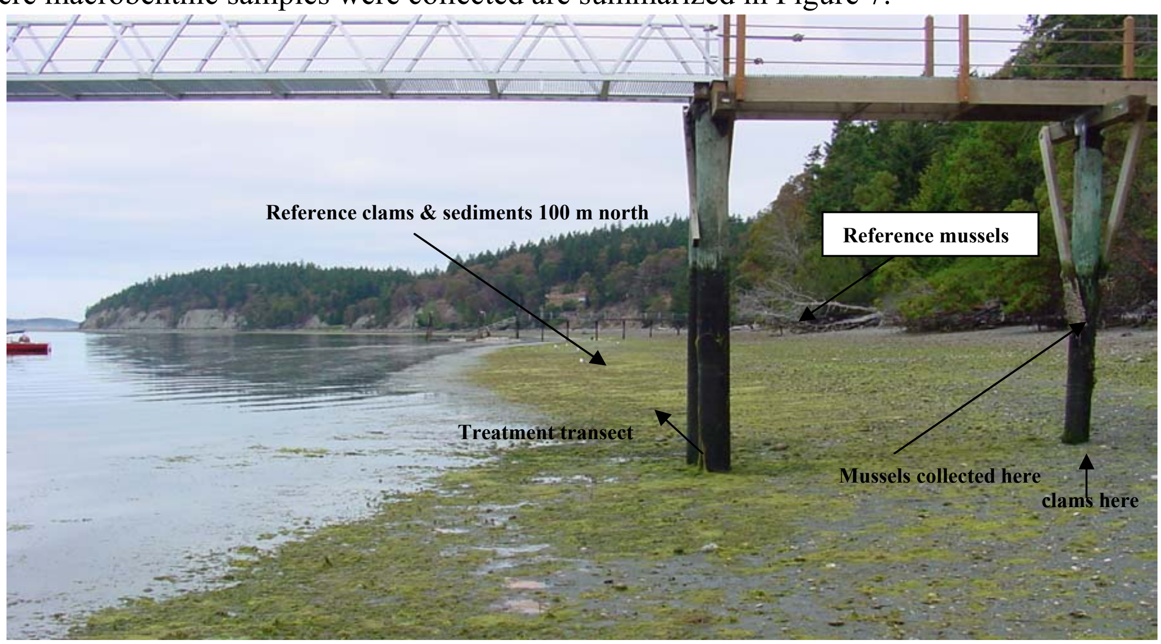

Figure 6. Location of sampling and reference transects and bivalve collection points in Sequim Bay, Washington.

Zinc concentrations were reduced in samples collected between 2.5 and 7.5 meters from the pier pilings in comparison with samples collected closer to the piling or at the reference location. These data suggest that metals lost from the piling were generally sedimented at distances not exceeding 2.5 m from the double piling. However, as previously noted all of the concentrations are low in comparison with SQC considered necessary to protect aquatic life. No adverse biological effects could reasonably be predicted at the observed metal concentrations.

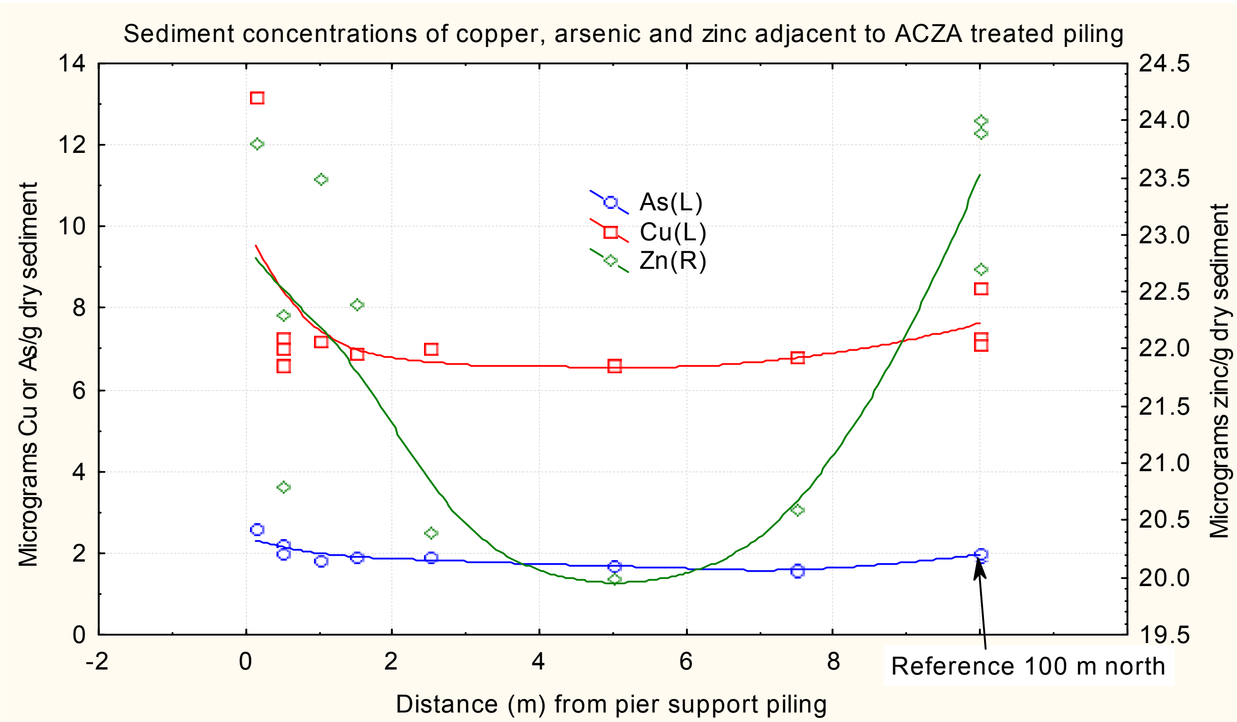

Figure 7. Sediment concentrations of copper, arsenic and zinc adjacent to ACZA treated piling supporting a pier constructed of ACZA treated lumber.

Macrobenthic samples were also collected in deeper water starting in the center of a four piling dolphin supporting the facilities mooring float. Figure 8 describes sediment concentrations of Cu, As and Zn as a function of distance from the center of the dolphin anchoring the North end of the float located over a bottom at ca. –7' MLLW (Figure 2). Both the float and the piling were constructed of ACZA treated wood and both would contribute metals to the water and sediments. Sediment concentrations were increased at those stations located ≤1.5 m from the closest piling near the center of the structure. The significance of differences between the triplicate samples collected at 0.5 meters (within the footprint of the dolphin) and those collected at the reference location is explored in Table 5 using a *t-test.* Differences in sediment concentrations of arsenic and zinc were small and not significant. The observed copper concentration in the center of the dolphin (13.9 µg/g) was significantly higher ($t = 6.03; p = 0.004$) than at the reference location (6.4 µg Cu/g). These data suggest that the structure, which was four years old when this survey was completed, had increased sediment concentrations within the dolphin's footprint from 6.4 to 13.9 µg/g, a final value that is only 0.35% of the SQC. However, it should be noted that a large sailboat, whose bottom is coated with copper based antifouling paint is moored here during the summer months. The contribution of copper from this source is unknown.

Variable	T-tests; Grouping: Distance (Sequim Bay) Group 1: 0.5; Group 2: Reference				
	Mean 0.5	Mean Reference	t-value	df	p
As	3.500	3.367	0.918	4.000	0.411
Cu	13.900	6.400	6.031	4.000	0.004
Zn	28.300	22.533	2.629	4.000	0.058

Table 5. Results of a *t-test* to examine the significance (α = 0.05) of differences in mean values of arsenic, copper and zinc (N = 3) in sediments collected from the center of an ACZA preserved four piling dolphin supporting an ACZA treated float and reference sediments in Sequim Bay, Washington.

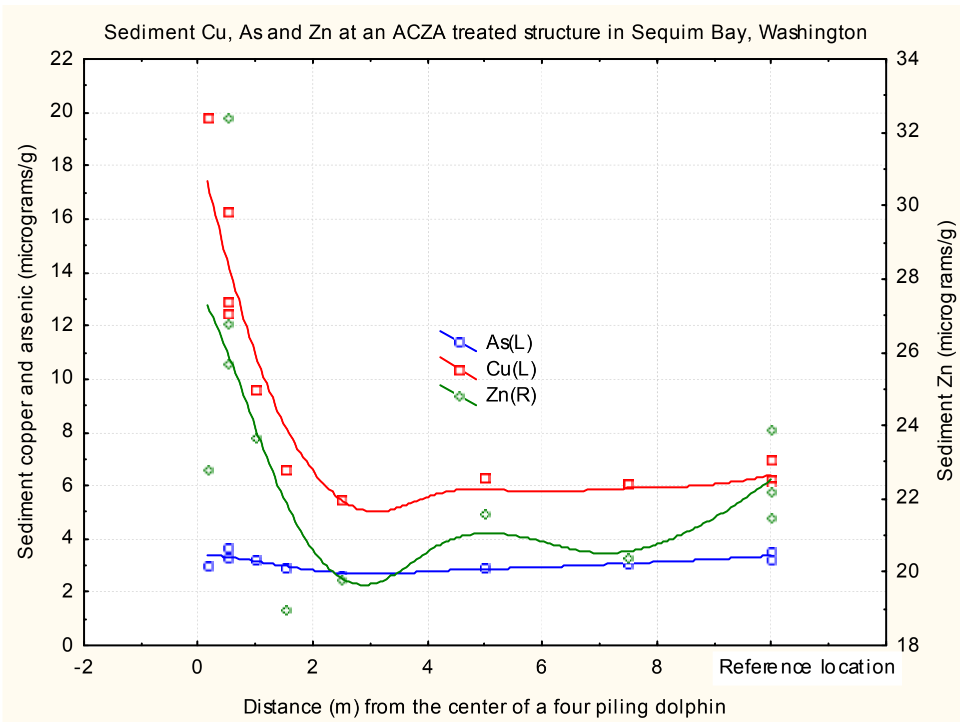

Figure 8. Sediment concentrations of copper, arsenic and zinc as a function of distance from the closest ACZA treated piling in a four piling dolphin supporting an ACZA treated float in Sequim Bay, Washington.

Sediment concentrations of copper, arsenic and zinc at the Port Townsend City and Fort Ward piers. Triplicate sediment samples were collected within 30 cm of one of three piling in a dolphin at the Port Townsend City pier and within 30 cm of one piling in a bent of pilings at the Fort Ward pier. Reference samples were collected at both locations. Analysis of variance followed by post hoc testing using Duncan's test with multiple ranges (Table 6) was used to assess differences in a) arsenic; b) copper; and c) zinc concentrations at the two treatment and two reference locations. Sediment concentrations of arsenic were significantly lower at Port Townsend's reference station than at all other locations. The observed concentration of 0.49 µg Cu/g is exceptionally low for any marine sediment. Other arsenic values were consistent with typical reference values and significant differences were not observed between other values.

Table 6. Results of analysis of variance followed by post hoc testing using Duncan's test to evaluate the significance of differences in sediment concentrations (µg/g dry sediment) of a) arsenic; b) copper; and c) zinc at wood structures in Port Townsend and Sequim Bay preserved with ACZA and at local reference stations.

Variable	Analysis of Variance (Sequim) Marked effects are significant at p < .05000							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
As	55.464	3.000	18.488	17.194	8.000	2.149	8.602	0.007

Site Distance	Duncan test; Variable: As (Sequim) Marked differences are significant at p < .05000			
	{1} M=4.4533	{2} M=.48667	{3} M=6.2867	{4} M=4.8433
Townsend 0.3 {1}		0.011	0.180	0.753
Townsend Reference {2}	0.011		0.002	0.008
Ward 0.3 {3}	0.180	0.002		0.263
Ward Reference {4}	0.753	0.008	0.263	

a) Arsenic

Variable	Analysis of Variance (Sequim) Marked effects are significant at p < .05000							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Cu	1633.832	3.000	544.611	2867.360	8.000	358.420	1.519	0.282

Site Distance	Duncan test; Variable: Cu (Sequim) Marked differences are significant at p < .05000			
	{1} M=34.400	{2} M=5.2533	{3} M=8.6333	{4} M=9.1367
Townsend 0.3 {1}		0.114	0.149	0.141
Townsend Reference {2}	0.114		0.833	0.816
Ward 0.3 {3}	0.149	0.833		0.975
Ward Reference {4}	0.141	0.816	0.975	

b) Copper

Variable	Analysis of Variance (Sequim) Marked effects are significant at p < .05000							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Zn	3480.489	3.000	1160.163	323.060	8.000	40.383	28.729	0.000

Site Distance	Duncan test; Variable: Zn (Sequim) Marked differences are significant at p < .05000			
	{1} M=45.567	{2} M=22.800	{3} M=68.333	{4} M=32.733
Townsend 0.3 {1}		0.003	0.002	0.039
Townsend Reference {2}	0.003		0.000	0.092
Ward 0.3 {3}	0.002	0.000		0.000
Ward Reference {4}	0.039	0.092	0.000	

c) Zinc

Differences in sediment copper were not significant. The single value of 77.9 µg Cu/g observed at Port Townsend increases the mean there, but it also increased the variance. Deleting this high value and reanalyzing the database indicated that copper at the Port Townsend City pier (12.65 µg Cu/g) was significantly higher ($F = 5.42$; $p = 0.03$) than at Port Townsend's reference location (5.25 µg Cu/g). Sediment Cu was not significantly higher at Fort Ward's pier than at either reference station.

Sediment zinc was significantly higher at the two treatment stations (45.6 and 68.3 µg/g) than at either reference station (22.8 and 32.7 µg/g). The two reference stations were not significantly different, but the value at Port Townsend, where some of the piling were zinc plated steel, were significantly higher than at Fort Ward, where all of the piling were wood preserved with creosote or ACZA.

Water column concentrations of arsenic, copper and zinc at the Sequim Bay structure. Triplicate water samples were collected at 0.5 m depth in the center of a four piling dolphin and at the Sequim Bay reference station. Three additional samples were collected under the dripline of the float at a depth of 15 cm during a slow steady rain in December, 2002. The results are summarized in Table 7. Highest mean values of all three metals were observed in the center of the four piling dolphin. Water under the drip line of the float contained less copper and zinc than observed at the reference station. Arsenic means were similar. The statistical significance of these values is explored in an analysis of variance summarized in Table 8. None of the differences were significant at $\alpha = 0.05$ or 0.10.

Table 7. Summary statistics describing concentrations of arsenic, copper and zinc in Sequim Bay marine water collected from the center of a four piling ACZA preserved dolphin supporting an ACZA treated float; under the drip line of the float and at a reference location. All values are in µg/L.

Breakdown Table of Descriptive Statistics (Sequim Water)
N=9 (No missing data in dep. var. list)

Location	As Means	Confidence -95.000%	Confidence +95.000%	Cu Means	Confidence -95.000%	Confidence +95.000%	Zn Means	Confidence -95.000%	Confidence +95.000%
Pier	2.257	-0.244	4.757	1.906	-1.244	5.056	7.233	-2.760	17.227
Float	1.293	1.179	1.408	0.775	0.564	0.985	2.113	1.745	2.482
C1	1.230	1.092	1.368	1.690	-0.033	3.413	4.120	0.270	7.970
All Grps	1.593	1.048	2.138	1.457	0.772	2.142	4.489	2.102	6.876

Table 8. Results of an analysis of variance assessing the significance of the data provided in Table 7.

Analysis of Variance (Body Water)
Marked effects are significant at p < .05000

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
As	1.986	2.000	0.993	2.037	6.000	0.339	2.926	0.130
Cu	2.164	2.000	1.082	4.192	6.000	0.699	1.549	0.287
Zn	39.934	2.000	19.967	37.215	6.000	6.202	3.219	0.112

Tissue concentrations of arsenic, copper and zinc in mussel and clam tissues. Three composite samples of 15 to 20 clams each were collected from within 0.5 meters distance within 120 degree sectors around the piling shown in Figure 6. Three composite samples of 15 mussels were collected from the ACZA preserved bracing seen in the same figure. Additional triplicate samples were collected at reference stations located $\geq$ 100 m north of the pier and float in Sequim Bay. Summary statistics describing the results of tissue analyses for arsenic, copper and zinc are provided in Table 9. The statistical significance of the differences in tissue concentrations of the three metals are explored in Table 10. Because of environmental and physiological differences between mussels and clams, the two species were treated separately using *t-tests*. The only significant (α = 0.05) difference was that mussel tissues from the reference location (14.7 μg Zn/g wet tissue) contained more zinc than mussels from the control (12.4 μg Zn/g). Tissue concentrations of arsenic were all less than the FDA (1993) level of concern of 130 μg As/g wet tissue.

Table 9. Summary statistics describing tissue concentrations of arsenic, copper and zinc in mussel (*Mytilus edulis trossulus*) and clam (*Protothaca staminea*) tissues collected at an ACZA treated structure and at a reference location in Sequim Bay, Washington.

Breakdown Table of Descriptive Statistics (Tissues)
N=12 (No missing data in dep. var. list)

Sample	As Means	Confidence +95.000%	Cu Means	Confidence +95.000%	Zn Means	Confidence +95.000%
Clams from treated piling	3.130	4.595	3.727	8.126	12.900	18.069
Clams from reference area	2.077	3.181	1.520	2.309	13.367	14.487
Mussels from treated piling	0.963	0.978	1.653	2.476	12.433	15.675
Mussels from reference area	0.983	1.238	1.647	2.318	14.733	15.737
All Grps	1.788	2.416	2.137	2.926	13.358	14.250

Table 10. Results of *t-tests* assessing differences in a) clam (*Protothaca staminea*) and b) mussel (*Mytilus edulis trossulus*) tissues collected at an ACZA treated wood structure (PC & PM) and at reference stations (CC & CM) in Sequim Bay, Washington.

T-tests; Grouping: Sample (Tissues)
Group 1: Clams from piling (PC)
Group 2: Clams from control (CC)

Variable	Mean PC	Mean CC	t-value	df	p	Valid N PC	Valid N CC	Std.Dev. PC	Std.Dev. CC	F-ratio Variances	p Variances
As	3.130	2.077	2.470	4.000	0.069	3.000	3.000	0.590	0.445	1.758	0.725
Cu	3.727	1.520	2.125	4.000	0.101	3.000	3.000	1.771	0.318	31.077	0.062
Zn	12.900	13.367	-0.380	4.000	0.724	3.000	3.000	2.081	0.451	21.295	0.090

a) clam tissues

T-tests; Grouping: Sample (Tissues)
Group 1: Pier mussels (PM)
Group 2: Control Mussels (CM)

Variable	Mean PM	Mean CM	t-value	df	p	Valid N PM & CM	Std.Dev. PM	Std.Dev. CM	F-ratio Variances	p Variances
As	0.963	0.983	-0.337	4.000	0.753	3.000	0.006	0.103	316.000	0.006
Cu	1.653	1.647	0.027	4.000	0.980	3.000	0.331	0.270	1.503	0.799
Zn	12.433	14.733	-2.916	4.000	0.043	3.000	1.305	0.404	10.429	0.175

b) mussel tissues

Macrobentbic response to the Sequim Bay pier and float. A total of 5,545 invertebrates were inventoried in the 24 samples, each of which had a footprint of 0.032 m^2. Statistics describing some of the biological endpoints evaluated in this study are provided in Table 11. Brooks (2001) has shown that macrobenthic communities are very sensitive to sediment free sulfides and redox potential. Analysis of variance indicated that redox potential was significantly lower ($p = 0.034$) and free sediment sulfides higher ($p < 0.00$) along the pier transect that ran through the eelgrass meadow than along the float transect located in deeper water outside the meadow. Therefore it is reasonable to expect differences in these two communities. Analysis of variance confirmed that the mean abundances and numbers of taxa were not significantly different between each transect and its reference station. However, a higher abundance was observed along the piling transect (401.2) than along the float transect (107.4) or either reference station (152.7 to 169.7) and the number of taxa was higher at the float control (29.7) than at other locations (17.7 at the pile control to 24.2 at the float).

Table 11. Summary statistics describing free sediment sulfides (μM), macrofaunal abundance (number/0.032 m^2), number of Taxa (number/0.032 m^2 sample), values of Shannon's Index and the Infaunal Trophic Index (ITI) observed on the intertidal Piling transect, subtidal Float transect and their two reference locations in Sequim Bay.

Breakdown Table of Descriptive Statistics (Sequim Bay) N=24 (No missing data in dep. var. list)									
Transect	Sulfide Means	Confidence +95.000%	Abundance Means	Confidence +95.000%	Taxa Means	Confidence +95.000%	Shannon Means	ITI Means	N
Piling	353.222	472.064	401.222	550.662	20.444	23.884	1.839	88.538	9
Float	57.011	69.724	107.444	134.147	24.222	27.433	2.690	67.624	9
Control Pile	241.000	432.472	152.667	368.834	17.667	25.256	2.205	76.860	3
Control Float	77.300	171.285	169.667	225.232	29.667	33.461	2.910	66.329	3
All Grps	193.625	264.759	231.042	307.600	22.667	24.844	2.338	76.459	24

Ninety-six (96) taxa were identified including 43 annelids, 35 mollusks, 17 arthropods and 1 "other" grouping that included colonial animals like bryozoans, sponges, hydroids. The mean observed abundance in the 24 samples was 231/0.032 m^2 sample, which is equivalent to an abundance of 719/0.1 m^2, the area upon which the Washington State Department of Ecology SEDQUAL database is constructed. Macrofaunal abundances and Shannon's Indices at all locations excepting the float transect (331/0.1 m^2) in Sequim Bay were as high or higher than observed generally for Puget Sound. The number of taxa observed in 0.032 m^2 samples at Sequim Bay was lower than observed in Puget Sound. However, this metric is not directly comparable between the two databases because the area included in each Puget Sound sample was three times larger than in Sequim Bay. These data indicate that macrobenthic communities in the studied area of Sequim Bay were more abundant, perhaps slightly less diverse, but more evenly distributed than observed in similar Puget Sound environments.

Macrobenthic endpoints are summarized as a function of distance from the double pier support piling in Figure 9. All of the fits were achieved using Statistica's Distance Weighted Least Squares routine. Macrofauna abundance was highest near the piling and declined with increasing distance. The significance of differences in biological endpoints assessed in triplicate samples collected at 0.5 m from the base of the ACZA treated piling and at the reference station was evaluated using *t-tests* (Table 13). The only significant ($\alpha = 0.05$) difference was in the Infaunal Trophic Index, which was significantly higher at 0.5 m than at the reference location.

Table 12. Summary statistics comparing macrobenthic community endpoints in Sequim Bay samples with mean values from Striplin (1996) for Puget Sound environments located in water depths <150' and sediments having <20% silt and clay. Abundance values for Sequim Bay were corrected to number/0.1 m^2 to correspond with Stripling (1996).

Site	Puget Sound Abundance	Puget Sound Taxa	Puget Sound Shannon	Sequim Bay Abundance	Sequim Bay Taxa	Sequim Bay Shannon
Sequim Piling	491.400	68.700	1.340	1251.700	46.000	2.440
Sequim Float	491.400	68.700	1.340	335.100	53.000	2.460
Pile Control	491.400	68.700	1.340	476.400	33.000	2.480
Float Control	491.400	68.700	1.340	529.400	64.000	2.820
All Groups	491.400	68.700	1.340	719.700	42.000	2.410

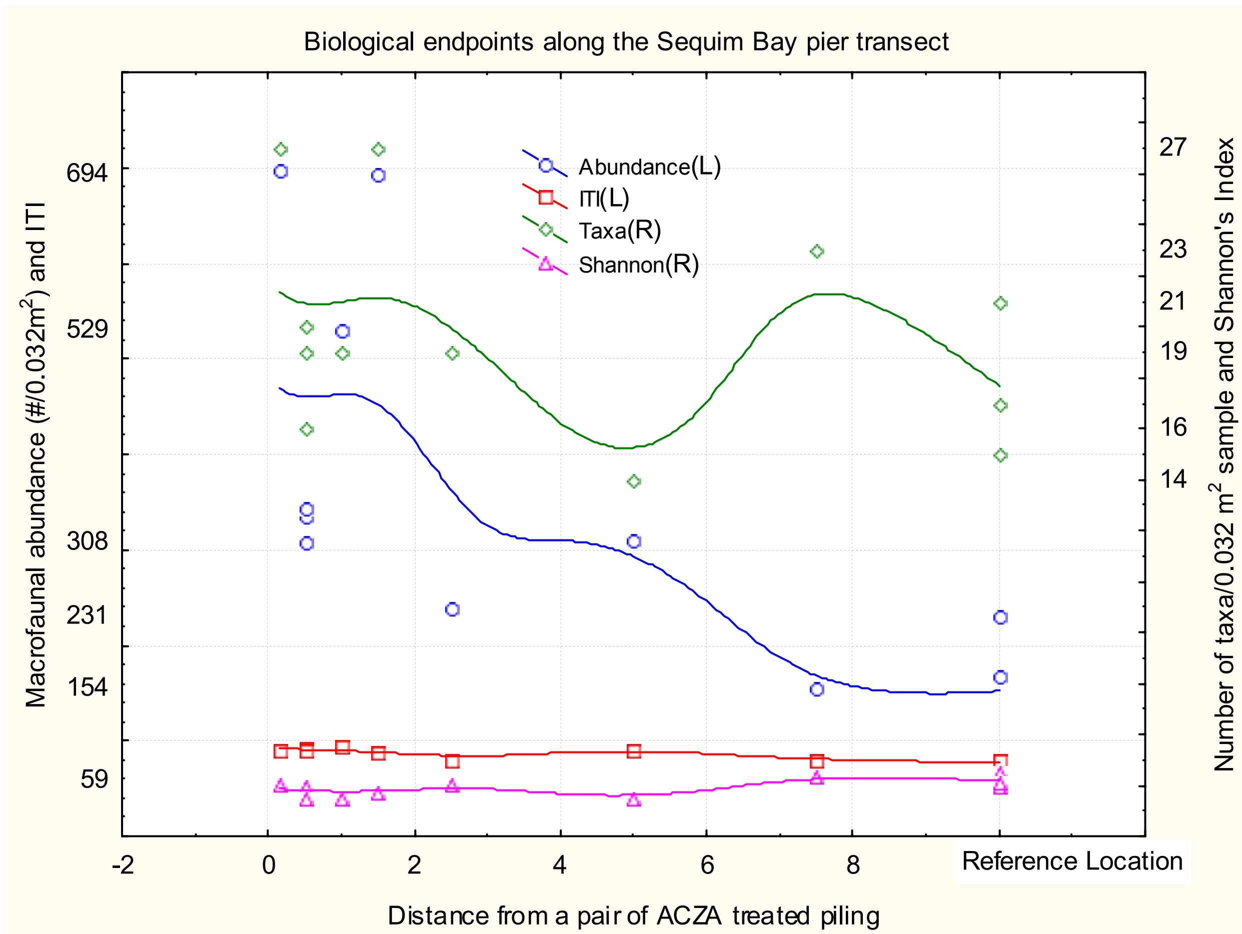

Figure 9. Biological endpoints as a function of distance from a pair of ACZA treated piling in Sequim Bay, Washington

Table 13. Results of *t-tests* assessing the significance of differences in four biological endpoints in triplicate samples collected 0.5 m from the base of a pair of ACZA treated pilings and at a local reference station in Sequim Bay, Washington. Abundance and number of taxa were transformed ($Log_{10}(N + 1)$ prior to the analysis. N = 3 at both stations.

	T-tests; Grouping: Distance (Sequim Bay) Group 1: 0.5 m Group 2: 100 m				
Variable	Mean 0.5	Mean 100	t-value	df	p
Shannon	1.79	2.20	-2.02	4	0.114
ITI	91.19	76.86	7.22	4	0.002
Abundance	2.52	2.12	2.22	4	0.091
Taxa	1.28	1.27	0.35	4	0.742

Differences in abundance were not significant when comparing the 0.5 m station with the reference location. However, abundance was nearly twice as high in the single samples collected at other stations located ≤ 1.5 m from the piling. Table 14 summarizes the abundance of those taxa representing ≥ 1% of the total abundance. Native littleneck clams (*Protothaca staminea*) dominated these samples. All of these clams had valve lengths < 1.0 cm and likely recruited during the year of the survey. Native little neck clams are typically found intertidally to heights of +5.0' MLLW. Their abundance in Puget Sounds is greatly reduced subtidally. Possible reasons for the observed distribution of members of the macrobenthic community were explored using Pearson correlation analysis in Table 15.

Table 14. Taxa found in an abundance ≥ 1% of the total abundance along the piling transect in Sequim Bay, Washington and at the reference stations. Values are numbers/0.032 m^2 sample. Values at 0.5 m and at the reference stations (100 m) are the mean of three replicate 0.032 m^2 samples. Other values are for single 0.032 m^2 samples.

	Distance (m) from the base of two ACZA treated pilings								
Species	0.2	0.5	1.0	1.5	2.5	5.0	7.5	100.0	Total
Protothaca staminea	376.0	197.6	325	349	68	176	50	45.7	1784
Polydora kempi	86.0	43	105	127	13	52	8	22.3	587
Spio butleri	57.0	43	105	127	13	52	8	27.3	351
Mysella tumida	58.0	19.7	21	43	39	7	13	23	275
Glycera sp.	8.0	1	17	56	51	24	8	1.5	169
Decamastus gracilis	0.0	9.3	10	17	14	17	1	0.7	89
Cirrepedia	46.0	3.7	1	4	19	0	2	0.7	85
Cooperilla subdiaphana	0.0	3.3	4	6	2	3	0	10	55
Macoma secta	7.0	3.7	4	4	2	3	5	5.3	49
Nereis juveniles	0.0	0.7	0	0	0	1	7	8.3	35
Hemigrapsus oregonensis	0.0	0	0	1	15	1	4	0	21
Clinocardium sp.	0.0	3	1	2	1	0	0	1.7	18

Table 15. Pearson correlation coefficients illustrating the correlation between dominant taxa along a transect originating at the base of two ACZA treated piling and proceeding north, away from the pier and physicochemical endpoints measured in sediments.

Variable	Correlations (Sequim Bay Pier) Marked correlations are significant at p < .05000 N=12 (Casewise deletion of missing data)							
	Distance	As	Cu	Zn	Fines	TVS	Sulfide	Redox
Decamastus gracilis	-0.45	-0.44	-0.49	-0.56	-0.23	0.11	0.08	-0.34
Glycera sp.	-0.35	0.06	-0.20	-0.17	0.13	0.20	0.69	-0.42
Nereis sp.	0.90	-0.31	-0.23	0.21	-0.11	-0.05	-0.22	0.48
Polydora kempi	-0.63	0.41	0.22	0.37	0.03	-0.04	0.33	-0.78
Spio Butleri	-0.21	0.35	0.23	0.46	-0.15	0.04	0.31	-0.59
Clinocardium sp.	-0.29	0.14	-0.36	0.13	-0.05	0.27	0.08	-0.61
Cooperilla subdiaphana	0.34	-0.21	-0.44	0.33	-0.03	0.02	-0.06	-0.05
Macoma secta	0.00	0.55	0.35	0.63	-0.04	0.14	0.15	-0.42
Mysella tumida	-0.64	0.46	0.32	0.10	-0.28	0.04	0.28	-0.23
Protothaca staminea	-0.75	0.23	0.09	0.00	-0.34	0.05	0.41	-0.75
Shannon	0.60	0.02	0.24	0.14	0.22	-0.21	-0.24	0.68
Pielou	0.72	-0.07	0.08	0.09	0.26	-0.25	-0.33	0.75
ITI	-0.86	0.22	0.01	-0.11	-0.13	0.10	0.53	-0.87
Abundance	-0.80	0.39	0.23	0.11	-0.27	0.00	0.37	-0.75
Number taxa	-0.32	0.28	0.43	0.18	-0.02	0.14	0.25	-0.22

No biological effects were anticipated in association with the low concentrations of copper, arsenic and zinc released from the ACZA treated piling that accumulated in sediments and none were observed. The only significant correlation between metals and biological endpoints was zinc and the bivalve *Macoma secta*, a surface deposit feeder and the correlation was positive. Of the 45 coefficients relating metal concentrations to biological endpoints, 12 were negative and 33 were positive.

Biological response in the subtidal habitat adjacent to the float structure in Sequim Bay. This transect and its reference station were located in subtidal substrates at –7' MLLW. The substrates were dominated by sand having relatively lower sulfide concentrations than were observed in the eelgrass meadow. A total of 1,476 macrobenthic organisms in 70 taxa were identified in twelve 0.032 m^2 samples. Mean abundance in this deeper water was 124/0.032 m^2 sample, which is equivalent to 384/0.1 m^2. The number of taxa observed here is less than the mean of 68 found in similar Puget Sound sediments. But as previously noted, the smaller grab size used in this study is at least partly responsible for that. Summary statistics for macroinvertebrates collected along the float transect and its reference station are provided in Table 16. Table 17 describes the abundance of individual taxa representing > 1% of the total abundance at this site. Juvenile native littleneck clams were present at this depth, but did not dominate the community the way they did higher in the intertidal. Higher abundances of annelids, gastropods and crustaceans were found in the deeper water. Figure 10 summarizes the biological endpoints evaluated in this study as a function of distance from the center of the ACZA treated dolphin.

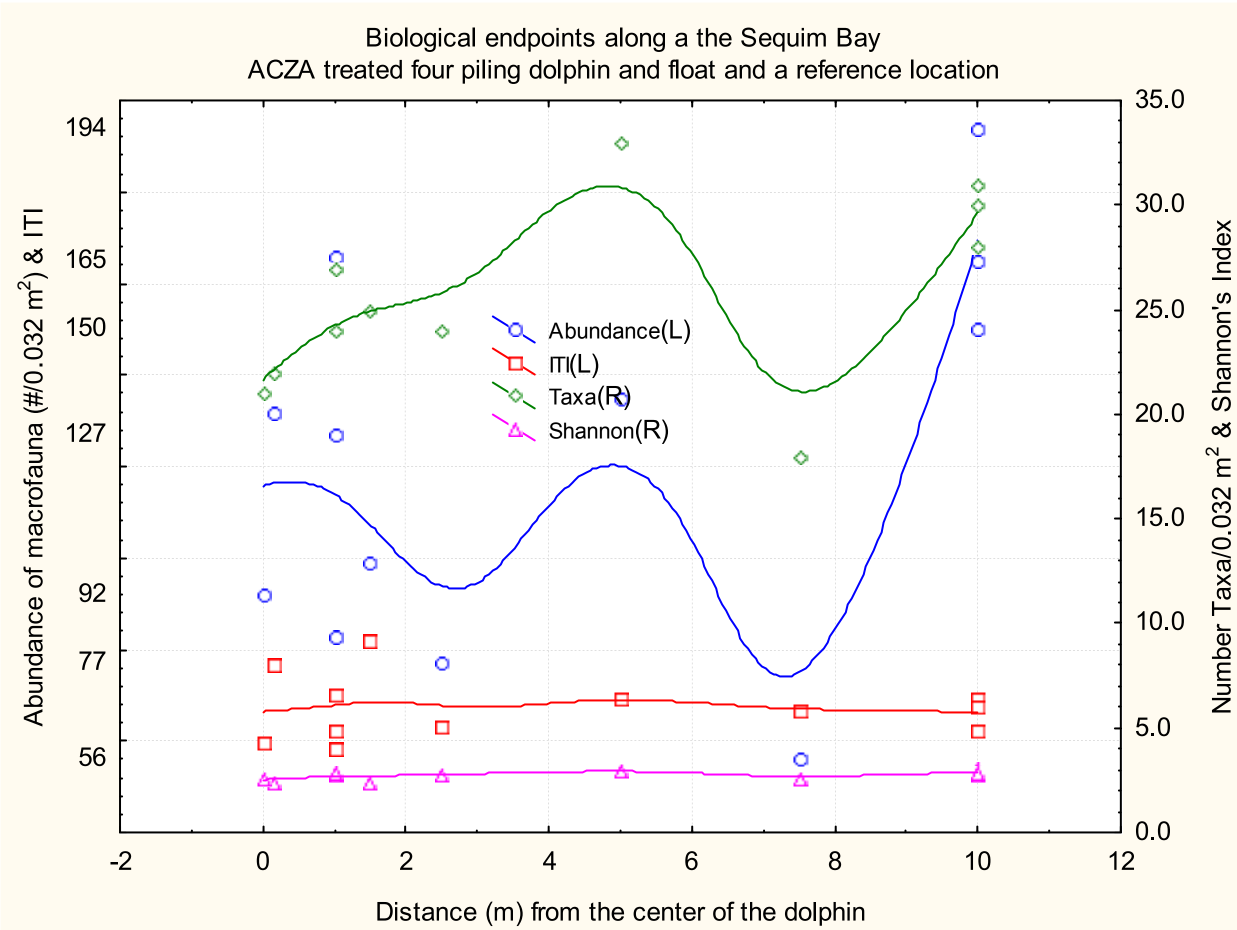

Figure 10. Biological endpoints as a function of distance from a four piling dolphin and float constructed of ACZA treated piling in Sequim Bay, Washington

Table 16. Summary statistics describing sediment sulfide concentrations and macrofaunal abundance, number of taxa, Shannon's Index and the ITI in 0.032 m^2 samples collected along a transect running north from the center of a four piling dolphin and float constructed of ACZA preserved wood and at a reference location.

Breakdown Table of Descriptive Statistics (Sequim Float)
N=12 (No missing data in dep. var. list)

Transect	Sulfide Means	Confidence +95.000%	Abundance Means	Confidence +95.000%	Taxa Means	Confidence +95.000%	Shannon Means	ITI Means	N
Float transect	57.011	69.724	107	134	24	27	2.690	67.624	9
Control Float	77.300	171.285	170	225	30	33	2.910	66.329	3
All Grps	62.083	76.894	123	150	26	28	2.745	67.300	12

Table 17. Taxa found in an abundance ≥ 1% of the total abundance along the transect running north from the center of a four piling dolphin and float treated with ACZA preservative in Sequim Bay, Washington and at the float's reference station. Values are numbers/0.032 m^2 sample. Values at 0.5 m and at the reference stations (100 m) are the mean of three replicate 0.032 m^2 samples.

Species/distance	0.2	0.5	1	1.5	2.5	5	7.5	100	Total
Owenia sp.	46	15	5	41	4	16	5	16	211
Mysella tumida	14	15	15	3	12	5	11	9	133
Nereis sp.	3	9	23	3	12	17	1	11	119
Macoma secta	13	6	3	0	0	15	6	19	113
Alvania sp.	4	7	6	8	4	8	3	19	110
Alia caurinata	2	6	7	3	6	6	4	9	72
Protothaca staminea	5	8	0	3	2	1	0	9	60
Alia gaussipata	3	4	5	1	3	2	3	10	60
Clinocardium sp.	7	2	0	4	2	1	2	12	58
Spiochaeptoteridae sp.	0	4	3	0	0	5	5	9	52
Goniada sp.	3	3	3	4	1	4	3	7	46
Armandia brevis	2	12	2	1	0	0	0	0	41
Pectinaria granulata	0	6	2	1	1	3	0	2	30
Platynereis bicanaliculata	1	1	0	0	0	5	2	6	29
Decamastus gracilis	0	4	0	1	2	8	0	0	23
Aoroides sp.	0	1	6	2	4	1	0	0	17
Photis sp.	0	1	0	1	4	4	0	1	16
Polydora sp.	2	1	0	4	0	1	0	2	15
Polydora kempi	0	2	0	4	1	0	0	1	12
Cooperilla subdiaphana	4	0	0	0	0	0	0	2	11
Macoma nasuta	2	1	0	0	0	1	1	1	11

The significance of differences between mean values in samples collected at 0.5 m near the center of the ACZA preserved dolphin and the reference location is explored using a two tailed *t-test* with $\alpha = 0.05$ in Table 18. Only the number of taxa was significantly different ($t = -3.48$; p = 0.025)

Table 18. Results of *t-tests* assessing the significance of differences in four biological endpoints in triplicate samples collected 0.5 m from the center of a four piling ACZA treated dolphin and at a local reference station in Sequim Bay, Washington. Abundance and number of taxa were transformed ($Log_{10}(N + 1)$ prior to the analysis. N = 3 at both stations.

	T-tests; Grouping: Distance (Sequim Float) Group 1: 0.5 m Group 2: 100 m (reference)						
Variable	Mean 0.5 m	Mean Reference	t-value	df	p	F-ratio Variances	p Variances
Sulfide	67.667	77.300	-0.409	4	0.703	6.199	0.278
Shannon	2.825	2.910	-0.878	4	0.429	4.489	0.364
ITI	63.636	66.329	-0.667	4	0.541	2.696	0.541
Abundance	2.085	2.230	-1.563	4	0.193	7.183	0.244
Number Taxa	1.414	1.486	-3.479	4	0.025	1.696	0.742

Even though the abundance values were not significantly different between the 0.5 m station and the control, there was an apparent trend toward increasing abundance and number of taxa with distance from the dolphin and float. Possible relationships between physicochemical and biological endpoints were explored using Pearson Correlation Coefficients in Table 19.

Table 19. Pearson coefficients describing the correlation between taxa and their summary statistics observed along the Sequim Bay float and its reference station. Only those variables for which at least one significant (α = 0.05) coefficient were found are reported.

	Correlations (Sequim Bay Float) Marked correlations are significant at p < .05000 N=12 (Casewise deletion of missing data)									
Variable	Distance	As	Cu	Zn	Gravel	Sand	Fines	TVS	Sulfide	Redox
Armandia brevis	-0.67	0.51	0.70	0.76	0.82	-0.88	0.58	0.50	0.16	0.22
Decamastus gracilis	-0.42	-0.16	0.03	0.14	0.68	-0.48	-0.07	0.01	-0.19	0.20
Eteone longa	-0.12	-0.60	-0.27	-0.30	-0.26	0.45	-0.54	-0.51	-0.26	0.32
Hesionidae	0.64	0.31	-0.22	0.24	-0.21	0.17	-0.03	0.43	0.21	-0.48
Maldanidae	-0.27	0.11	0.19	0.20	0.57	-0.61	0.30	0.18	0.01	0.25
Nephtys sp.	0.22	0.00	0.03	-0.12	-0.29	0.31	-0.23	-0.17	0.04	-0.58
Prionospio steenstrupi	0.61	0.14	-0.32	-0.19	-0.39	0.26	0.01	0.35	0.07	0.17
Platynereis bicanaliculata	0.74	0.24	-0.33	-0.17	-0.24	0.21	-0.11	0.27	0.12	-0.61
Polydora kempi	-0.32	-0.07	-0.01	0.03	0.19	-0.21	0.09	0.17	-0.39	0.61
Spio buttleri	0.35	0.51	-0.16	0.14	0.07	-0.11	0.08	0.33	0.55	-0.67
Spionidae	0.49	0.12	-0.06	-0.07	-0.22	0.28	-0.29	-0.08	0.40	-0.83
Spiochaetopterus sp.	0.66	0.43	-0.27	0.27	-0.24	0.00	0.33	0.45	0.25	-0.54
Syllis spongiphila	-0.27	0.31	0.45	0.78	0.25	-0.27	0.25	0.40	-0.09	0.00
Axinopsida serricata	-0.27	0.11	0.19	0.20	0.57	-0.61	0.30	0.18	0.01	0.25
Clinocardium nuttallii	0.60	-0.05	-0.14	-0.27	-0.47	0.48	-0.36	-0.03	0.05	-0.17
Macoma inquinata	-0.30	-0.19	0.68	-0.04	0.17	0.10	-0.42	-0.41	0.16	-0.06
Modiolus sp.	-0.30	-0.19	0.68	-0.04	0.17	0.10	-0.42	-0.41	0.16	-0.06
Tellina modesta	0.40	0.43	-0.23	0.06	0.05	-0.05	-0.00	0.26	0.60	-0.65
Arctomelon stearnsii	0.45	0.21	-0.17	0.05	-0.29	0.19	0.00	0.12	0.12	-0.72
Alia gaussipauta	0.58	0.55	-0.10	0.22	-0.24	-0.01	0.34	0.63	0.37	-0.17
Margarites pupillus	0.66	0.39	-0.29	-0.03	-0.43	0.28	0.01	0.15	0.65	-0.65
Opisthobranchiata	0.86	-0.25	-0.74	-0.50	-0.64	0.64	-0.42	-0.20	-0.08	-0.45
Volutidae	-0.27	0.11	0.19	0.20	0.57	-0.61	0.30	0.18	0.01	0.25
Unidentified Crustacea	0.45	0.21	-0.17	0.05	-0.29	0.19	0.00	0.12	0.12	-0.72
Leptochaelia savignyi	-0.27	0.31	0.45	0.78	0.25	-0.27	0.25	0.40	-0.09	0.00
Cirripedia	-0.40	0.06	0.73	0.10	0.35	-0.12	-0.25	-0.19	0.29	-0.06
Hemigrapsus oregonensis	-0.38	0.39	0.17	0.25	0.29	-0.47	0.59	0.36	0.28	0.26
Cancer gracilis	0.69	0.03	-0.46	-0.18	-0.22	0.27	-0.25	0.10	0.14	-0.60
Abundance	0.41	0.26	0.05	0.17	0.06	-0.09	0.02	0.42	0.24	-0.17
Taxa	0.46	0.06	-0.34	-0.05	0.01	0.07	-0.21	0.27	0.11	-0.25
Shannon	0.46	0.31	-0.32	0.23	0.11	-0.16	0.09	0.48	0.11	-0.40
Pielou	0.25	0.35	-0.16	0.32	0.12	-0.27	0.32	0.39	-0.00	-0.35
ITI	-0.07	-0.34	0.14	-0.24	-0.20	0.41	-0.51	-0.48	-0.17	-0.20
Transformed Abundance	0.30	0.27	0.12	0.22	0.13	-0.12	0.00	0.40	0.30	-0.15
Transformed Taxa	0.41	0.08	-0.30	-0.02	0.03	0.05	-0.20	0.28	0.15	-0.21

Significant negative correlations were observed between the annelid *Eteone longa* and sediment concentrations of arsenic and between Opistobranch mollusks and sediment copper. In addition to the two negative correlations, there are seven positive correlations between individual taxa and sediment concentrations of arsenic, copper or zinc. Of the 105 coefficients relating biological endpoints to sediment concentrations of metal, there were 2 significant negative and 7 significant positive. Of the total 37% were negative and 63% were positive indicating increased abundance or value with increasing concentrations of at least one of the metals. These data do not suggest any adverse effects associated with concentrations of arsenic, copper or zinc in Sequim Bay sediments.

None of the summary metrics were significantly correlated with any of the physicochemical endpoints. More negative correlations were associated with redox potential (9) than with any other physicochemical endpoint. These negative correlations indicate increasing abundance or value with decreasing redox potential. That result is not expected in productive environments, but is more indicative of areas where there is marginally reduced food (TVS). That possibility is supported by the positive (but not statistically significant) correlations between abundance and number of taxa with TVS and sulfide. Free sediment sulfide concentrations generally increase with increasing TVS (food), which increases biological oxygen demand and decreases redox potential. The float transect was located on the northern side of the structure and shaded the benthos along this entire transect. This shading could have reduced the production of benthic diatoms – a significant source of organic carbon in shallow benthic environments.

The differences in biological endpoints along the Float Transect were small and this study was not designed to assess sources of organic carbon to the benthos or of benthic primary productivity. Figure 11 compares sediment TVS and redox potential with the abundance of macrofauna and number of taxa. Goyette and Brooks (1998, 2001) and Brooks (2004) have described the epibenthic communities resident on creosote treated piling in the Pacific Northwest. These reports also described increases free sediment sulfides and decreased redox potential near the base of treated wood structures created by biodeposits from the fouling community, which enrich sediments.

High sediment sulfide and TVS concentrations were reported at the 0.5 m (1,870 μM $S^{=}$ and 6.090 % TOC) and 1.0 m (500.8 μM $S^{=}$ and 6.180% TOC) stations located within the footprint of a similar dolphin located at Fort Ward (see Figure 5). Note that Striplin Environmental Associates (1996) reported mean percent TOC in sediments containing 0 to 20% fines and water depths <150’ that varied between 0.14% in the northern parts of Puget Sound to 0.37% in southern Puget Sound. The TVS values reported at this site were converted to Total Organic Carbon (see Brooks and Mahnken, 2003a). A mean TOC concentration of 1.04% was observed at the 0.5 m station in Sequim Bay. This value is over seven times higher than the mean value for Northern Puget Sound, but less than reported for sediments around the highly fouled Fort Ward piling. The beginnings of an epibenthic community of mussels, barnacles and other undetermined invertebrates had settled on the Sequim Bay piling, but it was not as well developed as the communities reported by Brooks (2004). Biodeposits from this nascent community likely increased TOC near the dolphin in comparison with similar Puget Sound sediments. However, this newly established epibenthic community did not enrich the benthos to the degree seen at the older Fort Ward wharf. One interpretation of the TVS and sulfide record summarized in Figure 11 is that TVS within the footprint of the dolphin (i.e. distances $\leq$ 1.0 m) was enhanced by biodeposits from the piling’s epibenthic community to values equaling those found at the reference station. Sediments just north of the float and dolphin were shaded and this may have reduced benthic primary production, resulting in TVS concentrations that were less than those observed at the un-shaded reference location. The sulfide record reflects the labile nature of the animal waste associated with the dolphin's biodeposits and the lower BOD associated with plant detritus, which likely dominated organic carbon at the reference location. All of the differences seen in Figure 10 are subtle. However, there was a small but consistent reduction in the number of taxa where sulfide concentrations were increased and vice-versa. Highest abundances were observed where TVS was highest and somewhat reduced within the area shaded by the float where TVS was reduced.

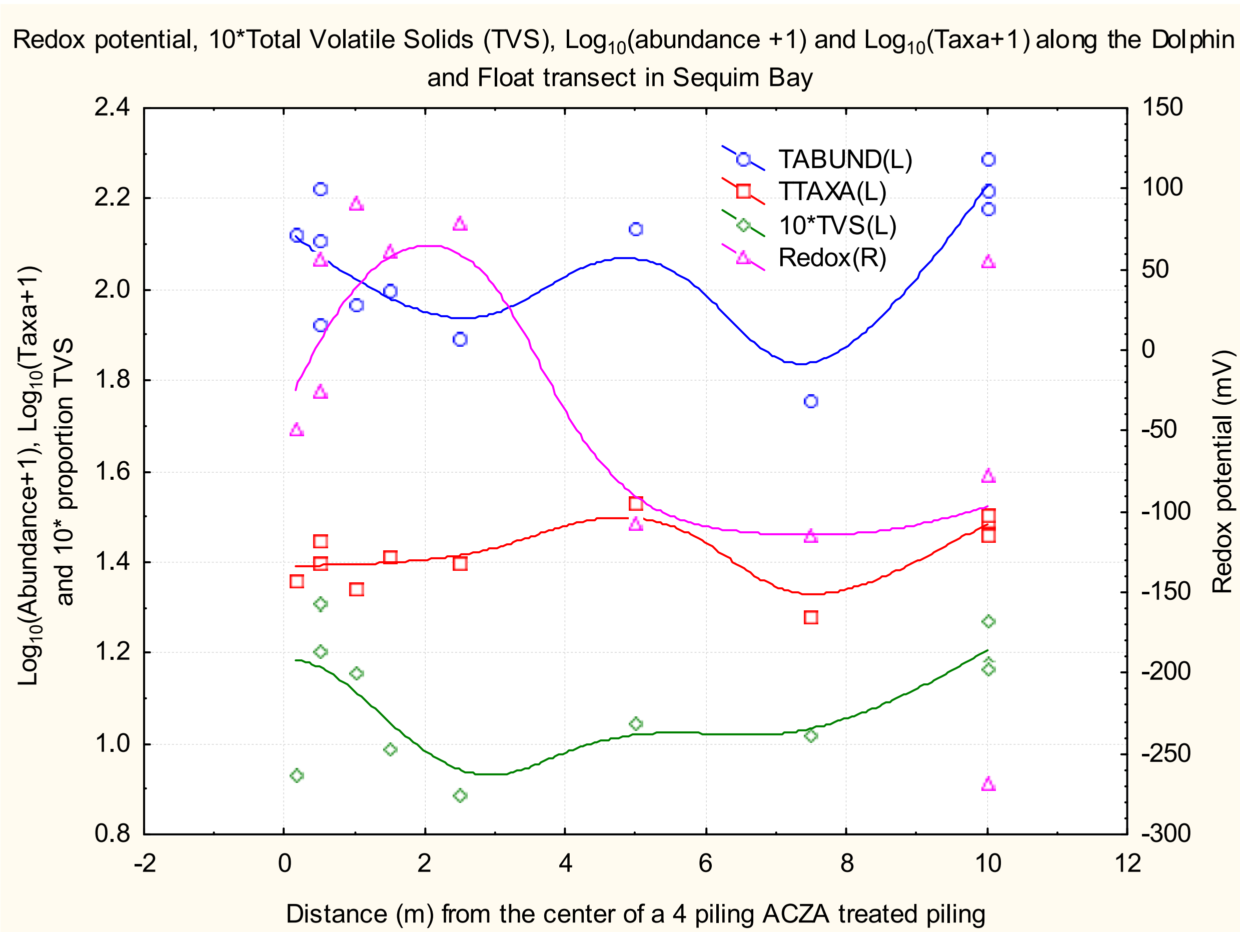

Figure 11. Redox potential (mV), 10 times the proportion sediment arcsin(sqrt) transformed TVS concentrations, Log_{10}(Abundance+1) and Log_{10}(Taxa+1) as a function of distance (meters) from the center of a four piling ACZA treated dolphin and float in Sequim Bay, Washington.

The bottom line in this analysis is that small, but not statistically significant, changes in the macrobenthic community were observed at this site. More taxa were positively correlated with sediment concentrations of metals lost from ACZA treated wood than were negatively correlated. While this study was not designed to determine cause and affect relationships and benthic primary production was not measured; a hypothesis has been developed that suggests that the subtle changes in the macrobenthic community were likely associated with biodeposits from the pilings' epibenthic community and with reduced primary production associated with shading by the float.

Summary. Metal concentrations in water and sediments from intertidal and subtidal areas of Sequim Bay adjacent to substantial ACZA preserved wood structures were determined and compared with sediment and water quality criteria and with macrobenthic communities near the structures and at local reference stations. To assess the potential for the bioconcentration of metals lost from ACZA and movement through the food chain, samples of clams and mussels growing on or as close to ACZA treated wood as possible were analyzed for arsenic, copper and zinc. Additional sediment samples were collected under marine structures decked with ACZA preserved wood at the Port Townsend City and Fort Warden piers. In all instances, samples were collected at locations where the highest concentrations of ACZA metals were expected to be found. In that sense, these are worst case assessments. The results indicate the following:

- Sediments at the Sequim Bay site revealed above average sediment concentrations of TVS and sulfide and reduced redox potential, particularly in the eelgrass meadow. The abundance of macrofauna was as high or higher in both areas as is than found in other areas of Puget Sound. Macrobenthic communities were different at the intertidal site located in an eelgrass meadow when compared with the subtidal site located outside the meadow. More bivalves, particularly native littleneck clams, were found in the intertidal, whereas more crustaceans and annelids were present in the subtidal area.

- Significantly more copper (13.9 μg Cu/g dry sediment) was observed in sediments from the center of the four piling dolphin supporting the float when compared with values at the subtidal reference (6.4 μg/g). However, the highest concentrations were a small proportion of Washington State's copper SQC of 390 μg/g. Significant differences in sediment copper were not observed between sediments collected within 0.3 m of ACZA treated structures and their reference locations at the other three locations examined in this study. All of the sediment copper values measured in this study were well below Washington State's SQC.

- Sediment concentrations of arsenic were all less than 3.7 μg As/g in Sequim Bay representing less than 10% of the 57 μg As/g Washington State SQC. Slighter higher concentrations of <7.44 μg/g were observed at Port Townsend and Fort Ward. The only statistically significant difference in sediment arsenic was the lower concentration at Port Townsend's reference station (0.49 μg/g) in comparison with all other stations in Port Townsend or Fort Ward.

- Sediment zinc was not significantly different between those stations closest to ACZA treated wood (20.8 to 32.4 μg/g) and reference locations in Sequim Bay (18.8 to 24.9 μg/g). Sediment zinc was significantly higher under the Port Townsend City pier (45.6 μg/g) when compared with the local reference station (22.8 μg/g). No other significant differences in sediment zinc were observed between paired treatment and reference locations. All of the observed zinc concentrations were well below Washington State's SQC of 410 μg Zn/g.

- Water column concentrations of arsenic, copper and zinc were not significantly different at the two Sequim Bay locations adjacent to ACZA treated structures in comparison with the local reference station and all of the metals were below their respective U.S. EPA water quality criteria. These samples were collected at slack tide during a light rain and likely represent the maximum concentrations observed at the site three years following construction. Highest metal concentrations would occur within a few day following construction and the long term concentrations observed in December 2002 were likely achieved by the end of the first week post construction.

- Tissue concentrations of arsenic, copper and zinc from mussels growing on ACZA treated wood and within half a meter of the base of an ACZA treated piling were not significantly increased above metals in the same species retrieved from reference areas. Zinc in mussels taken directly from ACZA treated piling was significantly **less** than found in mussels from the reference area. Arsenic concentrations in all samples (0.96 to 3.13 μg As/g wet tissue) were well below the Food and Drug Administration level of concern of 130 μg As/g wet tissue.

- As many or more macrofauna were found near the ACZA treated structures than were found at the local reference areas. The abundance of macrofauna, particularly native littleneck clams were nearly three times higher immediately adjacent to the ACZA treated piling than are generally found in similar Puget Sound sediments or at the intertidal control station in Sequim Bay. However, while the difference was significant at $\alpha = 0.1$, it was not significant at $\alpha = 0.05$. Significantly fewer taxa were found in the center of the float's four piling support dolphin when compared with the subtidal reference station. Significant differences were not found in macrofaunal abundance or in Shannon's Index or the Infaunal Trophic Index.

- Correlation analysis suggested that the subtle differences in biological end points near the float were not associated with metal concentrations in sediments. One hypothesis supported by the studies evidence is that shading by the float has reduced benthic diatom production leading to reduced TVS north of the float and therefore reduced macrobenthic production. However, it should be emphasized that the study was not designed to establish cause and effect relationships and that direct measurements of benthic pigments (chlorophyll, etc.) were not made.

In summary, the results of this study indicate very small, and in most cases, insignificant increases in either water or sediment concentrations of arsenic, copper or zinc associated with the use of ACZA preserved wood in Pacific Northwest marine environments. The evidence indicates minimal organismal uptake of these metals in close proximity to treated wood and very little or no potential for movement of these metals into the food chain. Arsenic concentrations in mussels growing directly on ACZA treated wood and in clams retrieved from within half a meter of ACZA treated piling were a small fraction of the FDA level of concern for arsenic in mollusks. Neither of the structures had any significant overall effect on macrobenthic communities resident as close as 0.3 m from ACZA treated wood. Subtle differences in the macrobenthic community north of the float appear to be associated with some factor other than sedimented metals and it is hypothesized that shading from the float may be the cause. The reason for the increased macrofaunal abundance near the pier's ACZA piling was not investigated. Overall, this study did not find any adverse effects associated with the use of ACZA preserved wood in the Pacific Northwest marine environments studied.

References

American Wood-Preservers' Association – Standards 2001. Published by the American Wood-Preservers' Association, P.O. Box 5690, Granbury, Texas 76049. 518 pp.

Brooks, K.M. 1997. Literature Review and Assessment of the Environmental Risks Associated with the use of ACZA treated wood products in aquatic environments. Produced for the Western Wood Preservers Association, 7017 N.E. Highway 99, Suite 108, Vancouver, WA 98665. 63 pp.

Brooks, K.M. 1999. Environmental effects associated with the use of CCA-C, ACZA and ACQ-B pressure treated wood used to construct boardwalks in wetland areas. U.S. Department of Agriculture - Forest Products Journal. 85 pp. plus appendices.

Brooks, K.M. 2001. An evaluation of the relationship between salmon farm biomass, organic inputs to sediments, physicochemical changes associated with those inputs and the infaunal response – with emphasis on total sediment sulfides, total volatile solids, and oxidation-reduction potential as surrogate endpoints for biological monitoring – Final Report. Technical report produced for the Technical Advisory Group (TAG) to the British Columbia Ministry of Environment, 2080-A Labieux Road, Nanaimo, British Columbia, Canada V9T 6J9. 186 pp. plus appendices.

Brooks, K.M. and C.V.W. Mahnken. 2003a. Interactions of Atlantic salmon in the Pacific northwest environment, II. Organic wastes. Fisheries Research 62, pp. 255-293.

Brooks, K.M. and C.V.W. Mahnken. 2003b. Interactions of Atlantic salmon in the Pacific Northwest Environment. III. Inorganic Compounds. Fisheries Research, Vol. 62, Issue 3, pp. 295-305.

Brooks, K.M. 2004. Environmental response to creosote treated wood structures in Puget Sound, Washington. Produced for Creosote Council II, 357 Browns Hill Road, Box 160, Valencia, PA 16059. 53 pp.

FDA, 1993. Guidance Document for Arsenic in Shellfish. Center for Food Safety and Applied Nutrition. United States Food and Drug Administration, 200 C St. S.W., Washington, D.C. 20204.

Goyette, D. and K. Brooks. 1998. Creosote Evaluation: Phase II – Sooke Basin Study – Baseline to 535 Days Post Construction (1995 – 1996). Regional Program Report PR00-03, Environment Canada, 224 West Esplanade, North Vancouver, British Columbia, Canada V7M 3H7. 563 pp.

Goyette, D. and K. M. Brooks. 2001. Continuation of the Sooke Basin Creosote Evaluation Study (Goyette and Brooks, 1998). Year Four – Day 1360 & Day 1540. Regional Program Report PR00-03, Environment Canada, 224 West Esplanade, North Vancouver, British Columbia, Canada V7M 3H7. 74 pp.

Plumb, R. H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers, Vicksburg, MS.

PSEP. 1986. Puget Sound Protocols. Prepared for U.S. Environmental Protection Agency, Region 10, Seattle, WA.

Striplin Environmental Associates, Inc. 1996. Final Report – Development of Reference Value Ranges for Benthic Infauna Assessment Endpoints in Puget Sound. Prepared for the Washington State Department of Ecology, Sediment Management Unit, P.O. Box 47600, Olympia, Washington 98504-7600. SEA Contract No. A94-01. 45 pp. plus appendices.