



REGIONAL
MONITORING
PROGRAM

Exposure and
Effects Pilot
Study

The Influence of Chemical and Physical Factors on Macrobenthos in the San Francisco Estuary

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Executive Summary

The objective of this study was to test a method for benthic stressor identification in the San Francisco Estuary. Benthic assessments often identify samples that are impacted for various reasons (*e.g.*, organic enrichment, disturbance, or contamination), but few studies have statistically associated specific contaminants or mixtures, with apparent impact on the benthos.

The sediment data used in this study were collected during several surveys of the Estuary. A common subset of variables that included trace metal and organic sediment contaminants, the mean ERM (effects range medium) quotient, salinity, total organic carbon, and fine sediments (< 63µm) were evaluated. The focus of this study was locations where benthic impacts have been previously identified using the San Francisco Estuary benthic assessment value (Thompson and Lowe 2004). The individual benthic metrics identified by Thompson and Lowe (2004) for calculation of assessment values were used as dependent variables. The areas selected for evaluation were those with a range in assessment metrics to optimize the likelihood of identifying relationships to chemical and/or physical factors. Six Estuary areas were identified as having adequate sample sizes for benthos and chemistry; Richmond Harbor, San Leandro Bay, San Pablo Bay Marshes, CCSF Wastewater Discharge Area, North Bay, and South Bay. A combination of correlation, multiple regression, and principle components analysis were used to evaluate each area.

Sediment contamination and other environmental factors (*i.e.*, salinity and/or sediment-type) were indicated as key factors in many (11 of 29, 38%) of the evaluations. The relative importance of chemical and physical factors was identical in these samples. In other evaluations, slight shifts in salinity or sediment-type appeared to strongly affect several benthic metrics, obscuring obvious correlations to sediment contaminants. This was most apparent in the San Pablo Bay Marshes, CCSF, and the North Bay. Sediment contamination alone was indicated as the key factor associated with benthic impacts in 6 of 29 (21%) evaluations. Richmond Harbor exhibited the most consistent results, as all five benthic metrics exceeded their respective reference ranges at many sites, and multivariate analysis strongly suggested benthic impact due to DDTs and dieldrin. Sediment contamination also appeared to be the key factor in several metrics from San Leandro Bay and San Pablo Bay Marshes. In Richmond Harbor and San Leandro Bay where contamination was an obvious key factor, effect thresholds were estimated.

Owing to the inherent covariation between sediment contaminants, identification of specific individual contaminants was generally not possible. However, where contaminants had a strong influence, they formed independent patterns that corresponded with benthic changes, such as in Richmond Harbor and San Leandro Bay. Mixtures of several sediment contaminants were shown to have influence on the benthos at 17 of 29 (58%) evaluations, and were significant model components in about half of them. These Estuary area evaluations suggest that benthic impacts are likely due to the interactions of sediment contaminants and environmental factors. The contaminant mixtures and thresholds identified are considered hypotheses that should be tested further. Understanding the causes of benthic impacts will provide for focused management actions in the future.

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INTRODUCTION

Macrobenthic samples have been widely used to assess sediment condition (Canfield *et al.* 1994, Hyland *et al.* 1999, Thompson *et al.* 2000). Benthic assessments often identify samples that are impacted for various reasons (*e.g.*, organic enrichment, disturbance, or contamination), but few studies have statistically associated specific contaminants or mixtures, with apparent impact on the benthos. Numerous benthic metrics are commonly used in assessments, and impacts to any single metric could be due to combinations of chemical and physical environmental factors, making the identification of them complicated.

The State of California is in the process of adopting regulatory sediment quality objectives (SQOs) for selected California bays and estuaries, which are scheduled for implementation in 2008. The SQO target resource for aquatic life protection is benthic macrofauna. SQOs will be based on extensive analysis and testing of a large sediment database for California bays that uses a co-occurrence and correlation among sediment contamination, toxicity, and benthos. The SQOs will provide a weight-of-evidence assessment of the degree of impact at a site. Although SQOs will indicate whether benthic impacts co-occur with elevated sediment contamination, they will not identify specific contaminants or mixtures of contaminants that could be causing any observed benthic impacts. The State Board's SQO Scoping Document (SWRCB 2006) suggests that additional stressor identification studies would be required to identify the specific cause of benthic impacts, and to initiate appropriate and effective management actions (*e.g.*, clean-up and loading reduction). Such studies will be recommended as part of the implementation plan for SQOs. Stressor identification studies for benthos are analogous to Toxicity Identification Evaluation (TIE) process for toxicity tests. However, no specific methods for benthos have been developed. The objective of this study was to test one method for benthic stressor identification in the San Francisco Estuary.

Conceptual Models of Benthic Response

Macrobenthos are expected to respond to gradients of sediment variables. Pearson and Rosenberg (1978) originally proposed a model for the response of macrobenthos to organic enrichment, which has recently been expanded for sediment contamination by Thompson and Lowe (2004). The benthic response curve is non-linear with intermediate concentrations resulting in elevated numbers (above reference values) of total number of taxa, abundances and tolerant taxa (Figure 1). These benthic metrics are assumed to respond positively as contaminant concentration increases until a biological threshold is reached. Conversely, sensitive taxa would begin to decrease as threshold values are exceeded. Above thresholds, levels of benthic metrics are expected to decline, eventually decreasing to zero survival in conditions of very high contamination, organic enrichment, or disturbance.

One difficulty in assessing the response of benthic indicators is identifying the position along the response curve that test samples correspond. Analyses of benthic response to organic enrichment and sediment contamination must account for the non-linearity of benthic responses. However, most datasets do not span the complete range of organic enrichment or contamination along the response curve. Subsequently, datasets commonly do not result in a strict curvilinear response. The response may be close to linear, or only slightly curvilinear. Since most analytical methods use linear correlations and regression, variable transformations are often

performed. Analyses of benthic response to sediment contamination should, therefore, include consideration of where on the conceptual response curve the samples fall.

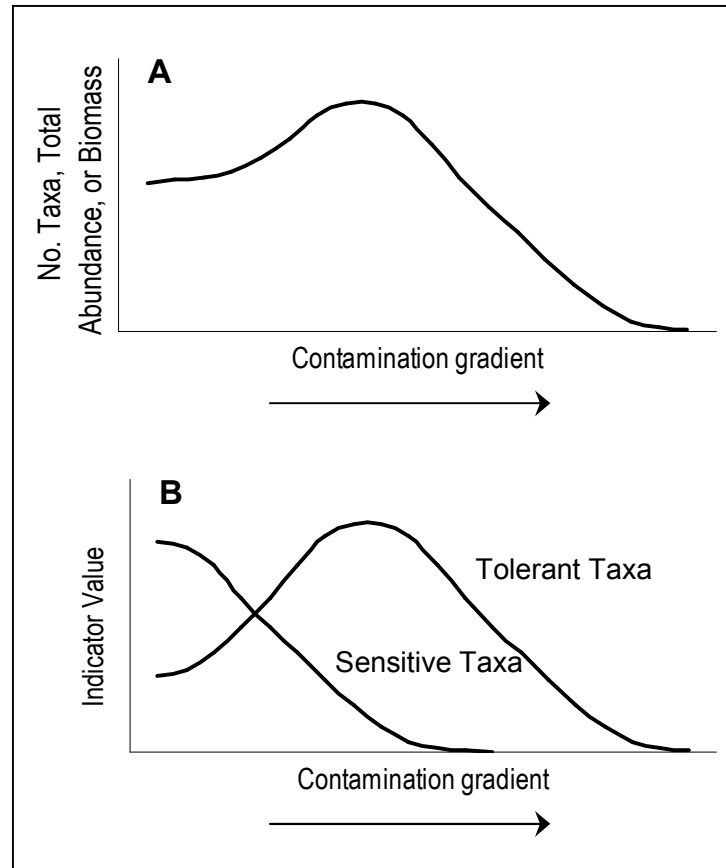


Figure 1. Conceptual models of benthic response to sediment contamination. (A) Generalized response for total number of taxa, total abundance, and higher taxon indicators. (B) Generalized response for contamination-sensitive and tolerant taxa. From Thompson and Lowe (2004).

METHODS

Data Used. The data used in this study were collected during several surveys in the Estuary (Table 1). Benthic samples were standardized to a 0.05 m² sampler-size, and were all screened through a 0.5 mm sieve.

Table 1. Sources of data used in this study. SFEI RMP is the San Francisco Estuary Institute's Regional Monitoring Program for Water Quality, BACWA LEMP is the Bay Area Clean Water Association's Local Effects Monitoring Program, DWR is the Department of Water Resources, BPTCP is the Bay Protection and Toxic Clean-up Program, EMAP is the Environmental Monitoring and Assessment Program, and CISNet was a multi-agency grant through UC Davis.

Program	Dates	Reference
SFEI RMP	1994-2000	Thompson <i>et al.</i> (2000); Thompson and Lowe (2004)
Richmond Harbor	1991	Ferraro and Cole (1997); Swartz <i>et al.</i> (1994).
BACWA LEMP	1994-97	Thompson <i>et al.</i> (1999b)
DWR	1994, 1996-97	DWR (1997)
BPTCP	1992, 1994, 1997	Hunt <i>et al.</i> (2001)
EMAP-NOAA	2000	<i>Not Available</i>
EPA CISNet	2000-01	Thompson and Lowe (2003)

Chemical and physical variables. A common subset of the contaminants measured by the Regional Monitoring Program for Water Quality (RMP) and other surveys (Table 1) was selected for use in this study (Table 2). This list includes trace metal and organic sediment contaminants, the mean ERM quotient (mERMq, an additive sediment contamination index; Long *et al.* 1995), salinity, total organic carbon (TOC), and fine sediments (< 63µm). Missing data were estimated by interpolation when one or two values were missing. Samples with more than two missing values were excluded from the analysis. Therefore, a major constraint of this study was the limited list of physical and chemical variables available for evaluation at each area. Certainly other contaminants may potentially cause benthic impacts, but they were not commonly measured at all sites. In particular, many pesticides (organochlorines and pyrethroids) have not been routinely measured in sediments.

Table 2. Chemical and physical variables evaluated in this study.

Trace Metals	Trace Organics	Other Variables
Silver (Ag)	Total DDTs	Salinity (psu)
Arsenic (As)	Total PCBs	TOC (%)
Cadmium (Cd)	Total Chlordanes	Fine sediment (% <63µm)
Chromium (Cr)	Total Low Molecular Weight PAHs	mERMq
Copper (Cu)	Total High Molecular Weight PAHs	
Mercury (Hg)		
Nickel (Ni)		
Lead (Pb)		
Zinc (Zn)		

Benthic Variables. This study focused on areas where benthic impacts have been previously identified using the San Francisco Estuary benthic assessment value (AV; Thompson and Lowe 2004). The AV is a numeric variable ranging between 0 – 5 that indicates the severity of apparent benthic impact. It is based on a multi-metric index of biotic integrity (IBI) method that uses four or five benthic metrics (depending on assemblage) to determine the AV, and it is intended to provide an evaluation of the condition of the benthos. However, since AVs are intended for general assessments, and only have a small range of values, they were not considered to be appropriate benthic endpoints for the analyses used in this paper. Instead, the individual benthic metrics identified by Thompson and Lowe (2004) for calculation of AVs were used (Table 3). These metrics are used in the assessment framework described below to indicate benthic impacts in the Estuary. The only area where these metrics could not be applied consistently was Richmond Harbor. Amphipod abundance (excluding *Grandidierella japonica*) was examined in lieu of *Streblospio benedicti* abundance, due to the low frequency of the polychaete. The amphipod *G. japonica* was excluded from the abundance of amphipods due to the evidence for this species being a relatively abundant contaminant-tolerant taxon compared to the other contaminant-sensitive amphipod species present. This metric was also selected by Swartz *et al.* (1994) in evaluation of sediment contamination in Richmond Harbor.

Table 3. Benthic metrics used in the evaluation of six areas of the San Francisco Estuary (From Thompson and Lowe 2004).

Polyhaline Assemblage	Mesohaline Assemblage
Total Number of Taxa	Total Number of Taxa
Total Abundance	Total Abundance
Number of Amphipod taxa	Number of Molluscan taxa
<i>Capitella capitata</i> (<i>C. capitata</i>) Abundance	Oligochaete Abundance
	<i>Streblospio benedicti</i> (<i>S. benedicti</i>) Abundance

Data Evaluation. The areas of the Estuary selected for evaluation were expected to have different physical and contaminant factors that could potentially be influencing the benthos.

However, combining data from an area that may not share common sources of contamination or common influences of salinity or sediment-type could have confounded our attempts to determine possible influences to the benthos. Therefore, data were excluded if they resulted in a different Estuary assemblage classification as defined by Thompson and Lowe (2004), than the remainder of samples from the area.

Selection of Areas. Areas of the Estuary selected for evaluation required a range in assessment metrics (Table 3) to optimize the likelihood of detecting relationships to chemical and/or physical factors. Since the main focus of this study was to identify contaminants that may be causing benthic impacts, the study areas also needed to have recognizable gradients of sediment contaminants. By examining a dataset with combined benthos and chemistry data, we were able to review the presence of gradients in the Estuary, with known benthic data available for correlations. A data exploration program was developed in the mathematical program Matlab (MathWorks, Natick, MA), which plotted contaminant concentrations on a regional basis. Our *a priori* assumptions were that regions such as Richmond Harbor, San Leandro Bay, Hunters Point, and Southern sloughs near Guadalupe River, may provide the necessary (individual or mixture) contaminant gradients required. The following areas were identified as having adequate sample sizes, a sufficient list of individual contaminant gradients, and were subsequently examined in the Estuary:

- Richmond Harbor (also used to test the analytical methods)
- San Leandro Bay
- San Pablo Bay Marshes
- City and County of San Francisco (CCSF) Wastewater Discharge Area
- North Bay
- South Bay

The raw chemical and benthic data used to evaluate each area are presented in Appendices I and II.

Correlation Analysis. Correlations of benthic variables to contaminants, salinity, and sediment-type, were used to evaluate the general relationships in each area. Correlations were evaluated using a combination of raw and transformed variables (*e.g.*, \log_{10} and arcsin-square-root). Trace organics were also evaluated on a TOC-normalized basis. Transformations were necessary owing to the possible non-linear benthic responses described above, and for subsequent multivariate analyses that used linear models.

Multivariate Analysis. Multivariate analysis was conducted in SAS 9.1 (SAS Institute, 2006) using similar methods as applied to evaluate relationships between sediment toxicity and contamination (Thompson *et al.* 1999a). The data analysis strategy was conducted in two steps. Step 1 used multiple regression analysis to determine the relative contribution of salinity, fine sediments, total organic carbon (TOC), and mERMq to the variation in each benthic metric in each area. The raw and transformed independent variables that were used in each evaluation were those that produced the highest correlations with each benthic metric (dependent variable). This analysis step was conducted to identify the independent variables that appeared to have the most influence on each benthic metric tested.

Step 2 consisted of Principal Components Analysis (PCA) combined with multiple regressions to determine which contaminant factors and physical variables (salinity, fine sediments, TOC) were highly associated with each benthic metric. The selection of the chemical variables to include in each area/metric evaluation included consideration of whether each contaminant was at least above its ERL (effects range low). If below the ERL, the contaminant was excluded because it was considered to be potentially non-causative. This elimination process was important for variable reduction, because including more variables than sites can lead to spurious results. However, in the few circumstances where this situation did occur, our examination of using greater numbers of variables than samples showed that the PCA was robust. Results were identical to when we arbitrarily reduced the number of variables to be less than samples. Therefore, we eliminated as many variables as possible in our PCA analyses without being arbitrary. PCA was applied using varimax rotation for contaminant variables only. Resulting factors with eigenvalues greater than 1 were retained.

The PCA factors were used as estimates of possible covarying contaminants. Multiple regressions using the SAS stepwise selection method was applied to determine which optimized independent variables provided the best model for each metric in each area. PCA factor scores, percent fines, TOC, and salinity were used as independent variables. TOC was not included as an independent variable in the analysis of Richmond Harbor and San Leandro Bay, since it was used to normalize several trace organic contaminants that were PCA factor components. Partial regression coefficients were used to evaluate the relative contributions of those components to the variability of the benthic metric. PCA statistics were not used to make the estimates of benthic stressor thresholds presented.

Interpretation. Sediment contamination variables shown to be significantly related to benthic metrics were further evaluated to assess the possibility that they could be key indicators of benthic impact. When a variable was repeatedly shown to be significantly related to the benthos at an area, and exceeded ERL and ERM values (Appendix III), this weight of evidence was interpreted as strong implication of the variable as a possible cause of benthic impact. In addition, graphical representation of proposed thresholds resulting from this study was compared to other known thresholds to help interpret the results. Specifically, other than the effect ranges for aquatic life (*i.e.*, ERL and ERM), mERMq thresholds for benthic effects in the Estuary have been suggested by Thompson and Lowe (2004). In this study, mERMq above 0.146 was used to reflect probable benthic impact in the Estuary.

RESULTS AND INTERPRETATION

The relationship of several benthic metrics to chemical and physical factors in six areas of the Estuary (Figure 2) is presented separately below.

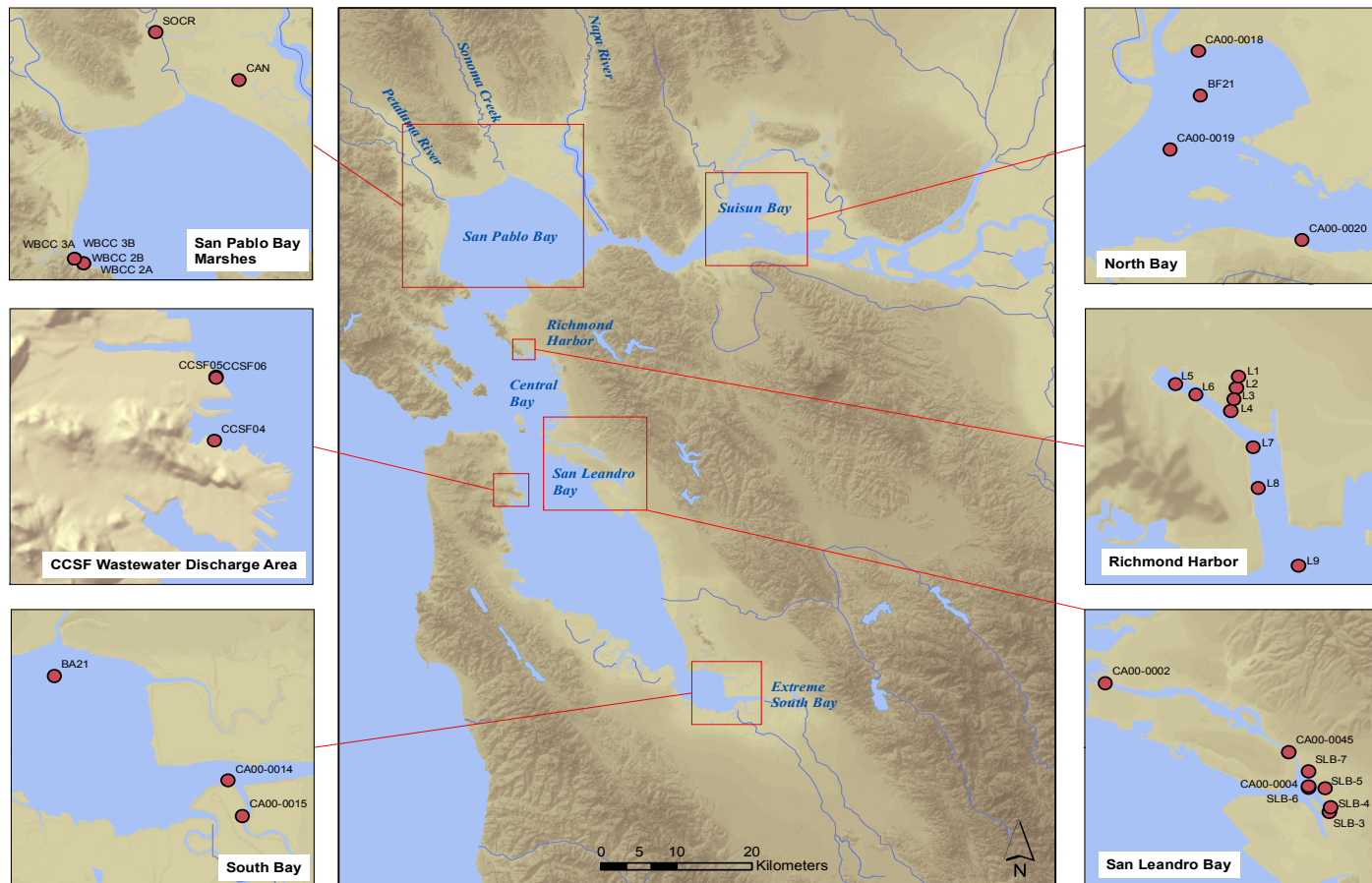


Figure 2. Map of San Francisco Estuary showing areas evaluated in this study.

Richmond Harbor

Richmond Harbor consists of three adjoining channels (Lauritzen, Santa Fe, and Richmond Harbor Channels) connected to San Francisco Bay near Brooks Island (Figure 2). Between 1947 and 1966, DDT and other pesticides were produced on the east bank of the Lauritzen Channel (Levine-Fricke 1990). This area is currently an EPA Superfund site. A strong sediment contamination gradient exists in Lauritzen and other adjoining channels. Nine sites in the channels (and an additional two in the Bay, but not included in our analyses) were sampled for benthos, sediment characteristics, and chemistry by the Environmental Protection Agency (EPA) in October 1991 (Ferraro and Cole 1997). The samples were located along the previously documented DDT contamination gradient in the area (Levine-Fricke 1990). We used these data to test and validate our analytical methods, which were then applied to other areas in the Estuary. We considered it important to identify similar benthic stressors in Richmond Harbor as the EPA team, to add further confidence to results shown in other Estuary areas.

The benthos was consistent with the main estuarine assemblage, dominated by the bivalve, *Theora lubrica*, and numerous species of polychaetes and oligochaetes. However, the opportunistic polychaete, *Streblospio benedicti*, was rare. Nine samples with both benthic and sediment contamination measurements were suitable for our analysis (Figure 2). All sediments were classified as silt, with moderate organic carbon content (Table 4). The salinity was constant at 30 psu, regardless of station location. Sediment contamination among stations varied from low to high, as the AV ranged from 0 to 4 and mERMq from 0.101 – 255. mERMq was highly weighted towards the high DDT concentrations (sum of o'p, and p'p isomers of DDE, DDT, and DDD) found at most locations (Appendix II). mERMq was not included in statistical analyses in Richmond Harbor as only 11 contaminants were measured, instead of the minimum of 24 for calculation of mERMq (Long *et al.* 1995). The pesticides (DDT and dieldrin) were always well above the ERM, while the concentrations for trace metals other than cadmium were often above the ERL. The majority of copper (5 of 9, 55.6%), nickel (7 of 9, 77.8%), and lead (9 of 9, 100%) samples were above the ERL. Zinc (3 of 9, 33.3%) and cadmium (1 of 9, 11.1%) concentrations, however, were not as elevated.

Table 4. General description of Richmond Harbor samples.

Site	Yr-Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq	AV*
L1	1991-10	30	93	2.4	254.6	3
L2	1991-10	30	85	1.8	157.9	4
L3	1991-10	30	86	1.7	88.7	4
L4	1991-10	30	90	1.5	9.3	4
L5	1991-10	30	100	1.5	1.521	3
L6	1991-10	30	79	3	8.959	2
L7	1991-10	30	83	1.1	1.479	3
L8	1991-10	30	95	1.2	0.427	0
L9	1991-10	30	82	0.9	0.101	4

* Assessment values calculated as proposed by Thompson and Lowe (2004).

Correlation analysis showed that two benthic metrics were related to chemical or physical factors in Richmond Harbor (Table 5). Percent fines was correlated to the number of molluscan

taxa, and both DDTs and dieldrin were significantly correlated with amphipod abundance. TOC was not significantly related to any of the benthic metrics. The lack of variability in salinity measurements meant that this factor was excluded from the analysis. The significant correlations indicated that benthic impairment would be likely in at least some benthic metrics, in future analysis steps.

Table 5. Significant correlations in Richmond Harbor (Pearson's r , $p < 0.05$); $n = 9$. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	None
Total Abundance	None
Number of Molluscan Taxa	Fines
Oligochaete Abundance.	None
Amphipod Abundance (excluding <i>G. japonica</i>)	DDTs, dieldrin

Step 1 of the multivariate analysis was not conducted for Richmond Harbor. Both salinity and mERMq values were not appropriate for this analysis, and performing Step 1 without these values would have been an incomplete evaluation of factors related to the benthos in this area.

In the Step 2 analysis, PCA produced three sediment contamination factors, each composed of covarying elements and compounds (Table 6). These factors were consistent for all benthic metrics. The trace metals were separated on two axes (Factors 1 and 3), while DDTs and dieldrin were both in Factor 2. These factors represent three independent sediment contamination patterns among the samples in Richmond Harbor.

Table 6. PCA Factor composition for Total Number of Taxa in Richmond Harbor.

Factor 1	Factor 2	Factor 3
Cu	DDT*	Ni
Pb	Dieldrin*	Cd
Zn		

* Normalized for organic carbon content

Multiple regression analysis revealed that DDT and dieldrin accounted for the majority of the variability in all Richmond Harbor benthic metrics. The contaminants composing PCA Factor 2 (DDT and dieldrin; Table 6) were selected in each benthic metric model run (Table 7). However, these contaminants were only significantly related (74.9%, $p = 0.003$) to oligochaete abundance. Percent fines were included in the final model for total number of taxa, total abundance, and number of molluscan taxa. However, this variable only contributed a significant amount of variation to the number of molluscan taxa (50.4%, $p = 0.03$). PCA Factor 1 (Cu, Pb, Zn; Table 6) was also selected in the molluscan taxa model, but was not a significant variable. These results suggest that the key stressors on benthos in Richmond Harbor are most likely DDTs and dieldrin, with a smaller influence of grain-size on certain metrics.

Table 7. Step 2 multiple regression results for Richmond Harbor; n = 9. Bold = significantly correlated to the benthic metric, $p < 0.05$. L = above ERL and M = above ERM.

Benthic Metric	Independent Variables	R-square	P
Total Number of Taxa	PCA Factor 2: DDT ^M , dieldrin ^M	0.329	0.068
	Fines	0.303	0.107
Total Abundance	PCA Factor 2: DDT ^M , dieldrin ^M	0.29	0.135
	Fines	0.236	0.134
Number of Molluscan taxa	Fines	0.504	0.032
	PCA Factor 2: DDT ^M , dieldrin ^M	0.202	0.089
	PCA Factor 1: Cd ^L , Zn ^L , Pb ^L	0.115	0.134
Oligochaete Abundance	PCA Factor 2: DDT^M , dieldrin^M	0.749	0.0026
Amphipod Abundance (excluding <i>G. japonica</i>)	PCA Factor 2: DDT ^M , dieldrin ^M	0.391	0.072

Apparent Benthic Effects Thresholds. Possible benthic effects thresholds for those sediment contaminants that were significantly associated with benthic metrics were evaluated graphically, using patterns of impact and concentration, and by considering ERL and ERM values for each implicated contaminant. Additionally, Richmond Harbor has been evaluated for benthic effects by Swartz *et al.* (1994) and their proposed thresholds were also considered.

Patterns in benthic impacts were associated with DDTs and dieldrin concentrations, but not with trace metals. This implication was also indicated during previous analyses of this dataset (Ferraro and Cole 1997). DDT concentrations of > 100 ppb (4 of 9 samples, 44%) were always associated with shifts in oligochaete abundance (Figure 3). This value is also well above the ERM of 46.1 ppb. The ERM for dieldrin is 8 ppb, but only three of nine samples (33%) were above concentrations of 3 ppb and they were associated with impact on oligochaete abundance.

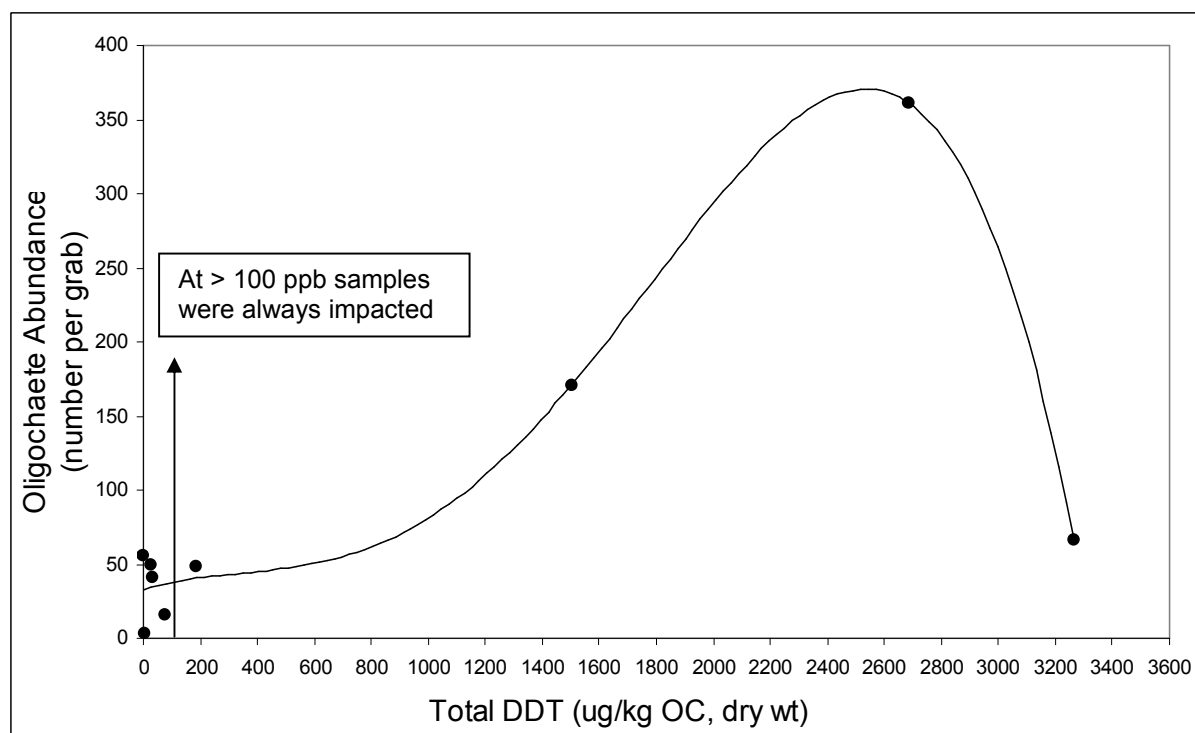


Figure 3. Effects threshold estimates for Richmond Harbor. Curve fit shown for illustrative purposes only.

Summary. Similar results were obtained from our method as summarized for Richmond Harbor by Ferraro and Cole (1997). This provides a validation of our method, and adds confidence to the results shown for other areas examined in this study. DDT and dieldrin accounted for most of the variation in the five benthic metrics evaluated. However, grain-size also contributed, although not significantly, to the variation in total abundance, total species, and number of molluscan taxa. Thresholds were identified using the relationship of sediment contaminants to oligochaete abundance. The abundance of oligochaetes were enhanced at total DDTs concentrations of more than 100 ppb and dieldrin concentrations of more than 3 ppb (Swartz *et al.* 1994).

San Leandro Bay

San Leandro Bay is a small, enclosed embayment located in the eastern Central Bay (Figure 2). It adjoins the Oakland Harbor to the north and outlets to the Central Bay at the south end of Alameda. Five sites were sampled in 1997 by the BPTCP (Hunt *et al.* 2001) and were considered to be slightly impacted. However, benthic assessments conducted by Thompson and Lowe (2004) suggested the benthos at all five sites were severely impacted. Additionally, three samples were collected in San Leandro Bay and Oakland Harbor during the EMAP – NOAA survey in 2000 (Table 23), and were evaluated in this study. The benthos at two of these sites were un-impacted, while the other site was slightly impacted (Ranasinghe *et al.* 2004).

The benthos at the BPTCP sites was consistent with the Estuary margin assemblage (Thompson et al. 2000), dominated by the opportunistic polychaete, *S. benedicti*, the cumacean, *Nippoleucon hinumensis*, and oligochaetes. The benthos was consistent with the mesohaline estuarine assemblage in the NOAA EMAP samples, and was dominated by oligochaetes, amphipods, and the opportunistic polychaete, *Heteromastus* spp. (Virnstein 1979). The mesohaline assemblage metrics were used to evaluate sediment contamination in this area.

Eight samples with both benthic and sediment contamination measurements were available for analysis. The Oakland Harbor sample was included to augment the number of samples available, and to allow inclusion of an un-impacted sample in the analysis. All sediments were characteristic of silty-clays, with elevated TOC content. Sediment contamination (as mERMq) was moderately elevated (Table 8), with values ranging between 0.121 – 0.838. Most of the trace metals had concentrations between the ERL and ERM, and mercury, nickel, and zinc had some concentrations above the ERM (Appendix I). All of the trace organic contaminants had at least one value above the ERM, except total PAHs. The salinity ranged between 24 – 29.1 psu.

Table 8. General description of San Leandro Bay samples.

Site	Yr-Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq	AV*
SLB-3	1997-04	24.0	80.7	3.82	0.378	5
SLB-4	1997-04	24.0	88.0	6.04	0.838	5
SLB-5	1997-04	24.0	85.4	2.14	0.248	5
SLB-6	1997-04	24.0	93.4	1.59	0.228	5
SLB-7	1997-04	24.0	72.9	2.76	0.688	5
CA00-0002	2000-07	29.4	96.4	1.2	0.121	0
CA00-0004	2000-07	29.1	84.9	2.13	0.201	1
CA00-0045	2000-07	29.1	49.6	2.25	0.197	2

* Assessment values calculated as proposed by Thompson and Lowe (2004).

Correlation analysis revealed the basic relationships between the chemical and physical factors and the benthos (Table 9). Salinity was significantly correlated with total abundance, oligochaete abundance and *S. benedicti* abundance. Neither TOC nor percent fines was significantly correlated to any of the benthic metrics. Several sediment contaminants were significantly correlated with total number of taxa, oligochaete abundance, and *S. benedicti* abundance. These relationships foreshadow the results of the Step 2 multivariate analysis presented below.

Table 9. Significant correlations (Pearson's r , $p < 0.05$); $n = 8$. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	As, Chlordanes
Total Abundance	Salinity
Number of Molluscan Taxa	None
Oligochaete Abundance	Zn, DDTs, Chlordanes, PCBs, mERMq, salinity
<i>S. benedicti</i> Abundance	DDTs, Chlordanes, salinity

Step 1 analysis suggested that salinity and percent fine sediments accounted for most of the variation of each benthic metric (Table 10). Sediment contamination (as mERMq) accounted for low to moderate proportions of the variance for all metrics, ranging between 1.7% for oligochaetes to 59.7% for total abundance. However, mERMq was not a significant model component for any of the benthic metrics.

Table 10. Step 1 multiple regression results for San Leandro Bay; n = 8. L = Log₁₀ transformation, A = Arcsin transformation, * = p < 0.05

Benthic Metric	Partial Coefficients Independent Variables				Total R ²
	Salinity	Fines	TOC	mERMq	
Total Number of Taxa	0.026 ^L	0.706*	0.018 ^A	0.350	0.817
Total Abundance ^L	0.874 ^{L*}	0.733 ^{A*}	0.089 ^A	0.597 ^L	0.988*
Number of Molluscan Taxa ^L	0.125	0.502 ^A	0.290 ^A	0.277 ^L	0.807
Oligochaete Abundance ^L	0.995 ^{L*}	0.002	0.008 ^A	0.017 ^L	0.995*
<i>S. benedicti</i> Abundance ^L	0.675 ^L	0.728*	0.037	0.247	0.936*

PCA produced four sediment contamination factors, each composed of covarying elements and compounds, except Factor 3, which was composed of mercury only (Table 11).

Table 11. PCA Factor composition for Total Number of Taxa in San Leandro Bay.

Factor 1	Factor 2	Factor 3	Factor 4
DDT	As	Hg	Cr
Zn	Chlordane		Cu
Pb			
HPAH*			
LPAH*			

* Normalized for organic content

Step 2 of the multivariate analysis indicated many significant relationships with potential key influences on benthic metrics. Differences in salinity accounted for the majority of the variability in total abundance, oligochaete abundance, and *S. benedicti* abundance. Salinity accounted for at least 67.5% of the variation in those variables and was a significant model variable (Table 12). PCA Factor 2 (chlordanes and As; Table 11) for total number of taxa and *S. benedicti* abundances was significantly associated, and accounted for over 90% and 25% of the variability, respectively. PCA Factor 2 (As, Hg) was also significantly associated with the number of molluscan taxa, and accounted for 55% of the variation. Finally, PCA Factor 4 (Cr, Cu; Table 11) was also selected, but was not a significant model variable.

Table 12. Step 2 multiple regression results for San Leandro Bay; n = 8. Bold = significantly correlated to the benthic metric; L = above ERL and M = above ERM.

Benthic Metric	Independent Variables	R-square	p
Total Number of Taxa	PCA Factor 2: As^L, chlordanes^M	0.902	0.0003
	Factor 4: Cr ^L , Cu ^L	0.05	0.072
Total Abundance	Salinity	0.875	0.0006
	Fines	0.091	0.014
Number of Molluscan taxa	PCA Factor 2: As^L, Hg^M	0.554	0.034
	TOC	0.237	0.063
Oligochaete Abundance	Salinity	0.995	<0.001
<i>S. benedicti</i> Abundance	Salinity	0.675	0.012
	PCA Factor 2: As^L, chlordanes^M	0.256	0.011

Apparent Benthic Effects Thresholds. Possible benthic effects thresholds for those sediment contaminants that were significantly associated with benthic metrics were evaluated graphically, using patterns of impact and concentration, and by considering ERL and ERM values for each implicated contaminant.

Patterns in benthic impact were evident in San Leandro Bay. Chlordane concentrations above 10.4 ppb were always associated with benthic impacts for total number of taxa and *S. benedicti* abundance (Figure 4). This value is very near the ERM of 6 ppb. For mercury, six of the seven samples (86%) with concentrations above 0.3 ppm were associated with benthic impacts. However, this concentration is below the ERM of 7.1 ppm, and is relatively low compared to the majority of recent sediment samples collected from the Estuary (SFEI 2006). Arsenic concentrations, on the other hand, showed no relationship to effects patterns. The highest sediment arsenic concentration (16.4 ppm) occurred at a site with no benthic impact (AV = 0). The ERL for arsenic is 8.2 ppm, and 67% of the samples above that level had impacts, but both samples below the ERL were impacted. Therefore, it was not possible to estimate a reliable effect threshold for arsenic.

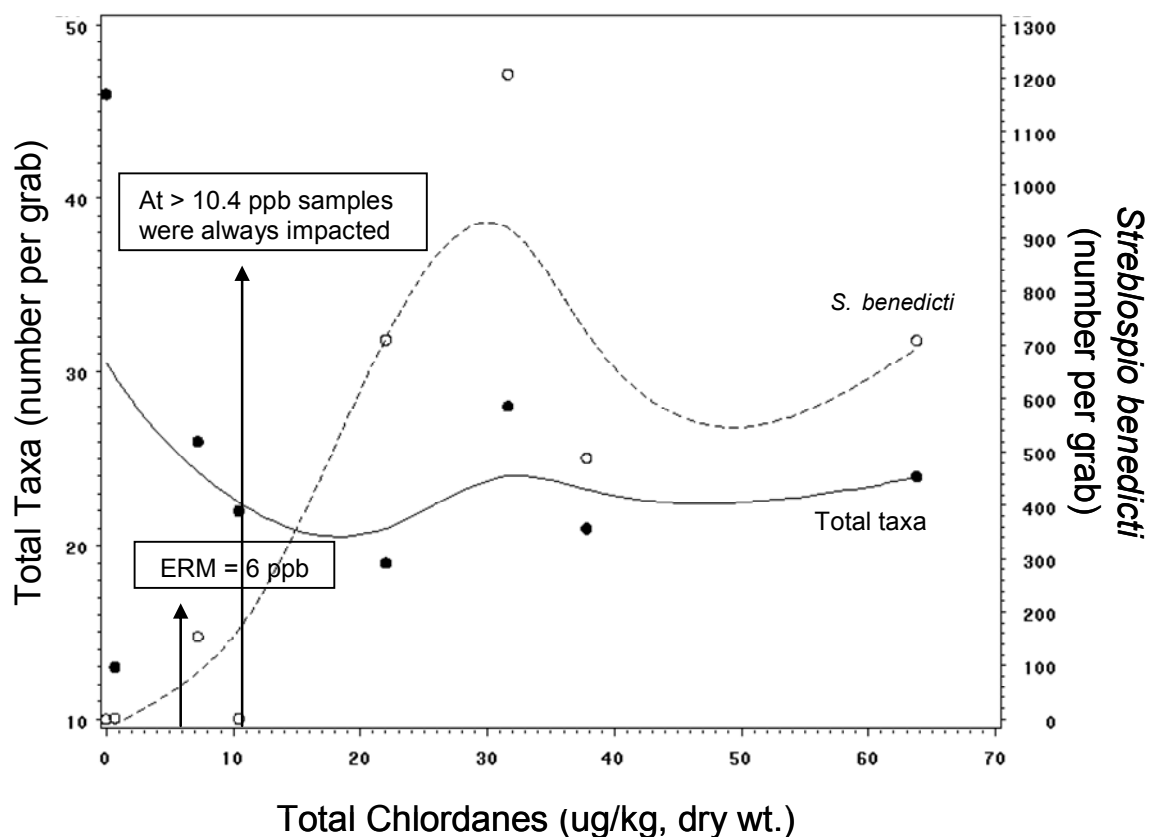


Figure 4. Effects threshold estimates for chlordanes in San Leandro Bay. Curve fits shown for illustrative purposes only.

Summary. Salinity and sediment grain-size accounted for most of the variation in the five benthic metrics evaluated. However, sediment contamination also contributed, both as mixtures, although not significantly compared to the salinity and grain-size. When examined further in Step 2 analysis arsenic, chlordanes, mercury, chromium, and copper were identified as possible sediment contaminants of concern that may affect total number of taxa, number of mollusca taxa, and *S. benedicti* abundance. A chlordane threshold of 10.4 ppb and a mercury threshold of 0.3 ppm were identified, but no apparent threshold for arsenic could be selected.

San Pablo Bay Marshes

San Pablo Bay has extensive tidal marshlands on its northern and western sides, bounded by several major tributaries (Figure 2). Benthic sampling was conducted at four sites in the China Camp marsh in 1995, as an RMP Pilot Study, and at two sites in the Napa–Sonoma Marsh in 2000–2001, as part of the EPA CISNet Project (Table 13).

The benthos at these sites were classified as the estuary margin sub-assemblage of the mesohaline main estuarine assemblage (Thompson *et al.* 2000, Thompson and Lowe 2003). The benthos consisted of elevated abundances of opportunistic and contaminant-tolerant taxa,

dominated by tubificid oligochaetes, the polychaete, *S. benedicti*, and the introduced cumacean, *Nippoleucon* sp. In general, a larger proportion of samples from the Estuary margin sub-assemblage were impacted compared to those from the main estuarine assemblage (Thompson and Lowe 2004). However, the possible causes of impacts were not investigated further. The San Pablo Bay marsh sites were assessed using the mesohaline assessment metrics, and resulted in three of the four China Camp sites, and the CAN CISNet samples having impacted benthos (Table 13). The un-impacted samples included corophiid amphipods and the introduced clam, *Corbula amurensis*.

Not all chemical and physical variables were measured at all sites. Salinity was not measured at the China Camp sites, and trace organic contaminants were not measured in the March 2000 CISNet samples (Appendix I). Therefore, the number of samples available for multivariate analyses was limited, including the ability to include salinity in the regression models.

Table 13. General description of San Pablo Bay Marsh samples. Salinity not measured in China Camp (WBCC) samples.

Site	Yr-Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq	AV*
CAN	2000-03	14.5	99.4	1.76	0.085	4
CAN	2000-07	21.0	98.7	1.83	0.067	3
CAN	2001-03	19.1	98.3	2.36	0.078	4
SOCR	2000-03	19.3	97.2	1.36	0.099	0
SOCR	2000-07	21.9	98.5	1.22	0.058	0
SOCR	2001-03	17.7	98.6	1.32	0.061	0
WBCC 2A	1995-02		99.0	2.3	0.106	2
WBCC 2B	1995-02		98.0	3.3	0.108	4
WBCC 3A	1995-02		99.0	2.0	0.099	1
WBCC 3B	1995-02		98.0	2.6	0.104	3

* Assessment values calculated as proposed by Thompson and Lowe (2004).

Salinity in the Napa-Sonoma Marsh samples ranged between 14.5 – 21.9 psu. However, salinity measured by RMP and DWR in the adjacent San Pablo Bay in February 1995 ranged between 3.35 – 7.5 psu. These values are considerably lower than those measured at the CISNet sites in 2001. Salinities in the China Camp samples were not estimated based on the San Pablo Bay measurements owing to possible differences between open bay and marsh channels. However, the possible differences in salinity between the CISNet and China Camp samples are noted as a potentially large source of influence on the benthos. All marsh sites consisted of very fine sediments, with moderate organic carbon content ranging between 1.2 – 3.3% (Table 13). Sediment contamination was generally low with mERMq values ranging between 0.058 – 0.108. Silver, cadmium, LPAH, and HPAH concentrations were all below ERL values, but nickel was usually above the ERM. The remaining contaminants had at least one measurement above its ERL value.

Correlation analysis indicated that TOC and PCBs were significantly correlated with oligochaete abundance, and DDTs and HPAHs were significantly correlated with *S. benedicti* abundance (Table 14). There were no significant correlations for total number of taxa, total abundances, or number of molluscan taxa.

Table 14. Significant correlations in San Pablo Bay Marshes (Pearson's r , $p < 0.05$); $n = 10$. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	None
Total Abundance	None
Number of Molluscan Taxa	None
Oligochaete Abundance	TOC, PCB
<i>S. benedicti</i> Abundance	DDTs, HPAHs

Step 1 multiple regression analysis was conducted excluding salinity, due to the missing values in China Camp. TOC accounted for most of the variability in all of the benthic metrics except number of molluscan taxa, which showed no strong relationships (Table 15). The only significant ($p < 0.05$) regression model was for oligochaete abundance, where all of the independent variables contributed significantly.

Table 15. Step 1 multiple regression results for San Pablo Bay Marshes; $n = 10$. L = Log_{10} transformation, A = Arcsin transformation, * = $p < 0.05$.

Benthic Metric	Partial Coefficients			Total R ²
	Fines	TOC	mERMq	
Total Number of Taxa	<0.001	0.281 ^A	0.112 ^L	0.362
Total Abundance ^L	0.043 ^A	0.434 ^A	0.085 ^L	0.505
Number of Molluscan Taxa ^L	0.027	0.008 ^A	0.188	0.216
Oligochaete Abundance ^L	0.371*	0.712 ^{A*}	0.577 ^{L*}	0.923*
<i>S. benedicti</i> Abundance	0.010 ^A	0.290 ^L	0.017 ^L	0.309

In the Step 2 analysis, PCA was conducted using 10 sediment contamination variables which produced two factors for total number of taxa and *S. benedicti* abundance (Table 16). Only one PCA Factor was produced for the other metrics as they used a different set of raw and transformed contaminant values.

Table 16. PCA Factor composition for Total Number of Taxa and *S. benedicti* Abundance in San Pablo Bay Marshes.

Factor 1	Factor 2
As	DDTs
Hg	
Cu	
Ni	
Chlordanes	
PCBs	

Physical and chemical factors were only related to total number of taxa and *S. benedicti* abundance (Table 17), as there was only one PCA Factor for the other metrics. TOC and PCA Factor 1 mixtures were significantly related to total number of taxa. PCA Factor 2 (DDTs; Table 16) was selected as the best variable for *S. benedicti* abundance, but did not form a significant model.

Table 17. Step 2 multiple regression results for San Pablo Bay Marshes; n = 8. Bold = significant correlated to the benthic metric, p < 0.05. L = above ERL and M = above ERM.

Benthic Metric	Independent Variables	R-square	p
Total Number of Taxa	TOC	0.428	0.078
	PCA Factor 1: As^L, Hg^L, Cu^L, Ni^M, chlordanes^L, PCBs^L	0.846	0.014
Total Abundance	No Stepwise Selection		
Number of Molluscan taxa	No Stepwise Selection		
Oligochaete Abundance	No Stepwise Selection		
<i>S. benedicti</i> Abundance	PCA Factor 2: DDTs ^L	0.326	0.139

Apparent Benthic Effects Thresholds. Step 1 analysis showed that contaminant mixtures (mERMq) contributed over half to model variation for oligochaete abundance. Contaminant mixtures (PCA Factor 1) was selected as a significant variable in the Step 2 multiple regression analysis for total number of taxa. Therefore, contaminant mixtures appeared to be a possible cause of observed benthic impacts. All of the mERMq values in San Pablo Bay marshes were below the threshold for benthic impacts (0.146) identified by Thompson and Lowe (2004). Additionally, 75% of the samples with mERMq above 0.067 were impacted, and all samples above 0.099 were impacted.

Summary. TOC was identified as key factor for benthos by correlation and Step 1 analyses, and as an important factor in the Step 2 analysis for total number of taxa. TOC and contaminant mixtures appeared to be the most probable cause of observed benthic impacts. Thompson and Lowe (2003) presented similar conclusions using the CISNet dataset, suggesting that elevated TOC and sediment contamination in these samples had more of an influence on benthic species composition and abundances than did differences in hydrodynamic regime (*e.g.*, river or marsh channel), or seasonal and tidal differences in salinity, flow, turbidity, or temperature. Furthermore, as part of the CISNet study, the clam *Macoma nasuta* was exposed to marsh sediments in laboratory exposures (Werner *et al.* 2004). Mortality, stress proteins (Hsp70) in gill tissues, tissue lesions in gonads, and lysosomal membrane damage were significantly correlated with clam tissue concentrations of DDT and/or its metabolites. Tissue concentrations of nickel, chromium, and copper were associated with macrophage aggregates in digestive gland and germ cell necrosis, whereas cadmium was linked to mortality and lysosomal damage.

In the present study, it was not possible to rule out salinity influence on benthic metrics due to missing values in the China Camp samples. Salinity in San Pablo Bay was within the range of the Estuary transition assemblage usually found in Suisun Bay. If similar salinities occurred in the China Camp channels, the benthos would be quite different from the CISNet benthos and would have had a primary influence.

CCSF Wastewater Discharge Area

The City and County of San Francisco (CCSF) wastewater discharge area is located on the western side of the Central Bay (Figure 2). CCSF sites were monitored by the Bay Area Clean Water Association's Local Effects Monitoring Program from 1994 to 1997, which was described in Thompson *et al.* (1999b). Three CCSF outfall sites were sampled in 1994 (once), and subsequently twice a year (1995-97) for sediment characteristics, contaminants, and benthos.

Eighteen samples were included in the evaluation of this area (Table 19). Three wet season samples collected in 1997 were excluded as they were more characteristics of a mesohaline estuarine assemblage, due to the flood flows in January. The remainder of the benthos was characteristic of a polyhaline assemblage (Thompson *et al.* 2000), represented by a large number of species, in relatively high abundance. The species were very similar to those at adjacent Central Bay sites, dominated by the amphipods *Monocorophium* spp. and *Ampelisca abdita*. The sediment grain-size was typically silty-clay, and indicated moderate organic carbon content and low sediment contamination (Table 18). Mean ERM_q values ranged between 0.1 – 0.35. AV values ranged from 0 – 3, although 12 of 18 (67%) samples exhibited an AV of zero. Arsenic, chromium, copper, and mercury had concentrations between the ERL and ERM (Appendix I). Nickel was consistently above the ERM, while cadmium, lead, silver, and zinc were nearly always below the ERL. The trace organic contaminants were low, with most values falling between the ERL and ERM.

Table 18. General description of CCSF samples.

Site	Yr-Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq	AV*
CCSF04	1994-09	30.6	55.5	0.644	0.30	0
CCSF04	1995-02	24.0	84.0	0.960	0.23	3
CCSF04	1995-08	30.6	91.0	1.183	0.15	0
CCSF04	1996-03	27.2	90.2	0.964	0.12	2
CCSF04	1996-08	33.2	73.4	1.050	0.15	0
CCSF04	1997-08	35.0	90.7	1.170	0.28	0
CCSF05	1994-09	30.8	46.3	0.573	0.35	0
CCSF05	1995-02	24.0	86.0	0.948	0.13	0
CCSF05	1995-08	30.6	82.0	1.166	0.13	1
CCSF05	1996-03	24.2	79.0	1.128	0.18	2
CCSF05	1996-08	33.2	59.4	0.961	0.20	0
CCSF05	1997-08	34.0	64.7	0.990	0.21	0
CCSF06	1994-09	31.0	56.6	0.562	0.12	0
CCSF06	1995-02	23.8	93.0	0.830	0.14	0
CCSF06	1995-08	30.7	95.0	1.074	0.12	0
CCSF06	1996-03	25.6	90.8	1.184	0.12	3
CCSF06	1996-08	33.3	80.3	1.185	0.16	1
CCSF06	1997-08	34.0	92.1	1.140	0.10	0

* Assessment values calculated as proposed by Thompson and Lowe (2004).

Correlation analysis showed that benthic metrics were related to several chemical and physical factors at CCSF sites (Table 19). Salinity and dieldrin were significantly correlated with all benthic metrics evaluated, while percent fines and TOC were only correlated to total number of taxa. Sediment contamination (as mERMq) was only significantly correlated to total number of taxa.

Table 19. Significant correlations for CCSF (Pearson's r , $p < 0.05$); $n = 18$, except $n = 9$ for dieldrin. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	Salinity, fines, TOC, Cr, Cu, Ni, Zn, dieldrin, LPAH, HPAH, mERMq
Total Abundance	Salinity, dieldrin
Number of Amphipod Taxa	Salinity, dieldrin, LPAH
<i>C. capitata</i> Abundance	Salinity, dieldrin

Step 1 of the multivariate analysis showed that a combination of physical factors accounted for most of the variation of each benthic metric (Table 20). Salinity explained most of the variation in total abundance, number of amphipod taxa, and *C. capitata* abundance. Both percent fines and TOC explained most of the variation in total number of taxa. Sediment contamination (as mERMq) was not significantly correlated to any of the benthic metrics, and explained a relatively small proportion of the variation due to the physical factors.

Table 20. Step 1 multiple regression results for CCSF; n = 18. L = Log₁₀ transformation, A = Arcsin transformation, * = p < 0.05

Benthic Metric	Partial Coefficients Independent Variables				Total R ²
	Salinity	Fines	TOC	mERMq	
Total Number of Taxa	0.142*	0.415	0.518 ^{A*}	0.112	0.785*
Total Abundance	0.620 ^{L*}	0.011	0.058 ^A	<0.001	0.647*
Number Amphipod Taxa	0.338 ^{L*}	0.118	0.070 ^A	0.041	0.479
<i>C. capitata</i> Abundance ^L	0.249 ^L	0.042	0.016 ^A	0.002	0.293

In the Step 2 analysis, PCA produced two sediment contamination factors. These factors were generally consistent for all benthic metrics, but since different suites of raw and transformed contaminants were used in the PCA for each metric (optimized correlation model), the order and composition of these factors was slightly different in the PCA for each benthic metric. Factor 1 was composed of covarying trace elements and PAHs, while Factor 2 was composed only of arsenic (Table 21).

Table 21. PCA Factor composition for Total Number of Taxa in CCSF.

Factor 1	Factor 2
Ni	As
Cr	
Cu	
Hg	
LPAH	
HPAH	

Step 2 of the multivariate analysis indicated that salinity was associated with all of the benthic metrics; the relationship was significant for total abundance, number of amphipod taxa, and *C. capitata* abundance (Table 22). The significant portion of the variation in total number of taxa ($R^2 = 0.56$, $p < 0.001$) was explained by the mixture of contaminants of Factor 1 (Ni, Cr, Cu, Hg, LPAH, HPAH; Table 21). Amphipod taxa was close to being significantly related to Factor 1, but the variance explained was low ($R^2 = 0.13$, $p = 0.08$). These results suggest that, in general, benthic impacts were mostly related to the influence of salinity, with some impact from a mixture of sediment contaminants.

Table 22. Step 2 multiple regression results for CCSF; n = 18, DDTs, dieldrin, chlordanes were excluded. Bold = significantly correlated to the benthic metric, $p < 0.05$. L = above ERL and M = above ERM.

Benthic Metric	Independent Variables	R-square	p
Total Number of Taxa	PCA Factor 1: Ni^M, Cr^L, Cu^L, Hg^M, LPAH^M, HPAH^M Salinity	0.555 0.077	<0.001 0.096
Total Abundance	Salinity	0.620	<0.001
Number of Amphipod taxa	Salinity PCA Factor 1: Ni^M, Cr^L, Cu^L, LPAH^M, HPAH^M	0.338 0.125	0.011 0.081
<i>C. capitata</i> Abundance	Salinity	0.249	0.035

Apparent Benthic Effects Thresholds. Benthic effects thresholds for the sediment contaminants (nickel, LPAH, and HPAH) that were significantly associated with benthic metrics were evaluated graphically, using patterns of impact and concentration, and by considering ERL and ERM values for each implicated contaminant.

Patterns in benthic impact were not indicated for CCSF sites. Graphical evaluation of the relationship between sediment contaminants of PCA Factor 1 and total number of taxa did not reveal shifts in benthic response. Also, the generally low incidence of threshold exceedances, and relatively low mERMq and AVs, suggested that sediment contamination was not a source of key influence to the benthos. For example, PAHs exhibited a linear relationship between total number of taxa and sediment contaminant concentration (not presented), as well as a lack of co-occurrence of high concentrations and benthic impact ($AV > 1$). The CCSF assessment highlights the difficulty in identifying benthic impact in areas that do not respond in a typical manner, particularly when areas lack true gradients in sediment chemistry. Some of the highest concentrations for LPAHs (> 1500 ppb) and HPAHs (> 4000 ppb) were found at CCSF sites that were relatively un-impacted ($AV < 1$).

Summary. Salinity accounted for most of the variation in the five benthic metrics evaluated at CCSF sites. A combination of physical factors, with salinity contributing the most, was identified through correlation and Step 1 analyses. Step 2 of the multivariate analysis suggested that a mixture of contaminants could play a secondary role in impacting benthic metrics, however, salinity was significantly related to all metrics other than total number of taxa. Although sediment contamination may have contributed to some benthic impacts, thresholds could not be identified for this area of the Estuary.

North Bay

Suisun and Grizzly Bays are the northernmost embayments of the San Francisco Estuary (Figure 2), and are referred to as the North Bay. It directly receives fresh water outflow from the Delta and 40% of California's total watershed area (Conomos 1979). Fifteen samples in the North Bay were included in this analysis. Twelve samples were from RMP station BF21 in Grizzly Bay collected between 1994 and 2000 (Table 8). Additionally, three samples were collected during the NOAA – EMAP survey of the Estuary in 2000. The benthos at these sites were characteristic of the Estuary oligohaline assemblage (Thompson *et al.* 2000), and consisted of the lowest number of taxa and individuals of any Estuary assemblage (Thompson and Lowe 2004). Dominant taxa included the amphipod, *Sinocorophium aliense*, the polychaete, *Marenzelleria viridis*, and the asian clam, *Corbula amurensis*.

A benthic assessment method for this assemblage was not included in Thompson and Lowe (2004) because the number of samples and sites with both benthic and sediment contamination data was not sufficient for the development and testing of such a method. Therefore, there was no *a priori* reason to believe that the samples analyzed here were impacted. However, the sediment at BA21 has been shown to be consistently toxic to amphipods and bivalve embryos (Anderson *et al.* 2006, in press). Five mesohaline assemblage metrics were selected to evaluate benthos in the North Bay.

Seasonal changes in salinity are considered to be the dominant abiotic Factor that affects all organisms in the North Bay (Jassby *et al.* 1995). Strong freshwater outflow from the Delta during the wet season may reduce salinity considerably. Conversely, in the dry season, outflows are minimal, resulting in saltwater intrusion into the Delta and elevated salinity. These fluctuations may occur rapidly, and few organisms are adapted to such changes (Thompson *et al.* 2000, Peterson *et al.* in prep). Therefore, oligohaline sites in the North Bay may often show reduced benthic diversity. The samples selected for this analysis demonstrated salinities ranging between 0.08 – 12.7 psu (Table 23). In addition, all samples had predominantly fine sediments with moderate to low organic carbon content (0.67 – 1.66%). Sediment contamination was generally low to moderate, with mERMq values ranging between 0.065 – 0.125. Silver, cadmium, and lead concentrations were all below ERL values, and nickel was above the ERM. All of the trace organic contaminants had at least one measurement above the ERL value, but all LPAH concentrations were below the ERL.

Table 23. General description of North Bay samples. AV method not proposed for the North Bay by Thompson and Lowe (2004) therefore not calculated for these samples.

Site	Yr-Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq
BF21	1994-02	5.8	99.0	1.46	0.125
BF21	1994-08	12.7	98.0	1.47	0.092
BF21	1995-02	0.2	99.0	1.38	0.081
BF21	1995-08	5.5	97.0	1.40	0.078
BF21	1996-02	0.1	98.0	1.37	0.094
BF21	1996-07	7.1	97.0	1.38	0.086
BF21	1997-01	0.1	99.1	1.38	0.088
BF21	1997-08	7.9	98.8	1.38	0.100
BF21	1998-02	0.1	99.3	1.66	0.081
BF21	1998-07	0.6	98.4	1.58	0.096
BF21	1999-02	0.1	99.1	1.53	0.102
BF21	1999-07	4.5	96.9	1.14	0.080
BF21	2000-07	4.6	97.5	1.44	0.065
CA00-0018	2000-07	6.2	90.5	1.21	0.090
CA00-0019	2000-07	7.3	61.3	0.67	0.069
CA00-0020	2000-07	3.4	98.6	1.52	0.102

Correlation analysis showed that salinity was significantly correlated with the total number of taxa and number of amphipod taxa (Table 24). Sediment grain-size and TOC content were significantly correlated with total number of taxa and oligochaete abundance. Various trace metals and organic contaminants were significantly correlated to the benthic metrics, except the number of molluscan taxa which was not significantly correlated with any chemical or physical variables tested.

Table 24. Significant correlations in North Bay (Pearson's r , $p < 0.05$); $n = 8$. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	Salinity, fines, TOC, Hg
Total Abundance	PCBs
Number of Molluscan Taxa	None
Number of Amphipod Taxa	Salinity, Cr, Hg, chlordanes
Oligochaete Abundance	Fines, TOC, Cu

Step 1 of the multivariate analysis showed that salinity and sediment characteristics accounted for most of the variability in the benthic metrics evaluated. Salinity was most important for total number of taxa and amphipod taxa, TOC accounted for most of the variability in total abundance and number of molluscan taxa, and fines for the variability in oligochaete abundance (Table 25). Sediment contamination (as mERMq) accounted for 40% and 34% of the model variance for total number of taxa and molluscan taxa respectively, but mERMq was not a significant model component for any of the benthic metrics.

Table 25. Step 1 multiple regression results for North Bay; n = 8. L = Log₁₀ transformation, A = Arcsin transformation, * = p < 0.05.

Benthic Metric	Partial Coefficients Independent Variables				Total R ²
	Salinity	Fines	TOC	mERMq	
Total Number of Taxa	0.601 ^{L*}	0.119 ^A	0.013	0.303	0.758*
Total Abundance	0.110	<0.001	0.212	<0.001 ^L	0.300
Number of Molluscan taxa ^L	0.054 ^L	0.077	0.288*	0.166	0.482
Number of Amphipod taxa	0.593 ^{L*}	0.002 ^A	0.073	0.076	0.652*
Oligochaete Abundance ^L	0.166 ^L	0.263 ^A	0.058	0.022	0.435

Step 2 of the multivariate analysis was conducted using 10 sediment contamination variables which produced four PCA factors. Each factor was composed of covarying sediment contaminants and compounds (Table 26). These factors were generally consistent for all benthic metrics, but since different suites of raw and transformed contaminants were used in the PCA for each metric (optimized correlation model), the order and composition of these factors was slightly different in the PCA for each benthic metric. They represent four independent sediment contamination patterns among the samples. Trace-metals grouped together on Factors 1 and 3, whereas the organic contaminants grouped on Factors 2 and 4.

Table 26. PCA factor composition for Total Number of Taxa in North Bay.

Factor 1	Factor 2	Factor 3	Factor 4
Zn	HPAH	As	Chlordanes
Ni	Dieldrin	Hg	DDTs
Cu			
Cr			

Step 2 multiple regression analysis showed that salinity changes in the North Bay were significantly related to the total number of taxa and number of amphipod taxa, and accounted for the majority of the variability in both metrics (Table 27). TOC was the most significant variable for oligochaete abundance. PCA contamination factors were the most important variables for total abundance (HPAHs and dieldrin) and were also included in the model selection for oligochaetes (DDTs and chlordanes), but those factors were not significant model components. None of the model variables were selected as significant for number of molluscan taxa.

Table 27. Step 2 multiple regression results for North Bay; n = 8. Bold = significantly correlated to the benthic metric, $p < 0.05$. L = above ERL and M = above ERM.

Benthic Metric	Independent Variables	R-square	p
Total Number of Taxa	Salinity	0.522	0.0007
	Fines	0.111	0.053
Total Abundance	PCA Factor 2: HPAH ^L , dieldrin ^L	0.162	0.122
Number of Molluscan Taxa	No selection		
Number of Amphipod Taxa	Salinity	0.593	0.0003
Oligochaete Abundance	TOC	0.377	0.011
	PCA Factor 4: DDTs ^L , chlordanes ^L	0.098	0.142

Apparent Benthic Effects Thresholds. The sediment contamination factors selected in Step 2 analysis were not significantly correlated with benthic metrics, and the factor components had concentrations below ERM values. Therefore, no effects thresholds were estimated. Studies of possible causes of sediment toxicity to mussel larvae observed at several North Bay RMP sites identified copper ions in sediment pore water as a probable factor (Phillips *et al.* 2003). Except for positive correlation between oligochaete abundance and copper ($R^2 = 0.50$, $p = 0.05$, $n = 16$), there has been no indication that copper is related to observed benthic impacts in the North Bay.

Summary. Salinity and/or sediment-type accounted for most of the variation in the five benthic metrics evaluated in the Step 1 and Step 2 analysis. The identification of salinity as a key determinant of benthic assemblages is consistent with the current understanding of key environmental drivers in the North Bay. Sediment contamination appeared to be of minor influence on the benthos. There was no clear identification of specific sediment contaminants that had consistent influence. Mixtures (mERMq) or PCA contamination factors were not significantly related to the benthos and concentrations were generally below levels of concern.

South Bay

The Southern-most portion of San Francisco Estuary, commonly referred to as the South Bay is a true mesohaline estuarine habitat generally characterized by moderate salinity. It receives freshwater inflows from the Santa Clara Valley, and greater San Jose area, including 'Silicon Valley'. Several major tributaries, as well as two major wastewater discharges discharge into this area of the Estuary.

Eleven samples collected from the South Bay (south of Dumbarton Bridge) between 1994 and 2000, were used for this analysis (Figure 2). The majority of samples were from RMP station BA21 collected twice annually since 1994. Two additional sites were sampled by EMAP-NOAA in 2000 and were included in our evaluation (Table 28).

Salinity in the thirteen samples ranged between 6.7 – 30.7 psu (Table 28). All sediments were characteristic of fine silty clays, and had carbon content ranging between 0.84 – 1.57%.

Sediment contamination was moderate to high with mERMq values ranging between 0.082 – 0.193. Only two of the samples had mERMq values above the 0.146, a probable benthic effects threshold (Thompson and Lowe 2004). Silver, cadmium, and lead were always below the ERL, and all but one sample of PCBs and LPAHs were also below the ERL. Nickel was consistently above the ERM value, and one mercury sample was above the ERM. The remaining contaminants were between the ERL and ERM values (Appendix I).

Table 28. General description of South Bay samples.

Site	Yr./ Mo.	Salinity (psu)	Fines (%)	TOC (%)	mERMq	AV*
BA21	1994-02	25.2	81.0	0.84	0.185	2
BA21	1994-08	30.7	92.0	1.26	0.107	0
BA21	1995-02	14.4	97.0	0.96	0.103	0
BA21	1995-08	23.4	98.0	1.33	0.107	0
BA21	1996-02	14.9	99.0	1.52	0.144	0
BA21	1996-07	22.4	97.0	1.44	0.117	0
BA21	1997-01	6.7	97.2	1.44	0.108	0
BA21	1997-08	28.4	90.9	1.41	0.111	0
BA21	1998-02	10.0	97.2	1.39	0.106	0
BA21	1998-07	20.6	97.1	1.35	0.097	1
BA21	2000-07	25.3	96.3	1.44	0.082	1
CA00-0014	2000-07	25.1	95.9	1.57	0.130	0
CA00-0015	2000-07	25.2	92.5	1.70	0.193	0

* Assessment values calculated as proposed by Thompson and Lowe (2004).

The benthos at these sites was classified as mesohaline (Thompson and Lowe, 2004). The assemblage was dominated by the introduced clam, *Corbula amurensis*, oligochaetes, and the clam, *Macoma* sp. Previous assessment of benthic condition showed that only one sample from February 1994 was slightly impacted (Thompson and Lowe 2004). The remaining samples were un-impacted. Therefore, there was little need, *a priori*, to examine possible causes of benthic impacts at these sites. However, Step 1 analyses were conducted to evaluate whether there was any obvious relationship with physical or chemical variables that might explain the observed incident of benthic impact.

Correlation analysis at the South Bay sites showed significant relationships between the benthic metrics and several physical and chemical variables (Table 29). Sediment-type (percent fines and/or TOC) was correlated with oligochaete abundance and *S. benedicti* abundance. The correlation of several metals and trace organic contaminants with each of the metrics suggests that contaminant mixtures could be important at these sites, and was further tested in Step 1 multivariate analysis.

Table 29. Significant correlations in the South Bay (Pearson's r , $p < 0.05$); $n = 13$. The individual correlations may have been made using raw or transformed variables.

Benthic Metric	Significantly correlated chemical and physical variables
Total Number of Taxa	Cd, Pb, chlordanes
Total Abundance	As, Cd, Pb, chlordanes
Number of Molluscan Taxa	Chlordanes, PCBs, LPAHs, mERMq
Oligochaete Abundance	TOC, Fines, Cd, DDTs, dieldrin, PCBs, LPAHs, HPAHs
<i>S. benedicti</i> Abundance	Fines, dieldrin, PCBs, HPAHs

Step 1 multiple regression was conducted to evaluate the relative contributions of physical and chemical variables to each benthic metric (Table 30). Salinity contributed most to the variation in total abundance, TOC contributed most to oligochaete abundance, and percent fine sediments contributed most to *S. benedicti* abundance, respectively. Sediment contaminant mixtures (mERMq) contributed between 5.7 – 92.1% of the variation of all benthic metrics, and contributed most towards total number of taxa and molluscan taxa, as a significant factor.

Table 30. Step 1 multiple regression results for South Bay; $n = 13$. L = Log_{10} transformation, A = Arcsin transformation, * = $p < 0.05$

Benthic Metric	Partial Coefficients				Total R ²
	Salinity	Fines	TOC	mERMq	
Total Number of Taxa ^L	0.017	0.033 ^A	0.279	0.531 ^{L*}	0.679*
Total Abundance ^L	0.098 ^L	0.006	0.094	0.076 ^L	0.250
Number of Molluscan Taxa ^L	0.002	0.141	0.019	0.649 ^{L*}	0.705*
Oligochaete Abundance	0.003 ^L	0.311	0.722 ^{A*}	0.142	0.836*
<i>S. benedicti</i> Abundance ^L	0.150 ^{L*}	0.815*	0.143 ^A	0.050	0.872*

Step 2 multiple regression was not conducted on the South Bay samples. With only one sample showing slight benthic impacts, it was felt that such an analysis, in search of specific contaminant causes, would be inappropriate.

Summary. Analyses suggest that contaminant mixtures may have a slight impact on the benthos in the South Bay samples. However, apparent benthic impact was only observed in one South Bay sample. In that sample, total abundance and oligochaete abundance were above the reference values, resulting in an AV of 2 and the mERMq was 0.185, above the benthic impact threshold. Correlation analyses indicated that a mixture of metals and trace organic sediment contaminants were significantly correlated with those, and other benthic metrics. Step 1 multiple regression indicated that salinity, percent fines and mERMq all contributed to variation in total abundances, and that TOC, percent fines, and mERMq contributed most to oligochaete abundance. Step 1 analysis also showed that mERMq contributed most to the numbers of total number of taxa and molluscan taxa, but those metrics were not outside of reference values in the AV assessment. Where contaminant mixtures were shown to be related to benthic metrics that





























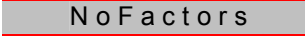



did not exceed reference ranges, the mixtures may not have been sufficiently high to cause an impact.

Comparison of Areas and Benthic Metrics

Sediment contamination combined with other environmental factors (*i.e.*, salinity and/or sediment-type) were indicated as key factors in many (11 of 29, 38%) of the area/metric evaluations. In these evaluations, the relative importance of chemical and physical factors was identical. Sediment contamination alone was indicated as the key factor associated with benthic impacts in 6 of 29 (21%) area/metric evaluations. An extreme contamination gradient was present in Richmond Harbor. Therefore, this was the only area where sediment contaminants were identified in all benthic metrics evaluated, and appeared to be the sole driver for oligochaete abundance and number of amphipod taxa. Total number of taxa was significantly influenced by contaminant mixtures in both San Leandro Bay and San Pablo Bay Marshes. Additionally, *S. benedicti* abundance in the San Pablo Bay Marshes appeared to be influenced by DDT alone. Other environmental factors appeared to have the most influence on the benthos in 10 of 29 (34%) area/metric evaluations.

There was general agreement within the areas among the benthic metrics evaluated in this study (Table 31). Richmond Harbor exhibited the most consistent results as all five benthic metrics exceeded their respective reference ranges at many sites, and Step 2 of the multivariate analysis strongly suggested benthic impact due to DDTs and dieldrin. However, sediment grain-size was also indicated as a source of probable influence on the benthos. The evaluation of San Leandro Bay suggested that the largest impact was from chlordane and arsenic, but this interpretation may have been obscured by the influence of salinity or sediment-type. In San Pablo Bay Marshes, contamination mixtures or DDTs (for *S. benedicti*) were indicated as probable sources of benthic impact. However, TOC was also indicated for many of the benthic metrics. In the CCSF evaluation, salinity was consistently indicated as the mostly likely source of benthic impact. In addition, contaminant mixtures and sediment-type contributed to responses in total number of taxa and number of amphipod taxa. In the North Bay, salinity and sediment-type appeared to have the most influence, with contaminants only having a large effect for total abundance. There were minimal benthic impacts in the South Bay. The two metrics that were significantly related to mERMq should be treated with caution given that there was only a single sample with an AV ≥ 2 . The remaining metrics appeared to most closely relate to physical factors.

Table 31. Comparison of factors related to benthic metrics in each Estuary area. Colors represent chemical and physical factors, where red is for contaminants, green is for grain size, brown is for organic carbon, and blue is for salinity. Note that South Bay analyses did not evaluate contaminant concentrations directly, only mERMq was used.

Estuary Area	Benthic Metric	Related Factors
Richmond Harbor	Total Number of Taxa	
	Total Abundance	
	Number of Molluscan Taxa	
	Oligochaete Abundance	
	Amphipod Abundance	
San Leandro Bay	Total Number of Taxa	
	Total Abundance	
	Number of Molluscan Taxa	
	Oligochaete Abundance	
	<i>S. benedicti</i> Abundance	 
San Pablo Bay Marshes	Total Number of Taxa	
	Total Abundance	
	Number of Molluscan Taxa	No Factors
	Oligochaete Abundance	 
	<i>S. benedicti</i> Abundance	
CCSF	Total Number of Taxa	 
	Total Abundance	
	Amphipod Taxa	
	<i>C. capitata</i> Abundance	
North Bay	Total Number of Taxa	
	Total Abundance	
	Number of Molluscan Taxa	
	Number of Amphipod Taxa	
	Oligochaete Abundance	 
South Bay	Total Number of Taxa	
	Total Abundance	No Factors
	Number of Molluscan Taxa	
	Oligochaete Abundance	
	<i>S. benedicti</i> Abundance	 

DISCUSSION and CONCLUSIONS

This study identified relationships between benthic assessment metrics and key physical and chemical variables in six areas of the San Francisco Estuary. Impacts to the benthic metrics evaluated in each area were associated with different sets of factors. However, the combination of salinity, sediment-type, and/or contaminants appeared to influence the benthos in all evaluated areas of the Estuary. This pattern prevailed regardless of the differing sediment patterns and proximity to sources between areas. In each area evaluated, at least one benthic metric was significantly related to a physical factor or contaminant mixture (Table 31). These results indicate that benthic impacts are likely the result of interactions of sediment contaminants and environmental factors. In other evaluation, slight shifts in salinity or sediment-type appeared to strongly affect certain benthic metrics, and obscured obvious correlations with sediment contaminants. This was most apparent in the San Pablo Bay Marshes, CCSF, and North Bay. In the majority of areas, grain-size was either not correlated, or was of similar relative importance as salinity or contaminant mixtures. Sediment contamination appeared to be the key factor in several metrics from Richmond Harbor and San Leandro Bay, and for total number of taxa and *S. benedicti* abundance in the San Pablo Bay Marshes. In Richmond Harbor and San Leandro Bay, where contamination was an obvious key factor, effects thresholds were estimated. Mixtures of several sediment contaminants were shown to have influence on the benthos at 17 of 29 (58%) area/metric evaluations, and were significant model components in about half of them. Salinity and/or sediment-type had key influence on several benthic metrics in some areas.

The multiple regression statistics (R-squared and partial coefficients) that were estimated are not intended to be used for predictive purposes, but simply to estimate relative contributions of the physical and chemical variables tested. We employed the 'best model' approach, where analyses identified the relative importance of several key physical and chemical factors. This approach was generally robust, maintaining the significance of specific variables or mixtures, regardless of which set of transformed or raw data we used, or order that variables were entered into model statements. Our analyses identified the factors that most likely explain apparent benthic impacts in the Estuary, but should not be used to predict impact for other regions. Owing to the inherent covariation in sediment contaminant data, identification of specific individual contaminants was generally not possible. However, where contaminants have strong influence, they formed independent patterns that corresponded with benthic changes, such as in Richmond Harbor and San Leandro Bay. Mixtures of sediment contaminants were consistently identified as factors in benthic impacts in nearly all areas evaluated.

Recent benthic assessments in the Estuary have indicated benthic response by evaluating deviations of metrics from reference values using AVs (Thompson and Lowe 2004). The AVs are useful for determining potential impacts, but not for correlating responses to key influences. Comparing the results presented in this study to previous AV results, sediment contamination was identified as a possible factor in 75% of the area/metric evaluations (Step 1 or 2) where AV values also identified impacts. Conversely, 25% of the area/metric evaluations where AV values suggested a benthic impact were not related to sediment contamination, but rather to other environmental factors.

Current TMDL (Total Maximum Daily Load) contaminants for the San Francisco Estuary (*e.g.*, mercury and PCBs) were components of some PCA factors. These priority contaminants were significantly correlated with total number of taxa in San Pablo Bay Marshes (Hg and PCBs) and at CCSF (Hg), and with number of molluscan taxa in San Leandro Bay (Hg).

The results from this study suggest that benthic impairment may be most closely linked to mixtures, due to their exposures to a suite of sediment-bound contaminants. However, near point-sources, such as the historic EPA Superfund site in Richmond Harbor, specific contaminants may be more important. Apparent benthic impairment in Richmond Harbor was likely attributed to both DDTs and dieldrin as a consequence of both pesticides being produced at the Superfund site, as was previously shown by Ferraro and Cole (1997). Chlordanes and PAHs may be the source of impairment in San Pablo Bay Marshes and San Leandro Bay because these contaminants have been so pervasive in the aquatic environment. Thompson *et al.* (1999a) previously associated these contaminants to sediment toxicity in the Estuary.

The contaminant mixtures and thresholds identified are considered hypotheses that should be tested further in the future. Although this study has identified correlations of physical and/or chemical factors to macrobenthos in the Estuary, this does not demonstrate the cause and effect of benthic impairment. Conclusive demonstration of the causes of impairment requires field and/or lab experiments designed specifically to examine cause and effect. These studies may include experiments along gradients of those contaminants shown to be possible stressors, where possible. For example, lab microcosm experiments could be used to examine the effect of the mixture of DDTs and dieldrin on the abundance of oligochaetes in sediments from Richmond Harbor.

The San Francisco Estuary benthic assessment methodology appears to have accurately identified sediment contamination in some areas of the Estuary. This study included 54% (26 of 48) of the benthic impacted sites ($AV > 2$; Thompson and Lowe 2004) previously identified. Locations not included in this analysis consisted of too few samples from discrete areas or gradients to be assessed. For example, the BPTCP identified benthic impacts in Islais Creek and Mission Creek on the eastern shore of San Francisco. However, there were only three samples at each location, which were each influenced by different runoff constituents. We did not conduct data analysis on regions with fewer than six samples. Other areas with reported benthic impacts and relatively high mERMq (Figure 5) were similarly excluded due to inadequate sample sizes, and because pooling data would have confounded sources and possible impacts. Only a few (< 8) stations have exhibited concentrations that are well above effect thresholds, as the majority of the Estuary has exhibited mERMq of less than 0.2 (Figure 5). Richmond Harbor exhibited the most obvious contamination in our analyses, and the mERMq data supports this, with much higher values than any other region of the Estuary (max mERMq = 255). South Bay indicated the least benthic impact due to sediment contamination in our analyses, and the mERMq map suggests that most of the stations in this region are below 0.15.

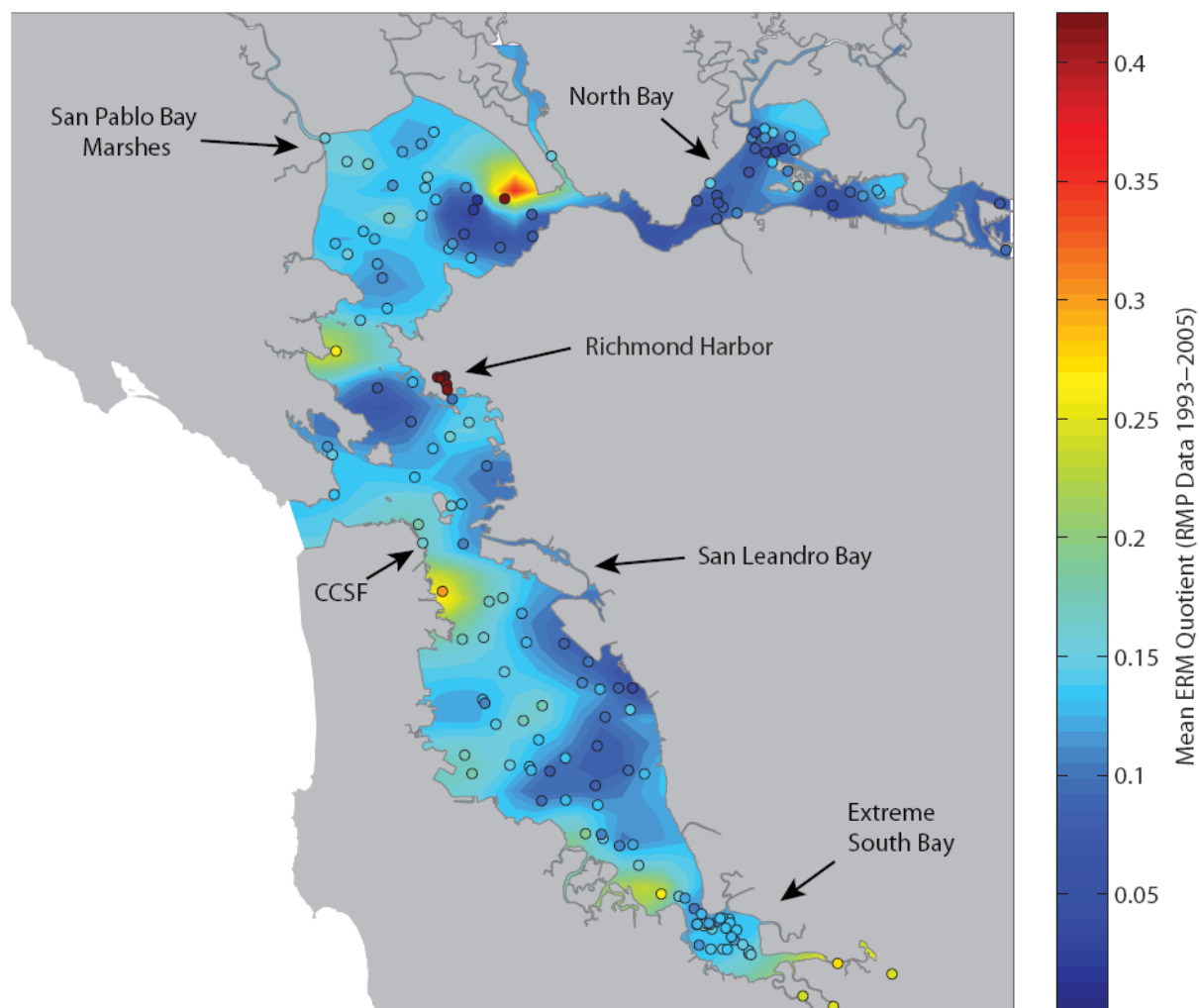


Figure 5. Contour map of mean ERM quotient (mERMq) for the San Francisco Estuary (1993 – 2005). Dots represent RMP (fixed) locations.

Throughout much of the Estuary, the assessment methodology showed that it was very difficult to adequately distinguish contaminant impact from other causes (*e.g.*, salinity, sediment-type) of apparent benthic impact. Therefore, refinements of assessment methods to clearly distinguish sediment contamination impacts may be useful in the future. Specific correlations could be used in a weight-of-evidence approach to identify the specific component metrics that would best explain contaminant responses only. As currently planned for use in SQOs, benthic assessments will be used to show possible impacts, which along with the weight-of-evidence from sediment contamination and toxicity, will provide the overall sediment assessment. The method presented in this paper may be used to provide a better understanding of the possible causes of the observed benthic impacts. These refinements in methodology may be most important in areas that do not show strong contaminant gradients. Benthic assessment in areas of strong contamination gradients (*e.g.*, Richmond Harbor) are easier to interpret as the contaminant effects so strongly outweigh the influence of changes in salinity or sediment-type. Understanding the causes of benthic impacts will allow for focused management actions in the future.

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Appendix I. Raw chemical data used in this study

Estuary Area	Site	Date	As	Ag	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Total DDTs	Dieldrin	Total Chlordanes	Total PCBs	LPAHs	HPAHs
CCSF	CCSF04	1994-09	0.48	10.56	72.67	0.22	24.67	1.08	56.85	19.33	65.98	0.96	0.92	0.92	4.41	2061.0	10395.7
CCSF	CCSF04	1995-02	0.33	11.33	101.61	0.21	51.63	0.33	84.14	17.75	118.00	0.72	0.72	0.72	0.72	1470.0	8700.0
CCSF	CCSF04	1995-08	0.30	7.00	85.38	0.20	40.50	0.19	75.22	17.05	88.80	5.30	1.35	10.50	1.70	993.0	4266.0
CCSF	CCSF04	1996-03	0.49	12.30	144.00	0.13	50.60	0.25	135.00	12.10	121.00				5.30	548.0	2637.0
CCSF	CCSF04	1996-08	0.43	7.90	133.00	0.43	36.20	0.20	99.30	32.10	112.00				6.00	779.0	4314.0
CCSF	CCSF04	1997-02	0.18	21.00	148.00	0.49	68.90	0.20	139.00	27.70	134.00				4.63	224.9	592.4
CCSF	CCSF04	1997-08	0.38	17.20	151.00	0.38	38.40	0.25	118.00	22.90	130.00				0.36	3201.6	6608.0
CCSF	CCSF05	1994-09	0.17	9.82	72.67	0.25	21.67	0.27	54.04	13.00	55.49	2.40	0.89	0.89	2.80	3945.2	13924.4
CCSF	CCSF05	1995-02	0.33	10.53	127.10	0.27	55.50	0.30	97.87	22.24	123.00	0.80	0.80	0.80	0.80	343.3	3893.3
CCSF	CCSF05	1995-08	0.10	9.00	130.80	0.13	60.20	0.23	107.10	6.25	125.00	1.30	1.30	5.00	1.90	812.0	4070.0
CCSF	CCSF05	1996-03	0.49	10.20	129.00	0.13	45.30	0.35	100.00	17.50	114.00				7.00	1073.0	5323.0
CCSF	CCSF05	1996-08	0.28	6.60	115.00	0.28	34.90	0.19	97.80	24.30	99.00				7.38	1431.8	6936.4
CCSF	CCSF05	1997-02	0.19	21.00	148.00	0.51	64.10	0.24	137.00	35.90	133.00				4.12	637.8	706.1
CCSF	CCSF05	1997-08	0.29	13.20	126.00	0.29	47.60	0.21	109.00	22.80	112.00				2.30	1793.8	6120.2
CCSF	CCSF06	1994-09	0.22	9.76	80.67	0.08	25.67	0.11	59.30	18.33	66.48	0.91	0.91	0.91	0.91	895.4	3848.0
CCSF	CCSF06	1995-02	0.33	7.46	123.60	0.27	54.47	0.25	106.44	21.31	117.33	21.05	0.76	0.76	0.76	246.7	4243.3
CCSF	CCSF06	1995-08	0.10	9.90	144.70	0.36	86.30	0.23	126.10	13.38	143.00	6.10	1.55	12.10	1.40	34.1	2136.0
CCSF	CCSF06	1996-03	0.49	11.20	122.00	0.13	48.70	0.23	112.00	19.90	120.00				6.70	648.0	2497.0
CCSF	CCSF06	1996-08	0.57	8.30	122.00	0.57	38.60	0.20	106.00	52.60	118.00				6.66	1035.0	3964.0
CCSF	CCSF06	1997-02	0.18	19.00	140.00	0.74	58.80	0.21	128.00	32.00	125.00				4.51	215.5	784.5
CCSF	CCSF06	1997-08	0.32	18.80	148.00	0.32	62.50	0.24	130.00	25.20	129.00				19.90	186.1	1031.7
North Bay	BF21	1994-02	0.33	12.10	70.01	0.26	67.15	0.36	114.60	23.01	130.59	10.35	0.80	0.00	15.82	383.5	2706.0
North Bay	BF21	1994-08	0.29	12.60	112.13	0.32	62.06	0.36	115.35	23.60	141.21	3.91	0.12	0.00	6.30	104.1	702.6
North Bay	BF21	1995-02	0.25	15.65	89.82	0.30	59.66	0.34	92.92	30.47	127.24	1.07	0.14	0.00	1.23	79.0	508.0
North Bay	BF21	1995-08	0.25	16.08	67.20	0.26	39.80	0.34	68.30	30.70	93.60	4.57	0.27	0.00	3.12	90.0	486.0
North Bay	BF21	1996-02	0.26	15.00	110.27	0.40	64.87	0.25	114.14	20.08	149.26	6.40	0.03	0.20	4.53	146.0	784.0
North Bay	BF21	1996-07	0.30	14.80	110.60	0.27	52.70	0.29	101.80	21.20	134.80	8.70	0.16	0.00	4.19	69.0	476.0
North Bay	BF21	1997-01	0.19	8.61	122.00	0.28	58.00	0.29	117.00	18.00	132.00	8.40	0.28	0.20	1.62	57.0	622.0
North Bay	BF21	1997-08	0.28	13.00	146.00	0.30	59.80	0.29	126.00	21.30	138.00	12.10	0.33	0.80	1.74	3.0	51.0
North Bay	BF21	1998-02	0.25	7.21	150.00	0.34	61.00	0.12	130.00	23.00	140.00	7.00	0.32	0.00	2.53	44.0	480.0
North Bay	BF21	1998-07	0.25	19.00	136.00	0.26	61.80	0.29	135.00	22.00	152.00	2.30	0.25	0.00	1.52	94.0	715.0
North Bay	BF21	1999-02	0.11	11.00	125.19	0.47	65.61	0.34	106.57	21.28	148.24	11.30	0.16	0.00	3.78	129.0	940.0
North Bay	BF21	1999-07	0.24	10.40	80.27	0.31	54.94	0.29	90.90	19.91	116.11	4.40	0.16	0.30	4.73	122.0	660.0
North Bay	BF21	2000-07	0.17	11.90	80.30	0.36	51.34	0.24	80.25	17.91	109.79	5.52	0.31	0.30	3.48	86.1	492.1
North Bay	BF21	2001-02															
North Bay	CA00-0018	2000-07	0.31	10.06	136.47	0.32	49.46	0.31	90.17	21.99	125.55	6.61	0.20	0.20	3.51	108.6	684.1
North Bay	CA00-0019	2000-07	0.16	8.50	129.76	0.20	35.66	0.27	92.99	16.97	113.33	3.40	0.12	0.00	1.88	52.8	308.3
North Bay	CA00-0020	2000-07	0.32	11.96	152.06	0.37	57.43	0.37	102.90	26.02	143.70	5.64	0.21	0.27	4.56	127.5	702.2
Richmond Harbor	L1	91-10				1.10	68.10		29.40	92.80	326.50	77736.80	748.00				
Richmond Harbor	L2	91-10				0.20	60.40		28.20	65.90	173.50	47828.60	527.90				
Richmond Harbor	L3	91-10				0.20	51.60		34.70	52.80	141.70	26004.90	441.80				
Richmond Harbor	L4	91-10				0.05	30.30		22.70	37.70	101.90	2744.40	35.70				
Richmond Harbor	L5	91-10				0.05	33.40		17.90	35.20	90.00	419.70	5.50				
Richmond Harbor	L6	91-10				1.40	47.60		25.30	110.60	298.50	2344.80	78.40				
Richmond Harbor	L7	91-10				0.10	38.90		27.90	41.70	118.60	368.30	9.50				
Richmond Harbor	L8	91-10				0.05	29.50		26.70	30.20	89.90	82.50	1.70				
Richmond Harbor	L9	91-10				0.05	10.10		8.00	11.20	49.90	12.20	0.60				

Appendix I. Raw chemical data used in this study

Estuary Area	Site	Date	As	Ag	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Total DDTs	Dieldrin	Total Chlordanes	Total PCBs	LPAHs	HPAHs
San Leandro Bay	CA00-0002	2000-07	0.41	16.37	178.39	0.23	52.29	0.30	95.38	25.98	154.59	4.97		0.69	9.10	317.0	1807.5
San Leandro Bay	CA00-0004	2000-07	0.06	10.70	118.70	0.70	62.73	1.12	81.66	78.80	215.65	18.22		10.44	47.54	274.8	1620.4
San Leandro Bay	CA00-0045	2000-07	0.30	4.22	82.90	0.58	65.00	0.52	77.00	90.60	188.00	27.70		0.00	61.10	232.0	2196.0
San Leandro Bay	SLB-3	1997-04	0.28	6.92	170.00	1.01	67.50	0.56		120.00	289.00	111.50		31.64	243.24	567.8	4216.5
San Leandro Bay	SLB-4	1997-04	1.85	11.10	111.00	2.78	110.00	0.76		185.00	604.00	113.20		63.82	630.27	3225.8	22448.0
San Leandro Bay	SLB-5	1997-04	0.81	11.90	122.00	0.84	131.00	0.59		84.00	364.00	30.76		22.02	133.07	240.2	2121.9
San Leandro Bay	SLB-6	1997-04	0.38	9.07	177.00	0.26	68.70	1.18		68.80	262.00	15.18		7.22	126.81	246.4	1895.9
San Leandro Bay	SLB-7	1997-04	0.62	9.62	274.00	3.91	85.60	0.59		191.00	836.00	211.23		37.83	428.97	1222.8	7188.4
San Pablo Bay Marshes	CAN	2000-03	0.08	7.32	20.30	1.05	19.00	0.01	41.90	26.40	75.90						
San Pablo Bay Marshes	CAN	2000-07	0.12	6.99	21.00	1.05	38.70	0.24	45.70	25.40	79.40	2.46		0.00	11.38	79.1	83.4
San Pablo Bay Marshes	CAN	2001-03	0.07	8.40	24.20	1.05	46.40	0.22	55.10	29.70	89.70	8.98		0.00	13.21	134.2	195.4
San Pablo Bay Marshes	SOCR	2000-03	0.09	5.05	20.70	1.05	39.00	0.06	43.60	26.10	73.40						
San Pablo Bay Marshes	SOCR	2000-07	0.09	5.14	18.60	1.05	19.00	0.11	47.20	21.70	73.50	1.00		0.00	17.55	122.4	614.2
San Pablo Bay Marshes	SOCR	2001-03	0.09	2.66	17.30	1.05	19.00	0.13	31.90	16.40	57.30	6.12		0.00	32.01	111.3	216.6
San Pablo Bay Marshes	WBCC 2A	1995-02	19.12	0.32	0.23	152.45	61.71	0.39	126.74	37.48	147.090	3.54	0.27	0.37	7.4	108.6	698.0
San Pablo Bay Marshes	WBCC 2B	1995-02	19.71	0.35	0.23	55.84	66.47	0.45	114.98	51.03	151.380	4.72	0.37	0.94	10.6	109.2	878.7
San Pablo Bay Marshes	WBCC 3A	1995-02	18.80	0.33	0.21	51.79	63.08	0.39	108.97	37.31	142.770	4.61	0.35	0.52	9.0	128.7	869.8
San Pablo Bay Marshes	WBCC 3B	1995-02	21.01	0.35	0.22	54.00	64.05	0.47	107.77	37.42	143.080	4.27	0.32	0.84	11.8	105.3	791.7
South Bay	BA21	1994-02	0.56	11.50	98.63	0.16	54.46	0.37	70.32	23.51	118.14	6.13	0.88	1.71	28.43	794.7	6837.4
South Bay	BA21	1994-08	0.38	9.90	93.69	0.15	42.24	0.36	99.99	22.87	130.21	3.35	0.22	0.22	14.05	245.8	2386.4
South Bay	BA21	1995-02	0.48	8.00	85.91	0.17	43.06	0.39	83.40	31.39	126.29	0.93	0.13	0.00	4.02	238.0	2102.0
South Bay	BA21	1995-08	0.38	11.61	89.80	0.16	38.30	0.37	86.30	30.10	114.80	5.60	0.33	0.94	13.41	314.0	1643.0
South Bay	BA21	1996-02	0.46	12.50	125.73	0.20	55.63	0.32	117.86	29.91	164.96	7.60	0.45	2.20	20.87	423.0	3512.0
South Bay	BA21	1996-07	0.46	8.93	112.20	0.15	40.20	0.33	96.70	26.10	130.50	4.70	0.30	1.40	19.86	298.0	2690.0
South Bay	BA21	1997-01	0.31	6.11	130.00	0.21	40.00	0.51	113.00	28.00	130.00	5.10	0.32	1.50	10.79	188.0	1455.0
South Bay	BA21	1997-08	0.40	7.72	137.00	0.20	42.80	0.28	111.00	26.10	130.00	14.80	0.31	0.00	10.25	272.0	1758.0
South Bay	BA21	1998-02	0.47	4.62	197.00	0.38	65.50	0.19	185.00	29.00	200.00	4.90	0.84	3.80	14.75	221.0	1182.0
South Bay	BA21	1998-07	0.34	11.00	135.00	0.17	43.80	0.34	124.00	9.30	149.00	2.90	0.35	0.00	3.38	239.0	1327.0
South Bay	BA21	2000-07	0.32	10.30	145.70	0.30	47.16	0.12	97.06	27.96	138.51	5.01	0.33	0.61	8.19	256.1	1606.8
South Bay	CA00-0014	2000-07	0.07	9.03	141.15	0.26	41.32	0.53	90.40	33.25	150.51	7.70	0.22	1.80	12.26	306.1	2186.6
South Bay	CA00-0015	2000-07	0.59	10.25	160.93	0.43	44.97	1.11	106.29	46.62	165.10	17.35	0.27	4.25	18.98	279.4	1882.0

Appendix II. Raw benthic data used in this study.

Estuary Area	Site	Date	Total Taxa	Total Abundance	Number of Amphipod Taxa	Number of Molluscan Taxa	Oligochaete Abundance	<i>Capitella capitata</i> Abundance	<i>Streblospio benedicti</i> Abundance
CCSF	CCSF04	1994-09	62	844	7			1	
CCSF	CCSF04	1995-02	13	27	1			0	
CCSF	CCSF04	1995-08	24	1594	5			0	
CCSF	CCSF04	1996-03	25	68	0			0	
CCSF	CCSF04	1996-08	24	957	3			0	
CCSF	CCSF04	1997-02	6	12	0			0	
CCSF	CCSF04	1997-08	48	2282	9			2	
CCSF	CCSF05	1994-09	79	2443	11			1	
CCSF	CCSF05	1995-02	39	357	4			0	
CCSF	CCSF05	1995-08	36	4073	10			1	
CCSF	CCSF05	1996-03	15	37	2			0	
CCSF	CCSF05	1996-08	41	858	9			3	
CCSF	CCSF05	1997-02	11	31	0			0	
CCSF	CCSF05	1997-08	29	1031	3			1	
CCSF	CCSF06	1994-09	65	1082	8			1	
CCSF	CCSF06	1995-02	27	115	2			0	
CCSF	CCSF06	1995-08	36	2016	7			2	
CCSF	CCSF06	1996-03	21	88	1			14	
CCSF	CCSF06	1996-08	26	784	3			0	
CCSF	CCSF06	1997-02	7	8	0			0	
CCSF	CCSF06	1997-08	33	594	7			2	
North Bay	BF21	1994-02	6	212	2	1	2		
North Bay	BF21	1994-08	6	216	1	1	2		
North Bay	BF21	1995-02	10	89	4	1	3		
North Bay	BF21	1995-08	7	12	1	2	0		
North Bay	BF21	1996-02	10	57	3	1	8		
North Bay	BF21	1996-07	8	23	2	2	2		
North Bay	BF21	1997-01	12	139	4	1	2		
North Bay	BF21	1997-08	6	63	0	2	1		
North Bay	BF21	1998-02	14	67	5	2	1		
North Bay	BF21	1998-07	8	41	1	3	9		
North Bay	BF21	1999-02	7	21	2	1	2		
North Bay	BF21	1999-07	8	300	2	1	0		
North Bay	BF21	2000-07	7	129	1	2	12		
North Bay	BF21	2001-02	10	346	3	1	21		
North Bay	CA00-0018	2000-07	5	47	0	2	0		
North Bay	CA00-0019	2000-07	4	139	1	1	0		
North Bay	CA00-0020	2000-07	5	17	0	2	0		
Richmond Harbor	RH1	1991-10	27	442	4	7	66	0	0
Richmond Harbor	RH2	1991-10	41	2012	6	14	361	0	0

Appendix II. Raw benthic data used in this study.

Estuary Area	Site	Date	Total Taxa	Total Abundance	Number of Amphipod Taxa	Number of Molluscan Taxa	Oligochaete Abundance	<i>Capitella capitata</i> Abundance	<i>Streblospio benedicti</i> Abundance
Richmond Harbor	RH3	1991-10	35	1041	2	16	171	0	1
Richmond Harbor	RH4	1991-10	32	1173	4	10	48	0	0
Richmond Harbor	RH5	1991-10	19	731	3	6	49	0	0
Richmond Harbor	RH6	1991-10	21	587	5	9	16	0	0
Richmond Harbor	RH7	1991-10	40	1219	9	14	41	0	0
Richmond Harbor	RH8	1991-10	10	79	1	3	3	0	0
Richmond Harbor	RH9	1991-10	47	1473	15	19	56	0	0
San Leandro Bay	CA00-0002	2000-07	13	73		3	0		2
San Leandro Bay	CA00-0004	2000-07	22	132		4	0		1
San Leandro Bay	CA00-0045	2000-07	46	732		7	0		0
San Leandro Bay	SLB-3	1997-04	28	5013		7	541	.	1208
San Leandro Bay	SLB-4	1997-04	24	3489		6	165		708
San Leandro Bay	SLB-5	1997-04	19	4847		4	205		710
San Leandro Bay	SLB-6	1997-04	26	4560		5	98		154
San Leandro Bay	SLB-7	1997-04	21	3844		5	394		490
San Pablo Bay Marshes	CAN	2000-03	21	4256		3	887		2672
San Pablo Bay Marshes	CAN	2000-07	18	2421		3	714		1434
San Pablo Bay Marshes	CAN	2001-03	21	3230		3	582		2124
San Pablo Bay Marshes	SOCR	2000-03	13	164		2	0		16
San Pablo Bay Marshes	SOCR	2000-07	9	139		2	11		0
San Pablo Bay Marshes	SOCR	2001-03	12	147		1	1		22
San Pablo Bay Marshes	WBCC 2A	1995-02	11	782		1	596		76
San Pablo Bay Marshes	WBCC 2B	1995-03	21	1793		2	965		209
San Pablo Bay Marshes	WBCC 3A	1995-03	10	156		1	85		0
San Pablo Bay Marshes	WBCC 3B	1995-03	15	1976		2	1583		298
South Bay	BA21	1994-02	13	1603		2	61		0
South Bay	BA21	1994-08	18	372		3	20		4
South Bay	BA21	1995-02	14	237		4	21		26
South Bay	BA21	1995-08	11	748		2	14		38
South Bay	BA21	1996-02	10	696		2	14		8
South Bay	BA21	1996-07	12	497		3	2		7
South Bay	BA21	1997-01	14	383		3	4		0
South Bay	BA21	1997-08	15	764		4	1		1
South Bay	BA21	1998-02	10	167		3	19		1
South Bay	BA21	1998-07	15	2890		4	5		4
South Bay	BA21	2000-07	14	1007		5	9		19
South Bay	CA00-0014	2000-07	8	367		2	0		10
South Bay	CA00-0015	2000-07	8	147		2	0		1

Appendix III. Effects Range Low (ERL) and Effects Range Medium (ERM) values used in this study.

Parameter	Unit	ERL	ERM
Arsenic	ppm	8.2	70
Cadmium	ppm	1.2	9.6
Chromium	ppm	81	370
Copper	ppm	34	270
Mercury	ppm	0.15	0.71
Nickel	ppm	20.9	51.6
Lead	ppm	46.7	218
Silver	ppm	1	3.7
Zinc	ppm	150	410
HPAHs	ppb	1700	9600
LPAHs	ppb	552	3160
Total Chlordanes	ppb	0.5	6
Total DDTs	ppb	1.58	46.1
Total PCBs	ppb	22.7	180

Comments by Dr. Fred Nichols (USGS, retired)

In the appendix that follows, comments received by Dr. Fred Nichols are provided. Dr. Nichols comments focused on the validity of the data manipulation, ecological interpretations, and overall conclusions. However, SFEI decided to not pursue changes to the report in response to these comments. Due to limited funding, some non-significant relationships, and the limitation of certain datasets, effort to further develop the statistical method presented in this report was not deemed possible at this time. Dr. Nichols comments should be considered when revising this method as more benthos and chemistry data from the Estuary become available through RMP and other monitoring efforts.

Nichols' Review of Melwani and Thompson
August 21, 2007

General Comments

- As Bruce well knows, I am not a contaminant expert. In my review I have focused attention mostly on the biology – i.e., do the study and its conclusions make sense from the perspective of what is known about the individual benthic species and communities found in San Francisco Bay, particularly from my own knowledge and experience. I have not checked your various statistical manipulations at all. I leave that to someone who is more familiar with these techniques.
- Overall, I am struck by three nervous reactions to this paper:
 - I cannot escape the concern that I am reading a new manipulation of already well manipulated data, i.e., not a re-evaluation of the original raw data. I wonder if this reworking of already worked information - an analysis that is even further removed from the raw data - affects the conclusions in a significant way. It is impossible for me to answer this question.
 - I worry about the fact that some information is intentionally not included in the analyses in order to reach the desired conclusions. The glaring example is the exclusion of *Grandidierella japonica* from the Richmond Harbor analysis solely because of “evidence for this species being a relatively abundant contaminant-tolerant taxon compared to the other contaminant sensitive amphipod species present.” This strikes me as an example of ignoring data that do not fit preconceived notions. The bigger concern is how much of this kind of approach is embedded in all of the existing analyses, both the previously published and present analyses?
 - Despite the apparent situation that (a) the majority of sampling sites show low levels of contaminants, (b) only a few sites show high concentrations, and (c) few if any locations have intermediate contaminant concentrations, the authors place emphasis on specific curvilinear relationships between contaminant concentrations and various measures of the biota that

assumes knowledge about the effects of intermediate contamination. Without such data, such emphasis is unwarranted. The curve fitting that appears in several of the figures is not based on sufficient data or valid statistical analysis. These graphics seem to me to represent wishful thinking.

- As Bruce also well knows, I have long stressed the need to understand the role of seasonal and interannual physical processes - for example those processes that in part are reflected in measures of salinity - on the composition of the benthic community. Unfortunately, the analysis in this paper continues the trend - seen in so many papers written about contaminant effects on the benthos - of assuming that the salinity measured at the time of sampling always has great relevance to the community found in the samples. My experience working with data sets that incorporate regular monthly, seasonal, and/or interannual sampling in river-dominated estuaries has clearly shown that salinity at the time of sampling is often much less important to the benthos on the day of sampling than the history of salinity changes at the site in the months *prior* to the sampling. For example, the physical conditions/processes - reflected in changing salinity - that control distribution and settlement of larvae or erosion/transport/deposition of sediment, often many months earlier, are probably very different from, and probably much more important to the determination of community structure than those conditions present at the time of sampling. Awareness of this fact, therefore, becomes very relevant when I try to estimate the veracity of conclusions drawn about the importance of contaminant effects from analyses that incorporate combining data collected at a site from different seasons and different years. What is presumed to be a linear relationship between community structure and salinity may be largely accidental or spurious. Thus, it is easy for me to understand why it has been difficult for you to draw definitive conclusions from these analyses.
- These analyses, the mostly unclear results, and the conclusions drawn in your "Discussion and Conclusions" section all suggest to me the overriding need for an altogether new study, focused for example at Richmond Harbor, San Leandro Bay, and the CCSF Wastewater Discharge Area, in which the sampling and analysis protocols are carefully designed to test specific contaminant effects hypotheses. What is needed, it seems to me, is a study employing a statistically sound sampling strategy based on collecting and evaluating information from an array of sampling locations in each of the three areas, extending from the most contaminated regions to the least contaminated regions ("background") in each area, including sites with intermediate levels of contamination. In short, the goal would be to collect benthos and contaminant samples along a *continuum* of contaminant exposure, i.e., to include samples from areas that represent the full range of contaminant loadings, especially the intermediate levels of contamination that seem to be mostly lacking in the data that you have analyzed. The purposes, obviously, would be to learn over what geographic distances from presumed "sources" one can demonstrate contaminant effects above some assumed background level and whether or not there are effects thresholds for individual

contaminants. The primary sampling effort should probably be carried out at one time to avoid the confounding effects of time. However, even more could be learned from collecting and evaluating information from a presumably smaller number of “representative” sampling locations in each area through repeated sampling (e.g., every two months?) over an extended period to begin to tease apart the natural effects of seasonal changes in salinity regime and sediment characteristics from the effects of contaminants. Without carefully designed studies to specifically address the problems that you raise in this paper, it does not seem to me that you will make any further progress in advancing the science or developing the understanding that is relevant to the agencies’ needs.

Specific Comments

- Page 2, line 18 and subsequent references – Where exactly in “North Bay” and “South Bay” do you mean? Given that both of these traditional terms represent broad areas, each with very different sub areas, it would help the reader greatly if you were more precise, e.g. Suisun/Grizzly Bay and Extreme South Bay below the Dumbarton Bridge. For example, the term “South Bay” has traditionally been used to describe that part of San Francisco Bay below the Bay Bridge, although more recently folks refer to the region south of the very important physical boundary of San Bruno Shoal. It is important that you use prevailing geographic terminology, not terminology developed for your own purposes.
- Page 8, figure 1A – From a biology perspective, the Pearson-Rosenberg model of the effects of increasing organic enrichment on the benthos – initial increase in abundance/biomass etc. in response to increasing food supply followed by a decrease as a result of organic overloading – makes perfect sense. [This was actually nicely demonstrated in San Francisco Bay in the 1950s in a field study carried out by Francis Filice and his students.] But, I still do not understand, from a biology perspective, why increasing contaminants should have the same initial enhancement effect. I worry that data might have been selectively used to try to make this case.
- Page 9, lines 12/13 – I do not have a clue what is meant by “Missing data were estimated by interpolation when one or two values were missing.” Which data? Missing over what space or time scales? Ditto “Samples with more than two missing values were excluded from the analysis.” What samples and values? I obviously have not tried to follow all of your computational steps, so this may be clearer to those who take the time to do this. Nonetheless, these statements about data manipulation contribute to my nervousness about the validity of the analyses and the resulting conclusions.
- Page 10, lines 13/14 – As I mention above, selectively removing from consideration the information about a dominant species makes it evident to this reader that you are manipulating the data to achieve a preconceived conclusion. Without any further explanation or presentation of all data, I conclude that this is an illegitimate application of the scientific method.
- Page 11, line 1 – re “combining data from an area...could have confounded our attempts to determine possible influences to the benthos.”, the same can be said

for the confounding probably caused by combining data from different seasons and years.

- Page 14 – I vaguely recall that Richmond was also a major source of PCBs; yet these are not mentioned here. Were PCB measurements not part of the sampling in this area?
- Page 15, lines 2/3 – You suggest that the lack of variability of salinity is a problem in your analysis. The fact that salinity was predictably the same at all locations during this one-time study should be viewed as a positive as it eliminates one variable. In other words, it seems to me that you could still include it in your table.
- Page 17, Figure 3 – I am puzzled by both the curve shown in the figure and the statement that “Curve fit shown for illustrative purposes only.” I have to ask the question, “What ‘illustrative purposes’ do you have in mind?” From my perspective, this figure illustrates (1) a lack of appreciation of basic statistical procedures: basing the specific shape of a curve largely on 3 widely scattered points is fantasy; and (2) an interpretation that has no explainable basis that I could detect. Unfortunately, I suspect that the intent was to produce a graphic that seemed similar to the hypothetical curve in Figure 1. I see no justification for this, and there is certainly no statistical basis for presenting this curve to readers as a legitimate/useful piece of “illustrative” information.
- Page 17, line 7/8 – In part because I am not familiar with the Ferraro and Cole paper, I do not understand this sentence.
- Page 17, lines 13-15 – To my eye, Figure 3 demonstrates (a) a tight clustering of most of the data at low DDT concentrations; (b) three erratic data points at high concentrations; (c) the lack of undoubtedly critical data between 200 and 1400 ug/kg that might allow for meaningful understanding of the true relationship and reveal the possibility of possible threshold contaminant levels; and (d) the curve “shown for illustrative purposes only” is spurious, i.e., has no basis in statistical reality. I think that you are greatly over-interpreting this set of data, i.e., implying much more than your data and any statistical analysis of them could support. You should not report any single fitted curve that tries to lump the small cluster of data points with the three widely separated and scattered points.
- Page 21, Figure 4 – Again, this curve fitting is imaginary. I suspect that simple least squares line drawing - a horizontal line [parallel to the x-axis, i.e., no relationship] for **total taxa**, and a straight line from lower left to upper right for *Streblospio benedicti* - would be the only statistically justifiable presentation. These large spreads of few data points allow no more detailed analysis or interpretation.
- Page 25, line 29 – Is this yet another example of more data manipulation to get better results?
- Page 32, line 21 – Again, you should be more specific about which region of South Bay you are talking about. It has been awhile since I have been involved in discussions of local nomenclature, but the region below Dumbarton Bridge used to be known as “Extreme South Bay.”