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Optimizing Transplanted Bivalve Studies for the Regional Monitoring Program for Trace Substances

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1 Introduction

The RMP Bioaccumulation Program originated in 1993 as one component of the RMP Status and Trends Monitoring Program, which also includes water and sediment quality monitoring. Over its history, the bioaccumulation program (the Program) has undergone numerous changes in response to program findings and identified needs. The purpose of this technical report is to document the various changes that have occurred in the Program, to present the justifications for these changes, and to identify ongoing investigations that may result in changes to the Program in the near term.

2 Objectives of the Bioaccumulation Program

The original objectives of the RMP (San Francisco Estuary Institute, 1994)were as follows:

- To obtain baseline data describing the concentration of toxic and potentially toxic trace element and organic contaminants in the water and sediment of the San Francisco Estuary;
- To determine seasonal and annual trends in chemical and biological water quality in the San Francisco Estuary;
- To continue to develop a data set that can be used to determine long-term trends in the concentrations of toxic and potentially toxic trace elements and organic contaminants in the water and sediments of the San Francisco Estuary;
- To determine whether water quality and sediment quality in the Estuary at large are in compliance with objectives established by the Basin Plan;
- To provide a data base on water quality and sediment quality in the Estuary which is compatible with data being developed in other ongoing studies in the region, including, but not limited to: wasteload allocation studies, model development, sediment quality objectives development, in-bay studies of dredged material disposal, Interagency Ecological Program water quality studies, primary productivity studies, local effects biomonitoring programs, and state and federal mussel watch programs.

In support of these programmatic objectives, the initial goals of the RMP bioaccumulation monitoring program component were to:

- Measure the bioavailable portion of contaminants in the water column;
- Evaluate which contaminants may be transferred to higher trophic levels of the food web, and thus to what extent certain contaminants may pose health risks to wildlife and humans.

These general goals implicitly address the overall original RMP objectives of determining seasonal and long-term trends in chemical and biological water quality. The bioaccumulation program component also complements the water and sediment sampling. Unlike the water quality sampling, which gives an indication of water quality at one particular point in time, contaminant concentrations measured in transplanted bivalves serve to integrate water quality over the period of deployment (typically 90 to 100 days). Also, while measurement of contaminant concentrations in water and sediment are useful for trend monitoring over time, they do not specifically reveal the extent to which various contaminants are able to transfer into the food web and pose risks to higher-order consumers.

3 Bioaccumulation Program History

The program was based upon numerous other transplanted bivalve monitoring programs that have been applied around the world (Phillips, 1980). Bivalves Methods were adopted that provided data that were comparable to the State Mussel Watch (SMW; *e.g.*, Phillips, 1988). The following section charts the history of the Program from its inception to the present.

3.1 Initial Program Design

The RMP Bioaccumulation Program was initiated in 1993 as a bivalve transplantation study in which bivalves were collected from "clean" locations (*i.e.*, those with relatively low concentrations of specific pollutants) and transplanted to fixed sites within the Estuary. The Program methodology was substantially based upon a pilot study (Stephenson, 1992), which examined variation in bioaccumulation according to deployment depth and duration. While transplanted bivalves have been widely used for measuring contaminants in coastal areas where temperature and salinity do not vary substantially from site-to-site or time-to-time, the major seasonal and spatial salinity and temperature variation in San Francisco Bay provided special challenges for designing a cohesive, estuary-wide transplanted bivalve bioaccumulation program. In order to address these varying parameters, the Program initially used three bivalve species, which were deployed according to salinity range expected at each site over the course of deployment:

- *Mytilus californianus*, the California mussel, were collected from Bodega Head and deployed at the most saline locations;
- *Crassostrea gigas*, the Japanese oyster, were collected from Tomales Bay and deployed at locations of intermediate salinity;
- *Corbicula fluminea*, a freshwater clam, were collected from Lake Isabella on the Kern River in central California and deployed at locations of lowest salinity.

Bivalves initially were deployed at eleven sites throughout the Estuary (Figure 1) to represent both the spine and margins of the Estuary. In 1994, four deployment sites were added, for a total of 15. Specific site locations were heavily influenced by the availability of a fixed structure to easily relocate the subsurface moorings, which remained in place year-around (Figure 2).

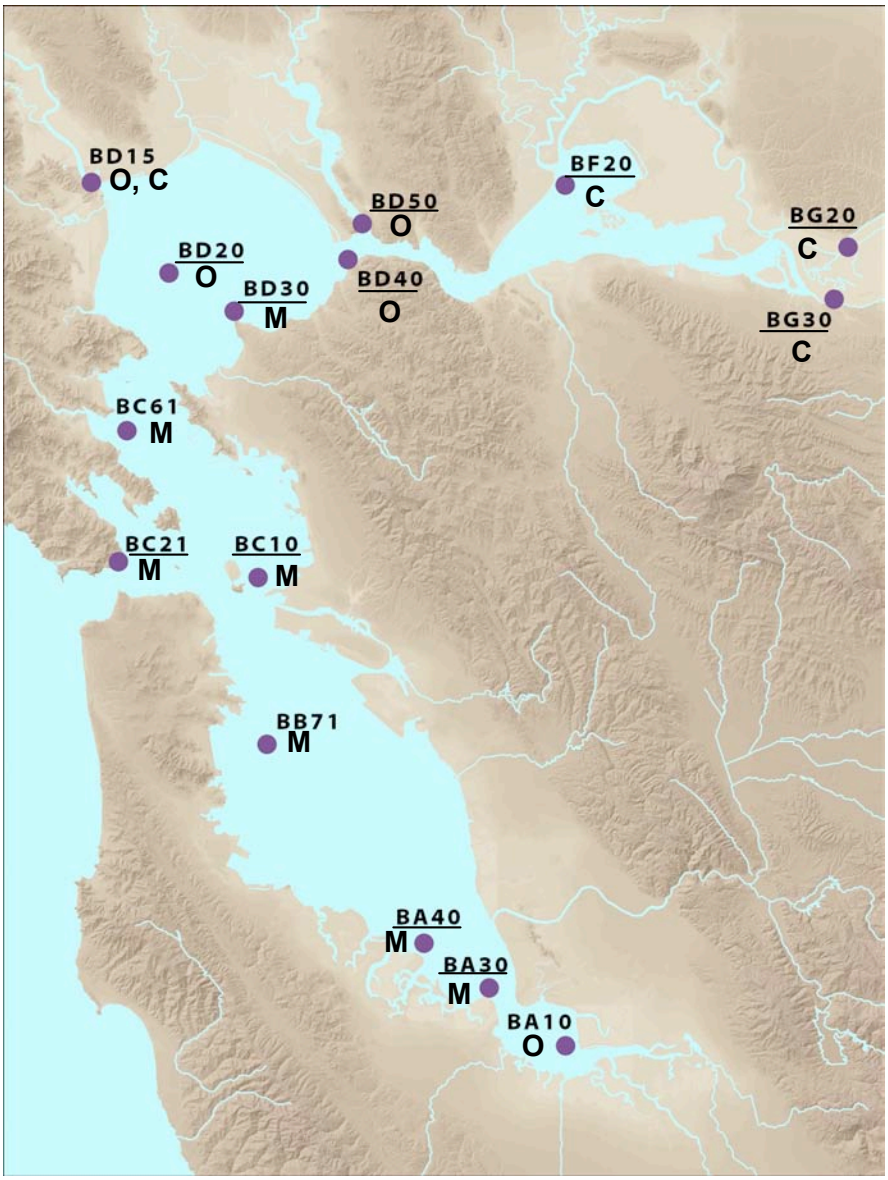

Figure 1. Regional Monitoring Program transplanted bivalve bioaccumulation deployment sites. Deployments at underlined sites began in 1993 and deployments at non-underlined sites began in 1994. Figure provided by SFEI. Bivalve species normally deployed at each site are indicated; C = freshwater calm (*C. fluminea*), O = Japanese oyster (*C. gigas*), M = California mussel (*M. californianus*). BD15 had clams deployed in the wet season and oysters deployed in the dry season.

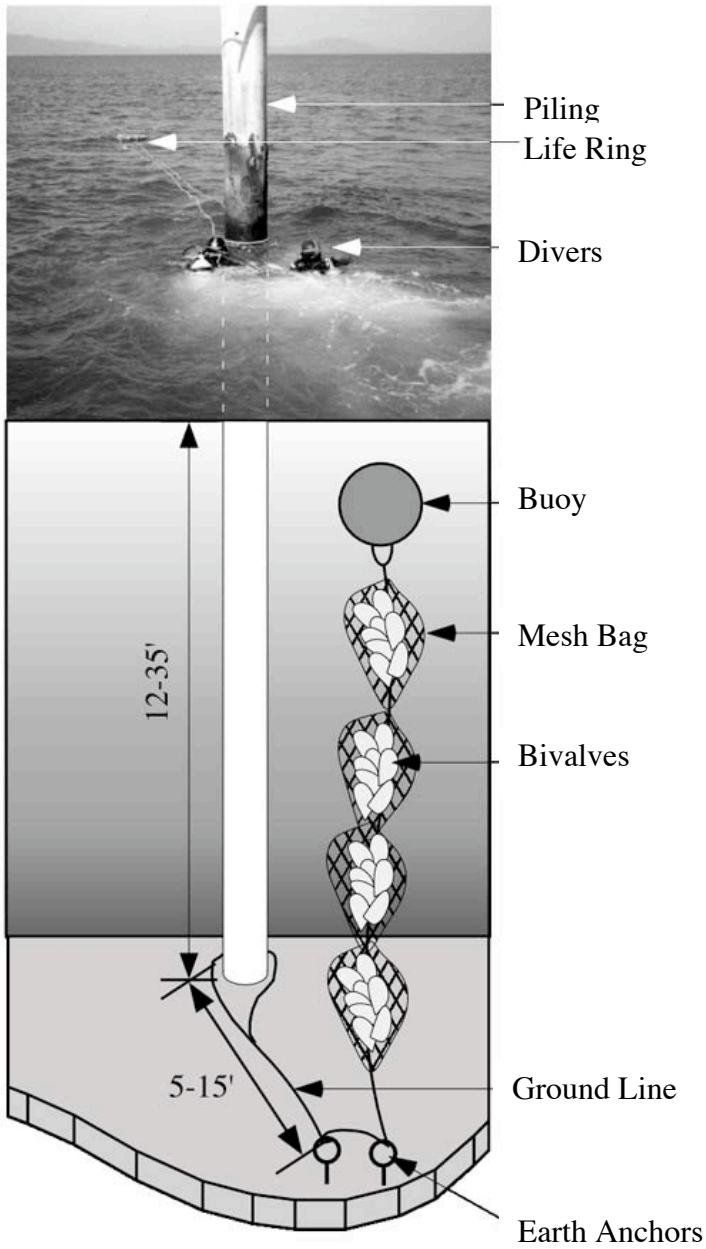

Figure 2. Typical bivalve deployment from initial program design.

Bivalves were deployed by scientific divers for 90 to 100-day periods beginning in approximately February and June, to coincide with typical wet and dry seasons, respectively. These deployment periods were chosen to encompass the range of hydrographic conditions in the Estuary and to allow comparisons of within-season variation in addition to trend monitoring over time. In most cases, the species deployed at an individual site were consistent between seasons. Nevertheless, site BD15 at the mouth of the Petaluma River exhibited such extreme seasonal variation in salinity that it was necessary to deploy *C. gigas* in the dry season and *C. fluminea* in the wet season to ensure sufficient numbers of surviving bivalves to allow all analyses to be performed.

At the conclusion of deployments, bivalves were retrieved, processed using clean techniques, and aliquoted for eventual analysis (David *et al.*, 2001). Bivalves designated for laboratory analysis were analyzed for a suite of trace metals and trace organics parameters. Laboratory processing prior to analysis was consistent with SMW. Bivalves were rinsed in Milli-Q water and removed from the shell while still frozen. As SMW had determined that the presence of gonads was a significant source of variation in trace metal concentrations, gonads were removed using stainless-steel scalpels before the bivalves were homogenized for trace metal analyses. Because the organic compounds to be measured in bivalves are lipophilic, gonads were not removed from bivalves being processed for organic analyses. Generally, 30–40 bivalves were composited from each site for each type of analysis, although mortality sometimes required fewer to be analyzed. Additional measurements associated with the Bioaccumulation Program included bivalve survival, an integrator of all causes of mortality, and condition index (Crosby & Gale, 1990), a measure of bivalve health.

Changing status of wild *C. fluminea* populations in Lake Isabella forced changes to the Program in 1996. In preparation for the 1996 wet season deployment cruise, AMS personnel were unable to locate sufficient numbers of *C. fluminea* within Lake Isabella to support deployment at any of the three stations. This coincided with an extended period during which the lake water level was significantly lower than usual. Attempts were made over the next two years to locate another population of uncontaminated clams, which involved collections from Putah Creek, Russian River, San Antonio Reservoir, Lake Sonoma, Lake Chabot, Del Valle Reservoir and Briones Reservoir, but a suitable population could not be found. Rather than discontinue gathering data from the least saline sections of the Estuary entirely, in 1998 the decision was made to collect and analyze resident *C. fluminea* from the BG20 (Sacramento River) and BG30 (San Joaquin River) sites. At that time, the BF20 (Grizzly Bay) site was discontinued because no resident clams could be found there.

3.2 Five Year Review / Redesign

A workshop (Bivalve Workshop) was held on March 30, 1999 to evaluate the bioaccumulation component of the RMP. This evaluation was performed within the context of revised overall program objectives for the RMP that were formulated as a result of the 5-year review (Bernstein & O'Connor, 1997). These revised RMP objectives are as follows:

- Describe patterns and trends in contaminant concentration and distribution,
- Describe general sources and loadings of contamination to the Estuary,
- Measure contamination effects on selected parts of the Estuary ecosystem,

- Compare monitoring information to relevant water quality objectives and other guidelines,
- Synthesize and distribute information from a range of sources to present a more complete picture of the sources, distribution, fates, and effects of contaminants in the Estuary ecosystem.

The revised program objectives formed the basis for revised objectives for the bioaccumulation component of the RMP. Two objectives continued from the initial Program design and three new objectives were added, as follows:

- Determine trends in tissue contamination,
- Measure the bioavailable portion of contaminants in the water column,
- Evaluate which contaminants may be transferred to higher trophic levels of the food web,
- Determine pathways and loadings of contaminants to the Estuary, and
- Determine effects of contaminants in the Estuary.

The goal of this workshop was to provide recommendations to the RMP Technical Review Committee for ways to improve the cost effectiveness and ability of the Bioaccumulation Program to address these objectives. Consequently, discussion at the workshop included consideration of whether the transplanted bivalve method currently used to measure bioaccumulation is the most appropriate way to achieve each of these objectives, as well as ways to improve the method. A report of the workshop is provided in Appendix A.

The following section describes the recommendations from the workshop and the actions taken to implement them, vis-à-vis the transplanted bivalve monitoring. Numerous recommendations were discussed that either did not pertain specifically to the transplanted bivalve monitoring or that were not subsequently implemented, and these are not discussed here. This discussion is organized according to program objective. In some cases, the Program modifications were implemented immediately following the five-year review. In others, the workshop identified areas requiring further investigation prior to any modifications. For each potential modification, we present a brief summary of findings considered as part of the five-year review, any follow-on investigations arising out of the review, and subsequent Program modifications.

3.2.1 Objective 1 - Determine Trends in Trends in Tissue Contamination

3.2.1.1 Recommendation – Continue transplanted bivalve monitoring using methods similar to California State Mussel Watch (SMW).

At the time of the five-year review, the SMW program had been in existence for almost two decades and represented an invaluable long-term database of bivalve bioaccumulation, to which the RMP was considered a key contributor. The SMW program employed mussels (*Mytilus californianus*) and clams (*Corbicula fluminea*), the latter as both transplants and residents. The RMP transplanted bivalves revealed trends that were not apparent from data on water contaminants, suggesting that bivalves may be especially valuable for tracking long-term changes in contaminant concentrations in the Estuary. For instance, analysis of data from

1993–1997 indicated significant increases in copper and decreases in PCBs in transplanted mussels that were not revealed in water data (Hardin *et al*., 1999). Moreover, bivalves integrate over the deployment period exposure to both dissolved and particulate phases of contaminants, as compared to semi-permeable membrane devices, which sample only the dissolved phase.

3.2.1.1.1 Action Taken

This recommendation was implemented and bioaccumulation monitoring using transplanted bivalves was continued.

3.2.1.2 Recommendation – Consider elimination of wet-season bioaccumulation monitoring.

During the period 1994–1997, trends in contaminant concentrations were more consistently observed in dry-season data than in wet-season data (Hardin *et al*., 1999). Variation within wet seasons was often lower than within dry seasons, suggesting that the bivalves are responding to real phenomena that vary from year-to-year on a non-linear basis. For example, recent analysis of transplanted bivalve data from 1980–2003 for Yerba Buena Island suggests that trends in legacy pesticides are influenced by discharges from the Sacramento-San Joaquin Delta (Connor *et al*., in preparation). While this wet season information is useful for determining processes and pathways for entry of contaminants into the Estuary (see Objective #4), year-to-year variation in wet-season transplanted bivalve data makes wet-season data less useful than dry-season data for detecting long-term trends.

3.2.1.2.1 Action Taken

The wet-season bivalve deployment was eliminated in 2000.

3.2.1.3 Recommendation – More than one site per bay segment is needed to detect trends in a reasonable time period.

When data for copper and total PAHs were examined as examples of the effect of sampling multiple sites per segment, very few significant trends were observed at individual sites, whereas numerous trends were observed when sites were combined into segments.

3.2.1.3.1 Action Taken

This recommendation was implemented and sampling at multiple sites per segment was continued.

3.2.1.4 Recommendation – Deploy a single species at all sites.

While the use of different species at different sites enables tracking trends at individual sites or within segments, the use of different species makes it very difficult to compare sites and segments. It is well known that different bivalve species accumulate contaminants different at rates (Hardin *et al*., 1999; O'Connor, 1999). Between 1993 and 1996, different species were

deployed side-by-side to determine which bivalve species survived best at certain sites (Table 1). These side-by-side comparisons included the oyster *Ostreola conchaphila*, which occurs naturally in San Francisco Bay. There was no consistent source of *O. conchaphila* for transplantation and, based upon its generally inferior survival rates, it was not studied as a possible species for deployment at all sites beyond 1996.

3.2.1.4.1 Action Taken

Beginning in 1999, numerous side-by-side deployments of *M. californianus*, *Mytilus edulis*, the bay mussel, and *C. gigas* were made to investigate whether one species could be deployed at all sites. Moreover, because much of the summer mortality in *C. gigas* was thought to be related to spawning, a triploid strain of *C. gigas*, which does not reproduce, also was used in these later side-by-side deployments.

3.2.1.5 Recommendation – Collect CTD Profiles at each Bivalve Site.

Analysis of variation in bivalve survival, condition and bioaccumulation according to water-quality parameters measured by USGS at sites near RMP bivalve sites suggested that non-contaminant environmental factors may affect bivalve bioaccumulation and health. Salinity, dissolved oxygen, temperature, and total suspended solids had the greatest effects. Moreover, periodic high mortality of transplanted bivalves was usually related to low salinities and high temperatures for mussels and oysters, respectively. Collection of CTD profiles would provide more site-specific data than are available from USGS, in order to help determine how non-contaminant environmental factors affect the transplanted bivalves.

3.2.1.5.1 Action Taken

This recommendation was implemented in the 1999 dry season deployments.

3.2.2 Objective 2 – Measure the Bioavailable Portion of Contaminants in the Water Column

3.2.2.1 Recommendation – Discontinue measurement of mercury and arsenic in bivalves.

Many trace metals do not appear to accumulate much above concentrations measured in the "clean" populations used as sources for transplants. In particular, mercury and arsenic were generally very similar in the transplanted bivalves and in the source populations. While it was not known whether the low accumulations in the transplants were due to poor bioaccumulation or ambient concentrations that were similar between the source locations and the estuary, trends apparent for tissue concentrations of some metals, such as copper, suggested that ambient conditions within the estuary are being reasonably well represented by the transplants. Moreover, in the case of mercury, there was evidence that bivalves were not the best indicators of bioavailability, especially for methylmercury.

Table 1. Survival results from side-by-side deployments of bivalve species in 1993–1996.

Site	Bivalve Retrieval Date	Species	% Survival
BA10	9/12/1996	*C. gigas*	74
		O. conchaphila	83
BA30	10/6/1993	*C. gigas*	37
		M. californianus	98
BA40	9/12/1996	*M. californianus*	99
		O. conchaphila	97
BB71	9/12/1996	*M. californianus*	99
		O. conchaphila	92
BC21	9/13/1995	*M. californianus*	98
		O. conchaphila	88
	9/11/1996	*M. californianus*	96
		O. conchaphila	89
BD15	10/6/1993	*C. gigas*	61
		M. californianus	98
	4/26/1995	*C. fluminea*	65
		C. gigas	0
	9/13/1995	*C. fluminea*	2
		C. gigas	25
		O. conchaphila	0
	9/11/1996	*C. gigas*	92
		O. conchaphila	80
BD30	9/13/1993	*M. californianus*	99
		O. conchaphila	97
	9/11/1996	*M. californianus*	100
		O. conchaphila	86
	4/23/1998	*C. gigas*	84
		M. californianus	0
BD40	10/7/1993	*C. gigas*	46
		M. californianus	97
	9/13/1995	*C. gigas*	64
		O. conchaphila	50
	9/10/1996	*C. gigas*	99
		O. conchaphila	89
BD50	10/6/1993	*C. gigas*	32
		M. californianus	16
BF20	4/27/1995	*C. fluminea*	89
		C. gigas	0
		O. conchaphila	0
	9/14/1995	*C. fluminea*	96
		O. conchaphila	0

3.2.2.1.1 Action Taken

The analysis of mercury and arsenic in bivalves was discontinued in 2000.

3.2.2.2 Recommendation – Reduce the frequency of trace metals analysis in bivalves.

This recommendation focused specifically on trace metals that are not on the 303(d) list or the Regional Board's "pollutants of concern" for San Francisco Bay. Nevertheless, although San Francisco Bay is listed as impaired due to selenium, USGS data on the temporal fluctuations in selenium in *Potamocorbula amurensis* suggest that the study design of the RMP transplanted bivalve bioaccumulation program cannot capture the short-term variation that dominates loads of selenium into the bay. Consequently, the transplanted bivalves were not considered to be the best method for measuring the bioavailable portion of selenium.

3.2.2.2.1 Action Taken

Trace metals were not measured annually in transplanted bivalves after 2001, but were to be analyzed every fifth year only.

3.2.3 Objective 3 – Evaluate which Contaminants May Be Transferred to Higher Trophic Levels of the Food Web

3.2.3.1 Recommendation – Develop additional ways to assess transfer of contaminants to higher trophic levels.

It was thought that the use of *M. californianus* as the primary organism for the transplanted bivalve component might limit achievement of this objective because this species does not normally occur in the estuary and it has no natural position within the food web. *Mytilus edulis*, on the other hand, does occur throughout the estuary and it was thought it might survive at a broader range of salinities than does *M. californianus*. Nevertheless, deployment of transplanted bivalves in the water column may not adequately represent transfer of contaminants from the sediments into benthos and higher trophic levels. Other types of organisms, such as benthos or fishes, may be the best way to assessing the transfer of contaminants to higher trophic levels of the food web.

3.2.3.1.1 Action Taken

This recommendation was implemented with a fish contamination pilot study beginning in 1999.

3.2.4 Objective 4 – Determine Pathways and Loadings of Contaminants to the Estuary

3.2.4.1 Recommendation – One species should be deployed at all sites to eliminate the difficulty of interpreting data from different species.

It was acknowledged that bivalve measurements are able to discern differences in contaminants over spatial scales ranging from tens of meters to kilometers and over temporal scales from months to years. Nevertheless, the then-current configuration of the transplanted bivalve component limited its ability to achieve this objective. The high variability of salinity in the estuary, especially during the wet season, necessitated deployment of three different species for bioaccumulation measurements. Because bivalve species differ in their bioaccumulation characteristics, site comparisons were limited to those with the same species. Side-by-side deployments of multiple species were needed to determine whether there was a single species suitable for wet season deployment at all sites west of Carquinez Strait.

3.2.4.1.1 Action Taken

As discussed for Objective 1, investigations in support of this recommendation began in 1999 to determine whether a single species could survive at all sites.

3.2.5 Objective 5 – Determine Effects of Contaminants in the Estuary

3.2.5.1 Recommendation – Investigate measurements of contaminant effects in transplanted bivalves.

Although the RMP bivalve monitoring program operates under the assumption that the bivalve species used are unlikely to be affected by contaminant levels found in the estuary, significant correlations exist between concentrations of tissue contaminants and indicators of bivalve health. While investigators in other areas have found significant biological effects of contaminants on bivalves (Salazar & Salazar, 1997; Salazar & Salazar, 1998), it is not known how non-contaminant environmental factors affect these biological indicators. Bivalve condition was being measured in the RMP transplanted bivalve monitoring as an indicator of bivalve health and as a potential indicator of contaminant effects. As the ratio of tissue weight to shell volume, condition is affected by both changes in shell size and changes in tissue mass. We have previously noted that transplanted *M. edulis* will gain in shell length while they are losing tissue weight (Hardin, pers. comm.). Consequently, it was proposed that a more direct measure of bivalve health would be to simply estimate growth by measuring tissue dry weight.

3.2.5.1.1 Action Taken

Beginning in 1999, a comparison of growth and condition was begun. Growth was estimated by subtracting the mean tissue dry weight for T-0 samples from the ending dry weight for each bivalve analyzed for condition.

3.3 Investigations Subsequent to Five-Year Review / Redesign

In this section, we present the results of investigations taken to address recommendations from the March 30, 1999 Bivalve Workshop, as well as the results of other investigations made in an effort to improve the transplanted bivalve monitoring. Many of these results were presented to the RMP Technical Review Committee (TRC) on September 19, 2002. In several cases, the TRC took action at the meeting, based upon presented results. This section is organized according to the issues being investigated.

3.3.1 Investigation of Tissue Weight Variability due to Reproductive State

Loss in transplanted bivalve tissue weight was frequently observed. During peer review of the bivalve results, questions arose concerning the possible effects of spawning on decreases in tissue weight. Growth metrics can be impacted by the reproductive stage of the bivalves in that release of gametes prior to bivalve retrieval can downwardly bias measurements of growth and condition. Moreover, release of lipid-rich gametes will also result in loss of accumulated lipophilic contaminants that are of particular interest to the RMP. In fish, reproduction also decreases methylmercury concentration in the parent as it is transferred to the offspring via gametes.

In an effort to reduce variability associated with reproduction, measurements were made to determine if animals were spawning during deployment. One of the common features of the transplanted bivalve data, especially for *M. californianus*, is a decrease in tissue weight during deployments at many sites in the Estuary. If the T-0 bivalve samples from Bodega Head had higher weights of gonadal tissue than the post-deployment samples, then declines in whole-body weights could be due to spawning. In an effort to examine this question, measurements of tissue weights for *M. californianus* were partitioned between somatic and gonadal tissues in 2000.

In the dry season 2000 deployment, tissue growth was calculated by subtracting the mean T-0 weight from the mean ending weight for each site (Table 2). Because the Bodega Head measurements were made for mussels of similar T-0 and end-of-deployment shell length, these data cannot be compared directly with those for mussels that would have grown in shell length during the deployment period.

The data suggest that losses of whole-body weight cannot be accounted for by losses of gonadal tissue related to spawning. At the two sites where losses of whole-body weight occurred, there were either a net increase in gonadal tissue or a decrease in gonadal tissue that was a small fraction of the lost whole-body weight. Conversely, at most sites where whole-body weights increased, greater than 50% of the increase was accounted for by gonadal tissue. The results

indicated that decreases in *M. californianus* tissue weight observed at many sites is probably due to lost of somatic tissue and not due to spawning.

Table 2. Growth in whole-body tissues (somatic + gonadal) and gonadal tissues in *Mytilus californianus* during dry-season deployments.

	Growth, g dry weight							
	1993	1995	1996	1997	1998	1999	2000	
Site	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body	Gonad
Bodega Head							0.76	0.18
Dumbarton Bridge	-0.24	-0.45	-0.20	-0.11	-0.14	-0.13		
Redwood Creek	-0.25	-0.50	-0.26	-0.09	0.24	-0.03	-0.03	0.02
Alameda		-0.12	0.20	0.09	0.00	0.30	0.50	0.30
Yerba Buena Island	0.25	1.22	0.28	0.30	0.16		0.82	0.46
Horseshoe Bay	0.88	0.27		1.06	1.05	1.17	1.45	0.82
Red Rock		-0.34	-0.26	0.04	0.00	0.21	0.15	0.09
Pinole Point	-0.18	-0.39	-0.26	-0.14	-0.20	-0.05	-0.15	-0.02

3.3.2 Test alternative measures of bivalve health

Prior to 2002, the RMP routinely measured condition index (CI) of deployed bivalves, a comparison of tissue dry weight to shell cavity volume. This measurement was made to verify that the bivalves were "healthy." When conditions are extreme, such as placing animals at the limit of their salinity tolerance, CI may be a useful indicator of health. When conditions are not extreme, however, there are many possible confounding factors that make interpretation of CI data difficult.

During the Bivalve Workshop held on March 30, 1999, measurement of tissue growth was proposed as a sensitive metric for indicating contaminant effects. Tissue growth, calculated as the difference between the mean dry weight of the T-0 bivalves and the mean dry weight of the sample after deployment, is a much less labor-intensive procedure compared to CI analysis, and therefore less expensive to the overall program.

An analysis was performed to determine if substituting growth for CI would result in a loss of power. Because statistical power varies according to the coefficient of variation ([standard deviation ÷ average] x 100) and the number of samples, the easiest comparison of power between CI and growth is based on the coefficient of variation. The coefficients of variation for growth (*i.e.*, changes in tissue weight) and changes in CI for *M. californianus* varied among years, with growth having the lowest coefficient of variation in two out of three years (Table 3). Tissue growth also had the lowest mean coefficient of variation over all three years, indicating

that use of tissue growth as the indicator of bivalve health would provide equivalent statistical power compared to CI. It was concluded from this analysis that the statistical power of growth is as good as, or better than, the statistical power of the CI for detecting trends or differences between years. Based on this conclusion, growth replaced the CI as the metric for bivalve health in 2002.

Table 3. Comparison of coefficients of variation between changes in CI and tissue growth for *Mytilus californianus* over three years.

	Coefficient of Variation	
Year	Condition Index	Tissue Growth
1999	343.5%	191.5%
2000	191.1%	166.5%
2001	294.8%	325.5%
Overall Mean	276.4%	227.8%

3.3.3 Transition to Use of Single Species for Deployments

From 1999 to 2002, several bivalve species were deployed in side-by-side experiments to evaluate their ability to survive and grow at all sites during dry-season deployments. *M. californianus* had been transplanted in the Estuary by the RMP in order to maintain consistency with previous SMW transplantation studies. As described earlier, studies of *O. conchaphila* as an alternate species were not continued past 1996 due to a lower survival rate than other species. The other bivalve species evaluated for deployment at all sites, in addition to *M. californianus* and *C. gigas*, were *M. edulis*, and a triploid version of *C. gigas*.

Each of the species offered advantages and disadvantages. *M. californianus*, while providing consistency with the SMW program, is normally restricted to exposed open-coast locations. The Bay mussel, which in California is commonly referred to as *M. edulis*, an Atlantic species that inhabits estuaries throughout the world. Nevertheless, there is substantial uncertainty regarding the taxonomy of Bay mussels that inhabit San Francisco Bay, as along the West Coast they are a hybrid of *M. trossulus* and *M. galloprovincialis* (Suchanek *et al.*, 1997). *M. edulis* is commonly used in monitoring programs in Europe and the East Coast of North America. The National Status and Trends Mussel Watch program samples *M. edulis* at three locations in the Estuary (O'Connor, 1999). If *M. edulis* survived better than *M. californianus*, contaminant studies conducted with the resident Bay mussel also could provide greater ecological relevance than those conducted with non-resident, open-coast mussels. *C. gigas* was investigated because it is an estuarine species with broad salinity tolerances. A triploid version of *C. gigas* also was investigated to see if prevention of spawning would reduce the summer mortality that had been observed in *C. gigas* in the RMP transplant monitoring.

The differences in survival among the species generally were not significant (Figure 3). Nevertheless, in the absence of predation that was observed at BC10 and BC21 (*i.e.*, bags torn and mussels broken by crabs), the triploid *C. gigas* and both species of mussels usually had higher survival than did *C. gigas*. Although the triploid *C. gigas* had good survival, it cost substantially more to obtain than the other species and was, therefore, not considered after 1999.

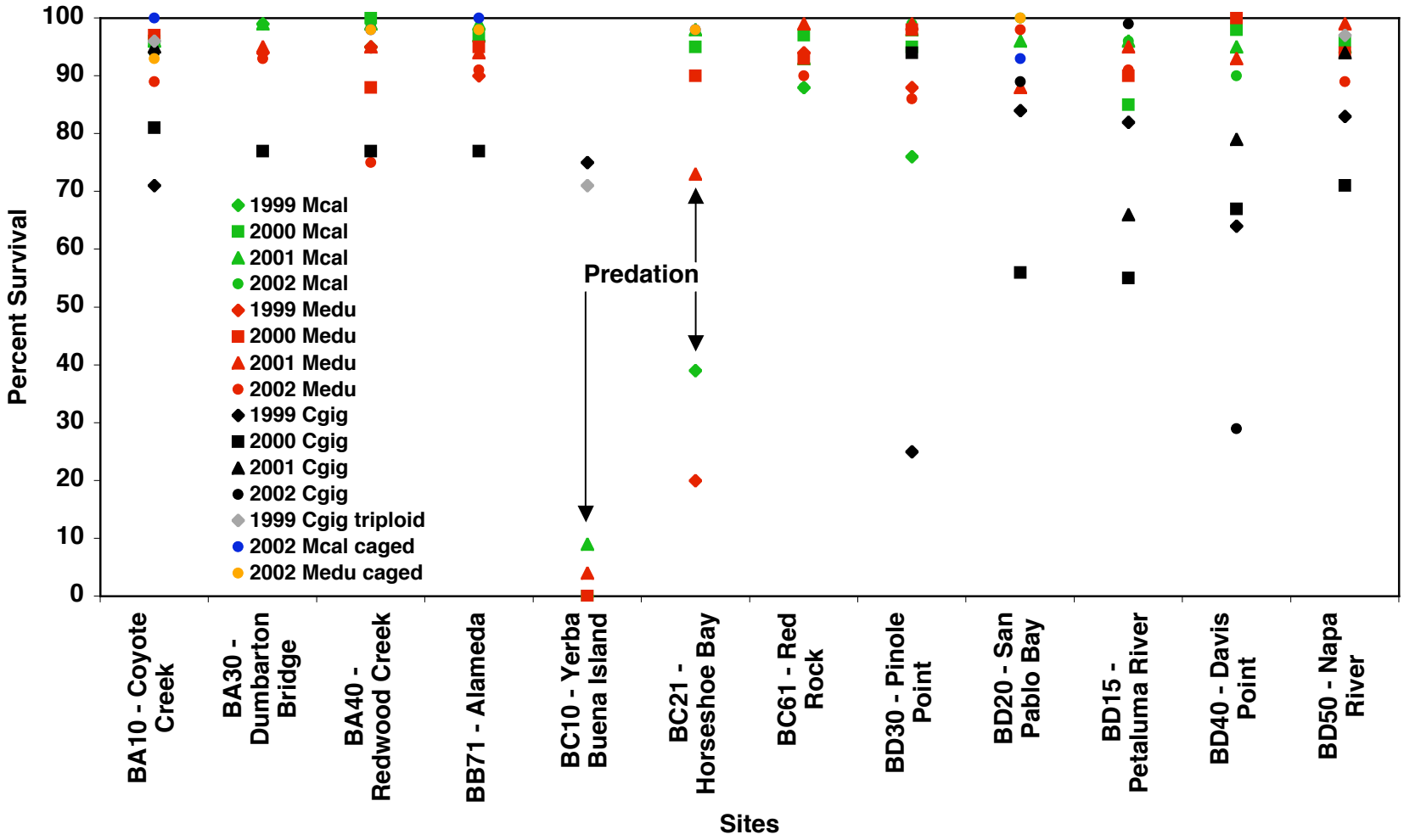

Figure 3. Survival of transplanted bivalves during dry-season deployments at 12 sites in San Francisco and San Pablo bays. All bivalves were maintained at the deployment mid-point by removing fouling organisms. Caged *M. californianus* and *M. edulis* denote a different kind of deployment container that was being tested to reduce predation on mussels.

Statistical comparison of survival among species using a paired comparison test indicated that few of the differences between species were significant (Table 4). Tests were divided according to site location, with sites where *M. californianus* was previously normally deployed in both wet seasons and dry seasons being considered main estuary sites and sites where *C. gigas* was previously normally deployed for both wet seasons and dry seasons being considered estuary margin sites. While *M. californianus* had higher survival than *M. edulis* at the main estuary sites, the converse was true at the estuary margin sites, with both results being marginally non-significant (*i.e.*, p = 0.0903 and p = 0.1194, respectively). Both mussel species survived better than *C. gigas* at both main estuary and estuary margin sites, with *M. edulis* having significantly higher survival at the estuary margins. Low sample numbers compromised results for triploid *C. gigas*.

Table 4. Results of paired comparisons for differences in survival between bivalve species during dry-season deployments in which predation did not affect mussel survival.

Region	Comparison		Mean % Survival	N	*p*
Main	*Mytilus californianus* Bay mussel (*M. edulis*)	vs	95.5 93.4	30	0.0903
	Mytilus californianus *Crassostrea gigas*	vs	90.3 65.3	3	0.2261
	Bay mussel (*M. edulis*) *Crassostrea gigas*	vs	91.3 65.3	3	0.2972
	Mytilus californianus Triploid *Crassostrea gigas*	vs	Predation 71	1	-
	Bay mussel (*M. edulis*) Triploid *Crassostrea gigas*	vs	Predation 71	1	-
	Crassostrea gigas Triploid *Crassostrea gigas*	vs	75 71	1	-
Margin	Bay mussel (*M. edulis*) *Mytilus californianus*	vs	93.8 85.0	23	0.1194
	Mytilus californianus *Crassostrea gigas*	vs	81.8 75.0	18	0.3248
	Bay mussel (*M. edulis*) *Crassostrea gigas*	vs	93.8 75.0	18	0.0016*
	Triploid *Crassostrea gigas* *Mytilus californianus*	vs	96.5 61.0	2	0.4196
	Triploid *Crassostrea gigas* Bay mussel (*M. edulis*)	vs	96.5 92.0	2	0.3228
	Triploid *Crassostrea gigas* *Crassostrea gigas*	vs	96.5 77.0	2	0.1750

* = Difference is statistically significant.

Measurements of growth also were compared as a secondary means of determining whether one species of mussel was more suitable than another for dry season deployments. Both *M.*

californianus and *M. edulis* generally grew better at sites nearest the open ocean (*i.e.*, BC10 and BC21 in Figure 4), with lower growth or weight loss observed at the other sites. Because *M. californianus* and *M. edulis* naturally could have differences in body tissue weight for animals of similar shell lengths, comparisons of growth were made with a statistical test for paired comparisons on the percent change in whole body dry weights. The proportional weight gain for *M. californianus* was significantly greater than that for *M. edulis* at the main estuary sites (Table 5). Both species lost weight at the estuary margins, with insignificantly greater losses for *M. edulis* than for *M. californianus*.

The comparisons of survival and growth were presented to the TRC on September 19, 2002. Based on this information, the TRC decided to deploy only *M. californianus* beginning in 2003. The main factors in the decision included the following:

- Lower survival of *C gigas*,
- Essentially equivalent survival between *M. californianus* and *M. edulis*,
- Better growth at many sites for *M. californianus*, and
- The extensive State Mussel Watch historic data set for transplanted *M. californianus* in San Francisco Bay.

3.3.4 Realignment of Sites in Conformance with Bay Biogeographical Segmentation Scheme

As part of the overall RMP five-year review, a biogeographic segmentation scheme was established for guiding sampling design for the water and sediment quality monitoring components of the RMP. Due to logistical constraints (*e.g.,* the need for a fixed structure on which to locate a mooring and need to access moorings with divers in a safe manner), it is not possible to implement a similarly randomized program for bivalve transplantation studies. Based on the new biogeographical delineation of the Estuary, however, it was apparent that the newly defined segments were not represented equally by the original 15-station deployment design (Table 6).

Consequently, an analysis was undertaken to determine the optimum number and distribution of sites to track trends in bioavailable contaminants in San Francisco Bay. Initially, the analysis focused on the number of sites per segment needed to detect statistically significant trends within a specified length of time. As statistical power is inversely related to the variation in the data, such an analysis must be contaminant specific. Polychlorinated biphenyls (PCBs) and Dieldrin are presented as examples. PCBs and Dieldrin had average within-segment coefficients of variation across all segments and times of 27 and 22, respectively. Using the formulae of Gerrodette (Gerrodette, 1987), we used the coefficients of variation to calculate the number of years that would be required to detect a 25% change in the lipid-normalized concentrations of PCBs and Dieldrin with 1–3 sites per segment. Fourteen, 10 and eight years would be required to detect a 25% change in the lipid-normalized concentrations of PCBs using one, two or three sites per segment, respectively (Figure 5). Approximately 11, eight and six years would be required to detect a 25% change in the lipid-normalized concentrations of Dieldrin using one, two or three sites per segment, respectively (Figure 5). For both contaminants, two or three sites per segment would enable detection of a 25% change in less than 10 years. Given the fixed costs involved

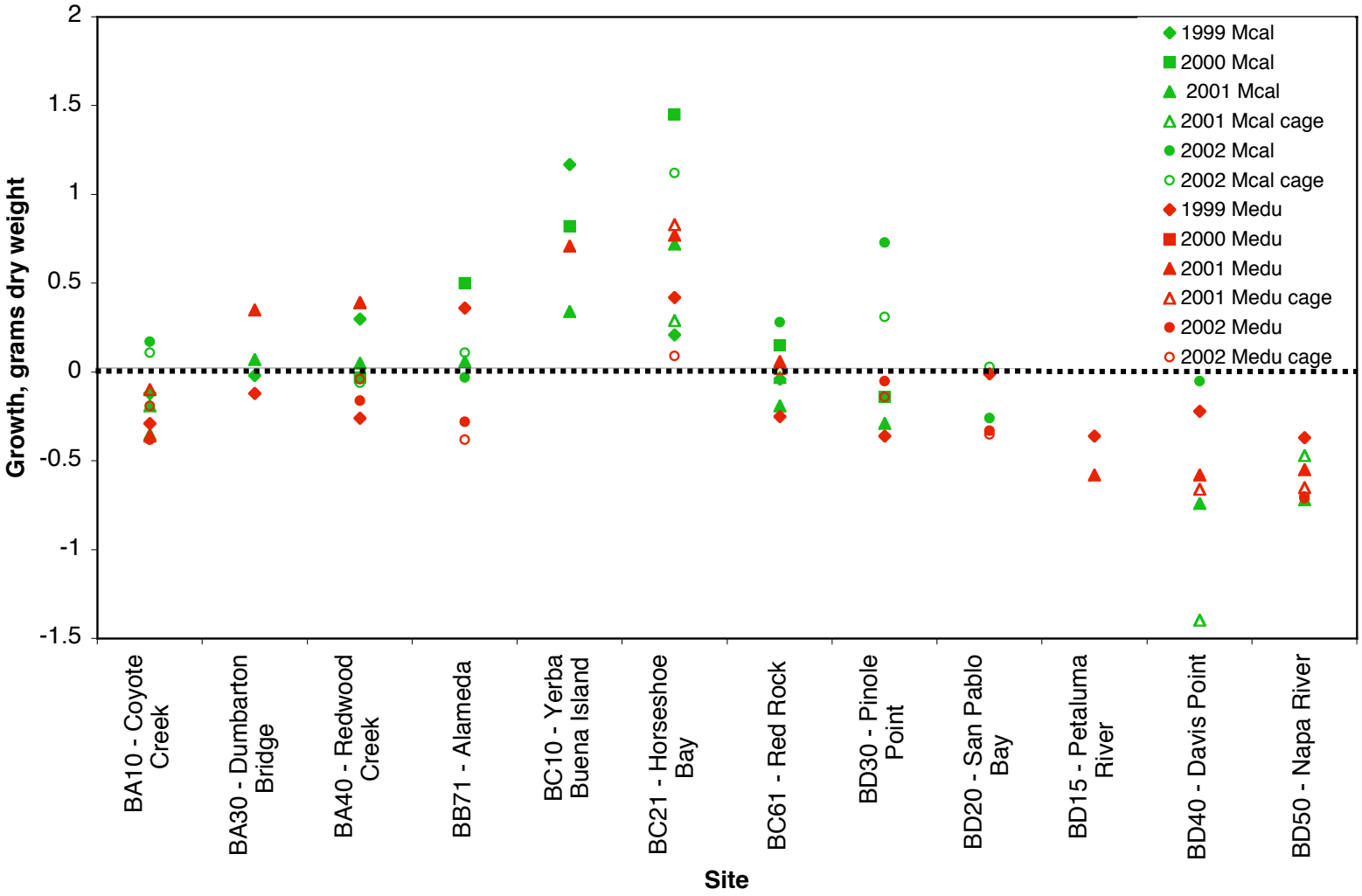

Figure 4. Comparisons of growth between *M. californianus* and *M. edulis* at 12 sites in San Francisco and San Pablo Bays. Caged *M. californianus* and *M. edulis* denote a different kind of deployment container that was being tested to reduce predation on mussels.

Table 5. Results of paired comparisons for differences in percent growth (change in weight ÷ average T-0 weight) between *M. californianus* and *M. edulis* during dry-season deployments in 1999, 2000, 2001 and 2002. Statistical significance is defined as P <0.05 and is denoted by an asterisk.

Region	Comparison		Mean % Growth	N	*p*
Main	*Mytilus californianus*	vs	26.2	26	0.0082*
	Bay mussel (*M. edulis*)		5.8		
Margin	*Mytilus californianus*	vs	-18.6	14	0.3643
	Bay mussel (*M. edulis)*		-25.2		

Table 6. Location of RMP bioaccumulation sites from original design relative to revised Bay biogeographical segmentation scheme

Segment	Existing Stations at Time of Segmentation Scheme
Lower South Bay	Coyote Creek
South Bay	Dumbarton Bridge, Redwood Creek
Central Bay	Alameda, Yerba Buena Island, Horseshoe Bay, Red Rock
San Pablo Bay	San Pablo Bay, Pinole Point, Petaluma River, Davis Point
Carquinez Strait	Napa River
Suisun Bay	None
Rivers	Sacramento River (residents), San Joaquin River (residents)

with collection, deployment and retrieval of the transplanted bivalves, efficiencies of scale achieved with three sites support sampling three sites per segment in order to achieve a 40–45% reduction in the time required to detect a 25% change in contaminant concentrations over that achieved with only one site.

Based on these findings, it was recommended at the September 19, 2002 TRC meeting that the following sites could be eliminated from the base Program and still maintain the ability of the transplanted bivalves to detect trends:

- Horseshoe Bay – This site is more representative of the open ocean waters than of the Central Bay region.
- Napa River and Petaluma River – These sites are more representative of tributaries to the Estuary than a specific region within the Estuary.

The recommendation to discontinue transplantation at the Horseshoe Bay, Napa River, and Petaluma River sites was approved by the TRC, which also decided to formalize analysis of resident *C. fluminea* in the Sacramento and San Joaquin River sites. Beginning with the 2003 deployments, the design of the Bioaccumulation Program study sites was modified to its current configuration, consisting of three transplant sites within the Lower South Bay-South Bay, Central Bay and San Pablo Bay Estuary segments and collection of resident bivalves at two sites within the Rivers segment (Figure 6).

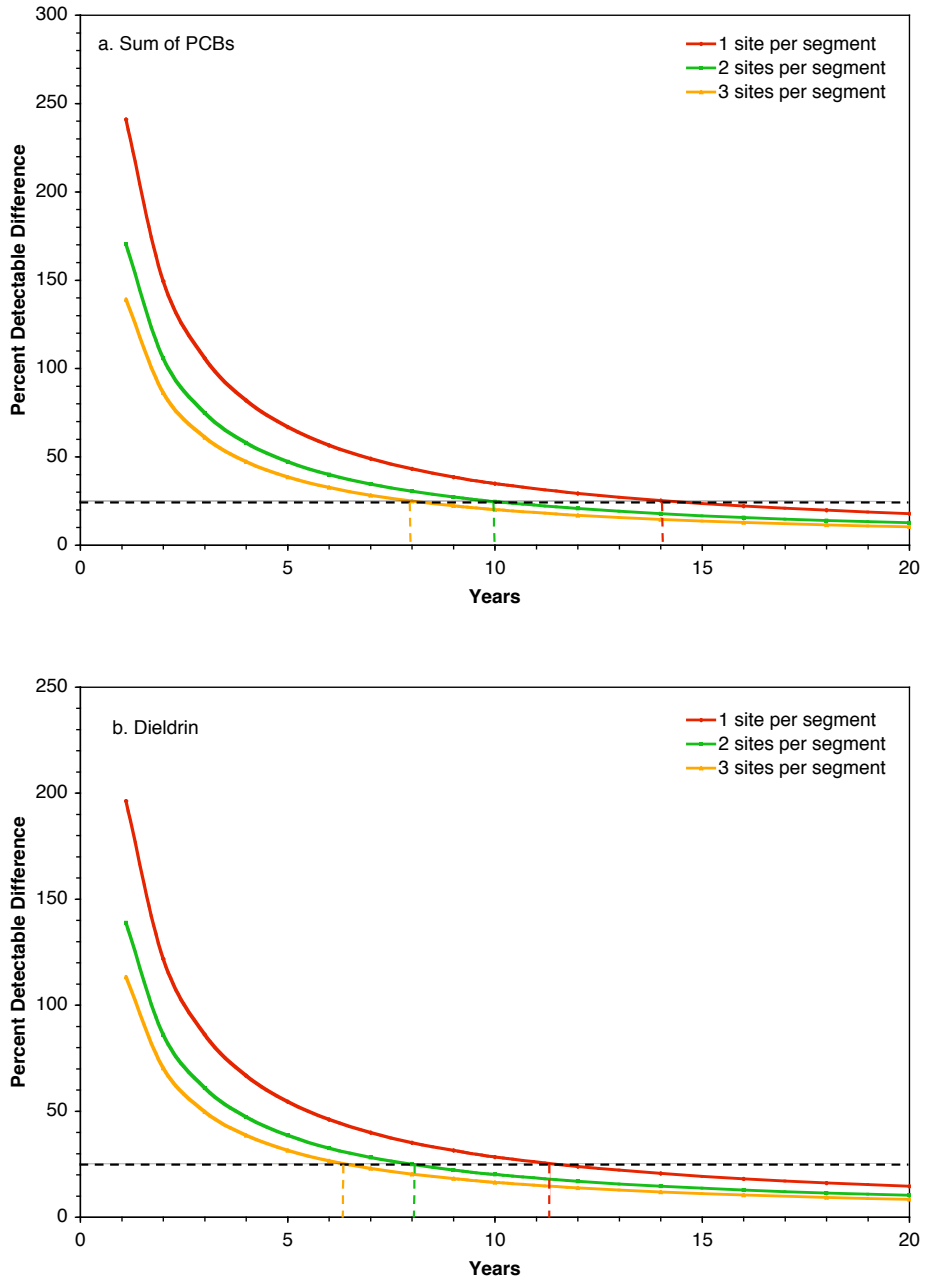

Figure 5. Number of years required to detect a 25% change in within-segment concentrations of lipid-normalized PCBs and Dieldrin in transplanted *M. californianus* using 1, 2 or 3 sites per segment. The within-segment coefficients of variation average 27 and 22 across all segments and times for PCBs and Dieldrin, respectively.

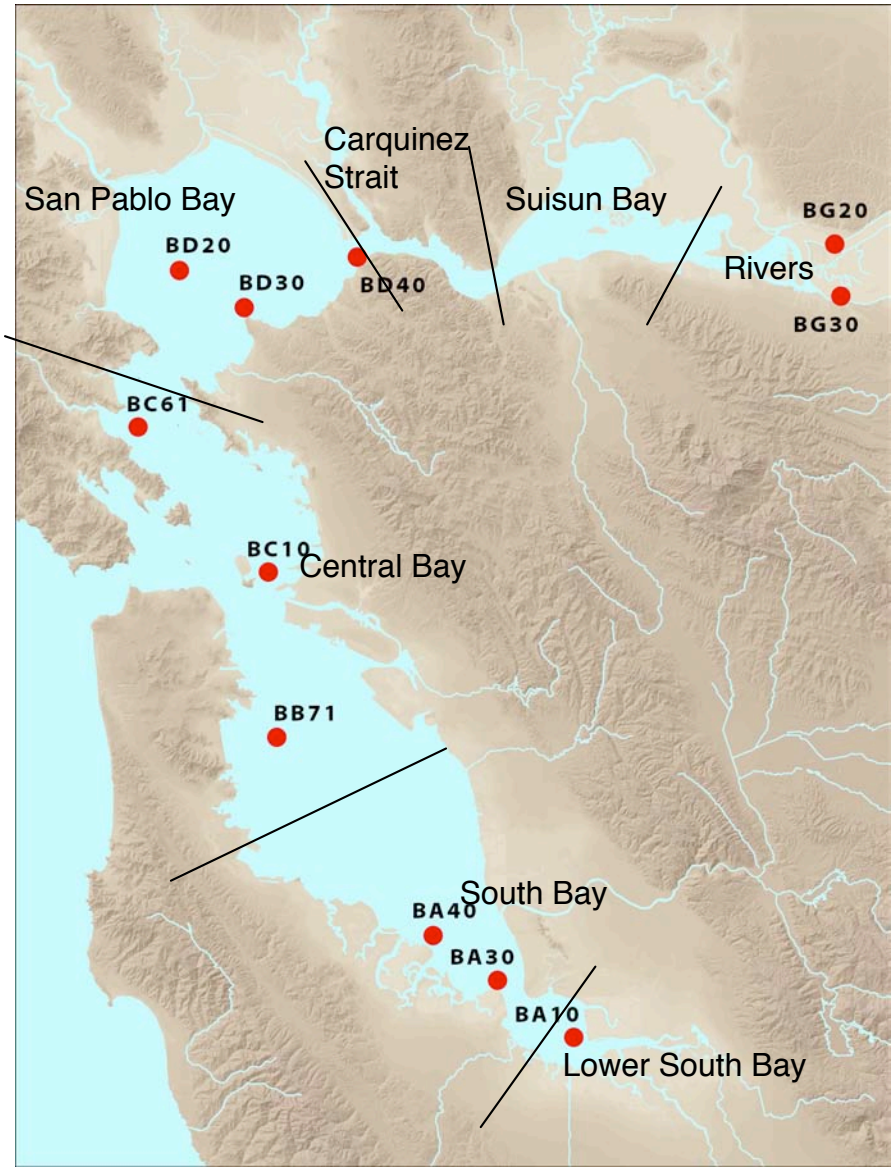

Figure 6. Current configuration of transplanted bivalve sampling sites. Resident *C. fluminea* are collected at the river sites. Figure provided by SFEI.

3.3.5 Modification of Containment Structure

Within the initial Program design, transplanted bivalves were deployed in plasticized nylon mesh bags, attached to mooring systems on the Estuary bottom (Figure 2). At Yerba Buena Island and Horseshoe Bay, mortality was sometimes significant during dry-season deployments due to predation by crabs and sea stars, as indicated by torn mesh bags and broken mussel shells (Figure 7). At times, this predation led to an insufficient number of bivalves to support all desired analyses and at other times causing loss of entire deployments at a site.

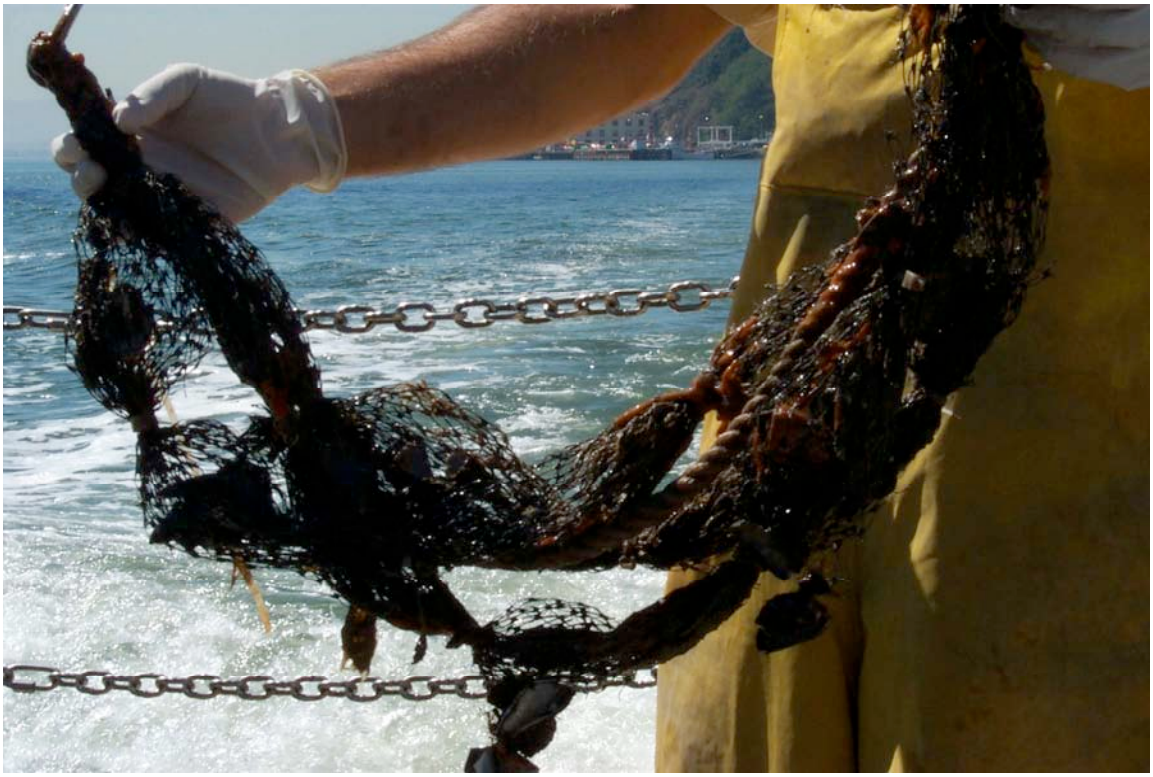

Figure 7. Nylon mesh bags from a bivalve deployment at Yerba Buena Island showing evidence of predation.

To reduce predation, cages were developed and deployed on a pilot basis beginning at one station in 1999 and followed by widespread testing beginning in 2000. The cages are made of a section of 6-in. diameter PVC pipe from which large sections are cut out and replaced with a very heavy-gauge plasticized nylon mesh (Figure 8). Cages were soaked for a minimum of three days in tap water before initial use to remove potential contamination from the cages themselves.

To test the effect of the new cages on survival and growth, side-by-side deployments of bagged and caged bivalves were conducted during the 2000 through 2002 deployments. The results of these experiments (Table 7) indicated that bivalves deployed in cages showed growth rates that did not differ significantly from those deployed in bags. Although use of cages reduced bivalve mortality, the difference in mortality was not significant when compared across all sites. Nevertheless, the effectiveness of the cages at reducing serious mortality due to predation at two sites led to the decision to deploy all transplanted bivalves in cage-type structures beginning with the 2003 deployment.

Figure 8. Bivalve containment cages from Napa River, post-deployment. The cage on the left was cleared of fouling organisms midway through deployment (maintained) and the cage on the right was not (unmaintained).

Table 7. Results of paired comparisons for differences in percent survival and growth between *M. californianus* deployed in cages and bags.

			% Survival			Growth, g dry wt	
Species	Comparison	N	Mean	*P*	N	Mean	*P*
Mytilus californianus	Cage vs. Bag	13	88.4 76.4	0.1393	11	-0.130 -0.053	0.4271

3.3.6 Elimination of Maintenance Cruise

The original design of the RMP transplanted bivalve program implemented in 1993 included a maintenance cruise near the midpoint of the bivalve deployments. The objectives of the maintenance cruise were as follows:

- Reduce mooring loses by checking their integrity,
- Improve bivalve survival and health by removing biological and physical fouling.

While moorings have been destroyed between deployment and the maintenance cruises by boating activity or replacement of navigation aids by the USCG, there never has been a case where maintenance activities have detected mooring problems whose repair prevented the loss of

the mooring. Consequently, the maintenance cruises have not been instrumental in achieving their first objective. Moreover, the efficacy of the maintenance cruise in achieving its second objective had not been tested prior to 2002. In an effort to improve the cost-effectiveness of the Program, side-by-side deployments were begun in 2002, in which some cages were maintained and some were not maintained in order to test whether elimination of the maintenance cruise would impair bivalve survival or growth. The effects of cage maintenance at one site are shown in Figure 7.

Paired comparisons between maintained and unmaintained cages indicate slight differences in the survival or growth of *M. californianus* (Table 8). There was greater survival in the unmaintained cages, which was marginally nonsignificant, and the slightly less weight lost in the unmaintained cages was not significant.

Table 8. Results of paired comparisons for differences in percent survival and growth between bivalves deployed in maintained and unmaintained cages (tested with matched-sample t-tests and verified with Wilcoxon matched sample tests).

				% Survival		Growth (g dry wt)	
Species	Comparison		N	Mean	*P*	Mean	*P*
Mytilus californianus	Maintained	vs.	22	88.4	0.0817	-0.052	0.4844
	Unmaintained			90.1		-0.032	

Side-by-side comparisons of maintained and unmaintained cages are being performed in the 2005 Program. If those results substantiate these findings that the maintenance activities do not improve bivalve survival or growth, the maintenance cruise should be considered for elimination in future years.

4 Conclusions

Transplanted bivalves provide an effective method for measuring spatial and temporal patterns in bioaccumulative pollutants. Bivalves integrate contaminant concentrations over the period of exposure, differing from non-living sampling methods, such as semi-permeable membrane devices, by incorporating bioavailable contaminants from both the dissolved and particulate phases in the water column. From its inception in 1993, the Program has exemplified adaptive management, as it has been modified based upon findings from various studies aimed at optimizing its ability to measure the status and trends in bioavailable pollutants in San Francisco Bay.

Further optimization of the Program should consider two additional modifications supported by this report and other recent work. As described in Section 3.3.6, elimination of the maintenance cruise should be considered following the 2005 project year. Also, as indicated in Section 3.2.1.2, wet-season data provide valuable information regarding causes of variation in long-term trends of legacy pesticides and perhaps other organic pollutants in San Francisco Bay. In an analysis of temporal trends (Figure 9) in legacy pesticides (DDTs, chlordanes and dieldrin) in transplanted bivalves, mean daily delta outflow and date were significant variables for explaining variation in all three legacy pesticides (Table 9). In each case, lipid-normalized

pesticide concentrations exhibited significant positive associations with mean daily delta outflow and significant negative associations with time. Partial correlations suggested that time had a larger effect on each pesticide than did delta outflow and additional analyses suggested that the effects of delta outflow are diminishing with time. Re-instituting a wet-season deployment of transplanted *M. californianus* at BC10 (Yerba Buena Island), where salinity remains relatively high during the wet season, would enable continued monitoring of the effect of delta outflow on contaminant concentrations in transplanted mussels, while minimizing the effects of wet-season salinity variation on mussel survival and growth. If the maintenance cruise can be eliminated, those cost savings could be used to offset the cost of the wet-season deployment at Yerba Buena Island.

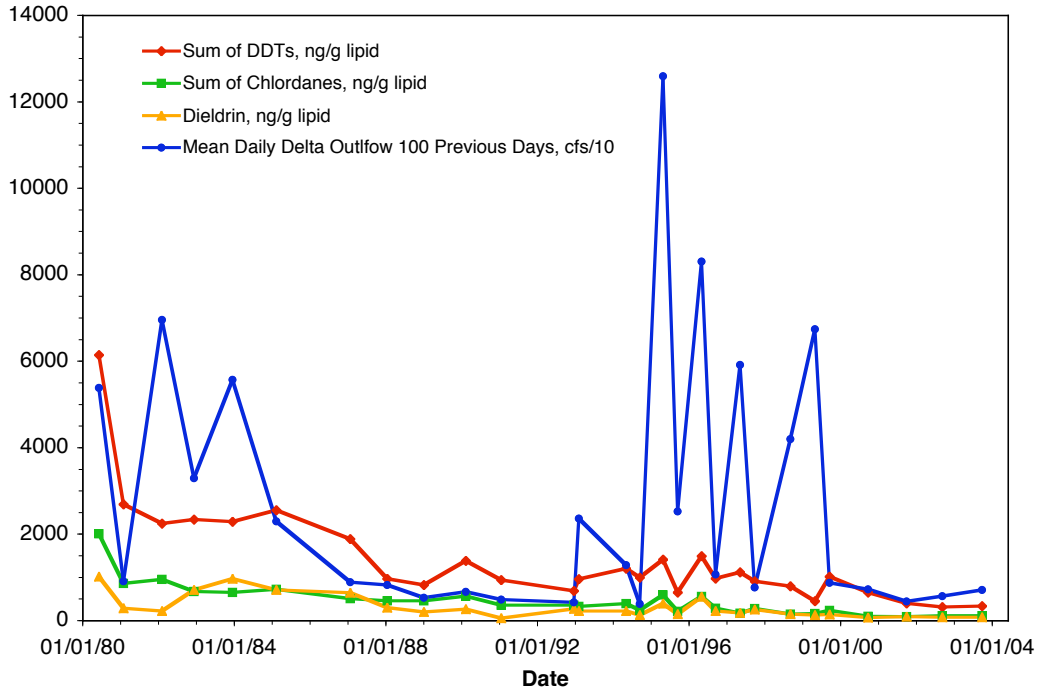

Figure 9. Lipid-normalized concentrations DDTs, Chlordanes and Dieldrin in *Mytilus californianus* transplanted adjacent to Yerba Buena Island displayed with mean daily delta outflow during the four months prior to mussel retrieval.

Table 9. Results of stepwise linear regressions between log-transformed (natural log) time and mean daily delta outflow (independent variables) and lipid-normalized DDTs, Chlordanes and Dieldrin (dependent variables) in *Mytilus californianus* transplanted to sites adjacent to Yerba Buena Island.

Pesticide	r^2	p	Equation
DDTs	0.7958	<0.0001	log DDTs = 0.65 log delta outflow – 6.80 log date + 153
Chlordanes	0.8360	<0.0001	log Chlordanes = 0.121 log delta outflow – 8.17 log date + 183
Dieldrin	0.6161	<0.0001	log Dieldrin = 0.259 log delta outflow – 6.32 log date + 140

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6 Appendix A

Report of the Bioaccumulation Workshop

1.0 Introduction

A workshop was held on March 30, 1999 to evaluate the bioaccumulation component of the RMP. This evaluation was performed within the context of revised overall program objectives that were formulated as a result of the 5-year review (Bernstein and O'Connor, 1997). These revised program objectives are as follows:

1. describe patterns and trends in contaminant concentration and distribution,
2. describe general sources and loading of contamination to the estuary,
3. measure contamination effects on selected parts of the estuary ecosystem,
4. compare monitoring information to relevant water quality objectives and other guidelines,
5. synthesize and distribute information from a range of sources to present a more complete picture of the sources, distribution, fates, and effects of contaminants in the estuary ecosystem.

The revised program objectives, in turn, formed the basis for revised objectives for the bioaccumulation component of the RMP, as follows:

1. determine trends in tissue contamination,
2. measure the bioavailable portion of contaminants in the water column,
3. evaluate which contaminants may be transferred to higher trophic levels of the food web,
4. determine pathways and loadings of contaminants to the estuary,
5. determine effects of contaminants in the estuary.

The goal of this workshop was to provide recommendations to the Program Technical Review Committee for ways to improve the ability of the program to address these objectives. Consequently, discussion at the workshop included consideration of whether the transplanted bivalve method currently used to measure bioaccumulation is the most appropriate way to achieve each of these objectives, as well as ways to improve the transplanted bivalve method. Workshop participants are shown in Table 1.

The discussions at the workshop were wide-ranging and provided numerous opinions regarding the bioaccumulation component and the best ways to achieve the program objectives. Consensus on the various opinions and recommendations was not necessarily achieved at the workshop and this document seeks to synthesize the current state of knowledge concerning the transplanted bivalve method and provides recommendations for improving and streamlining the program that are consistent with the general direction of discussions at the workshop. In some cases, recommendations are contingent upon additional information or analyses of the existing transplanted bivalve data.

Table 1. Bioaccumulation Workshop participants.

Name	Affiliation
Ray Arnold	Exxon Biomedical Sciences
David Bell	Applied Marine Science
Cynthia Brown,	United States Geological Survey
Jay Davis	San Francisco Estuary Institute
Jordan Gold	Applied Marine Sciences
Andy Gunther	Applied Marine Sciences
Dane Hardin	Applied Marine Sciences
Rainer Hoenicke	San Francisco Estuary Institute
Michael Kellogg	City and County of San Francisco
Henry Lee	U.S. Environmental Protection Agency
Allison Luengen	U.C. Santa Cruz
Michael May	San Francisco Estuary Institute
Michael Salazar	Applied Biomonitoring
Karen Taberski	Regional Water Quality Control Board
Bruce Thompson	San Francisco Estuary Institute
Inge Werner	U.C. Davis

2.0 Current Program Configuration

Currently, the bioaccumulation component of the RMP has the following configuration:

- Bivalves are obtained from historically clean locations for transplantation into the estuary. *Mytilus californianus* are obtained from Bodega Head, *Crassostrea gigas* are obtained from a commercial grower in Tomales Bay, and *Corbicula fluminea* were obtained from Lake Isabella, until the population crashed in 1996. Currently, resident *C. fluminea* are collected from RMP sampling sites for analysis, because new transplant populations have not been found in clean locations.
- Bivalves have been transplanted to 15 sites (Figure 1).
- Bivalves are deployed for two 90-day periods each year, one during the wet season (January-April) and one during the dry season (June-September).
- Bivalves are analyzed for condition, trace metals, and trace organic contaminants.

3.0 Recommendations for Redesign

This section is organized according to program objective. We present a brief summary of findings and recommendations for redesign associated with each objective.

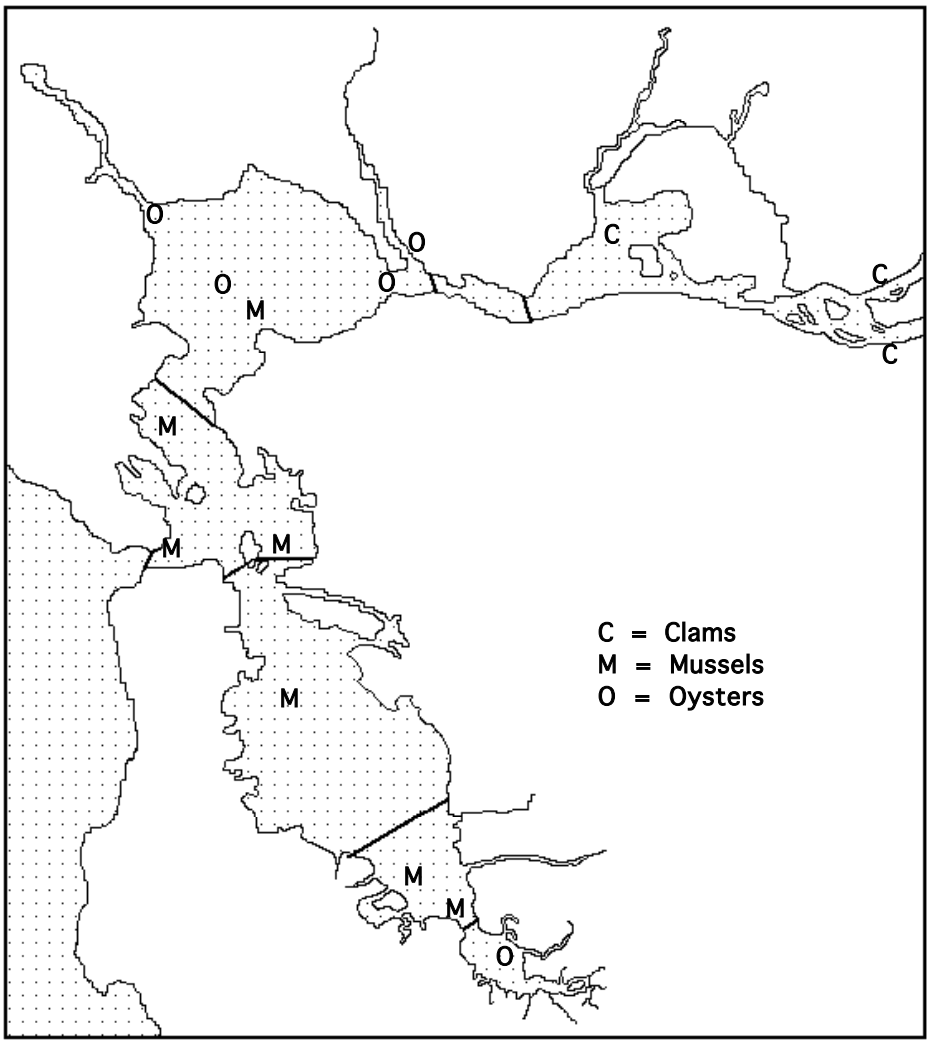

Figure 1. Sites for transplantation of bivalves.

3.1 Objective #1 - Determine Trends in Tissue Contamination

Findings

Bivalves respond to changes in water concentrations of contaminants, and they integrate contaminants from the water column over time (Gunther, *et al.* 1999; Gunther and Davis, 1997; De Kock and Kramer, 1994). The State Mussel Watch program has been in existence for almost two decades and represents an invaluable long-term database of bivalve bioaccumulation that should be continued by the RMP. The SMW program has employed mussels, *Mytilus californianus*, and clams, *Corbicula fluminea* (the latter as both transplants and residents). The RMP transplanted bivalves reveal trends that are not apparent from data on water contaminants, suggesting that bivalves may be especially valuable for tracking long-term changes in contaminant concentrations in the estuary. For instance, recent analysis of data from 1993–1997 indicated significant increases in copper and decreases in PCBs in transplanted mussels that were not revealed in water data.

Nevertheless, these findings and interpretation of the transplanted bivalve data are complicated by several facts:
Contaminant trends are not consistent between bivalve species. Significant estuary-wide trends in mussels were not observed in oysters.
The mussel trends for different contaminants were more or less apparent depending on the season.
Regression analyses suggest that non-contaminant environmental factors may affect bivalve bioaccumulation and indicators of health. Salinity, dissolved oxygen, temperature, and total suspended solids had the greatest effects, but statistical procedures allow adjustments to data to account for these effects.
Periodic high mortality of transplanted bivalves is usually related to low salinities and high temperatures for mussels and oysters, respectively.
Populations of *Corbicula fluminea* at clean sites recently have declined dramatically and we do not current know of an alternate clean site to obtain clams for transplanting to the river sites.

Additional data analyses were recently undertaken using mussel data to help determine the optimum design for achieving this objective. The first step in these analyses consisted of assessing the presence of site groupings that would provide the basis for characterizing the estuary with less than the current number of sampling sites (Figure 2). The Bray-Curtis similarity index (Bray and Curtis, 1957), which is normally used to determine site similarities based on organism abundances, was calculated to determine the similarities between sites based on mean concentrations of trace metals, PAHs, and PCBs. This index can range from 0.0, in which case the sites share no contaminants in common, to 100.0, in which case the sites share all of the contaminants in the common and have identical mean concentrations. These similarities were then clustered using an unweighted pair-group method (Swartz, 1978) to graphically represent the affinities among the sites for each group of contaminants.

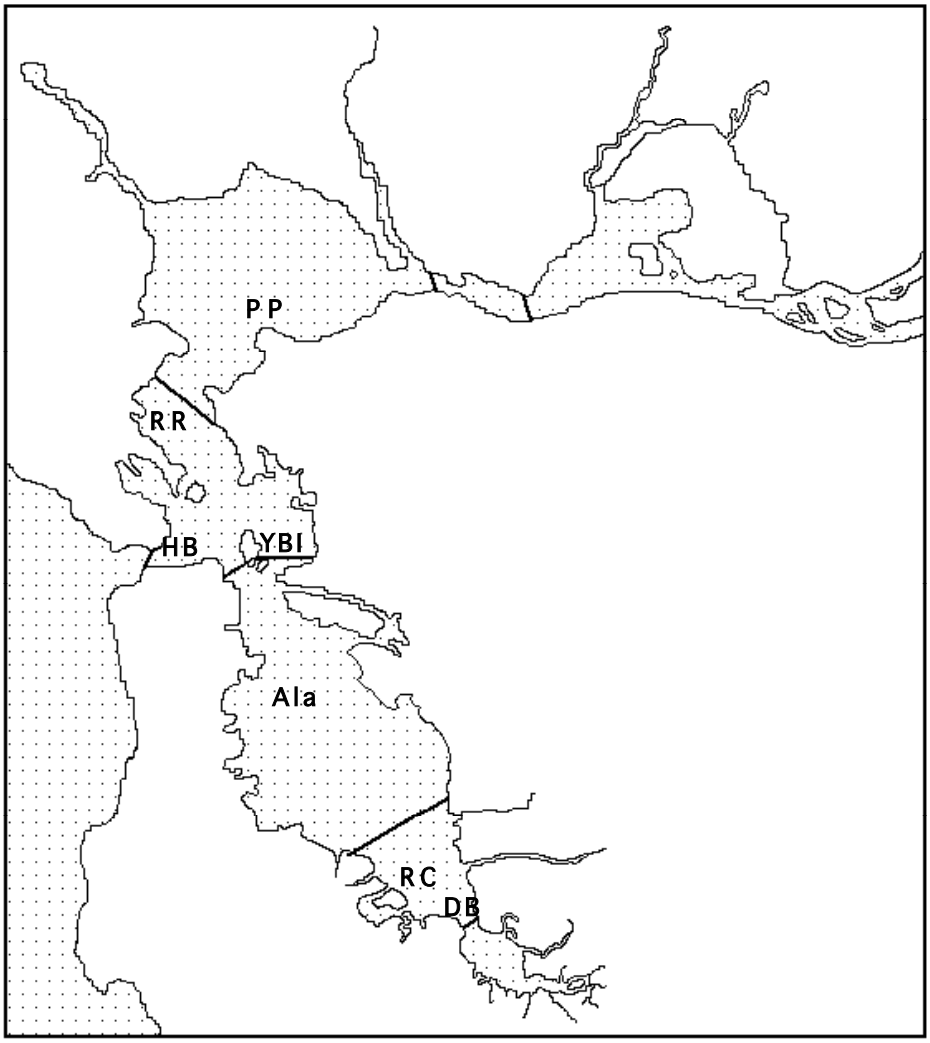

Figure 2. Mussel deployment sites. DB = Dumbarton Bridge, RC = Redwood Creek, Ala = Alameda, YBI = Yerba Buena Island, HB = Horseshoe Bay, RR = Red Rock, PP = Pinole Point.

The second step in these analyses consisted of predicting the ability of the transplanted bivalve method to detect changes over time and differences between reaches of the estuary under various sample reduction scenarios. The percentage of change that could be detected with regression analysis over a five-year period in each reach of the estuary was estimated for each season separately and for both seasons together using the methods of Gerrodette (1987). This method is based upon the coefficient of variation (CV, the percentage of the mean represented by the standard deviation) within sets of samples from each point in time. This method also assumes that samples are distributed over time and that the change over time (*i.e.*, trend) is linear. The amount of difference between reaches that could be detected with analysis of variance (ANOVA) was also estimated using the method of Sokal and Rohlf (1995). Because the results of these analyses vary according to analyte, we focused our efforts on copper, mercury, nickel, selenium, total lipid-normalized PAHs and total lipid-normalized PCBs.

The cluster analyses revealed different patterns for each group of contaminants (Figures 3-5). Although delineation of clusters is somewhat arbitrary, trace metals provided relatively little definition of site groupings, with high similarities among all site/season combinations except wet season samples from Dumbarton Bridge and Red Rock (Figure 3). Similarities based on mean concentrations of PAH analytes revealed several clusters that separated generally along seasonal and regional lines (Figure 4). Cluster 1 consisted of dry season samples from sites between Redwood Creek and Yerba Buena Island, cluster 2 consisted of dry season samples from Dumbarton Bridge and Pinole Point plus both seasons from Horseshoe Bay. Cluster 3 consisted of the remainder of the wet season samples, except for Redwood Creek, and cluster 5 consisted of the Bodega Head samples. These clusters generally differed according to mean concentrations of total lipid-normalized PAHs, with the dry season samples from each estuary site having the highest concentrations. Similarities based on mean concentrations of PCB congeners revealed several clusters that were generally based on regions (Figure 5). Clusters 1 and 2 included both seasons for all sites from Yerba Buena Island south to Dumbarton Bridge, and cluster 3 included both seasons for all sites from Horseshoe Bay to Pinole Point. Cluster 4 included both seasons from Bodega Head. These clusters also generally differed according to mean concentrations of total lipid-normalized total PCBs, with southern dry season samples have higher concentrations. The different clustering patterns for the three groups of contaminants suggest that there is no single strategy for delineating groups of sites that is applicable to all contaminants.

The best across-the-board strategy for grouping sites will probably be based on arbitrary geographic definitions of estuary reaches. For the following analyses of power in regression analyses and ANOVA, the South Reach includes Dumbarton Bridge, Redwood Creek, and Alameda, the Central Reach includes Yerba Buena Island and Horseshoe Bay, and the North Reach includes Red Rock and Pinole Point (Figure 2).

Figure 3. Clusters based on concentrations of trace metals. Ala = Alameda, BH = Bodega Head, DB = Dumbarton Bridge, HB = Horseshoe Bay, PP = Pinole Point, RC = Redwood Creek, RR = Red Rock, YBI = Yerba Buena Island.

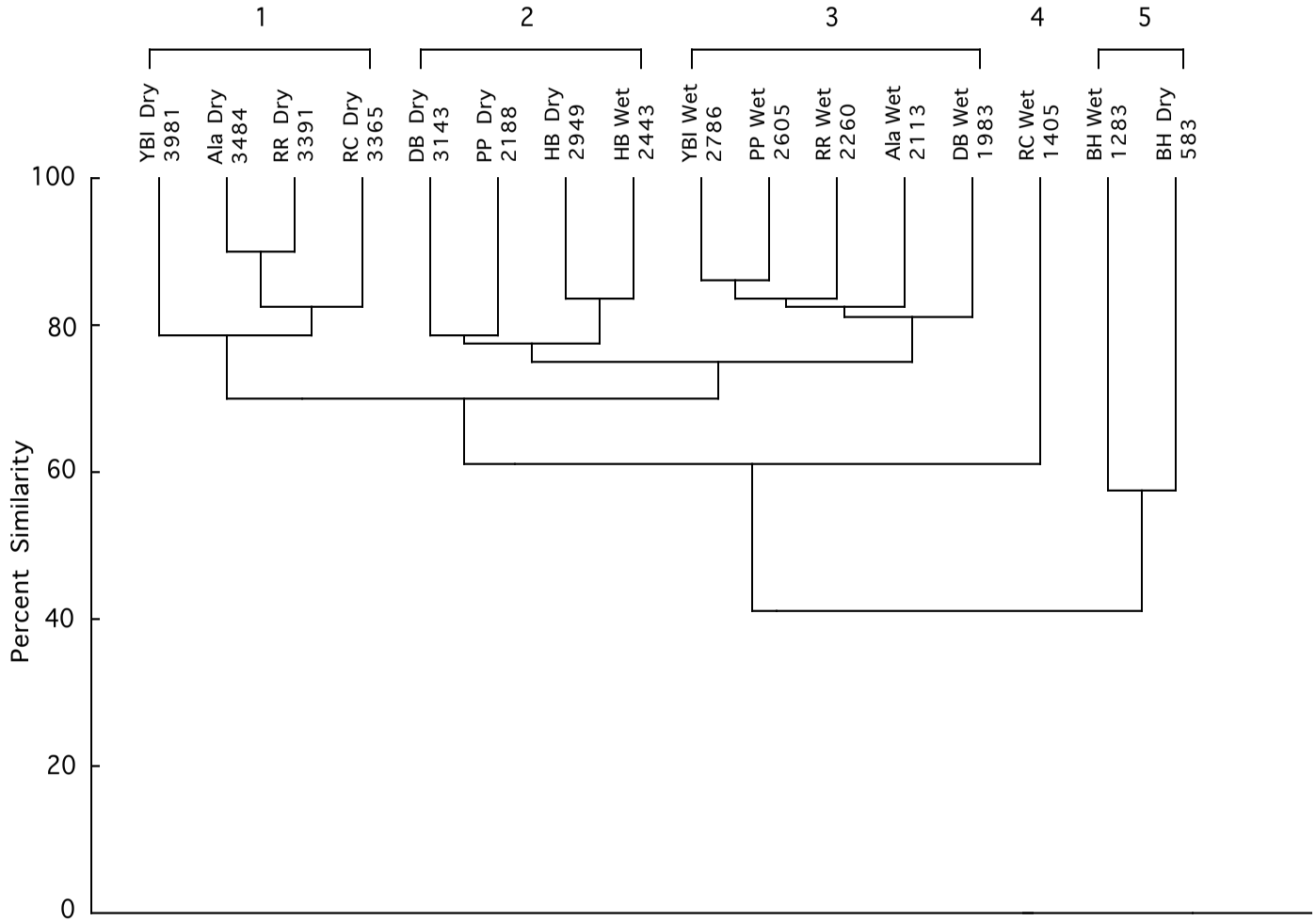

Figure 4. Clusters based on concentrations of PAH analytes. Ala = Alameda, BH = Bodega Head, DB = Dumbarton Bridge, HB = Horseshoe Bay, PP = Pinole Point, RC = Redwood Creek, RR = Red Rock, YBI = Yerba Buena Island. Numbers are lipid-normalized concentrations of total PAHs (micrograms of PAH/kilogram of lipid).

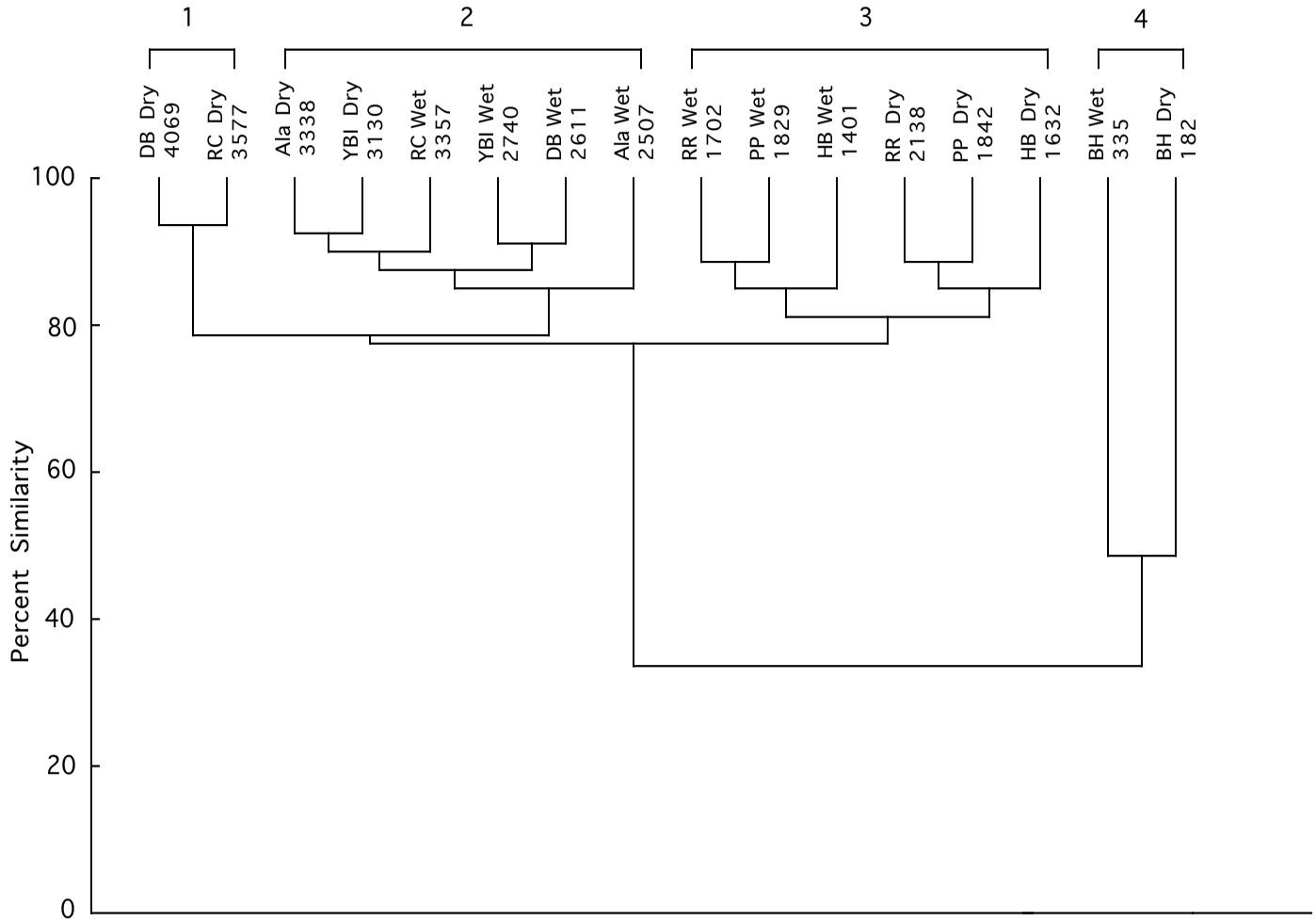

Figure 5. Clusters based on concentrations of PCB congeners. Ala = Alameda, BH = Bodega Head, DB = Dumbarton Bridge, HB = Horseshoe Bay, PP = Pinole Point, RC = Redwood Creek, RR = Red Rock, YBI = Yerba Buena Island. Numbers are lipid-normalized concentrations of total PCBs (micrograms of PCB/kilogram of lipid).

Based on the average CV within sampling periods, the predicted percentage change that might be detectable with five samples (*e.g.*, annual samples over five years) varied from 6.48 in wet season mercury from the Central Reach to 78.46 in wet season nickel from the North Reach (Table 2). The combination of wet and dry season samples to provide 10 samples over time decreased the estimated percentage change that could be detected for each analyte in each reach, often by substantial amounts. With 10 samples, the predicted percentage change that might be detectable ranged from 4.78 for mercury in the Central Reach to 20.50 for nickel in the North Reach.

The differences in predicted detectable percentage change for wet season and dry season samples varied inconsistently among reaches and analytes. There were six cases in which high power (*i.e.*, <15% predicted detectable change) was indicated for wet season samples and one case in which high power was indicated for dry season samples. Nevertheless, the predicted results were often not reflected in the actual regressions, because trends did not always accompany low predicted detectable percentage changes. In these cases, the low CVs indicate that all the sites within the reach had similar concentrations of contaminants, but the concentrations either varied irregularly through time or did not vary with time. Moreover, the actual regression results indicated several cases in which very significant regressions occurred when the predicted detectable percentage change was high (*e.g.*, dry season PAHs in every reach), indicating especially strong trends. There were eight and 10 cases in which significant actual regressions ($P < 0.10$) occurred for wet and dry seasons, respectively. There were seven significant regressions for both seasons combined. These results suggest that there is not an optimum season for detecting trends that applies to all contaminants but that sampling in both seasons might not always improve detection of actual trends.

Although the influence of replicate samples was not determined for the analysis of predicted detectable percentage change, actual regression results were used to evaluate this issue. The inclusion of 3, 2, and 2 sites to characterize the South, Central, and North reaches, respectively, resulted in the detection of numerous significant regressions (Table 2). But when individual sites were analyzed for seasonal copper and total PAHs, the two analytes with the strongest actual regressions, few significant regressions occurred (Table 3). Only in the case of wet season copper in the Central Reach and dry season copper in the North Reach did both sites exhibit significant regressions consistent with the overall trend for that reach. Moreover, where significant reach-wide regressions occurred, the probability was always lower than for any individual site. These results suggest that five-year trends in reaches cannot generally be adequately described using single sites.

The ability to detect differences between reaches using ANOVA is also strongly affected by the number of sites analyzed per reach. In the case of ANOVA comparisons, each site represents a replicate sample within its reach. The predicted percentage differences between reaches that could be detected for each analyte was substantially less (*i.e.*, greater power) for three samples than for two samples (Table 4). The percentage difference that can be detected using three samples ranged from 37.23 for wet season mercury to 150.85 for wet season nickel.

Table 2. Results of statistical analysis to determine the predicted minimum percentage change detectable over the period covered by the samples using regression analysis (*i.e.*, statistically significant trends over time), compared to actual regression results. The predicted results are the minimum percentage change detectable because they do not account for the increase in power due to analyzing multiple replicate samples (*i.e.*, more than one site) from each sampling time. The predicted results also assume that the change is linear with time and that the samples are spread over time. Actual regression results are based on analysis of three samples per time for the South Reach and two samples per time for the Central Reach and North Reach.

				Predicted Results		Actual Regression Results	
Analyte	**Estuary Reach**	**Season**	**Average C.V.**	**N[a]**	**Minimum % Change Detectable**	**R^2**	***P***
Copper	South	Wet	13.4	5	14.97	0.441	0.0259**
		Dry	33.2	5	37.09	0.568	0.0019***
		Both	23.3	10	9.20	0.373	0.0012***
	Central	Wet	11.6	5	12.96	0.576	0.0177**
		Dry	19.3	5	21.56	0.297	0.1033
		Both	15.4	10	6.08	0.291	0.0170**
	North	Wet	33.2	5	37.09	0.031	0.7049
		Dry	21.4	5	23.91	0.697	0.0070***
		Both	27.3	10	10.78	0.135	0.1216
Mercury	South	Wet	25.4	5	28.38	0.379	0.0437**
		Dry	29.3	5	32.73	0.136	0.1939
		Both	27.3	10	10.78	0.076	0.1832
	Central	Wet	5.8	5	6.48	0.554	0.0214**
		Dry	18.5	5	20.67	0.302	0.0997*
		Both	12.1	10	4.78	0.297	0.0158**
	North	Wet	9.7	5	10.84	0.608	0.0386**
		Dry	35.7	5	39.88	0.045	0.4849
		Both	22.7	10	8.97	0.031	0.4596
Nickel	South	Wet	50.0	5	55.86	0.079	0.4029
		Dry	39.8	5	44.46	0.0003	0.9527
		Both	44.9	10	17.73	0.007	0.6863
	Central	Wet	44.9	5	50.16	0.022	0.7027
		Dry	30.6	5	34.18	0.118	0.3303
		Both	37.8	10	14.93	0.012	0.6557
	North	Wet	70.5	5	78.76	0.004	0.8990
		Dry	33.3	5	37.20	0.004	0.8512
		Both	51.9	10	20.50	0.008	0.7185

Table 2. Continued.

Analyte	Estuary Reach	Season	Average C.V.	Predicted Results N[a]	Predicted Results Minimum % Change Detectable	Actual Regression Results R^2	Actual Regression Results *P*
Selenium	South	Wet	20.7	5	23.12	0.099	0.3454
		Dry	8.2	5	9.16	0.262	0.0614*
		Both	14.4	10	5.69	0.041	0.3339
	Central	Wet	20.6	5	23.01	0.003	0.8864
		Dry	14.3	5	15.98	0.333	0.0808*
		Both	17.4	10	6.87	0.131	0.1278
	North	Wet	23.4	5	26.14	0.014	0.8018
		Dry	25.7	5	28.71	0.263	0.0734*
		Both	24.5	10	9.68	0.145	0.0974*
Total PAHs	South	Wet	58.4	5	65.24	0.019	0.7062
		Dry	27.0	5	30.16	0.624	0.0022***
		Both	42.7	10	18.66	0.307	0.0075**
	Central	Wet	12.0	5	13.41	0.114	0.4136
		Dry	24.3	5	27.15	0.697	0.0099***
		Both	18.1	10	7.15	0.111	0.2080
	North	Wet	33.7	5	37.65	0.091	0.5609
		Dry	36.4	5	40.66	0.630	0.0106**
		Both	35.0	10	13.82	0.255	0.0549*
Total PCBs	South	Wet	28.0	5	31.30	0.509	0.0205**
		Dry	22.7	5	25.36	0.416	0.0235**
		Both	25.3	10	9.99	0.256	0.0162
	Central	Wet	46.0	5	51.39	0.387	0.0997*
		Dry	42.9	5	47.93	0.182	0.2917
		Both	44.4	10	17.54	0.238	0.0553*
	North	Wet	8.5	5	9.50	0.883	0.0053**
		Dry	16.4	5	18.32	0.132	0.3369
		Both	12.4	10	4.90	0.144	0.1630

[a] = N is the number of sampling times, not the number of replicate samples in each time.
* = Regression is significant at the 0.10 level.
** = Regression is significant at the 0.05 level.
*** = Regression is significant at the 0.01 level.

Table 3. Significance of regressions for each site compared with those for sites combined into reaches.

Analyte	Site or Reach	Regression *P* for Each Season Wet	Dry
Copper	South Reach	0.0259**	0.0019***
	Dumbarton Bridge	0.0648*	0.0820*
	Redwood Creek	0.2527	0.1553
	Alameda	0.8134	0.2368
	Central Reach	0.0177**	0.1033
	Yerba Buena Island	0.0565*	0.1002
	Horseshoe Bay	0.0576*	0.3031
	North Reach	0.7049	0.0070***
	Red Rock	0.7505	0.0429**
	Pinole Point	0.3290	0.0449**
Total PAHs	South Reach	0.7062	0.0022***
	Dumbarton Bridge	0.5592	0.2213
	Redwood Creek	0.8146	0.2925
	Alameda	0.1473	0.0626*
	Central Reach	0.4136	0.0099***
	Yerba Buena Island	0.6939	0.1094
	Horseshoe Bay	0.5839	0.0952*
	North Reach	0.5609	0.0106**
	Red Rock	0.0106**	0.1177
	Pinole Point	-[a]	0.2072

[a] = Only two points available for regression.
* = Regression is significant at the 0.10 level.
** = Regression is significant at the 0.05 level.
*** = Regression is significant at the 0.01 level.

Table 4. The predicted percent difference detectable between reaches of the estuary based upon analysis of variance using two or three sites (*i.e.*, N) per reach.

Analyte	Season	Average C.V.	N	Predicted % Difference Detectable
Copper	Wet	19.4	3	53.11
			2	80.71
	Dry	24.6	3	67.35
			2	102.34
Mercury	Wet	13.6	3	37.23
			2	56.57
	Dry	27.8	3	76.11
			2	115.65
Nickel	Wet	55.1	3	150.85
			2	229.22
	Dry	34.6	3	94.72
			2	143.94
Selenium	Wet	21.6	3	59.13
			2	89.86
	Dry	16.1	3	44.08
			2	66.98
Total PAHs	Wet	34.7	3	95.00
			2	144.35
	Dry	29.2	3	79.94
			2	121.47
Total PCBs	Wet	27.5	3	75.29
			2	114.40
	Dry	27.3	3	74.74
			2	113.57

The percentage difference that can be detected using two samples ranged from 56.57 for wet season mercury to 229.22 for wet season nickel. The decrease in the predicted detectable percentage difference was proportional to the number of samples, with three samples being able to detect a difference that was two-thirds the difference detectable with two samples.

Recommendations

In order to track trends, the RMP should maintain sites and methods comparable to SMW and the last six years of RMP data collection. Although long-term trends are more apparent in the dry season than in the wet season, the low CVs in many wet season samples suggest that the bivalves are responding to real phenomena that vary from year-to-year on a non-linear basis. While this wet season information is useful for determining processes and pathways for entry of contaminants into the estuary (see Objective #4), transplanting bivalves in the wet season could perhaps be suspended without seriously affecting the program's ability to achieve Objective #1. Bivalves should be deployed at more than one site to characterize trends within and differences among reaches and three sites are recommended. These sites should be distributed as widely as possible to adequately represent all the variation within the reach. Reaches should be defined using geographical criteria.

One species should be deployed at all sites to eliminate the difficulty of interpreting data from different species. If deployment of bivalves in both the wet season and dry season is maintained, side-by-side deployments of several species should be continued for several years to determine whether a suitable species is available for deployment in the wet season at all sites west of Carquinez Strait. CTD profiles should be recorded during each visit to deployment sites to allow further examination of the effects of non-contaminant factors on bioaccumulation and bivalve health. If cost savings are required while maintaining deployments in both seasons, elimination of mid-deployment maintenance cruises should be examined.

3.2 Objective #2 – Measure the Bioavailable Portion of Contaminants in the Water Column

Findings

Many trace metals do not appear to accumulate much above concentrations measured in the "clean" populations used as sources for transplants. It is not known whether the low accumulations in the transplants are due to poor bioaccumulation or ambient concentrations that are similar between the source locations and the estuary. Nevertheless, trends apparent for tissue concentrations of some metals, such as copper, suggest that ambient conditions within the estuary are being reasonably well represented by the transplants.

In the case of mercury, however, there is evidence that bivalves may not be the best indicators of bioavailability, especially of the most toxic form of this element. Mercury is a contaminant of concern that is found in very high concentrations in many fishes in the estuary, most likely in the methylated form. Mercury concentrations in fishes are sufficiently high that health advisories have been issued warning people to limit the amount of fish they consume from the estuary.

Although mercury concentrations have declined significantly in mussels since 1993, primarily in the wet season, it does not occur in very high concentrations in the transplanted bivalves and the best available information suggests that mussels are not efficient accumulators of methylated mercury.

The transplanted bivalve method, as it is currently employed, also does not seem to capture ecologically important short-term trends in organo-selenium presence in the estuary. Although RMP data indicate increases in dry season selenium are approaching statistical significance, USGS data from resident bivalves near Carquinez Strait suggest that selenium fluxes to the estuary may occur over periods of less than one month. The time-integration design used in the RMP transplanted bivalve program (90–100 day deployments) does not capture such short-term events. The current design also can meet the objective of assessing the bioaccumulation potential of substances heretofore not identified. At least three workgroups have proposed recommendations related to new pollutant identification or diagnostic monitoring.

Recommendations

Some analytes should no longer be analyzed in bivalves. Mercury and arsenic, in particular, appear to not provide information that is helpful to environmental or risk managers. While trends in mercury have been noted in the transplanted bivalves, it would be prudent to add a resident or transplanted bivalve component that is more sensitive to methylated mercury and the temporal scales of selenium fluctuation. The frequency of analysis of metals not on the 303(d) or the Regional Board's "pollutants of concern" list should be reduced to once every 3-5 years in order to maintain the trend database.

Assemble a database on known bioaccumulative substances and the current state of knowledge on environmental effects (*e.g.*, flame retardants). Identify peaks on existing chromatograms according to Bob Risebrough's proposal and determine what is known about potential environmental effects of those compounds still in use today. Determine which (potential) pollutants that are currently not on the RMP analyte list ought to be tracked. This would add a proactive element to the RMP, which would enable the Regional Board to work with other agencies (EPA pesticide registration, DHS, etc.) to determine whether or not additional studies are needed prior to use restrictions.

3.3 Objective #3 – Evaluate which Contaminants May Be Transferred to Higher Trophic Levels of the Food Web

Findings

The findings for Objective #2 also apply to this objective. Use of the California Mussel, *Mytilus californianus*, as the primary organism for the transplanted bivalve component may also limit achievement of this objective because this species does not normally occur in the estuary and it has no natural position within the food web. The Bay Mussel, on the other hand, does occur throughout the estuary and may survive at a broader range of salinities than does *M. californianus*. If the current side-by-side deployments indicate it is a suitable transplant organism, the Bay Mussel may improve achievement of this objective. Nevertheless, deployment

of transplanted bivalves in the water column may not adequately represent transfer of contaminants from the sediments into benthos and higher trophic levels. Other types of organisms, such as benthos or fishes, may be the best way to assessing the transfer of contaminants to higher trophic levels of the food web.

Recommendations

Develop additional ways to assess transfer of contaminants to higher trophic levels. These should include a benthic bivalve, other invertebrates, or fishes to evaluate bioavailability and transfer of sediment contaminants to higher trophic levels.

3.4 Objective #4 – Determine Pathways and Loadings of Contaminants to the Estuary

Findings

Bivalve measurements are able to discern differences in contaminants over spatial scales ranging from tens of meters to kilometers and over temporal scales from months to years. They can be used to assess the relative magnitude of contaminant problems at the terminus of watersheds and in front of outfalls or other point sources. Bivalves may also be used within this context to measure the response to clean-up efforts or other management action within watersheds that have been identified as pollutant contributors to the estuary.

The patterns in contaminant concentrations indicated by Figures 4 and 5 suggest that substantial seasonal and spatial differences exist in contaminant input or build-up in the estuary. Although additional analyses should be performed to determine whether the high PAH concentrations in dry season samples from Yerba Buena Island to Redwood Creek are primarily petrogenic or pyrogenic, they suggest the importance of aerial fallout from the busy motor vehicle corridors that border this part of the estuary. The regional differences in PCB congeners may also indicate contaminant sources that vary spatially.

The current configuration of the transplanted bivalve component limits its ability to achieve this objective. The high variability of salinity in the estuary, especially during the wet season, necessitates deployment of three different species for bioaccumulation measurements. Because bivalve species differ in their bioaccumulation characteristics, site comparisons are limited to those with the same species and sites with the same species do not necessarily overlap with the geographic definition of reach. Side-by-side deployments of multiple species currently being performed may determine whether there is a single species suitable for wet season deployment at all sites west of Carquinez Strait.

Recommendations

One species should be deployed at all sites to eliminate the difficulty of interpreting data from different species. If deployment of bivalves in both the wet season and dry season is maintained, side-by-side deployments of several species should be continued to determine whether a suitable species is available for deployment at all sites in the wet season. If a single species cannot be

found that survives at all sites during the wet season, a subset of sites with more limited salinity variation should be used. If cost savings are required while maintaining deployments in both seasons, elimination of mid-deployment maintenance cruises should be examined.

As the examination of contaminant pathways into the estuary focuses on smaller spatial scales, methods other than transplanted bivalves may be more appropriate. For instance, tracing PCBs to upstream sources may be best accomplished by using sediment sampling.

3.5 Objective #5 – Determine Effects of Contaminants in the Estuary

Findings

Although the RMP bivalve monitoring component operates under the assumption that the bivalve species used are unlikely to be affected by contaminant levels found in the estuary, this assumption has not been tested. Significant correlations exist between concentrations of tissue contaminants and indicators of bivalve health, but it has not been determined how either of these factors affects the other. Investigators in other areas have found significant biological effects of contaminants on bivalves, but it is not known how non-contaminant environmental factors affect these biological indicators.

Other organisms, such as fishes or birds, may be better indicators of contaminant effects in the estuary. Previous studies have suggested that contaminants in the estuary are at or above the threshold for effects on some vertebrates (Spies *et al.*, 1988; Spies and Rice, 1988; Davis 1997; Davis *et al.*, 1997).

Recommendations

Bivalve growth may be the best indicator of contaminant effects in the current transplanted bivalve program. Tissue dry weight is measured as part of the condition measurements and changes is tissue weight can be easily determined from differences in tissue weight between the T-0 (predeployment) bivalves and postdeployment bivalves. Tissue growth and contaminant concentrations that have been adjusted for the effects of environmental factors can be statistically compared to determine whether contaminants might be affecting growth.

Although bivalve growth may be an indicator of contaminant effects, fishes, birds, and mammals may be more suitable for this purpose. These forms are more likely to show effects than are bivalves because they occupy higher positions in the food web and will contain higher concentrations of contaminants that biomagnify. A special study should be developed to examine the effects of contaminants on fishes, birds, or mammals.

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