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**Sublethal Effects of the November 2007 Cosco Busan Oil Spill on Native Oysters
DRAFT REPORT**

**Chela Zabin, Ted Grosholz and Sarikka Attoe
Dept. of Environmental Science and Policy
University of California, Davis
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Project Background

The results presented here are based on data collected as part of a larger study addressing the status and health of the native Olympia oyster, *Ostrea lurida*, in San Francisco Bay. The demographic information from the oyster populations was not collected specifically with the intent of analyzing potential impacts of the Cosco Busan oil spill. However, our data were gathered at dates both before and after this oil spill and include sites that were lightly oiled as well as ones that were not oiled. In addition, we had a small amount of data from a heavily oiled site. Therefore, our approach is one of retrospective data analysis to determine if there was a measurable effect of the Cosco Busan oil spill on the native oysters and other benthic organisms in our study.

Data summary

Study Sites

As part of the larger study of native oyster population biology and ecology conducted from summer 2006 through December 2008, we identified sites throughout San Francisco Bay that had measurable Olympia oyster populations. In 2006, our work included 13 sites; in 2007-2008, we focused on a subset of eight sites. Based on this work and the circumstances of the oil spill, we identified five sites for which we would be able to assess the potential sublethal effects of the November 2007 Cosco Busan oil spill. The classification of these sites was based on either a definitive signal of Cosco Busan oil detected from the oyster tissue collected at a site and the nearby shoreline having been determined to have been oiled during the Cosco Busan spill by Shoreline Cleanup Assessment Technique (SCAT) teams (oiled) or because tissue analysis indicated no Cosco Busan oil detected and the nearby shoreline had been classified as “no oil observed” during the Cosco Busan spill by SCAT teams (control). One of our sites, Point Orient, is included here as a control with the following caveats: 1) one tissue sample collected there contained PAHs with an “indeterminate” signal for Cosco Busan oil; 2) overflight maps showed tar balls in the water near the site, but not onshore (November 12 and 13, 2007); 3) simulations by NOAA showed that oil could have reached the site.

A sixth study site (Alameda) was considered very lightly oiled; analysis of data from this site was not part of our original contract. However, where the data could be easily extracted from our existing data sets, we included Alameda in our analyses. In addition, we had a small data set on survivorship from one of the heavily oiled sites, San Francisco Aquatic Park, which we have included. These sites are described in Table 1 below.

Photos of the main study sites are included as Appendix 1 and a map of the sites is included as Appendix 2.

Table 1. UC Davis study sites included in this analysis.

Site	Substrate Type	Oiled/control	SCAT Shoreline Oiling Category
Alameda (Encinal Boat Launch)	rip-rap	Oiled	Very lightly oiled
	natural cobble and seawall	Oiled	
Angel Island (Ayala Cove)	rip-rap	Oiled	Very Lightly oiled
Berkeley, Shorebird Park	rip-rap	Oiled	Lightly oiled
Oyster Point	rip-rap	Control	No oil observed
Point Orient	natural cobble	Control	No oil observed**
		Oiled	High pressure, hot water treated;
San Francisco Aquatic Park	Rip-rap		Very lightly oiled
Tiburon-Belvedere	cement cobble	Oiled	Very lightly oiled

**One indeterminate sample was reported from this site

At the above sites, we collected data on recruitment, growth and mortality of oysters along permanent 50 m transects parallel to shore in the middle of the oyster zone at approximately ± 0.5 ft. MLLW. We also photographed permanently marked quadrats along the transect lines on a quarterly basis over the study period. Field methods are described briefly below.

Field Methods

Recruitment. We measured larval recruitment of oysters to 10 recruitment collectors placed every 5 m along our permanent, 50 m transect lines. Recruitment collectors in 2006 and 2007 consisted of 10 x 10 cm PVC plates attached to cement pavers. In 2008, cement pavers alone were used (Fig 1). Collectors were deployed in June and retrieved in November or December each year.



Figure 1. Cement recruitment collector.

Growth. Oysters were tagged in 2006, 2007, and 2008 using cyanoacrylic or marine epoxy and Floy shellfish tags (Fig. 2). Initial sizes of oysters were taken, and sites were revisited periodically to re-measure oysters. Tagged oysters were sometimes lost due to mortality and the difficulty of re-finding mud-covered oysters. New oysters were tagged each visit to keep the number of total tagged oysters near 50.



Figure 2. Marked oysters, shown here with orange numbered tags (center of photo) were periodically measured to track growth. Tagged oysters were also used for mortality calculations.

Mortality. At each site visit, we recorded the number of live and dead tagged oysters. When tagged oysters were not found, they were recorded as “missing” and not included in mortality rate calculations.

Community composition. To determine whether competition for space might be a limiting factor for native oysters at our study sites, we assessed the abundance of other sessile organisms in the oyster zone using 10 permanent 100 cm² photoquadrats along our transect lines (Fig. 3). In the field we recorded the presence of all organisms in the quadrats to assist in interpreting the photoquadrat images. The images were downloaded and percent cover of each type of organism was determined by projecting a grid of 25 points onto the computer screen.

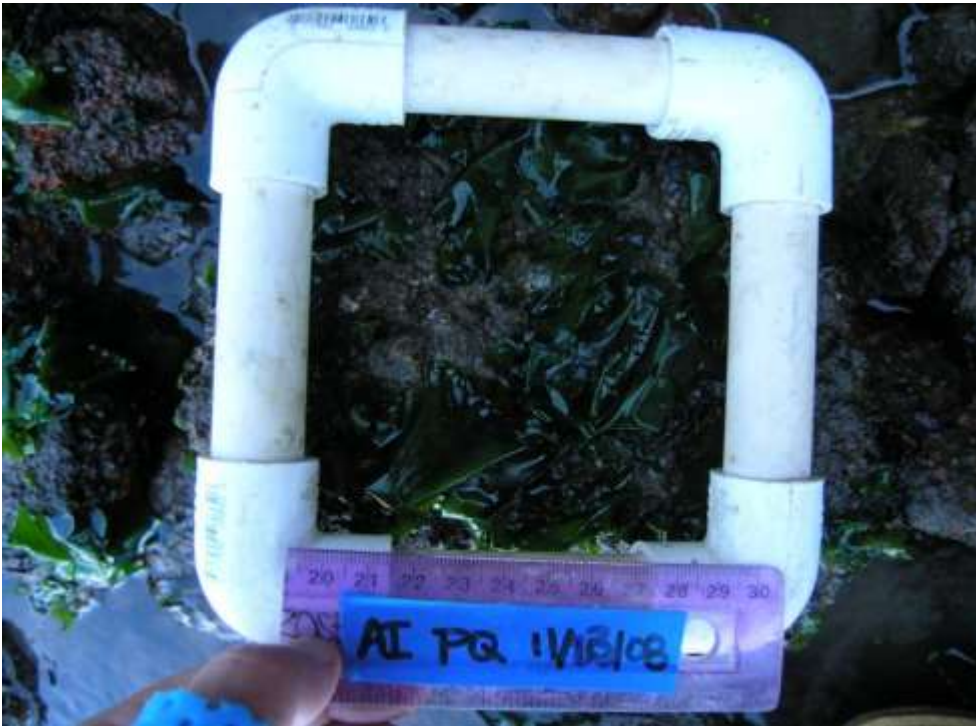


Figure 3. A photoquadrat taken at the Angel Island site.

Data analysis

Power Analysis. The first phase of data analysis involved a determination of whether we had sufficient power to detect a 20 percent change in a given parameter (i.e. recruitment, mortality, growth, percent cover of organisms in photoquadrats) with both alpha and beta = 0.2. Where this was the case, we looked at whether the change in a given parameter pre- and post-spill was different between oiled and control sites. We present the initial results of the power analysis below within each data section.

Recruitment. Recruitment was calculated as the mean number of recruits per recruitment unit (standardized to #/m²) over the entire recruitment period. Recruitment was highly variable both spatially and temporally. Our power analysis showed we did not have sufficient sample size to meet the power requirements. Recruitment data showed substantial spatial variation, as well as being very sparse, making the detection of

differences among sites difficult. Data from 2007 and 2008 only were considered as there was virtually no recruitment in 2006, likely due to extreme lowered salinity in the Bay following late spring rains

Growth. Because mean monthly growth rates were low, we calculated growth over two 6-month periods: warm season (May through Oct) and cold season (Nov-April). Growth rates were calculated as follows: the difference between the last measured size of a tagged oyster minus its initial size, divided by the time period over which the oyster was tracked. As small oysters grow more quickly than large oysters, growth rate was then divided by the oyster's initial size to calculate proportional growth for each oyster. We used data from all sites to generate a Model I regression of proportional growth on oyster size to generate an estimate of expected proportional growth based on initial size. We then calculated a residual value of proportional growth for each oyster by subtracting this expected proportional growth from that observed. This way we could determine whether growth at a given site was more or less than predicted (the residual difference) based on the size of oysters at a particular site. We used these residual data to test for differences in growth among sites.

We found that cold season growth rates were negligible, so in this analysis we used only warm season growth rates for the warm season preceding the Cosco Busan oil spill and the warm season following the spill. For Tiburon, Point Orient and Oyster Point, we used 2006 and 2007 warm season growth (May through October) as the pre-spill measures. No data were collected at the Berkeley site in 2007 and at Angel Island no data were collected in 2006; in these cases we used only one year of pre-spill growth. Alameda data were not included.

The power analysis indicated that we had sufficient power to detect change at the specified levels. An analysis of covariance indicated there was no difference in growth between the oiled sites for which data are available (Angel Island, Berkeley, and Tiburon) and the control sites (Point Orient and Oyster Point) site (Fig. 4).

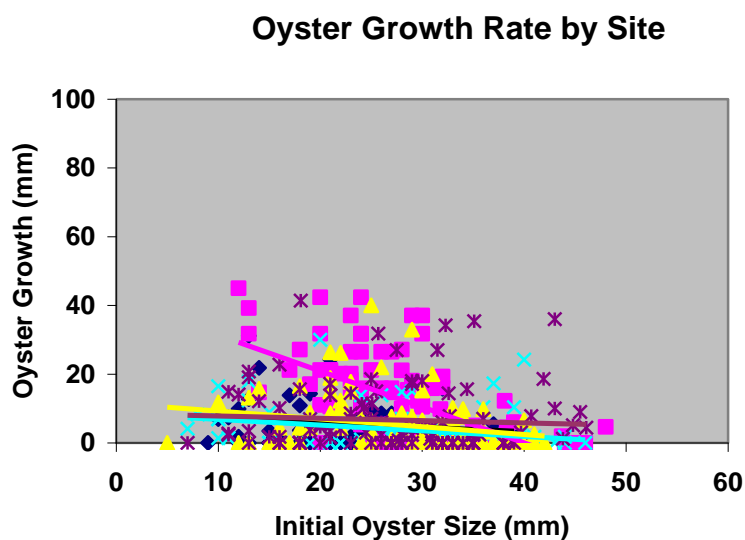


Figure 4. Oyster growth rates for study sites from 2006-2008 (2007-2008 for Angel Island, 2006 and 2008 for Berkeley). Linear regressions of growth on initial size are shown for each site and were used to calculate residual growth. Berkeley shows higher growth rates than other sites, but there was no overall difference between oiled and control sites.

Mortality

Mortality was calculated as the percent of tagged oysters that were dead at a given time period. Mortality varied seasonally and between sites. Thus, it made the most sense to compare pre-spill mortality with post-spill mortality for each site. We had sufficient data to compare 2-3 time points for each site (except for San Francisco Marina, which had only 1 comparison), pairing a post-spill date with data from the same site ~12 months earlier. Mortality decreased post-spill at Oyster Point, but increased at 3 of the 5 other sites (Fig. 5).

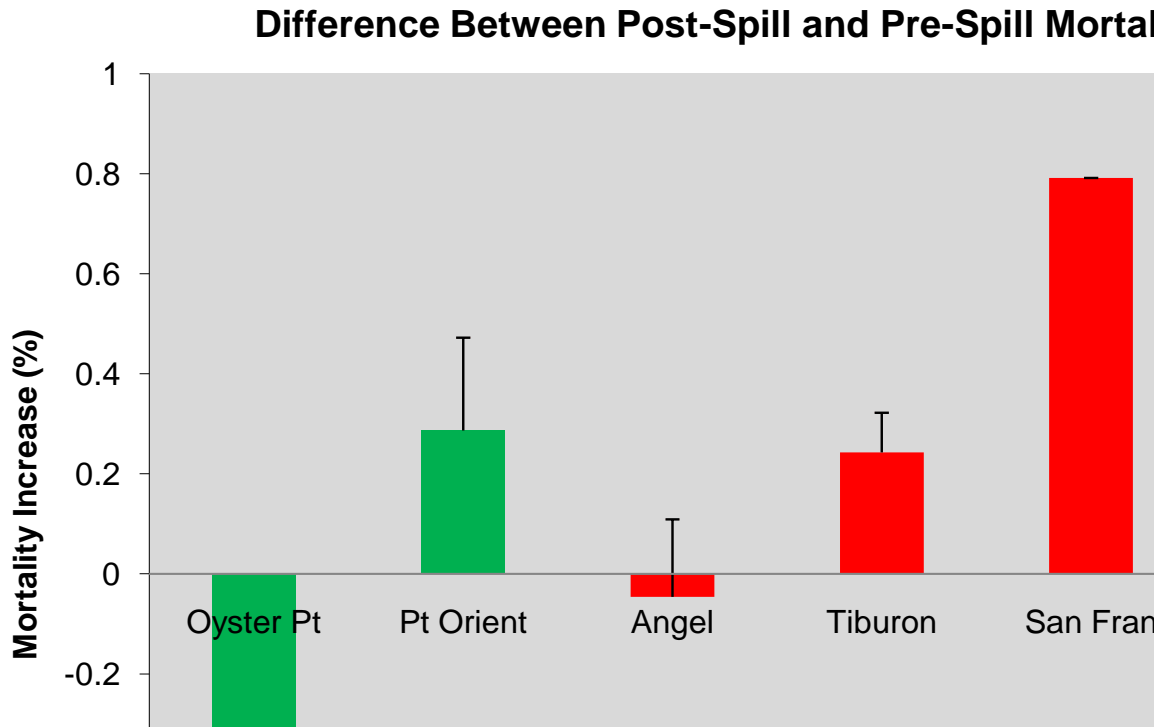


Figure 5. Change in mortality rates for each study site between 2007 and 2008. Mortality did not change or increased at the oiled sites (red) and showed mixed results at the control sites (green), Oyster Point and Pt. Orient. Bar heights are mean values with error bars representing one standard error.

We analyzed these data using a Kruskal-Wallis test, which makes no assumptions about normality or heterogeneity of variances. The test is based on ranks; thus it is extremely conservative. We included Alameda and San Francisco Aquatic Park in this analysis.

The change in mortality between years is not different between oiled and control sites (Mann Whitney U = 16, p = 0.386, df = 1). Because San Francisco Aquatic Park has a high background mortality rate, we also ran these tests with and without these data; excluding this site makes no difference in the qualitative outcome of this test.

Community composition. While more than 18 taxa were present in the photoquadrats, percent cover of any single species was very low for most quadrats on most dates. Even the most abundant taxa such as the ephemeral green alga *Ulva* sp., *Mytilus* sp. (*M. trosellus* and *galloprovincialis* complex) and barnacles (*Balanus* spp.) typically amounted to only 15 to 10 percent cover (Fig. 6 and 7). Thus, it was unlikely that an analysis focused on a single species, even the most abundant groups, would be meaningful.

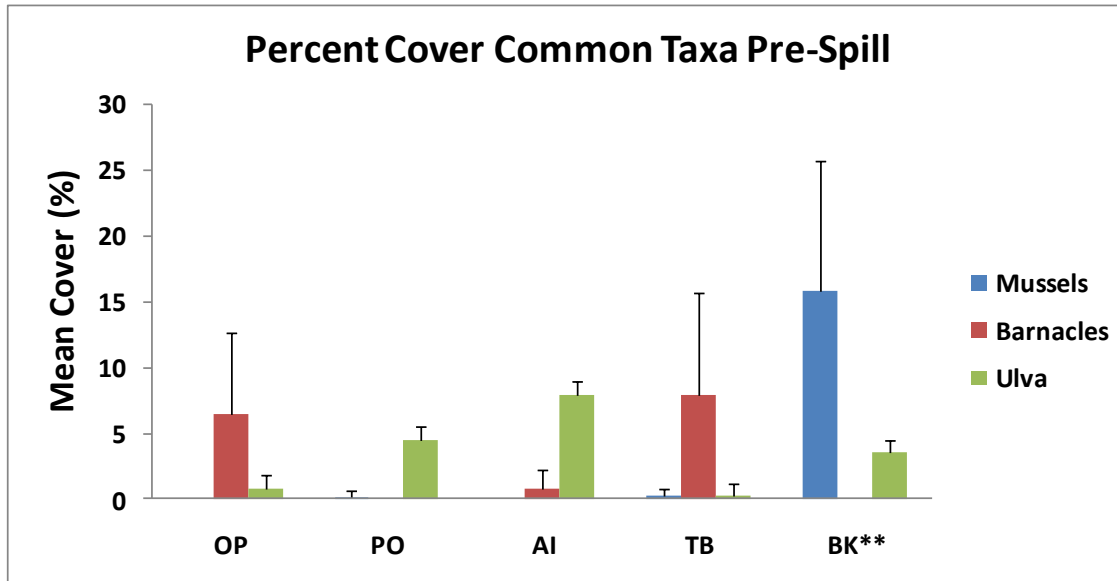


Figure 6. Mean percent cover of the three most common taxa within photoquadrats for each study site during fall 2007 (pre-spill). Despite among site differences, there were no significant differences between oiled and control sites.

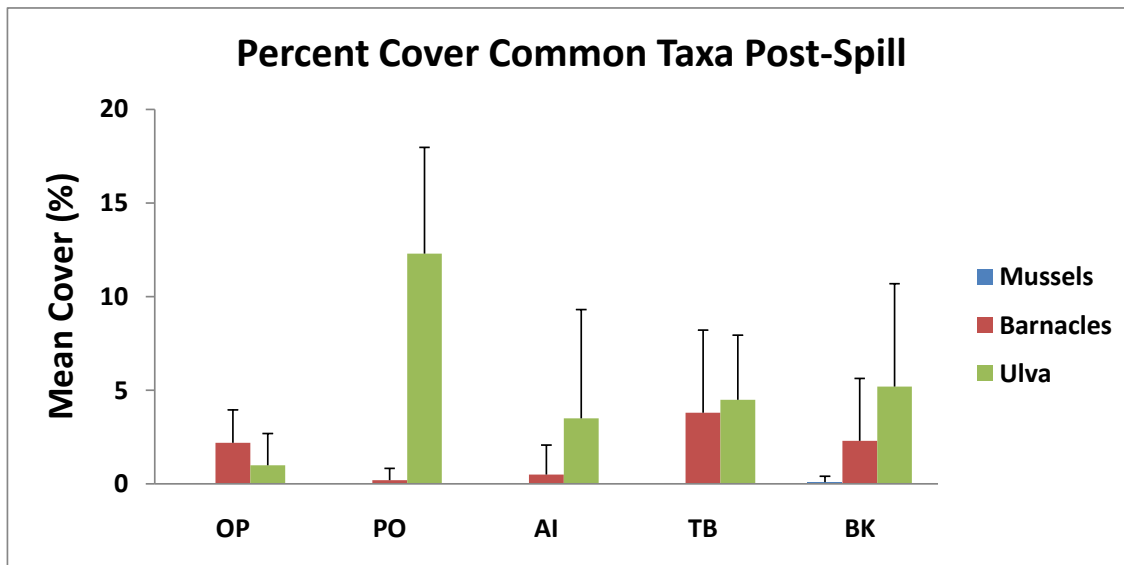


Figure 7. Mean percent cover of the three most common taxa within photoquadrats for each study site during fall 2008 (post-spill). Despite among site differences, there were no significant differences between oiled and control sites.

Because of the low abundance of most taxa, we focused our analysis on the percent cover of all taxa combined, and asked whether there was a difference pre- and post-spill

between oiled and control site(s). For this analysis, we used photographs taken in November 2007 (pre-spill) for Point Orient, Angel Island, Tiburon and Oyster Point and compared these to photographs taken in November 2008. At Berkeley Marina we used photographs taken in June 2007, as we had not photographed it in the fall that year. Spring photographs typically had extremely low cover and we did not have enough photographs in the summer to capture both pre- and post spill at all sites. Cover was determined using the point-intercept method with 25 contact points overlaid each quadrat and the number of points for each taxa (out of 25) tallied. These point counts were then square-root transformed to meet assumptions of normality for analysis of variance. Graphic presentation of percent cover uses standardized values based on 25 points equal to 100 percent cover.

As a broad stroke approach, we compared all pre-spill cover to all post-spill cover in a two way ANOVA with year, oil status (oiled vs. control) and the interaction of year and oil status as fixed factors. This is a conservative approach, as any between-site variability is incorporated into the overall variability, potentially obscuring differences due to oil exposure. We had sufficient power to detect a change in cover of total taxa at the specified level. There was a significant interaction in nearly every case as total cover increased in 2008 for the non-oiled sites and decreased in 2008 for two of three oiled sites (Fig. 8). The full model with the interaction of year (before vs. after the spill) vs. oiled status (oiled vs. control) was statistically significant ($F = 7.62, p = 0.0001, df = 105$).

Percent Cover of All Taxa

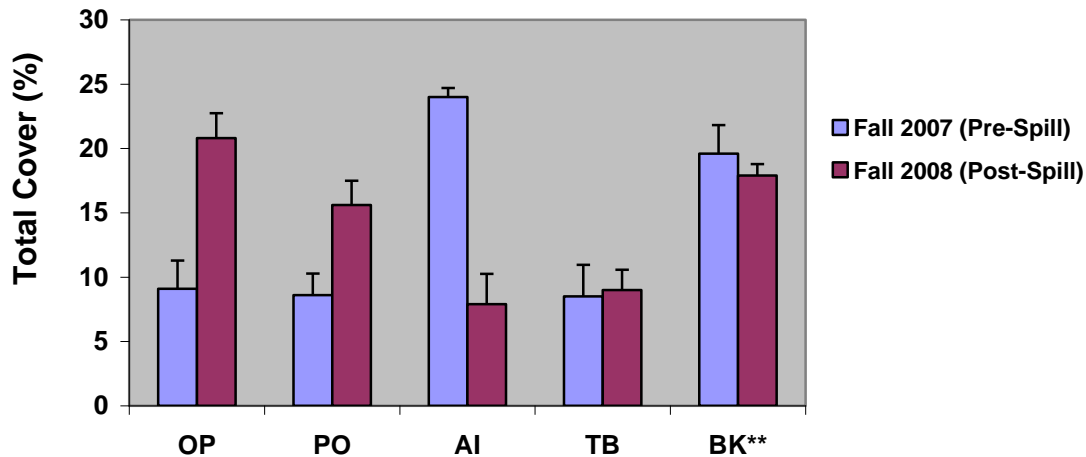


Figure 8. Differences in total percent cover (all species combined) within photoquadrats for each study site, fall 2007 and fall 2008.

We also compared the full community composition among sites to determine if there were differences among oiled and control sites either before or after the spill in terms of overall community composition. We began by using non-metric multidimensional scaling (PRIMER v. 5), which provides a visual representation of similarities between

ecological communities along synthetic axes. Communities which are more alike in terms of shared species group more closely together than those that have fewer species in common. Percent cover data were arcsine squared root transformed to improve normality, and a Bray-Curtis similarity measure was used to create these graphs. If there was a shift in the community composition at oiled sites post-spill, these data points should cluster separately from those of the control site post-spill and from the pre-spill data points. Sites cluster by year (Fig. 9, sites in 2007 are more alike than sites in 2008) and indicate no difference in community composition between oiled and control sites.

SF Bay PhotoQuads

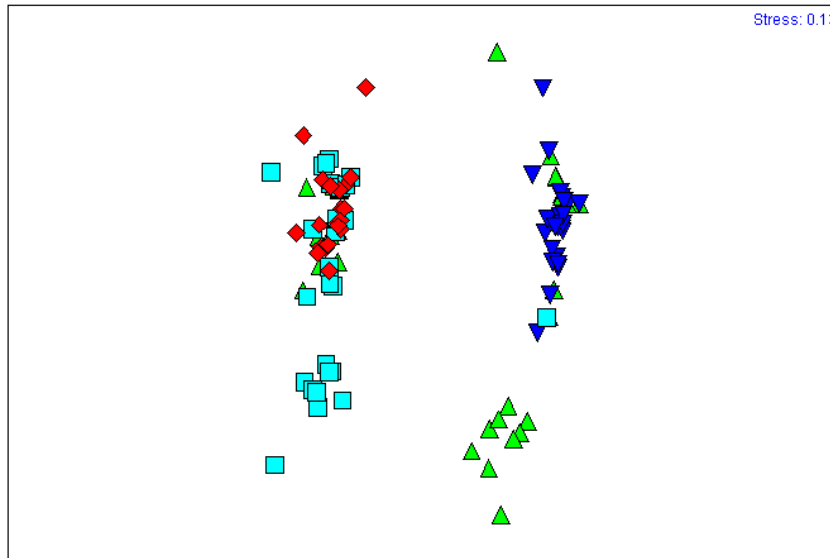


Figure 9. Non-metric multidimensional scaling plot of the similarities in community composition between oiled and control sites pre- and post spill. Data are coded as follows: dark blue triangles = control sites, pre-spill; red diamonds = control sites post spill; green triangles = oiled sites, pre-spill; blue squares = oiled sites, post-spill. Community composition clusters by year, but not by oiled vs. control status.

While this is not a formal statistical test, the complete lack of separation of the post-spill oiled sites from the control sites makes such a test unnecessary. This differs from the finding of a difference in overall cover and is likely a result of overall low abundance of most species, with only a few species driving the overall change in cover.

Summary

Other than a change in total cover, the data summarized here do not indicate a difference between the control sites and the lightly or very lightly oiled sites for which we had data. For oysters, there is no indication of an impact on growth or mortality, and recruitment of spat was too variable between years and sites to allow for analysis. It should be noted,

however, that none of our main study sites received moderate or heavy oiling, so we cannot draw conclusions on the potential effects on oysters of greater amounts of oil. It is also important to note that while Point Orient has been included here as a control site, there are issues that undermine the confidence with which we make this assignment. Of particular concern is that when Point Orient is either excluded from the analysis or included as an oiled site, there is a statistically significant difference in oyster mortality post-spill at oiled sites vs. the one non-oiled control (Oyster Point).

Intertidal benthic community composition does not appear to differ between oiled and the non-oiled sites and cover of individual taxa was too low and variable to provide useful information. However, total cover of all organisms (i.e., the opposite of bare space) did decrease post spill at two of the oiled sites and increase at the control sites. (Notably, this change remains whether Point Orient is excluded or included as an oiled site.) The increase in bare space was due mainly to a mean decrease in mussels and diatoms at the oiled sites and an increase in diatom and *Ulva* sp. cover at the control sites. This suggests there may have been a moderate effect of oil on mussels, but additional data would need to be considered before any definitive conclusion could be drawn.

Appendix 1 Main Study Sites



Figure 10. Tiburon site (near border with Belvedere). This site was categorized by SCAT teams as very lightly oiled.



Figure 11. Ayala Cove, Angel Island. This site was categorized by SCAT teams as very lightly oiled.



Figure 12. Ayala Cove, Angel Island: an oiled oyster on shoreline armoring at our study site.



Figure 13. Berkeley Shorebird Park. This site was categorized by SCAT teams as lightly oiled.



Figure 14. Oyster Point. This site was categorized by SCAT teams as no oil observed.



Figure 15. Point Orient. This site was categorized by SCAT teams as no oil observed and is considered not oiled in this analysis.



Figure 16. Alameda (Encinal Boat Launch) study site, classified as very lightly oiled. This site was closed following the oil spill.

Appendix 2. Map of study sites.

