

# Demographic and photosynthetic characteristics of eelgrass after an oil spill in San Francisco Bay, USA

Mark S. Fonseca<sup>a</sup>, Christine M. Addison<sup>a</sup>, Gregory A. Piniak<sup>a</sup>, and Natalie Cosentino-Manning<sup>b</sup>

<sup>a</sup>NOAA Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Road, Beaufort, NC 28516 USA

<sup>b</sup>NOAA Restoration Center, NOAA Fisheries, Santa Rosa, CA 95404 USA

\*[Mark.Fonseca@noaa.gov](mailto:Mark.Fonseca@noaa.gov), fax 252-728-8784, phone 252-728-8729

## Table of Contents

1. Introduction.....	3
1.1 Review of oiling effects on seagrass.....	3
1.2 Summary of Cosco Busan Spill.....	5
1.3 Sampling Objectives.....	6
2. Methods.....	6
2.1. Site description .....	6
2.2. Demographic characteristics.....	11
2.3. Seagrass photosynthesis.....	12
3. Results and discussion .....	14
3.1. Site description .....	14
3.2. Demographic characteristics.....	15
3.3. Seagrass photosynthesis.....	17
3.4. Summary .....	18
Acknowledgements.....	19
References.....	19
Figures and Tables .....	23

## 1. Introduction

### 1.1 Review of oiling effects on seagrass

Many studies have examined the effects of oil on seagrasses (e.g, den Hartog and Jacobs 1980, Hatcher and Larkum 1982, Zieman et al. 1984, Thorhaug et al. 1986), yet consensus of impacts to the overall health of individuals within and among seagrass species is lacking due to a high degree of variability in response to oiling. For example, benthic respiration rates of *Posidonia australis* increased with oiling, indicating stress, yet, leaf turnover - a response averaging plant health over longer timer periods - was not affected (Hatcher and Larkum 1982). An examination of lethal dose limits ( $LD_{50}$ ) to several Caribbean seagrass species identified plant death was less affected by oiling type and varied more among species; for three Caribbean seagrass species,  $LD_{50}$  ranged from 75ml – 125ml in 100L seawater for a 100hour exposure period (Thorhaug et al. 1986). In the case of the oil spill in Bahia Las Minas, Panama, a thick covering of oil resulted in complete loss of seagrasses (Jackson et al. 1989; Marshall 1990). Eight months after the sinking of the tanker *Haven*, near Arenzano, Italy, dying *Posidonia oceanica* plants were identified (Sandulli et al. 1998). Nine years later, no traces of oil were found but the absence of rhizomes older than 8 years was consistent with a massive shoot mortality; analysis of rhizome growth curves, agrees with expectations of suppression of growth under stressed conditions (Peirano et al. 2005).

Conversely, other studies detected significant impacts to seagrasses arising from oiling. No appreciable impact to *Zostera marina* was determined from the *Exxon Valdez* spill (Dean et al. 1998), considered one of the most devastating human caused environmental

disasters to occur at sea. No significant photosynthetic impacts were detected for *Z. capricorni* (Wilson and Ralph 2008; but see Macinnis-Ng and Ralph 2003) and for *Halophila ovalis*, *H. stipulacea*, and *H. uninervis* (Durako et al. 1993).

Oil dispersants were also found to have detrimental effects on seagrass photosynthesis (Ralph and Burchett 1998; Scarlett et al. 2005; Macinnis-Ng and Ralph 2003) and at elevated concentrations were lethal to some seagrass species (Thorhaug and Marcus 1987; Scarlett et al. 2005). Use of oil dispersants appears to negatively affect seagrass more universally than oiling alone. A likely cause for this negative response is that researchers believe dispersants irreversibly affect respiratory organs and depending on exposure time, they reversibly affect the nervous system (Scarlett et al. 2005).

Studies documenting impacts to seagrasses can be grouped into three general categories, based on duration of effects: short term, long term, and resulting from cleanup efforts. Historically, it was believed that seagrasses were not highly susceptible to oil effects, except when physically covered with oil or when dispersants were used. When oil is affixed to plants, it reduces gas exchange, photosynthetic rate, and rhizome expansion rates. Leaf fouling produces more immediate effects to above ground vegetation than does fouling of the sediment surface. However, soil fouling may subject below ground plant tissue to more persistent petro-chemical toxin exposure. Accumulated petro-chemicals within the sediment reduce gas exchange by killing microbes and creating elevated anaerobic soil conditions (Hester and Mendelssohn 2000). Such conditions may be toxic to below ground tissues resulting in reduced rhizome expansion rates and below

ground biomass. Reproduction may be affected by oiling - marsh flowers that are oiled rarely produce viable seed, but it is not known if eelgrass flowers also abort seeds when oiled. Moreover, clean-up efforts may increase turbidity directly reducing light availability to seagrass plants and may compact or disturb sediment.

Given the lack of consistent responses of seagrasses to oiling events and the absence of a standard assessment protocol for determining seagrass impacts related to oiling events, the *Cosco Busan* oil spill required a thorough evaluation to establish whether impacts to seagrass (*Z. marina*) occurred in San Francisco. The information collected as part of this impact assessment could help shape the restoration process based on the degree of injury, if any, to seagrass beds in the Bay.

## **1.2 Summary of Cosco Busan Spill**

On November 7, 2007, the container ship *Cosco Busan* struck the Oakland-Bay Bridge in San Francisco Bay spilling an estimated 58,000 gallons of bunker fuel. The spill occurred during a neap tide cycle near the height of the incoming tide. Strong winds and an outgoing tide transported surface oil out of the mouth of the Bay and northward along the coastline yet, some of the oil was retained within the Bay. Shoreline cleanup assessment teams (SCAT) and clean-up responses by private, government, and public organizations provided near real-time documentation of the oiling extent and enhanced control over the affected areas potentially reducing the overall distribution of the oil.

During the week of November 26, a team of scientists from NOAA's National Centers for Coastal Ocean Science (NCCOS), NOAA's Restoration Center and Office of Response and Restoration, and researchers from San Francisco State University evaluated oil impacts to seagrass plants and associated fauna in San Francisco Bay. This report is a synopsis of NCCOS' findings of oiling impacts to eelgrass meadows throughout the Bay 19 days after the *Cosco Busan* oil spill.

### **1.3 Sampling Objectives**

- Compare shoot densities and reproductive status of eelgrass at oiled and un-oiled sites.
- Compare recent rhizome growth curves to evaluate short term rhizome expansion at oiled and un-oiled sites.
- Evaluate photosynthetic response of seagrass plants at oiled and un-oiled sites.

## **2. Methods**

### **2.1. Site description**

Site selection was guided by an effort to sample eelgrass from oiled (impacted) and un-oiled (control) area while taking care to survey sites that were representative of the diversity of bed morphologies, elevations, exposure, and sediment types of eelgrass beds around the Bay. Sites surveyed from November 25-27, 2007 are identified in Fig. 1 with site characteristics listed in Table 1. Oil and control site designation was based on detailed aerial and boat reconnaissance data delineating oil dispersal.

Degree of oiling assignments for eelgrass beds was calculated separately for intertidal (0-4 ft.) and subtidal (greater than 4 ft.) meadows. Using SCAT survey data, the intertidal beds were assigned a degree of oiling equivalent to the most prominent maximum oiling observed, as defined by SCAT data, on the closest adjacent shoreline to the intertidal bed. The subtidal beds were assigned an oiling one degree lighter than the oiling assigned to the adjacent intertidal beds.

Qualitative information from each site was recorded describing the general characteristics of the seagrass bed and nearby habitats as well as the type, condition, and extent of observed oil. Within the seagrass meadow at each site the visible presence and description of sediment or plant surface oil (SO) was recorded. Randomly distributed pits were dug along the survey transect to determine if subsurface oil (SSO) was released (using methods described in Taylor and Reimer 2008; Table 1). This section describes, qualitatively and at a coarse macroscopic level, the meadow characteristics and sediment and plant surface and subsurface conditions observed during the November sampling period. Any oil or tarballs found on the plant leaf surface were recorded. Any non-visible oiling, such as PAH absorption into plant or animal tissue, was not detectable using these methods thus are not described here.

Point San Pablo (hereafter PSP): This is one of the Bay's largest seagrass beds encompassing approximately 400 hectares and extending from the subtidal to low-mid intertidal region. It is an offshore persistent submersed and partially intertidal site thus no shoreline or higher intertidal observations were made. Sampling was conducted at the

highest elevation in which seagrass occurs, overlapping with areas of past eelgrass surveys (authors unpubl. data).

Point Orient (hereafter PO): Seagrass at this site exists in a narrow band located less than a meter offshore of a rip rap shoreline. Seagrass is patchily distributed and grows in fine, very soft sediments; this site is very close to areas of past eelgrass surveys (authors unpubl. data).

Keller Beach (hereafter KB): This site is accessible by shore, located adjacent to the Miller/Knox Regional Shoreline Park. Clean up activities were conducted in the upper intertidal region of this beach, above eelgrass distribution, prior to this sampling. The seagrass meadow has been surveyed here over a number of years and contains a persistent perennial bed, composed of patchy and continuous areas of seagrass; this site is also quite near areas of past eelgrass surveys (authors unpubl. data).

Cozy Cove (hereafter CC): Located approximately 200m northwest of Keller Beach, this site is a narrow band of seagrass extending along shore approximately 100m. To our knowledge, clean up activities were not conducted here.

Emeryville (hereafter EV): Located offshore between the Berkeley Pier and Berkeley Marina, the Emeryville seagrass bed is a small stretch of seagrass growing on the windward side of a small sand berm. Sediments are firm, fine grain sand with patchy seagrass distribution. Although SCAT data identified oil on the shoreline, no SO or SSO

was observed within the surveyed region of this offshore seagrass bed; this site is also quite near areas of past eelgrass surveys (authors unpubl. data).

Crown Beach (hereafter CB): Located along the Carlsbad State Beach, this site is a unique seagrass bed to San Francisco Bay because it is a well documented annual bed. At the time of sampling, this site had already experienced a winter decline in shoot abundance. The cause of this decline is unknown, but suspected to be due to natural processes including flower senescence and disturbance by rays and geese. At peak growth, during summer months, this site contains nearly six hectares of seagrass, with large clones of vegetative and flowering shoots distributed throughout the site. At the time of sampling, shoot densities were low and nearly exclusively vegetative due to a recent heavy grazing event by migrating geese. Sediments are firm, fine grain sand; this site has been a focus of past eelgrass surveys (authors unpubl. data).

Bay Farm Island (hereafter BF): Eelgrass at this site is located nearly 700m offshore of Oakland, along the ferry channel on a slightly elevated sand mound. Historic surveys at this site documented a lush, dense eelgrass bed supporting a large number of infauna. Sediments here are firm sand, with softer sandy mud at the deeper margins of the bed; this site has been a focus of past eelgrass surveys (authors unpubl. data).

Horseshoe Cove (hereafter HC): Seagrass here extends along the east side of the cove in a narrow band composed of small, sparse clones. This cove contains a small marina that provides mooring and anchorage for approximately 100 vessels. A film of heavy oiling

was observed on the rip rap, clearly delineating the tide level at the time of oil impact. Attempts at removing the oil from rip rap were evident; we were told cold water spray was the treatment used. This cleaning attempt does not appear successful. Oil sheen was visible on the water and sediment surface within the eelgrass region. A water sample was collected and submitted for petrochemical analysis.

Angel Island (hereafter AI): Located within a sandy, rock cobble cove on the North side of Angel Island, this site has historically been an eelgrass bed with continuous to patchy cover extending parallel to the shore out approximately 20 m. Where eelgrass is found, sediments are firm with an occasional boulder. At higher tidal levels the beach is primarily gravel and small cobble. Traces of black tar/oil were found on the sides of some upper intertidal boulders; this site has been a focus of past eelgrass surveys (authors unpubl. data).

Keil Cove (hereafter KC): A coat of oil extended along the rocky edge of the shoreline, encompassing an area of more than 100m long and 20cm wide. Clean up attempts at this site were evident, rock scrubbing was attempted, heavily oiled larger rocks were placed in piles for apparent removal later. In addition to the rock piles, a coat of oil remained on bedrock and smaller gravel/cobble. Offshore approximately 3meters, within seagrass habitats, no oil was observed however a researcher exited this site with a tarball on their field pants. Seagrass within this cove has one of the longest historical documentations of eelgrass in North America, beginning in the early 20<sup>th</sup> century (Setchell 1929); this site has also been a focus of past eelgrass surveys (authors unpubl. data).

Paradise Cove (hereafter PC): Seagrass at this site exists in a narrow band located along shore in the shallow subtidal. Seagrass is patchily distributed but can be found as large clones and grows in fine to firm sediment; this site has been a focus of past eelgrass surveys (authors unpubl. data)..

## **2.2. Demographic characteristics**

Seagrass leaf production coincides with the below ground production of rhizome nodes, thus if plants are experiencing stress, such as that due to heavy oiling, it would be manifested as a reduction of growth to above and below ground structures of the plant. Observations of seagrass meadow distribution and condition as well as quantifying above ground (shoot density) and below ground plant metrics (rhizome expansion) were recorded to document the condition of each surveyed site.

Above ground seagrass characteristics surveyed include quantitative measurements of shoot densities and qualitative descriptions of leaf conditions as well as presence/absence of oil on or around plants. At each site, a 100m transect tape was placed within eelgrass beds at the highest elevation of eelgrass distribution; overlapping the area most likely affected by oiling. Ten quadrats ( $0.25\text{m}^2$ ) were randomly placed along a transect where eelgrass shoots were counted and plants were inspected for evidence of surface oil.

During the time of our survey, no flowering shoots were encountered within the random quadrats, thus all data reported are densities of vegetative shoots. Thirty randomly selected plants, with above and below ground structures intact, were collected for below

ground analysis and closer examination of above ground surfaces for oil presence at all sites except HC and CB, due to patchy nature of the bed and low shoot densities. At sites with exposed sediment during field surveys, the sediment surface was examined for oil or tar ball presence. Any oil observed was measured and recorded.

Potential effects of below ground oiling were evaluated by analyzing recent rhizome node production as well as examining the area for subsurface oil. Previous studies conducted in San Francisco indicate node formation occurs approximately every 15 days (M. Fonseca, unpublished data). We measured the four youngest complete internode lengths, which would document growth for approximately 60 days, encompassing the time period immediately before and after the *Cosco Busan* spill.

Internode measurements were collected between root nodes using a digital caliper, measuring to the nearest hundredth mm. Numbering is as follows: internode 1 = most recent (post spill) to internode 4 = oldest (approximately 2 months old). Due to natural variability in plant size, in which larger, more robust rhizomes produce longer internode lengths, fractional growth rates were used in this analysis to standardize growth among plants. Fractional growth rates were calculated as follows:

$$\text{Fractional Length of Internode} = \text{Internode Length} / (\text{sum of internode lengths of nodes 1-4})$$

### **2.3. Seagrass photosynthesis**

Intact *Z. marina* were collected, held in seawater, and returned to the Romberg Tiburon Center for Environmental Studies for fluorometric analysis. Three plants were collected

from each of 10 quadrats at the initial sites (PSP, PO, AI, KC). Due to excessive processing time, one plant per quadrat was assessed at the remaining sites (PC, EV, BF, KB, CC). Plants were held in seawater at ambient temperature in an outdoor shaded (~13% of ambient light) aquarium facility. The flow-through seawater system was not functioning due to the spill, so plants were kept in open Ziploc bags filled with seawater, with water changes completed as necessary. All measurements were made within 24 h of plant collection.

All plants were standardized to a light exposure of  $500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at ambient temperature for at least 20 min prior to measurements. Photosynthetic characteristics of *Z. marina* were then assayed using rapid light curve (RLC) techniques adapted from Ralph and Gademann (2005). The optical probe of a DIVING-PAM (Walz GmbH) fluorometer was fixed orthogonal to and 4 mm from the leaf blade; fluorescence measurements were made on the second youngest leaf 2-5 cm from where it emerged from the sheath. Plants were subjected to 10 s of quasi-darkness, followed by RLCs using stepped actinic illumination 10 s in duration (Ralph and Gademann 2005). Light levels ranged from  $56\text{-}862 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ , as measured with the PAM's light meter calibrated against a Licor LI-192 SA quantum sensor.

Analysis of the RLC data focused on two parameters: the initial effective quantum yield (pseudo-dark adapted  $\Delta F/F_m'$ ), and relative electron transport rate (rETR) at the last irradiance step (used as a proxy for the maximum rETR, as little evidence of photoinhibition was seen in the data). The rETR have not been corrected for leaf

absorption (though leaf absorption measurements were made). The  $\Delta F/F_m'$  data did not meet parametric assumptions after arcsin transformation, and were analyzed with non-parametric statistics. The rETR data were square root transformed to meet parametric assumptions.

RLCs can be quantitatively compared by treating the data as a photosynthetic-irradiance (P-E) curve and estimating curve characteristics  $\alpha$ ,  $E_k$ , and rETRmax using a Marquardt-Levenberg regression algorithm (Ralph and Gademann 2005). While these calculations would shed additional light on the ecological condition of the plants, quantum yields may be sufficient to detect the effects of petrochemicals on seagrass photosynthesis (Macinnis-Ng and Ralph 2003), so the RLC parameters were not calculated for this study.

### 3. Results and discussion

#### 3.1. Site description

Five of the surveyed sites experienced some degree of oiling and SCAT surveys confirmed our control sites, PO and PSP, did not receive detectable levels of oiling due to the *Cosco Busan* spill. Visual estimates of surface and subsurface oil were at five sites, due to submersion of eelgrass plants (identified as nd on Table 1); here, no oil was detected on the sediment or plant surface within the seagrass meadow. A surface sheen was observed at HC within the area of seagrass distribution, a water sample of the sheen was collected and submitted for analysis.

Outside the area of seagrass distribution, but within the cove or embayment of surveyed seagrass, heavy oiling was observed at two sites HC and KB. HC contained a band of oil extending along the rip rap approximately 100 m long by 15 cm wide. Here, the band of oil was approximately 2 m above the depth at which seagrass occurs. Oiled seagrass wrack was found at the high tide line but intact plants appear free of oil. At KB, the oil band, 20 cm wide, extended nearly 200 m along shore, covering 51-90% of the affected area. The upper edge of the KB seagrass meadow is approximately 1 meter below the observed oil band. Cleanup activities occurred prior to the time of eelgrass sampling, yet we observed a heavy oil film on the beach cobble and bedrock as well as three small tarballs on a boulder above the distribution of eelgrass (approximately 1 m). At all other sites, no evidence of tar or oil was observed by us within or adjacent to the eelgrass meadow surveyed.

### **3.2. Demographic characteristics**

Average shoot densities for the nine sites ranged from 12 - 64 vegetative shoots/m<sup>2</sup> (Fig. 2). Highest shoot densities were found at EV, HC and PO. These sites are distributed throughout the Bay (Fig. 1) and experienced a range of oiling (Table 1). Lowest shoot densities were recorded at CB; a very light oiled site. Shoot densities recorded at CB were not representative of historic average densities within this site. The low densities recorded were likely due to concomitant grazing of migratory brant geese and dominance of annual plants within the meadow, causing plants to senesce in the fall; this site is typically re-established by seedlings the following spring. Significant differences of shoot densities were found among sites (Table 2). However, the variability in shoot

density did not appear to correlate with oiling, as demonstrated by the lack of pattern of differences in shoot density by oiling category (Fig. 2; Table 2). For example, shoot densities from a heavy oiled site (KB) were not significantly different from those with light to very light oiling (Fig. 2).

If below ground tissue was affected by oiling, we hypothesized that a decline in rhizome growth would occur in response to oiling. Although, among-site difference in total rhizome length (most recent 4 internodes, approximating 60 days of growth) were detected (ANOVA  $p=0.0010$ ,  $F\text{-ratio}=4.8$ ,  $DF=4$ ) there was no apparent pattern of rhizome size and oiling (Fig. 3). In fact, the shortest rhizome fragments occurred at PO, a control site, whereas the longest rhizome lengths were found in the moderate and heavy oiling categories (CC, KB, KC). Rhizome lengths from heavy and moderate oiling sites were significantly greater than those from light oiling, and all other categories were not significantly different.

Rhizome extension displayed a variety of responses from increasing, decreasing, to no change over the most recent two month period (Fig. 4). Except for an increasing trend in rhizome growth at PO, rhizome growth generally declined during the study period at sites with little oiling (PSP, BF, PC, AI; Fig. 4), which was counter to a hypothesis of negative impacts from oiling. There was no significant within-site change in rhizome expansion over the last two months at three sites (EV, KC, CC; Fig. 4; Table 3). A significant reduction in growth was observed at four sites, AI, PC, BF, and PSP but appeared to be a linear trend beginning before the oil spill. Previous eelgrass research in this area (authors

unpubl. data) indicates a decline in rhizome extension in winter month which was consistent with what we observed at these sites. Eelgrass at PO and KB exhibited increased rhizome growth rates, signaling an increase in the rate of lateral expansion within the meadow.

### 3.3. Seagrass photosynthesis

Average effective quantum yields at the nine sites ranged from 0.745 to 0.692 (Fig. 5).  $\Delta F/F_m'$  varied significantly with oiling category (Kruskal-Wallis  $H = 29.79$ ,  $df = 4$ ,  $p < 0.001$ ). However, post-hoc comparisons indicated the categorical differences were not due to the degree of oiling. For example, lightly oiled sites had significantly higher yields than both moderately oiled sites and sites with no oil (both  $p < 0.001$ ). There was also no difference in  $\Delta F/F_m'$  between sites with no oil and those with moderate or heavy oil. Differences between the oiling categories further complicated by significant differences in  $\Delta F/F_m'$  between sites (Fig. 5; Kruskal-Wallis  $H = 39.50$ ,  $df = 8$ ,  $p < 0.001$ ). For example, lightly-oiled AI had a higher yield than moderately oiled CC ( $p = 0.001$ ) and KB ( $p = 0.015$ ), but not moderately oiled EV ( $p = 1.0$ ).

The relative electron transport rates at the highest light intensity were used as a proxy for maximum rETR, as photoinhibition appeared minimal at the highest intensity used (Fig. 6). Average maximum ranged from 53.7-82.4 (a.u.), and varied with oiling category ( $F_{4, 169} = 30.54$ ,  $p < 0.001$ ) in a way that showed greater coherence with oiling severity than the differences in yield. Sites with no oil had significantly higher rETR than all other sites (Tukey's unequal n HSD, all  $p < 0.001$ ). There was generally no difference in rETR

among oiled sites, regardless of the degree of oiling. The two significant differences that did occur were unrelated to oiling degree—highly oiled sites had lower rETR than lightly oiled sites ( $p = 0.002$ ) but not very lightly oiled sites ( $p = 0.656$ ). There were also site-specific effects on rETR ( $F_{4, 169} = 16.90$ ,  $p < 0.001$ ). This was largely due to high rETR at PSP and PO. These sites did not differ from each other; however PSP had significantly higher rETR than all the oiled sites (Tukey unequal n HSD;  $p$  ranged from 0.043 to  $< 0.001$ ), while PO had higher rETR than PC ( $p < 0.001$ ), KC ( $p < 0.001$ ), and AI ( $p = 0.002$ ). The only other significant comparison was that lightly oiled AI had a higher rETR than heavily-oiled KC ( $p = 0.005$ ).

### 3.4. Summary

Variability in eelgrass demographics and photosynthesis were detected among study sites. However, differences in plant demographics (shoot density, total rhizome length, and rhizome expansion) and photosynthetic characteristics (fluorescence yield, electron transport) did not correlate with oiling intensity. Across the study sites, there was no evidence of a short term impact to below ground or above ground eelgrass health using these metrics. However, if oil remains in the sediment, it is known to detrimentally affect microbe communities by shifting sediments toward severely anoxic conditions. Such environments can become highly stressful for eelgrass, thus further monitoring is recommended to evaluate any potential long term impacts to eelgrass bed health and growth.

## Acknowledgements

Tbd

## References

- Ballou, T.G., R.E. Dodge, S.C. Hess, A.H. Knap, T.D. Sleeter. 1987. Effects of a dispersed and undispersed crude oil on mangroves, seagrasses, and corals. American Petroleum Institute, publication number 4460. American Petroleum Institute, Washington, D.C.
- den Hartog, C., and R. Jacobs. 1980. Effects of the “Amoco Cadiz ” oil spill on an eelgrass community at Roscoff (France) with special reference to the mobile benthic fauna. *Helgolander Marine Research* 33:182.
- Dean, T.A., M.S. Stekoll, S.C. Jewett, R.O. Smith, J.E. Hose. 1998. Eelgrass (*Zostera marina* L.) in Prince William Sound, Alaska: Effects of the Exxon Valdez oil spill. *Marine Pollution Bulletin* 36: 201-210.
- Durako, M. J., W. J. Kenworthy, S. M. R. Fatemy, H. Valavi, and G. W. Thayer. 1993. Assessment of the toxicity of Kuwait crude oil on the photosynthesis and respiration of seagrasses of the northern Gulf. *Marine Pollution Bulletin* 27:223.
- Hatcher, A. I., and A. W. D. Larkum. 1982. The effects of short term exposure to Bass Strait crude oil and Corexit 8667 on benthic community metabolism in *Posidonia australis* Hook.f. dominated microcosms. *Aquatic Botany* 12:219.
- Hester, M.W., and I.A. Mendelsohn. 2000. Long-term recovery of a Louisiana brackish marsh plant community from oil-spill impact: vegetation response and mitigating effects of marsh surface elevation. *Marine Environmental Research* 49:233-254.

- Jackson, J. B. C., J. D. Cubit, B. D. Keller, V. Batista, K. Burns, H. M. Caffey, R. L. Caldwell, S. D. Garrity, C. D. Getter, C. Gonzalez, H. M. Guzman, K. W. Kaufmann, A. H. Knap, S. C. Levings, M. J. Marshall, R. Steger, R. C. Thompson, and E. Weil. 1989. Ecological Effects of a Major Oil Spill on Panamanian Coastal Marine Communities. *Science* 243:37.
- Macinnis-Ng, C. M. O., and P. J. Ralph. 2003. In situ impact of petrochemicals on the photosynthesis of the seagrass *Zostera capricorni*. *Marine Pollution Bulletin* 46:1395-1407.
- Marshall, M. J. 1990. Subtidal seagrass communities. Pages 261-286 in Long-term assessment of the oil spill at Bahia la Minas, Panama: interim report, Volume II. U.S. Department of the Interior, Minerals Management Service, publication number MMS 90-0031. Gulf of Mexico OCS Region, New Orleans.
- Peirano, A., V. Damasso, M. Montefalcone, C. Morri, and C. N. Bianchi. 2005. Effects of climate, invasive species and anthropogenic impacts on the growth of the seagrass *Posidonia oceanica* (L.) Delile in Liguria (NW Mediterranean Sea). *Marine Pollution Bulletin* 50:817-822.
- Ralph, P. J., and M. D. Burchett. 1998. Impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Marine Pollution Bulletin* 36:429-436.
- Ralph, P.J., R. Gademann. 2005. Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany* 82, 222-237.

- Sandulli, R., C.N. Bianchi, S. Cocito, C. Morri, A. Peirano, S. Sgorbini. 1998. An experience of 'baslisage' in monitoring the effects of the *Haven* oil spill on some Ligurian *Posidoina oceanic* meadows. *Oebalia* 24:3-15.
- Scarlett, A., T. S. Galloway, M. Canty, E. L. Smith, J. Nilsson, and S. J. Rowland. 2005. Comparative toxicity of two oil dispersants, superdispersant-25 and corexit 9527, to a range of coastal species. *Environmental Toxicology And Chemistry* 24:1219-1227.
- Setchell, W.A. 1929. Morphological and phonological notes on *Zostera marina* L. *University of California Publications in Botany* 14: 389-452.
- Taylor, E., and D. Reimer. 2008. Oil persistence on beaches in Prince William Sound – A review of SCAT surveys conducted from 1989 to 2002. *Marine Pollution Bulletin* 56: 458-474.
- Thorhaug, A., and J. Marcus. 1987. Oil spill clean-up: The effect of three dispersants on three subtropical/tropical seagrasses. *Marine Pollution Bulletin* 18:124.
- Thorhaug, A., J. Marcus, and F. Booker. 1986. Oil and dispersed oil on subtropical and tropical seagrasses in laboratory studies. *Marine Pollution Bulletin* 17:357.
- Thorhaug, A., B. Miller, B. Jupp, and F. Booker. 1985. Effects of a variety of impacts on seagrass restoration in Jamaica. *Marine Pollution Bulletin* 16:355.
- Wilson, K. G., and P. J. Ralph. 2008. A comparison of the effects of Tapis crude oil and dispersed crude oil on subtidal *Zostera capricorni*. Pages 85 in 2008 International Oil Spill Conference. American Petroleum Institute, Savannah, GA (USA).
- Zieman, J. C., R. J. Orth, R. C. Phillips, G. W. Thayer, and A. Thorhaug. 1984. The effects of oil on seagrass ecosystems. Pages 37-64 in J. Cairns and A. L. Buikema,

editors. Restoration of habitats impacted by oil spills. Butterworth Publishers, Boston, Mass.

AE

## Figures and Tables

Fig. 1. Eelgrass sites surveyed following the *M/V Cosco Busan* oil spill and location of the spill site (black circle). Site abbreviations: PSP, Point San Pablo; PO, Point Orient; CC, Cozy Cove; KB, Keller Beach; EV, Emeryville; CB, Crown Beach; BF, Bay Farm Island; PC, Paradise Cove; KC, Keil Cove; AI, Angel Island; HC, Horseshoe Cove.

Fig. 2. Mean ( $\pm 1$  S.E.) vegetative shoot density ( $0.25\text{m}^2$ ) at each site by oiling category ( $n=10$  per site). Grayscale coloring reflects oiling status: hollow bars = no oil, solid black = heavily oiled.

Fig. 3. Mean ( $\pm 1$  S.E.) total rhizome length (4 complete nodes) at each site by oiling category.

Fig. 4. Mean rhizome fractional growth of four most recent complete nodes (node1 = < 10 days old, node 4 = aprx. 60 days old) at each site by oiling categories ( $n=10$  per site). Vertical reference line denotes approximate time of spill. No rhizome samples were collected from CB and HC due to low plant density.

Fig. 5. Mean ( $\pm 1$  S.E.) quantum yield ( $\Delta F/F_m'$ ) of *Zostera marina* leaves. Grayscale coloring reflects oiling status: hollow bars = no oil, solid black = heavily oiled. Lower-case letters indicate differences among categories detected by post-hoc statistical comparisons.

Fig. 6. Rapid light curves for *Zostera marina*. A = unoiled sites, B = lightly oiled, C = moderately oiled, D = heavily oiled. Error bars are  $\pm 1$  S.E of relative electron transport rates at each fixed light level.

Fig. 1.

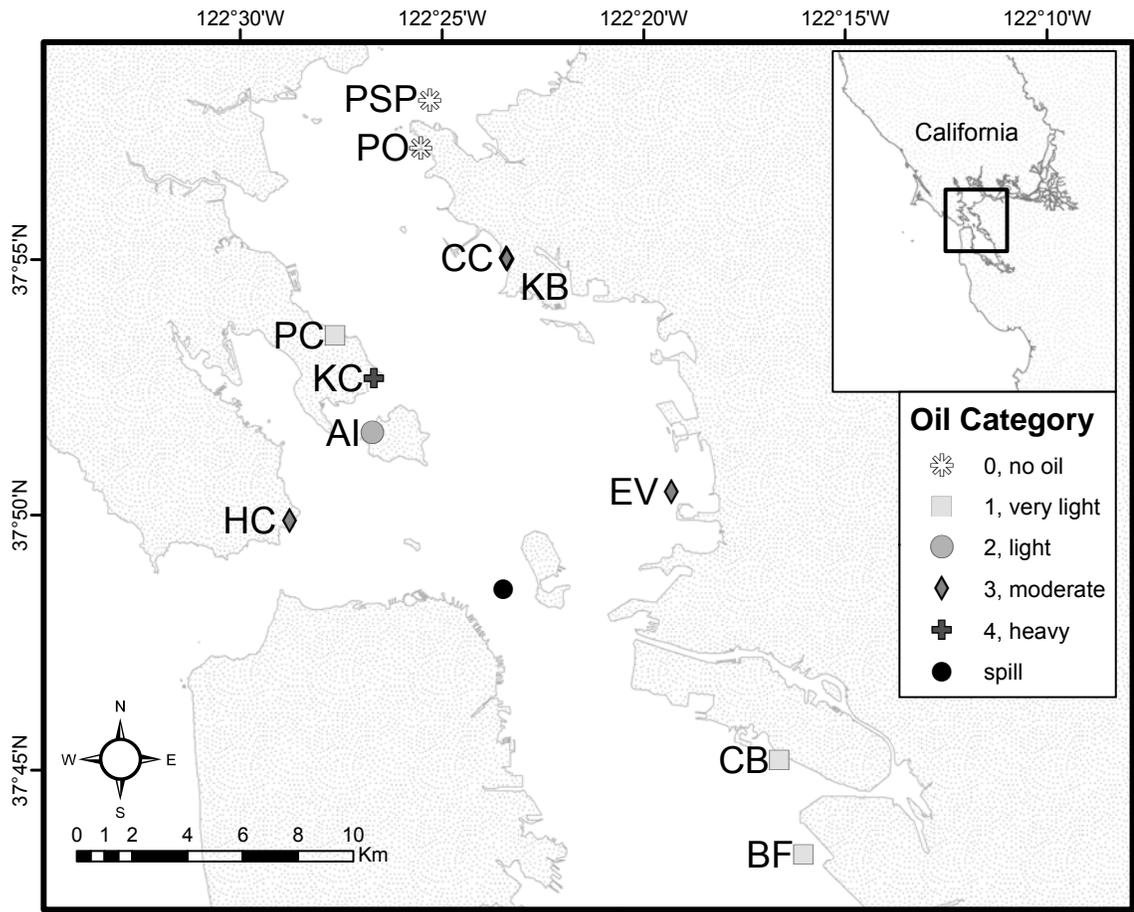


Fig. 2.

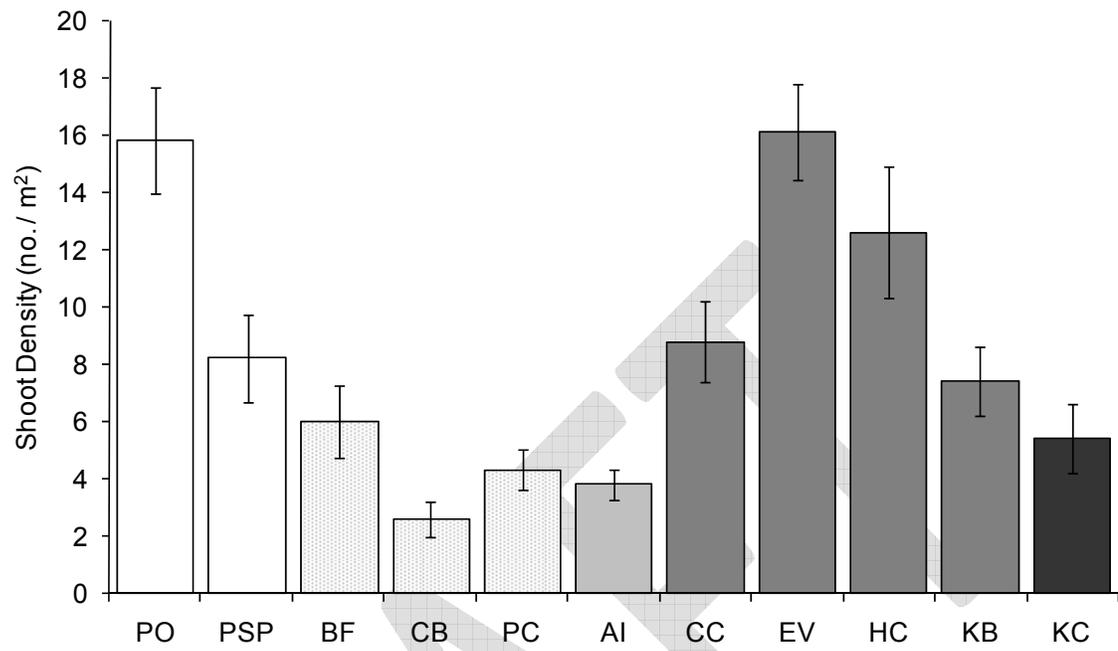


Fig. 3.

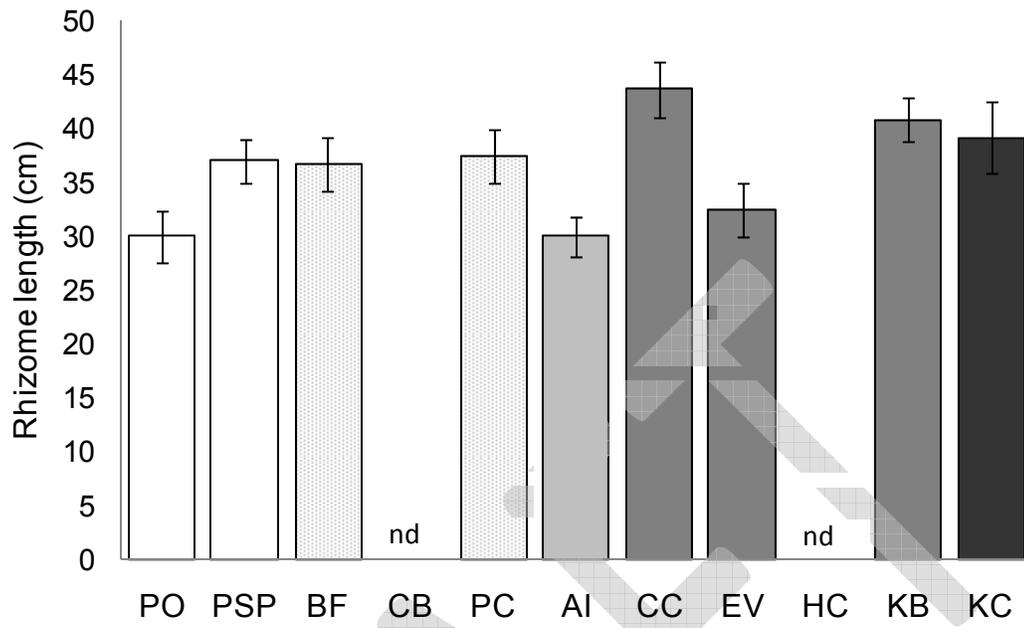


Fig. 4.

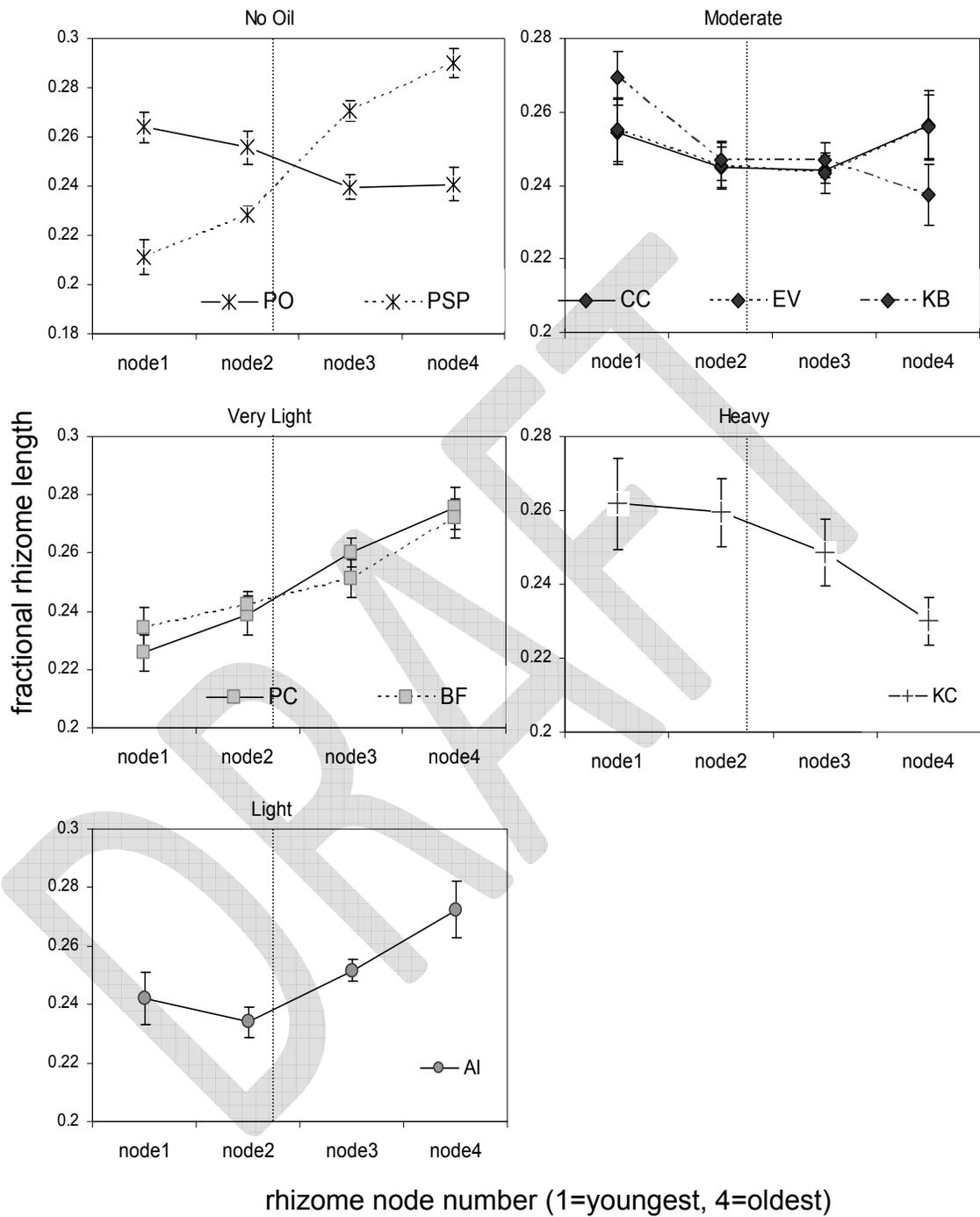


Fig. 5.

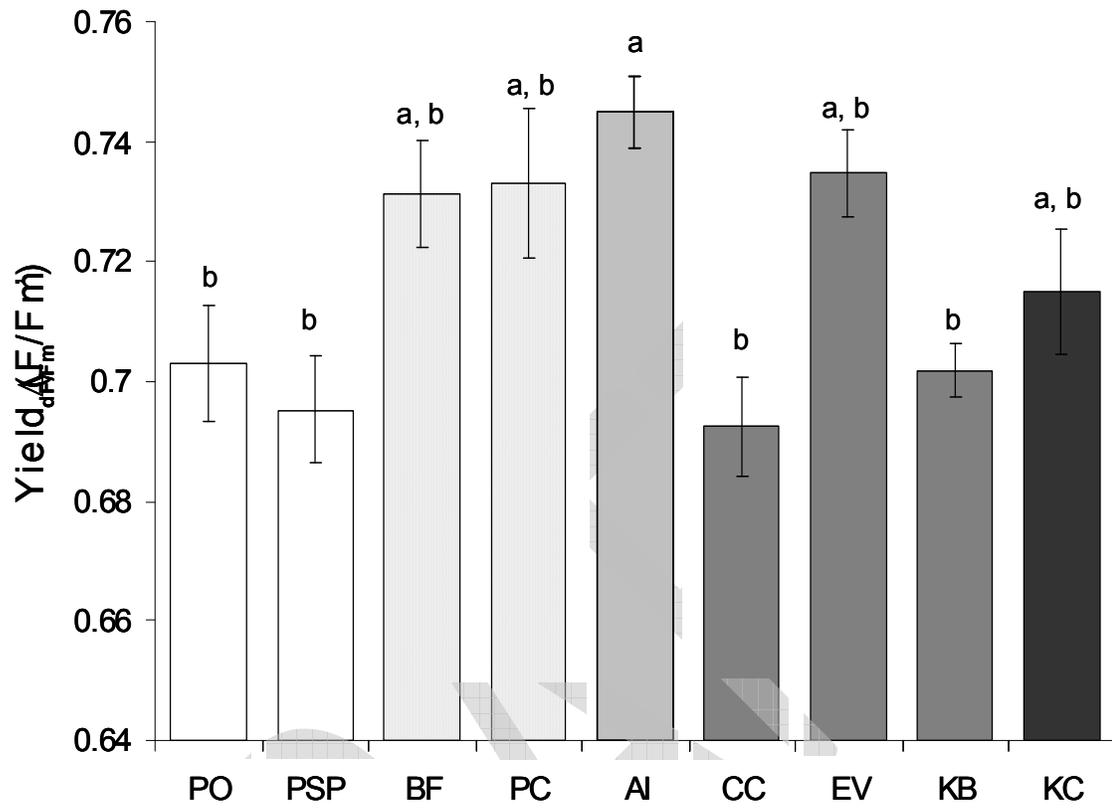


Fig. 6.

Relative ETR

Relative ETR



Table 1.

Sample sites from post-spill eelgrass survey, conducted November 2007

Site	Oiling status	SG Surface Oil	SG Subsurface Oil	Oil of surrounding area
PO	No Oil	none	none	none
PSP	No Oil	none	none	none
BF	Very Light	none	none	none
CB	Very Light	none	none	none
PC	Very Light	nd	nd	none
AI	Light	nd	nd	tar balls on boulder
CC	Moderate	none	none	none
EV	Moderate	none	none	none
HC	Moderate	sheen	none	oil mat on shoreline
KB	Moderate	none	none	post-cleanup
KC	Heavy	nd	nd	oil mat on shoreline

*Notes:* Surface oil, oiling observed on sediment or plant surface within seagrass meadow; Subsurface oil, oil released when sediment surface disturbed within seagrass meadow; nd, no data available.

Table 2. Shoot density comparison by site using post-hoc sequential bonferroni comparison (-, non-significant; +, significant difference where adjusted  $\alpha = 0.05$ ).

		no oil		very light			light	moderate				heavy
		PO	PSP	BF	CB	PC	AI	CC	EV	HC	KB	KC
no oil	PO		+	+	+	+	+	+	-		+	+
	PSP	+		-	+	-	-	-	+	-	-	-
very light	BF	+	-		-	-	-	-	+	+	-	-
	CB	+	+	-		-	-	+	+	+	+	-
	PC	+	-	-	-		-	-	+	+	-	-
light	AI	+	-	-	-	-		+	+	+	-	-
moderate	CC	+	-	-	+	-	+		+	-	-	-
	EV	-	+	+	+	+	+	+		-	+	+
	HC	-	-	+	+	+	+	-	-		+	+
	KB	+	-	-	+	-	-	-	+	+		-
heavy	KC	+	-	-	-	-	-	-	+	+	-	

Table 3. Analysis of approximately 60 days of rhizome growth (4 complete internodes)

Oiling status	Site	significance.	P	Internode expansion: (old to young)
no oil	PO	+	0.0164	Increasing
	PSP	+	<0.001	Decreasing
very light	BF	+	0.0003	Decreasing
	PC	+	0.0069	Decreasing
light	AI	+	0.0025	Decreasing
moderate	CC	-	0.4	Uniform
	EV	-	0.5098	Uniform
	KB	+	0.0068	Increasing
heavy	KC	-	0.0779	Uniform