

Changes in the Condition of Surfgrass (*Phyllospadix torreyi*) and Macroalgae Following the Refugio Oil Spill, Santa Barbara County

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Summary

The May 19, 2015 crude oil spill into the ocean from a pipeline rupture on the coastal terrace above the shoreline near Refugio State Beach, Santa Barbara County, California resulted in shores along the coast being oiled where surfgrass (*Phyllospadix torreyi*), a marine angiosperm aquatic seagrass grows abundantly in the low-intertidal and shallow-subtidal zone.

Two months after the spill, leaves of surfgrass in areas exposed to the spill shifted in color from being normal emerald green/light-green to yellow, brown, or black, and leaves became brittle and broke apart easily when pulled. Macroalgae occurring with the surfgrass also became discolored. The discoloration was from tissue damage involving the loss of functioning chlorophyll pigment essential in photosynthesis for growth and production. Intertidal and subtidal surveys ensued to further assess the magnitude and extent of the discoloration observed shortly following the spill.

Surfgrass habitat in the low-intertidal zone is only accessible for brief periods of time, even during the best low tides. Therefore, photographing quadrats of one quarter square meter in size (0.25 m²) and analyzing the photos later for discoloration was chosen as the best method to collect data quickly on surfgrass condition for the study. Although eight sites were considered, five sites were chosen for repeated sampling. The five sites differed in oiling exposure, and were analyzed for the magnitude of the discoloration associated with the spill.

At each site, quadrats were placed and photographed along transects deployed between fixed GPS waypoints. In the lab, the color and coverage of surfgrass and macroalgae in each photographed quadrat was quantified using the point-contact sampling method where each sampling point (of a grid of 100 points) contacting surfgrass and macroalgae was recorded (scored) for presence and color condition.



Analysis of the August 2015 survey photographs revealed Corral Canyon had the highest proportion of discolored surfgrass and discolored macroalgae among the five sites sampled and analyzed; Corral Canyon was also among the most heavily oiled sites, based on shoreline cleanup assessment technique data (SCAT data). Approximately 82.0% of the surfgrass sampled at Corral Canyon was discolored. Macroalgae were less abundant but more discolored in proportion to all of the macroalgal species sampled; approximately 99.2% of the macroalgae sampled at Corral Canyon was discolored. As a result, the total coverage of discolored surfgrass and macroalgae combined was greatest at Corral Canyon (approximately 84.5% cover).

The amount of discoloration in surfgrass and in the macroalgae followed a gradient along the shore of oiling exposure corresponding to the SCAT data. Most discoloration was at Corral Canyon. There was less discoloration in surfgrass and in the macroalgae at three of the four other sites (Arroyo Hondo, Refugio West, Coal Oil Point), and essentially no discolored surfgrass or discolored macroalgae was observed at Mussel Shoals, an unoiled area but within the overall spill range based on the SCAT data.

The offshore distance of the discoloration in surfgrass and macroalgae was determined by snorkel, paddleboard, and SCUBA surveys. The maximum distance of the discoloration from shore was approximately 100 m (328 ft), and the maximum depth was generally between -2.1 m and -3.1 m (-7 and -10 ft) mean lower low water (MLLW). Approximately one year after the spill (June 2016) the continued monitoring found the discoloration in surfgrass and macroalgae was appreciably less than observed at the onset in summer 2015.

Introduction

On May 19, 2015, an underground pipeline conveying oil along the cliff bluff in Santa Barbara County, California near Refugio State Beach ruptured. An estimated 2,934 barrels (123,228 gallons) of heavy crude oil was released from the broken pipe (U.S. Dept. of Transportation 2016). A large volume subsequently reached the ocean. Surfgrass (*Phyllospadix torreyi*) was among the many marine species exposed to the spilled oil.

Phyllospadix torreyi is a habitat-forming marine angiosperm that grows on wave exposed sand-swept rocky habitats in the low-intertidal/shallow-subtidal zone between the 0.0 and -3.1 m (0 ft and -10 ft) MLLW tide levels. It is abundant along the Santa Barbara County coastline growing as dense beds/meadows. Rhizomes hold the plants to rocky substrates, and the narrow leaves (blades) are uniformly emerald green/light green and can be up to 1.5 m (4.9 ft) long.

Macroalgal species occur within and next to surfgrass beds. These commonly include the feather boa kelp (*Egregia menziesii*), bladder chain kelp (*Sargassum muticum*), and the red macroalgae *Chondracanthus canaliculatus* and *Corallina vancouveriensis*, including many others. In addition, the red algae *Smithora naiadum* and *Melobesia mediocris* are macroalgal species that are exclusively epiphytic on surfgrass (Abbott and Hollenberg 1976).

Surfgrass beds also provide important nursery habitats, refuge, and foraging areas for many species of fishes (DeMartini 1981, Heck et al. 2003, Galst and Anderson 2008). These include topsmelt (*Antherinops affinis*), señoritas (*Oxyjulis californica*), blacksmith (*Girella nigricans*),



and black surfperch (*Embiotoca jacksoni*), among many others. Additionally, surfgrass beds provide critical nursery habitat for juvenile California spiny lobsters (Engle 1979). Many other invertebrates are also abundant in surfgrass beds (Holbrook et al. 2000, Heck et al. 2003).

The first intertidal surveys in response to the Refugio oil spill were in May and June 2015, and were completed by marine biologists of the University of California, Santa Cruz and TENERA Environmental Services of San Luis Obispo, California. Surfgrass in the low-intertidal zone was observed with spots of oil on the leaves (**Figure 1**). However, no unusual discoloration in surfgrass was apparent during the initial surveys.

In early July 2015, nearly two months after the spill, visits to El Capitan and Corral Canyon found that intertidal surfgrass and species of macroalgae were unusually discolored (**Figure 1**). Surveys then followed to document the change suspected to be an impact from the spill and to document subsequent changes. The discoloration in the surfgrass and macroalgae was of similar nature to that found in prior oil spills; the January 1969 crude oil spill in the Santa Barbara Channel found surfgrass along the mainland shore that had been oiled turned brown and gradually disintegrated, and macroalgae in the affected surfgrass beds also became discolored (Foster et al. 1969, Mitchell et al. 1970, Nicholson and Cimberg 1971). Discoloration in surfgrass and macroalgae and subsequent abundance declines have occurred from other oil spills (Washington State Department of Ecology 1975, O'Brien and Dixon 1976, Clark et al. 1978, Floch and Diouris 1980, Antrim et al. 1995). The condition of surfgrass and macroalgae observed following the 2015 Refugio oil spill was therefore studied to assess the apparent impact from the spill, and the findings from the quantitative intertidal surveys and qualitative observations are described here.

Methods

Pull Tests

In July 2015 when surfgrass was first observed to be in a discolored condition, leaves were felt and held in hand. The leaves were found to be brittle and broke apart easily. This led to “pull tests” to evaluate tensile strengths. The pull tests consisted of reaching a hand into the surfgrass bed, closing the hand on a handful leaves, and gently pulling on the leaves. The leaf sections that broke off were measured to the nearest centimeter.

Whole Leaf Measurements

Whole leaves of surfgrass were collected and measured for length to the nearest centimeter. The whole leaves were collected by reaching indiscriminately at random locations into the surfgrass bed and feeling where a leaf was attached to the rhizome base and then breaking the leaf off at the rhizome base. The color of the breakage point of each leaf was also noted when measuring the leaf's length (green, yellow, or brown).





Figure 1. Examples of oil contact and surfgrass and macroalgae discoloration: a) oil on surfgrass; b) normal emerald green surfgrass; c) discolored surfgrass; and d) discolored feather boa kelp that is normally brown, not orange-red. Photos from east of Refugio State Beach on August 28, 2015.

Intertidal Quadrat Photo-Surveys and Laboratory Photo-Scoring

Data on the condition and abundance of intertidal surfgrass were collected by photographing quadrats (0.25 m²; 2.7 ft²) in the low-intertidal zone, the upper vertical range extent of surfgrass. This sampling was completed at eight sampling sites (**Figure 2**) in five surveys over the study period (**Table 1**). Photographing quadrats enabled data to be collected quickly as possible in the low-intertidal zone where there are only short time windows to sample during low tides.



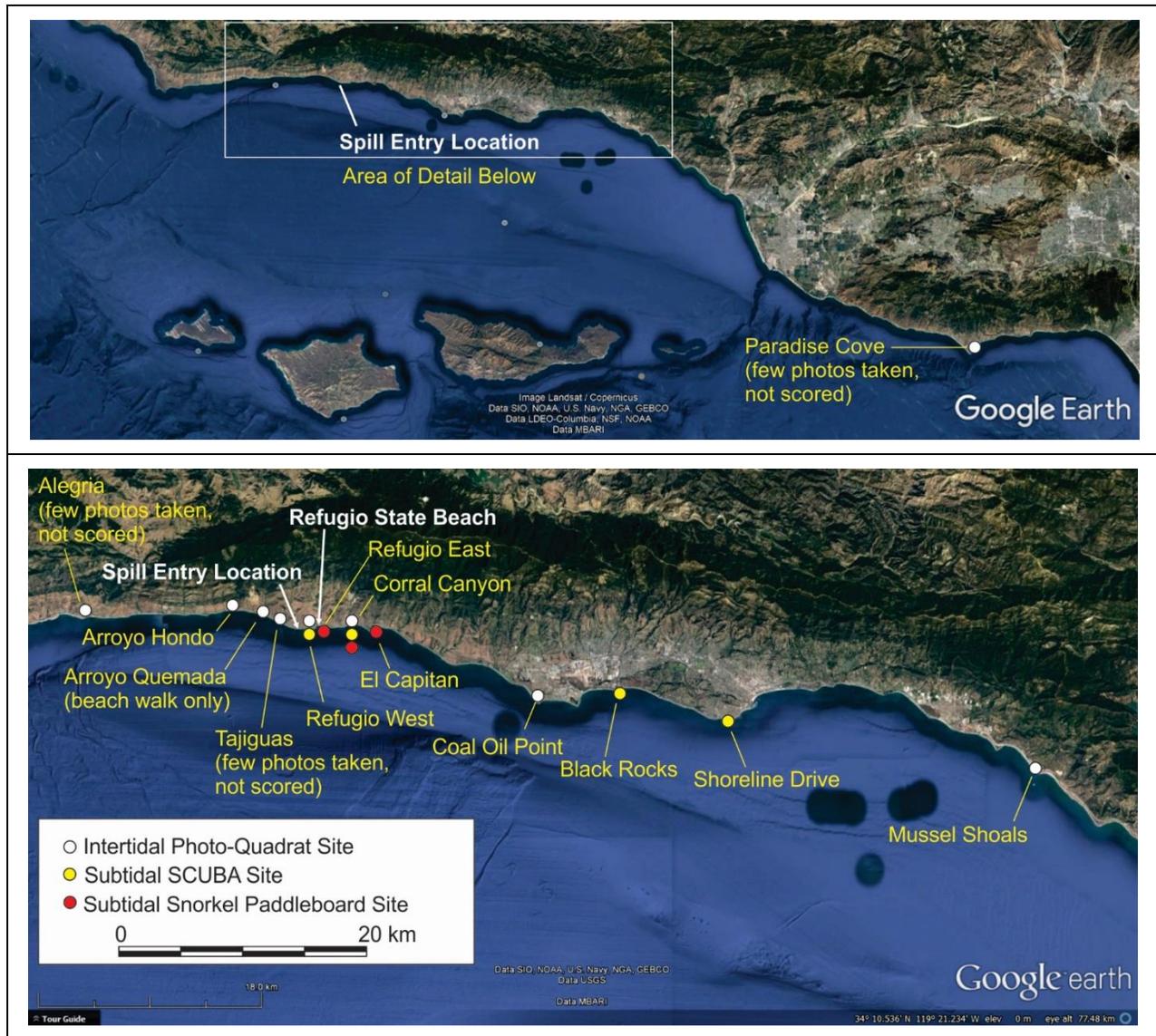


Figure 2. Sites sampled for surfgrass and macroalgae condition.

The sampling sites spanned much of the geographical range of the spill, and included sites that had heavy to no observed oil, based on SCAT data (**Figure 3**). However, due to limited tides sufficiently low to sample, the timing of the low tides, and swell constraints, including labor resources, not all of the intertidal sites could be sampled together in the same survey, and not all of the sites had equivalent large sample sizes. Sites that were sampled and analyzed are shown in **Table 1**, those being Arroyo Hondo, Refugio West, Corral Canyon, Coal Oil Point, and Mussel Shoals (**Figure 2**).

At each sampling site, multiple 10 m (33 ft) transects were deployed during extreme low tides in the accessible intertidal surfgrass zone between about the 0.0 m and -0.3 m (-1.0 ft) MLLW tide levels. Quadrats (0.25 m²) were then spaced and photographed every 1–2 m (3.3–6.6 ft) along each transect. Most transects were oriented parallel-to-shore. Wave run-up and surf prevented



sampling in lower intertidal zones. At three of the sites (Arroyo Hondo, Coal Oil Point, Mussel Shoals), surfgrass was also sampled along pre-existing transects established and sampled by MARINE (Multi-Agency Rocky Intertidal Network) (Engle 2008). Generally, the entire sampling area at each site was approximately 10 m wide (33 ft) perpendicular to shore by 50-100 m (164-328 ft) along the shore.

Table 1. Intertidal survey sites with numbers of transects and quadrat photographs taken at each site. Site locations are shown in Figure 2. The numbers of quadrats that were determined to have readable (scorable) data and the numbers of quadrats scored at each location are also shown.

		Site Scored							
		Alegria	Arroyo Hondo	Tajiguas	Refugio West	Corral Canyon	Coal Oil Point	Mussel Shoals	Paradise Cove
		10 m Transects Quads Photographed Scorable Quads Quad Scored	10 m Transects Quads Photographed Scorable Quads Quads Scored						
Initial Survey									
Jul. 31, 2015					7 72 59 35		9 89 89 45		
Aug. 1, 2015									
Aug. 2, 2015						11 111 100 55			
Aug. 3, 2015								8 79 70 42	
Aug. 15, 2015			5 29 29 29						
Intermediate Surveys									
Aug. 17, 2015				0 4 4 0					
Aug. 29, 2015					5 52 47 0				
Aug. 30, 2015						5 37 11 0			
Aug. 31, 2015								5 36 27 0	
Sep. 1, 2015									0 3 3 0
Oct. 25, 2015							9 97 88 0		
Oct. 26, 2015	3 34 33 0			0 3 3 0					
Oct. 27, 2015					5 55 47 0	1* 10 4 0			
Oct. 28, 2015						9* 91 36 0			
Oct. 29, 2015								8 80 75 0	
Oct. 30, 2015		4 19 18 0							
Nov. 23, 2015		5 54 54 0							
Nov. 24, 2015						8* 80 37 0			
Jan. 21, 2016						6 60 52 27			
Jan. 24, 2016						9 100 85 29			
Jan. 25, 2016								5 55 39 35	
Final Survey									
May 23, 2016	0 16 15 0								
May 24, 2016				0 4 4 0					
Jun. 5, 2016					7 78 76 34				
Jun. 6, 2016						17 175 173 50			
Jun. 7, 2016							7 93 89 45		
Jun. 8, 2016								8 81 81 40	
Jun. 9, 2016		5 38 38 28							

* very marginal conditions



The transect origin and terminus latitude-longitude coordinates were recorded using a consumer-grade Garmin GPSmap 76C. The coordinates were used to re-deploy the transects in the same locations in subsequent surveys. Mostly, all transects at a site were able to be re-deployed for re-photographing quadrats when revisited each survey.

The photographs from the initial survey (August 2015) and final survey (June 2016) were analyzed because they had the largest number of photographs across the most sites (same five sites) within a few days or weeks of each other. Four of the five sites were oiled (Arroyo Hondo, Refugio West, Corral Canyon, and Coal Oil Point), of which Corral Canyon was among the most heavily oiled based on SCAT data (**Figure 3**). The fifth site, Mussel Shoals that was located furthest from the spill provided reference data from a site that was least exposed or not exposed to oil (**Figure 3**). Even though Corral Canyon and Mussel Shoals were the only sites sampled in the January 2016 survey (**Table 1**), the quadrat photographs from that survey were also included in the analysis, because surfgrass discoloration was most extensive at Corral Canyon (most oiled) and could be compared to Mussel Shoals (reference) over three versus two surveys.

In the laboratory, the quadrat photographs were first selected for clarity; those that were not in focus were eliminated. After this quality check, quadrat photographs were randomly selected and scored for each site and survey. Generally, 30-50 photo-quadrats were scored for each survey at each site (**Table 1**), which was approximately one-half of the scoreable quadrats at each site per survey.

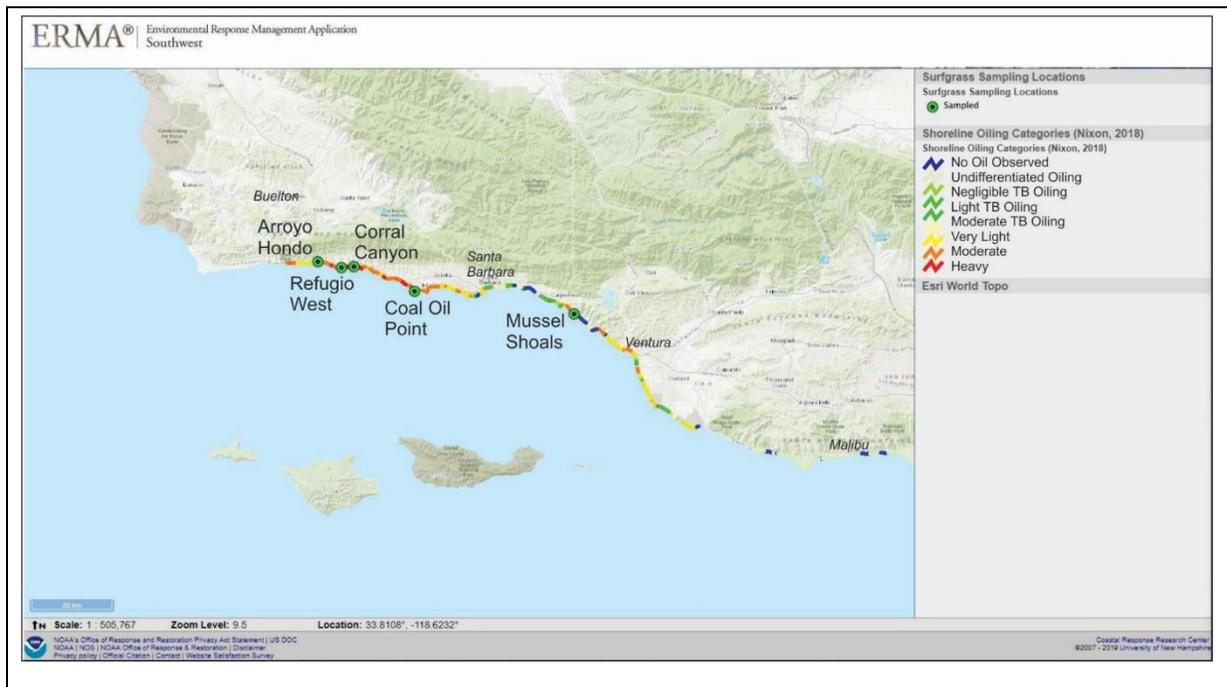


Figure 3. Oil exposure at intertidal surfgrass sampling sites based on SCAT maximum oiling data; Arroyo Hondo and Coal Oil Point moderately oiled, Mussel Shoals not oiled, and Refugio West and Corral Canyon heavily oiled. However, Corral Canyon was most heavily oiled in the low-intertidal, due to tide level and currents at the time of the spill. See *Discussion* section for explanation.



The MARINE point-contact sampling method was used in the laboratory to record (score) the photographs for species occurrences and color condition (Engle 2008, UCSC 2019). A grid of 100 evenly spaced points was superimposed over each photograph (using Adobe Photoshop™), and the species directly beneath each sampling point was scored for color. The scoring provided quantitative data on intertidal surfgrass and macroalgae color, condition, abundance, and proportions based on the contacts of the top-most layer in the photographs.

A color chart was used to score colors consistently across the quadrat photographs (Figure 4). The Photoshop™ eyedropper tool was used to capture the range of surfgrass colors in photographs taken in the first survey in August 2015 at Mussel Shoals (reference site) and at Corral Canyon (a heavily oiled site). The range of colors was used to create a color library chart with each color being assigned a label/name. During the scoring, the color chart was viewed alongside the quadrat photograph on the computer monitor. The same computer and monitor were used in scoring all photographs. Green and light-green surfgrass leaves were scored and analyzed as normal color. All other

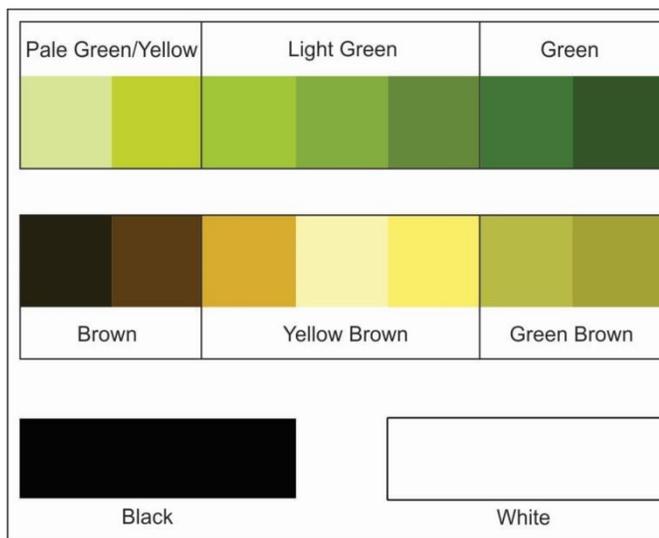


Figure 4. Color chart to score surfgrass leaf colors in the quadrat photographs.

surfgrass colors were considered unusual (discolored/injured tissues). The macroalgae were scored with fewer color categories than shown in Figure 4: “normal color”, “bleached”, or “discolored”. The color and condition categories are listed further in Table 2.

The abundance (percent cover) of each scored category in a quadrat was determined by the number of contacts out of the 100 points sampled. The total percent cover of surfgrass, for example, was the total number of contacts to surfgrass (regardless of color). The proportion of discolored surfgrass to all surfgrass in a quadrat was the number of point contacts scored as discolored surfgrass divided by the total points contacting surfgrass and multiplied by 100. Sample points scored as “unknown color” (from shadowing effects), and “epiphytes” (for surfgrass) were not included in the total points scored for discoloration. Algal species were scored for abundance and discoloration in the same manner.

Line-Point Contact Intertidal Field Measurements

The 10 m transects for placing and photographing the 0.25 m² quadrats were sampled in the field at every 10 cm (4 in.) interval (point) for the presence/absence of surfgrass and other species (line-point contact field sampling method). Five to 15 transects were sampled per site yielding contact data for 500 to 1,500 sample points per site for each survey.



Table 2. Taxa and condition categories scored in the quadrat photos.

Taxon	Condition	Taxon	Condition
<i>Phyllospadix torreyi</i>	green	<i>Egregia menziesii</i>	normal
	light green		bleached, discolored
	yellow		unknown color
	black	<i>Gastroclonium subarticulatum</i>	normal
	brown		bleached, discolored
	yellow-rown		unknown color
	green-brown	<i>Macrocystis pyrifera</i>	normal
	unknown color		
	<i>Smithora</i> on leaf	<i>Mazzaella leptorhynchos</i>	normal
	<i>Melobesia</i> /white crust on leaf		bleached, discolored
	unid. filamentous macroalgae on leaf	<i>Prionitis</i> spp.	unknown color
	unid. gelatanous eggs? on leaf		
	snagged drift macroalgae on leaf	<i>Sargassum muticum</i>	normal
	pink <i>Melobesia</i> on leaf		bleached, discolored
unid. foliose macroalgae on leaf	<i>Stephanocystis osmundacea</i> /S. <i>dioica</i>	normal	
<i>Ulva</i> spp. on leaf		bleached, discolored	
limpet on leaf	<i>Ulva</i> spp.	unknown color	
hydroid on leaf		normal	
rhizome normal	<i>Zonaria farlowii</i>	bleached, discolored	
rhizome discolored		unknown color	
rhizome with broken leaves			
coralline crust	normal	branched red macroalgae (unid.)	normal
macroalgae	bleached, discolored		bleached, discolored
non-coralline crust	normal		unknown color
macroalgae	unknown color	filamentous red macroalgae (unid.)	normal
articulated coralline	normal		bleached, discolored
macroalgae	bleached, discolored		unknown color
	unknown color	brown macroalgae (unid.)	normal
<i>Chondranthus canaliculatus</i>	normal		bleached, discolored
	bleached, discolored		unknown color
<i>Chondracanthus spinosus</i>	normal	foliose/fleshy red macroalgae (unid.)	unknown color
	bleached, discolored		normal
	unknown color		bleached, discolored
<i>Colpomenia</i> spp.	normal	anaerobic stain/bare substrate anemone bare bed rock bare boulder bare cobble barnacle encrusting invertebrate (unid.) mussel <i>Phragmatopoma californica</i> sand/gravel/pebble unid. sample point	
	unknown color		
<i>Corallina vancouveriensis</i>	normal		
	bleached, discolored		
	unknown color		
<i>Desmarestia ligulata</i>	normal		
	unknown color		

At each sample point, the occurrence of surfgrass (leaves) was recorded (scored) regardless of whether surfgrass was the top-most layer or was underneath layers of macroalgae. The latter situation was determined by brushing the macroalgae aside to confirm the presence/absence of surfgrass leaves underneath the sample point. In contrast, other species (taxa) underneath the overstory surfgrass and macroalgae layers, such as sea anemones, barnacles, and surfgrass rhizomes were not scored as a (secondary) layer in this sampling method, even if present. Also,



the color and condition of the surfgrass and macroalgae were not scored for each point in this field sampling method. However, the appearance and condition of surfgrass and macroalgae was summarized for each transect and described in field notes and supplemented with overview panoramic photos to help document conditions.

Leaf Density and Biomass

The priority intertidal sampling during the limited low tide sampling windows was quadrat photography for assessing surfgrass and macroalgae condition, the line-point contact field sampling method for percent cover abundance and collecting surfgrass leaves for measuring leaf lengths. Leaf density and biomass data would have provided additional information to assess the health and condition of surfgrass, but these data were not collected.

Snorkel and Paddleboard Surveys

The offshore extent and depths of discolored surfgrass were initially determined at several sites in snorkel and paddleboard surveys at Refugio East, El Capitan, Corral Canyon (**Figure 2**). Refugio East was surveyed on August 28, 2015, followed by El Capitan on September 3, 2015 and Corral Canyon on September 4, 2015. The offshore (subtidal) outer boundaries of surfgrass beds were determined by deploying meter tapes from shore to offshore and using GPS to record locations. Depths were determined by lowering a weighted meter tape from the surface but was estimated in some cases. Depths were corrected to MLLW based on National Oceanic and Atmospheric Administration (NOAA) predicted tide heights and times for Gaviota Pier, the closest tide gauge to the sampling sites. Arroyo Quemada (**Figure 2**) was another site planned for noting the depths and distances of discolored surfgrass from shore, which was visited on September 3, 2015. However, waves were too large to enter the water so only beach walk observations were made.

SCUBA Surveys

Information from the snorkel and paddleboard surveys was used to plan and conduct SCUBA surveys to further assess the subtidal extents of the discolored/injured surfgrass. The SCUBA surveys were conducted from a dive boat on October 21, 2015. Locations were Refugio West, Corral Canyon, Black Rocks, and Shoreline Drive, sites across a geographic range of the spill (**Figure 2**).

The SCUBA surveys could not incorporate the same sampling methods used in the intertidal surveys, mainly because the work was underwater in the surf zone, a difficult place to sample. The observations were qualitative, and were made by a single two-person dive team at each site.

One transect was surveyed at each site. The anchor weight of the transect origin (marker buoy) was lowered to the seabed offshore of the surfgrass zone. Due to very clear water, the offshore boundary of surfgrass was visible from the dive boat in all areas surveyed. The origin marker coordinates were recorded using GPS. The divers then attached the end of a meter tape to the origin marker weight and spooled the tape out underwater on a compass heading directly towards



shore to define the transect. The divers recorded distance intervals and color condition of surfgrass and macroalgae along each transect. All transects terminated in the low-intertidal zone of the shore that was confirmed by seeing macroalgae species that are strictly intertidal in occurrence (e.g., *Corallina vancouveriensis*). Depths were corrected for tidal height based on NOAA predicted tidal heights and times for Santa Barbara Harbor.

Results

Initial Observations of Discoloration in Surfgrass

On July 8, 2015, 53 days after the spill, El Capitan State Beach (**Figure 2**) was visited. Tar balls were observed on the rocky shore, and while dense surfgrass was present along the shore, many of the leaves were unusual in color and condition; leaves were yellowish-tan in color, brittle, and broke off easily when pulled. The discoloration observed from shore was present in plants at depths of approximately -1.2 m to -1.8 m (-4 ft to -6 ft) MLLW.

On July 10, 2015, the surfgrass bed at Corral Canyon (**Figure 2**) was observed by walking and wading along the shoreline. Oil was present along the shoreline underneath cobbles. Like El Capitan State Beach, surfgrass was noticeably discolored; the surfgrass was more tan than green, and leaves broke off easily when pulled. The discolored surfgrass was observed out to a depth of approximately -0.5 m (-1.5 ft) MLLW. Observations could not be made further offshore in deeper water due to waves and wave run-up on the shore preventing access.

On July 17, 2015, observations were made on a paddleboard along the shoreline west of Refugio State Beach up to the spill point (**Figure 2**). The surfgrass was not as uniformly discolored as the surfgrass at Corral Canyon. However, the surfgrass was unusually slimy in texture and tan in color in some areas.

Leaf Measurements

Leaf length measurements from the pull tests and measurements of whole leaves at Corral Canyon in the August 2015 survey found the leaf sections from the pull test (all leaf break point color categories combined) were nearly the same lengths of whole leaves (**Figure 5**); the leaves in the pull tests broke off near the rhizome bases. Similar results occurred in the January 2016 survey. Overall, whole leaves were shortest at Corral Canyon in the initial August 2015 survey compared to Mussel Shoals.

In contrast, whole leaves were longer at Mussel Shoals in the August 2015 survey, and pull tests found less leaf breakage (**Figure 5**). The summer (June 2016) survey found whole leaves at both Corral Canyon and Mussel Shoals had increased in length from the prior winter survey (January 2016), and there was no leaf breakage in pull tests.



Abundance: Line-Point Contact Field Sampling

The field transect line-point contact sampling method provided more complete data on surfgrass abundance and changes over time in cover than data from the quadrat photographs. This was because any surfgrass present at a field sampling point was scored regardless of whether the leaves were over or underneath an overstory layer of macroalgae. In contrast, the quadrat-photo method did not score surfgrass if underneath an overstory macroalgae layer.

Surfgrass was abundant at all of the sites in the initial survey completed in August 2015 and was most abundant at Corral Canyon (88.5% cover, **Figure 6**). Surfgrass then declined in cover from August 2015 to January 2016 and then increased by June 2016. However, the final abundance in June 2016 was lower than initially observed in 2015.

Surfgrass was also less abundant in the final versus initial survey at Refugio West and Arroyo Hondo. In contrast, surfgrass was slightly more abundant in the final versus initial survey at Coal Oil Point and Mussel Shoals. Coal Oil Point was the farthest sampling site exposed to the spill, and Mussel Shoals, the reference site, was furthest from the spill.

Rhizomes bearing only leaf stubs (bare rhizomes) could be another indication of an oil spill effect, as discolored leaves broke apart more easily than normal emerald green leaves; all breakage could tend to leave rhizomes with only leaf stubs. Bare rhizomes were twice as common at Corral Canyon than at Mussel Shoals, but low in occurrence overall (less than 6.0% cover based on data from the quadrat photo and field line-point contact sampling methods). The estimate, however, is most likely an underestimate, as bare rhizomes were scored only if there was no overstory layer of macroalgae or surfgrass on top of the rhizomes at the sampling points. The qualitative field observations, however, further noted bare rhizomes were also black in color and peeling off rocks, a condition that was more extensive at Corral Canyon than at Mussel Shoals.

The field line-point contact sampling data also revealed that macroalgae were less abundant than surfgrass (**Figure 6**), although the abundance of macroalgae from this method were also likely underestimated, due to the algae often occurring underneath surfgrass and thus not scored. The

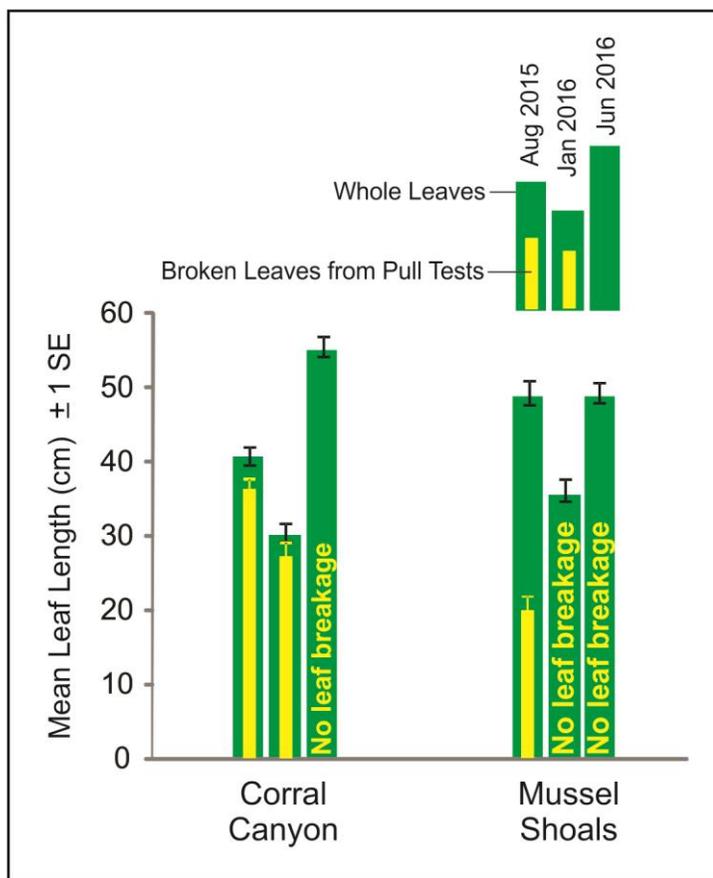


Figure 5. Surfgrass whole leaf lengths and lengths of leaves that broke off from pull tests.

method, however, detected increases in both surfgrass and in macroalgal abundance by the final June 2016 survey at Corral Canyon. Most of the increase in macroalgal abundance at Corral Canyon was from *Sargassum muticum*, a large naturalized non-native brown algal that was often scored in this method, due to being an overstory species.

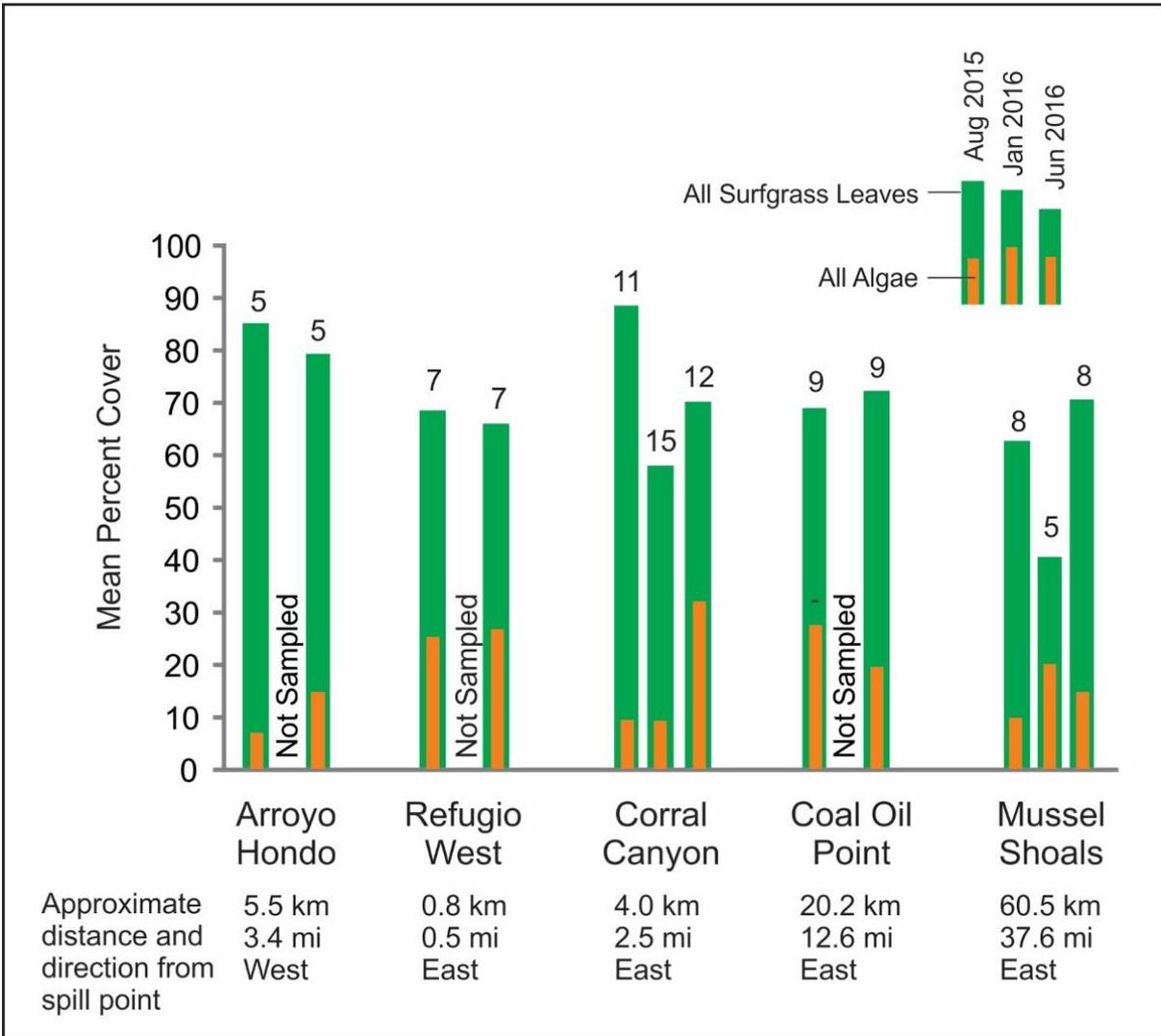


Figure 6. Percent cover changes by the line -point contact field sampling results for surfgrass and macroalgae (all colors and conditions combined for each taxon). The macroalgae do not include crustose species. The approximate distances of the sampling sites from the spill point are shown. Numbers above bars are the number of 10-m transects sampled.

Invertebrate data from the line-point contact sampling method are not included here, as invertebrates were typically the bottom-most layer and therefore not scored when there was an overstory layer present.



Discoloration: Photo-Quadrat Scoring

The largest proportion of surfgrass discoloration was observed at Corral Canyon (82.0%) during the (initial) August 2015 survey (**Table 3a**). This was followed by Coal Oil Point and Refugio West in the same survey where approximately half of the surfgrass leaves scored were discolored. At Arroyo Hondo, approximately one third of the leaves scored were discolored. Mussel Shoals had the smallest proportion of discolored leaves scored (less than 3%).

Macroalgal species were also discolored in the initial (August 2015) survey, and to a greater degree than surfgrass; nearly 100% of the macroalgae sampled was discolored at all sites, except at Mussel Shoals (**Table 3b**). Very little discoloration of the macroalgae (and surfgrass) was observed in the final survey in June 2016 at any of the sites (**Table 3a** and **3b**).

Table 3. Proportion of surfgrass discoloration to total cover at sites over time: a) surfgrass; b) algae.

	Arroyo Hondo	Refugio West	Corral Canyon	Coal Oil Point	Mussel Shoals
a) Surfgrass					
August 2015	37.4	46.2	82.0	54.3	2.2
January 2016	ns	ns	34.3	ns	19.7
June 2016	16.9	2.1	9.5	2.4	1.9
b) Algae					
August 2015	92.3	92.2	99.2	86.1	6.1
January 2016	ns	ns	2.0	ns	15.1
June 2016	1.9	0.8	0.2	11.2	0.0

ns: not sampled due to insufficient tides and labor resources

The combined coverage of discolored surfgrass and macroalgae scored as the top-most layer in the quadrat photographs was greatest in the August 2015 survey at all sites, and mostly at Corral Canyon at 84.5% cover (**Table 4**). This was followed by Refugio West, Coal Oil Point, and Arroyo Hondo. The least amount of discolored surfgrass and macroalgae was at Mussel Shoals. Nearly all of the discoloration in surfgrass and macroalgae had diminished by the final (June 2016) survey at all of the sites.

Table 4. Percent cover of top layer discoloration at sites over time, surfgrass and macroalgae combined.

	Arroyo Hondo	Refugio West	Corral Canyon	Coal Oil Point	Mussel Shoals
August 2015	35.3	61.2	84.5	52.0	3.4
January 2016	ns	ns	18.9	ns	12.5
June 2016	7.1	1.3	4.6	4.1	1.1

ns: not sampled due to insufficient tides and labor resources



Snorkel and Paddleboard Findings

Refugio East, Corral Canyon, and El Capitan (**Figure 2**) were surveyed in snorkel swims and from a paddleboard. The Refugio East site located at Refugio State Beach campground was searched in this manner on August 28, 2015 where an extensive subtidal surfgrass bed extended for hundreds of meters parallel-to-shore. A sand channel separated most of the surfgrass area from the deeper offshore kelp forest. The discoloration in surfgrass at Refugio East was observed in some areas as bands of discolored surfgrass leaves oriented parallel-to-shore alternating with bands of green leaves also oriented parallel-to-shore (**Figure 7a**). The discoloration was also observed to be near the distal ends of surfgrass leaves with leaves being more green closer the rhizome base (**Figure 7b**).

At Transect 1 at Refugio East (**Figure 8**), the outer depth boundary of surfgrass was -2.0 m (-6.5 ft) MLLW and was 88 m (289 ft) from shore. The depth at the outer boundary of surfgrass along Transect 2 was -1.8 (-5.8 ft) MLLW where it transitioned into a kelp forest. The outer depth boundaries of surfgrass at Transects 4 and 5 were -0.9 m (-2.8 ft) and -1.4 m (-4.5 ft) MLLW, respectively, with surfgrass occurring out to approximately 100 m (328 ft) from shore at both transects. Transect 3 (**Figure 8**) was not deployed due to breaking surf and was therefore not sampled; underwater visibility was also poor in that location.

Arroyo Quemada (**Figure 2**) was visited on September 3, 2015, but waves were too large to enter the water, and underwater visibility was poor. Paddleboard and snorkel surveys were not attempted, but beach walk observations found that drift surfgrass stranded along the high tide line on the shore was abundant. The leaves were yellowish-tan and brittle.

El Capitan State Beach cove (**Figure 2**) was also visited on September 3, 2015. Surfgrass did not occur in the intertidal zone along the sand beach but occurred on offshore emergent rocky outcroppings. In the subtidal zone, surfgrass was widespread, but not a solid bed. Snorkeling observations were able to be completed only outside the breaking surf zone. The depth reached was approximately -1.8 m (-6 ft) MLLW, which was approximately 50 m (164 ft) from shore. The surfgrass seen was pale with both light green and greenish-yellow leaves. Some patches were distinctly yellow/tan/brown. None of the surfgrass observed was bright emerald green. Discolored leaves were also brittle and broke apart easily when pulled by hand.

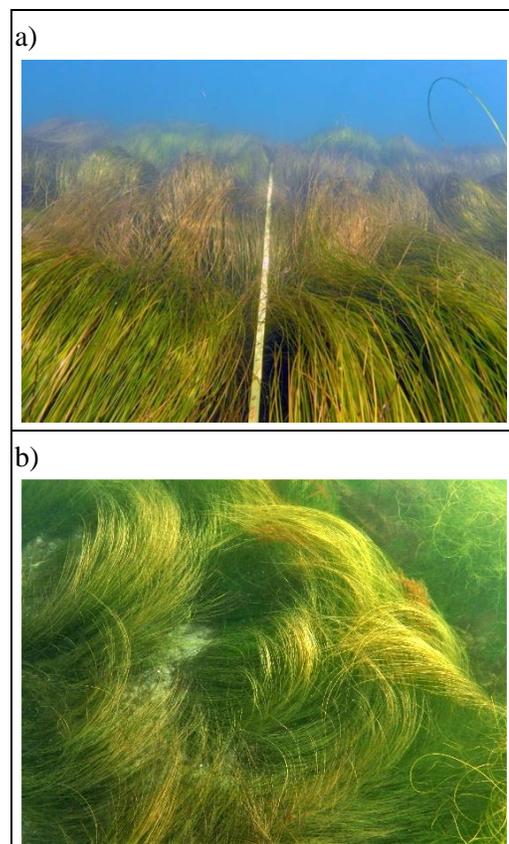


Figure 7. Discoloration in subtidal surfgrass appearing as: a) bands of non-green leaves alternating with bands of green leaves; and b) non-green leaf sections at the distal ends with green leaf sections closer to the rhizome base. Photos taken at Refugio East on August 28, 2015.



Corral Canyon (**Figure 2**) was surveyed on September 4, 2015, but surveys were hampered by strong southerly swells and waves. The outer extent of the bed was mapped using a GPS on a paddleboard and with a meter tape anchored at the 0.0 m MLLW tide level on the shore and deployed offshore. The surfgrass bed extended approximately 100 m from shore where depths were -1.5 m (-5 ft) to -2.1 m (-7 ft) MLLW. All of the surfgrass over this distance was pale, tan, black, and yellow-green. No surfgrass was bright emerald green.

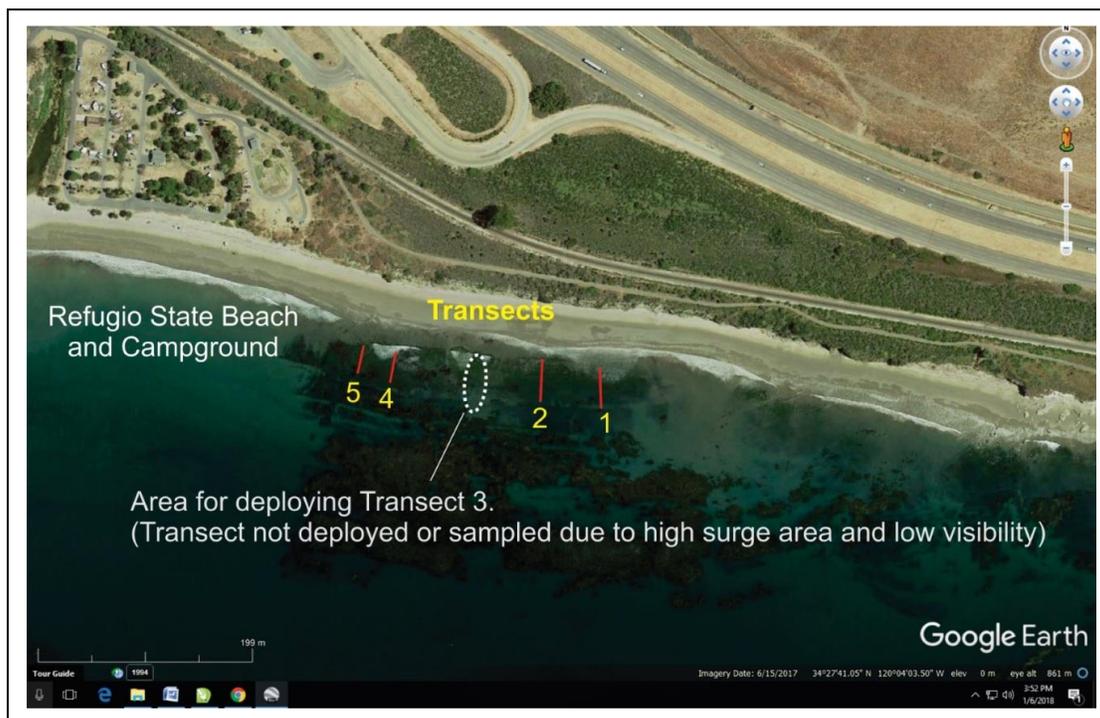


Figure 8. Transects at Refugio East where surfgrass was observed in paddleboard and snorkel surveys on August 28, 2015.

SCUBA Findings

SCUBA surveys were completed on October 21, 2015, five months after the spill and approximately seven weeks after the snorkel and paddleboard surveys. Underwater visibility was 6-10 m (20-30 ft) at all four sites, and waves in the surf zone were 0.6–0.9 m (2-3 ft).

The findings from the SCUBA observations were similar to the earlier snorkel and paddleboard findings on depths, extent, and surfgrass discoloration (**Figures 9** and **10**). The maximum offshore depth of surfgrass was between -1.5 m to -3.1 m MLLW, and surfgrass extended offshore no greater than approximately 100 m at the sites surveyed.

At all four sites, yellow-green surfgrass was common at all of the depths and distances from shore surveyed, and discolored algae occurred with the discolored surfgrass. A quantitative assessment of the amount of discoloration in the algae and surfgrass (i.e., aerial coverage) was not completed, due to difficulties in sampling underwater in the surf zone; the purpose was to mainly confirm whether the discoloration occurred in the subtidal.



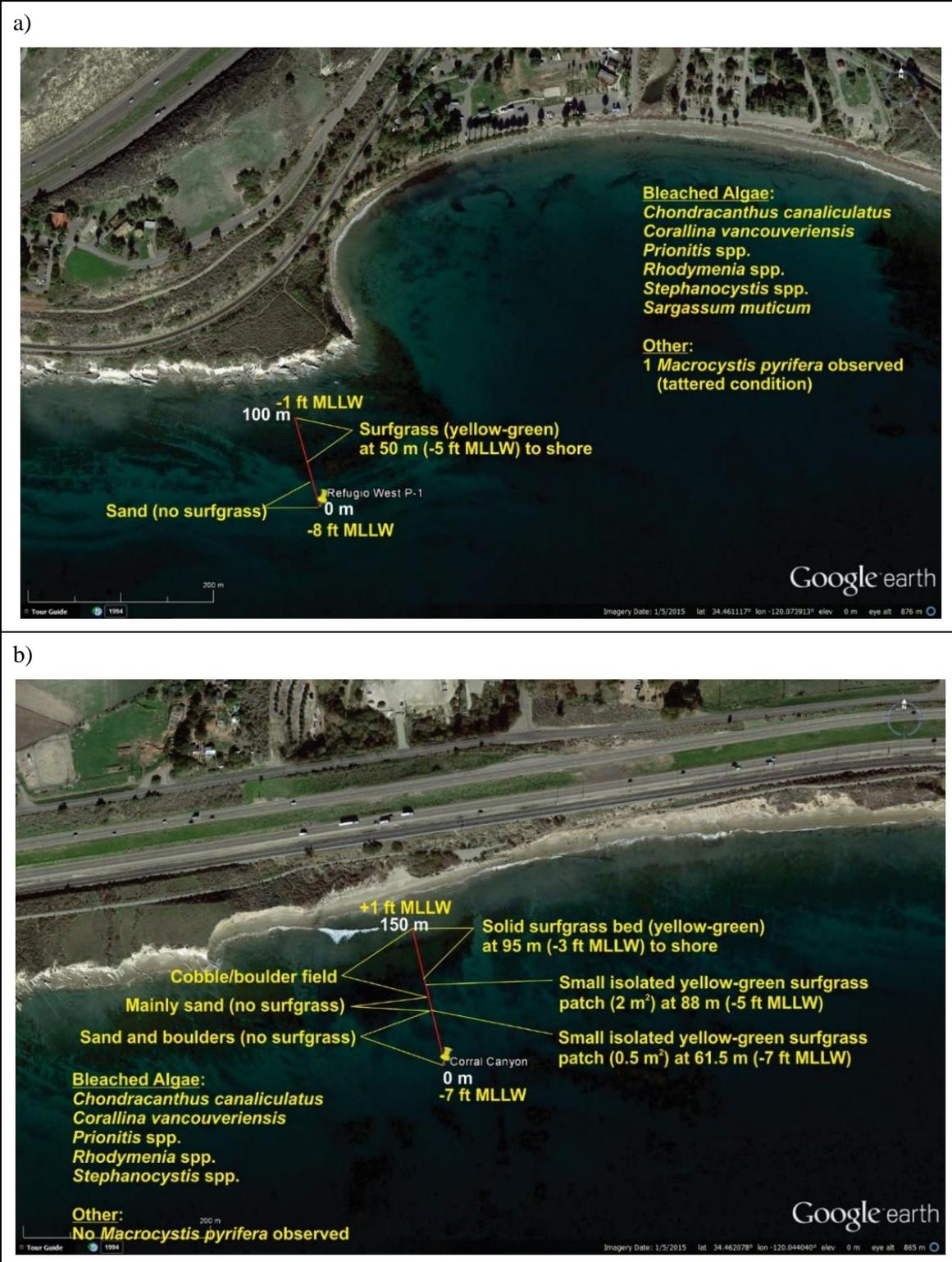


Figure 9. Subtidal (SCUBA) observations at: a) Refugio West; and b) Corral Canyon on October 21, 2015.



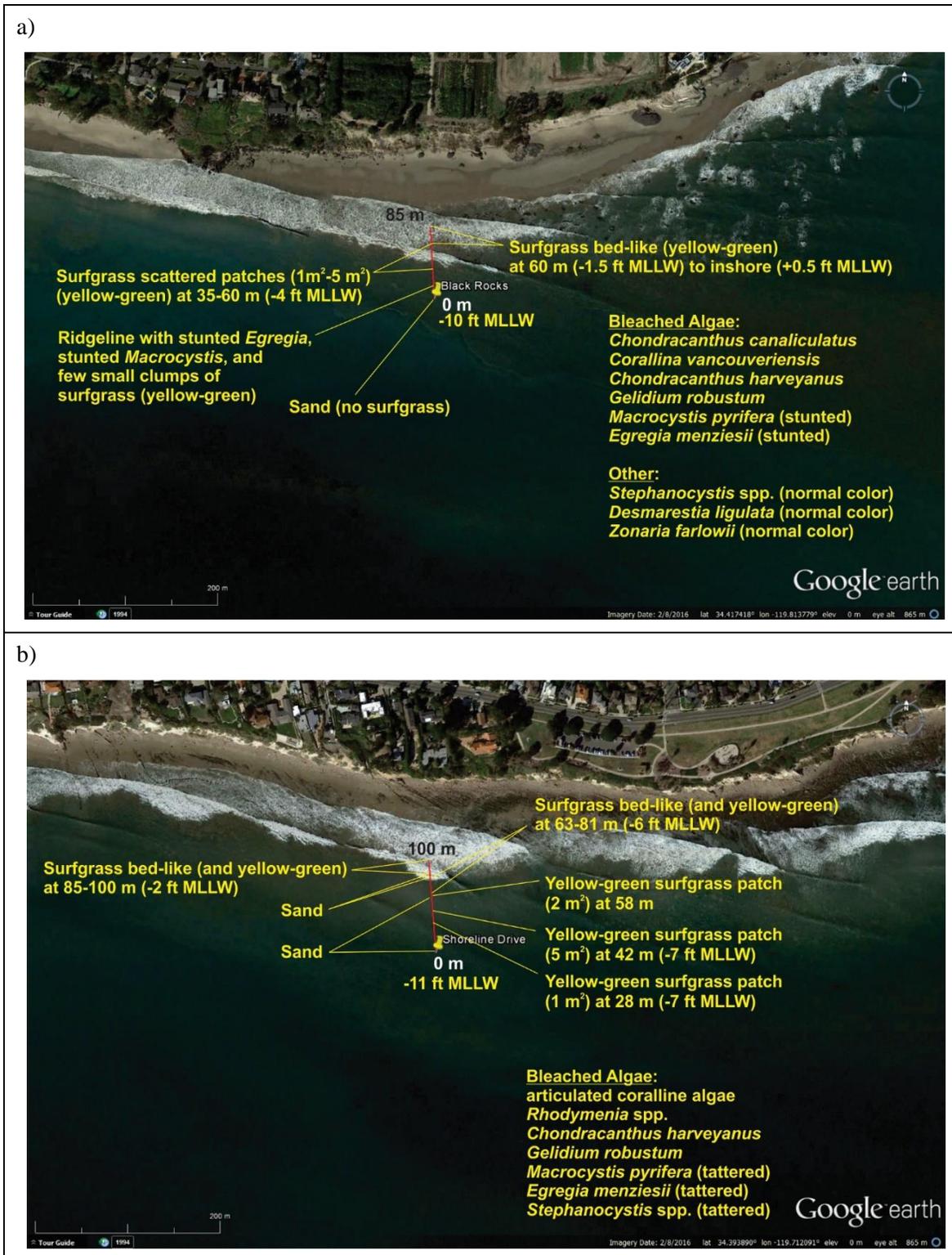


Figure 10. Subtidal (SCUBA) observations at: a) Black Rocks; and b) Shoreline Drive on October 21, 2015.



Discussion

Magnitude and Extent of Surfgrass and Macroalgae Discoloration

The intertidal quadrat photo-surveys found the discoloration in surfgrass, expressed as the proportion of discolored (i.e., dead and dying) surfgrass compared to all the surfgrass sampled at a site, was greatest at Corral Canyon (82.0%) over the other sites sampled (**Table 3a**). Results for the macroalgae were similar with the greatest discoloration found at Corral Canyon (99.2%, **Table 3b**). Even though the immediate shore at Refugio State Beach Campground was highlighted in news media as a heavily oiled shore, which was closer to the spill point to the ocean (~0.8 km; ~0.5 mi) than Corral Canyon (~5 km; ~3 mi), surfgrass and macroalgae at the Corral Canyon sampling site were more affected by the spill than at the Refugio West sampling site that was immediately at the Refugio State Beach Campground. A possible explanation for the discrepancy is the tide level and currents at the time of the oil spill.

The spill occurred during a high tide when the ocean current and wind were in an eastward trajectory (downcoast). As a result, the Refugio West sampling site and Refugio State Beach Campground shores were contacted by the buoyant oil plume primarily in the upper intertidal zone on the day of the spill where the oil eventually dried as black bands coating the vertical rock (cliff) faces along the high splash zone of the high intertidal zone. The high tide condition thus limited the oiling exposure to the low-intertidal surfgrass occurring beneath the buoyant plume. As the oil plume continued to move east down the coastline, the tide level was dropping. Hours later, the oil plume reached Corral Canyon when the tide level was lower, which increased the exposure of the low-intertidal occurring surfgrass and macroalgae to the oil plume.

Using surfgrass discoloration/tissue damage as criteria to rank oiling exposure and injury, oiling exposure and injury was greatest at Corral Canyon, followed by Refugio West, Coal Oil Point, and Arroyo Hondo, and with Mussel Shoals being unoiled or least oiled. This is consistent with SCAT oil ranking data (**Figure 3**). This is also consistent with the first post-spill studies completed by the University of California, Santa Cruz, which included sites sampled in similar locations as in the present study and where oil scored in quadrats revealed the same gradient of oiling exposure; most oil was in quadrats sampled at Corral Canyon (Raimondi et al. 2019). Another hypothesis for why Corral Canyon in the present study was more affected by the spill than the other sites is that the low-intertidal zone of surfgrass at Corral Canyon is a habitat of boulders and cobbles rather than bench rock; oil tends to persist and remain entrapped in a liquid state in the open spaces between boulders and cobbles and can then re-suspend and re-oil the shore with the next incoming tide.

The upper-most elevation of the discoloration in surfgrass was found to be approximately 0.0 m MLLW, and the lowest elevation was found to be approximately -3.1 m MLLW underwater. This was also the full intertidal-subtidal vertical range of surfgrass in the areas surveyed. The maximum distance offshore of the surfgrass discoloration was approximately 100 m. Macroalgae co-occurring with the intertidal and subtidal surfgrass were also discolored in the same areas. The discoloration in surfgrass and in the macroalgae was appreciably less by the end of the study at all of the sites exposed to the oil spill.



Tensile Strength of Surfgrass and Leaf Lengths

The surfgrass leaf measurements of whole leaves at Corral Canyon and Mussel Shoals provide evidence of an effect from the oil spill and then recovery. Whole leaves at Corral Canyon were appreciably shorter in the initial survey compared to the final survey (**Figure 5**). In contrast, whole leaf lengths at Mussel Shoals were nearly identical between the initial and final surveys. Without the oil spill, one hypothesis is whole leaf lengths at Corral Canyon should have been more similar to each other between the initial and final surveys, as found at Mussel Shoals. As such, one explanation why surfgrass leaves at Corral Canyon were considerably shorter in the initial survey compared to the final survey is that the discolored and weakened leaf sections had already broken off leaving mostly shorter leaves to be measured. No further leaf breakage from pull tests was evident in the final survey.

The initial decline in surfgrass leaf lengths at Mussel Shoals, the control/reference site where the decline was followed by an increase in leaf lengths, may also help to explain some of the changes at Corral Canyon (**Figure 5**). The decline at Mussel Shoals from the initial survey (August 2015) to the mid-winter survey (January 2016) can be considered a natural change consisting of leaf growth slowing with the shorter day lengths and the leaves becoming shorter from winter storm waves eroding leaves. This may also explain all or part of the decline at Corral Canyon over the same time period. Leaf lengths and percent cover then increased at both Corral Canyon and Mussel Shoals (**Figure 6**). The increases at both sites represent positive growth responses to the returning spring/summer growth conditions.

Rhizome Condition

Bare rhizomes were only observed in the winter sample period (January 2016), and were sampled by the photo-quadrat and field line-point contact sampling methods at Corral Canyon and Mussel Shoals, the only sites sampled in the January 2016 survey. The occurrence of bare rhizomes at Mussel Shoals indicates bare rhizomes occur naturally, possibly related to winter storm waves and shorter day lengths causing leaf die-back and sloughing leaving bare rhizomes. On this basis, it is not possible to confirm whether all or a portion of the bare rhizome condition sampled at Corral Canyon was from the oil spill or from natural causes because bare rhizomes were also sampled at Mussel Shoals. However, other information suggests eelgrass rhizome condition was affected by the spill more than what the quantitative sampling revealed.

Bare rhizomes comprised less than 6% cover at both sites, as determined by the photo-quadrat and field line-point contact sampling methods, but were twice as common at Corral Canyon than Mussel Shoals. However, both sampling methods likely underestimated bare rhizome cover at both sites, due to rhizomes being the bottom-most layer in both sampling methods and therefore not scored if overstory species (surfgrass and macroalgae) were present.

In contrast, qualitative observations of general site conditions found that bare rhizomes, damaged/black rhizomes, and rhizomes partially pulled away from rocks were much more common at Corral Canyon than at Mussel Shoals. Without more quantitative data, however, it is not possible to describe the total amounts involved.



Discoloration and Oiling Exposure

The term “discoloration” in the present study describes the unusual condition of surfgrass leaves and macroalgae blades and branches occurring at the oiled sites in July–August 2015 after the oil spill. “Bleaching” is another term commonly used to describe the appearance of damaged tissues, but bleaching is from tissues becoming desiccated and changing to white in color. Natural tissue bleaching and desiccation generally occurs during extreme low tides when the intertidal zone is exposed to air, strong sunlight, and a consistent warm air breeze blowing across the intertidal zone (Scrosati and DeWreede 1998, Irving et al. 2004, Keough and Quinn 1998, Martone et al. 2010, Tenera Environmental, unpublished observations).

Bleaching along the central California coast tends to occur more often in winter than in the other seasons, associated with the timing of low tides and low tide levels. Winter is the season of the most extreme low tides that occur during daylight hours, which results in the low-intertidal zone becoming exposed to air and direct sunlight for longer hours than other seasons and to offshore winds that can be warm with low humidity. Extreme low tides also occur in spring and summer, but these low tides occur most often during night and early morning when the low-intertidal zone is not exposed as much to strong sunlight and warm air temperatures. Low tides also occur in fall, and can be during strong daylight, but the tide levels during the daylight hours are generally not as low in fall compared to winter. As such, the low-intertidal zone in fall is exposed less to warm air conditions than in winter. Thus, the timing when low tides occur and how low in elevation the tides reach largely explains why bleaching in intertidal surfgrass and macroalgae tends to occur more in winter than in any other season along the central California coast.

As such, and based on timing, the discoloration seen in surfgrass that was first observed in summer 2015 was not from exposure to direct sunlight and warm air temperatures, but rather the discoloration can be better explained as an impact from the oil spill; the discoloration did not occur suddenly at the time of the spill but instead was a delayed change. Supporting the assessment of being an oil impact is the discoloration also occurred in the subtidal, which is never exposed to direct sunlight and warm air temperatures, and essentially no discoloration was observed at Mussel Shoals, the reference/control site. The unusual color combined with the brittle condition of leaves further supports the determination that the tissue injury was caused by a harmful substance, not damage from sun exposure.

The observed discoloration and tissue injury could have also been due all or in part from natural oil seeps in the region, but no unusual discoloration was immediately apparent during the first post-spill surveys completed in May and June 2015 by University of California, Santa Cruz and Tenera Environmental biologists; no discoloration was noted because it was not observed. Additionally, the Coal Oil Point site with its high exposure to natural oil seeps (NOAA 2015) would have been the highest candidate site to observe such discoloration, but none was seen at Coal Oil Point during the May and June (first) surveys in 2015. Instead, the discoloration in surfgrass and macroalgae was first observed later in July 2015 at multiple locations contacted by the spill. The greatest discoloration occurred at Corral Canyon, one of the most heavily oiled sites from the spill. The timing of the discoloration would suggest an oiling dose higher than natural oil seeps is necessary to elicit a discoloration response in surfgrass.



The Refugio oil spill occurred during the 2014-2016 El Niño (Thompson 2015, Becker 2016). Accordingly, the El Niño event, rather than the oil spill, could explain the observed discoloration and tattered condition seen in surfgrass and in the macroalgae. If this was the case, one could expect the discoloration to be relatively uniform within and across sites. However, the magnitude of discoloration was not uniform within and across sites, but instead corresponded more closely to the amount of oiling exposure, in accordance with the SCAT data. In addition, an El Niño effect was not found in other local studies to cause changes outside the range of normal variation in species components of *Macrocystis pyrifera* (giant kelp) forests in the Santa Barbara Channel (Cohen 2016, Reed et.al. 2016). This would suggest the oil spill had a greater influence on the discoloration than a possible effect from the El Niño that occurred in the present study.

An additional observation supporting the hypothesis that oil contact caused the discoloration and injury to surfgrass was the discoloration was mostly at the distal ends of leaves in subtidal surfgrass (**Figure 7**). An explanation for this condition is surfgrass leaves wafting in the water column were contacted by the buoyant oil plume in the troughs of waves as waves passed by.

Other supportive evidence that the discoloration seen in the present study was from the oil spill is that similar observations have been made following other oil spills, in particular, the Santa Barbara oil spill that occurred in 1969 (Foster et al. 1969). Surfgrass with oil adhered to leaves from the spill turned yellow then brown (Nicholson and Cimberg 1971). In another study, discolored and burnt leaves of surfgrass (referred to as false eelgrass) resulted from a diesel fuel spill and exposure incident in Puget Sound, Washington that occurred in 1972 (Washington State Department of Ecology 1975). Also, other oil spills have been linked to causing marine algal tissues becoming discolored (O'Brien and Dixon 1976, Clark et al. 1978, Floch and Diouris 1980, Antrim et al. 1995). These past observations are consistent with the present observations of discoloration occurring in surfgrass and macroalgae as a response to the oil spill.

Conclusions

Surfgrass and macroalgae discoloration was observed in intertidal habitats following the Refugio oil spill. The discoloration from cell damage and loss of chlorophyll pigment necessary for primary production and growth represents a direct spill injury. Similar discoloration and injury associated with the spill was observed in subtidal eelgrass in snorkel, SCUBA, and paddleboard surveys. The magnitude of discoloration injury was consistent with oiling exposure. The study also provides evidence of recovery one year after the spill.

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