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Journal

San Francisco Estuary and Watershed Science, 15(3)

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Publication Date

2017

DOI

10.15447/sfews.2017v15iss3art4

Copyright Information

Peer reviewed

RESEARCH

Distribution and Genetic Structure of *Fucus distichus* Linnaeus 1953 (formerly *F. gardneri*) within Central San Francisco Bay

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ABSTRACT

Fucus distichus, a rockweed common to the mid-intertidal shoreline within the San Francisco Estuary (previously known as *F. gardneri*), was injured during the Cosco Busan oil spill in November 2007 and subsequent clean-up actions. Restoration planning activities are underway to help recover *F. distichus* at sites within central San Francisco Bay where damage occurred. As a first step, we conducted shoreline surveys during the summers of 2012–2013 to map the occurrence of this rockweed. Of the 151.73 km of rocky shoreline within the central bay, *F. distichus* covered 32.16 km of shoreline. The alga generally occurred in narrow bands but formed expansive beds at locations with natural, flat bedrock benches. We also observed *F. distichus* on artificial substrata such as seawalls and riprap, but not on pilings. Samples of *F. distichus* from 11 sites throughout the central/east San Francisco Bay were genetically analyzed (microsatellite genotyping). The populations analyzed (1) had low genetic diversity, (2) the frequency of homozygotes was higher than expected (suggesting high inbreeding), and (3) also displayed geographic population structure, in part driven by very small differences in the midst of extremely low within-population genetic diversity. However, these genetic data do not raise concerns for restoration methods in terms of choosing donor populations and mixing *F. distichus* from different sites within the central bay. The choice of donor populations should be based on practical criteria for effective restoration; individuals will nonetheless be taken from locations as nearby to donor sites as possible. Various locations throughout the central San Francisco Bay are composed of cobble or small riprap that are populated with *F. distichus*, which could provide efficient means of translocating rockweed for future restoration activities.

KEY WORDS

INTRODUCTION

The freighter, M/V Cosco Busan, collided with the San Francisco–Oakland Bay Bridge on November 7, 2007, causing 202.8 cubic meters (53,569 gallons) of Intermediate Fuel Oil (IFO-380) to spill into the San Francisco Bay (bay) (CBOST 2012). Most of the oil affected the waters and shoreline of the central portion of the bay, but some oil reached areas outside of the Bay, from approximately Half Moon Bay to Limantour Beach at Point Reyes (Figure 1).

According to a natural resource damage assessment, 1,363 hectares (3,367 acres) of shoreline were damaged as a result of the spilled oil and/or clean-up efforts (CBOST 2012). A proportion of the affected shoreline was rocky intertidal habitat composed of artificial surfaces such as riprap and seawalls, as well as naturally-occurring hard substrata, including bedrock, boulder, and coarse sediments that varied in size from gravel to cobble–pebble. A total of 155.5 hectares (384.3 acres) of rocky shoreline in San Francisco Bay was affected by the spill (CBOST 2012).

Rocky intertidal habitats support a rich diversity of species that depend on a number of variables including, but not limited to, geological composition, the degree of wave exposure, salinity, temperature, and influence from currents. Most locations within the Bay experience considerably different conditions than the open-coast environments outside of the Bay, but the Golden Gate Straits and the western shorelines of Angel and Alcatraz islands experience similar conditions to outer-coast sites since they are affected by westerly swells (Silva 1979). Sites that are influenced by similar abiotic conditions are typically characterized as having comparable biological communities.
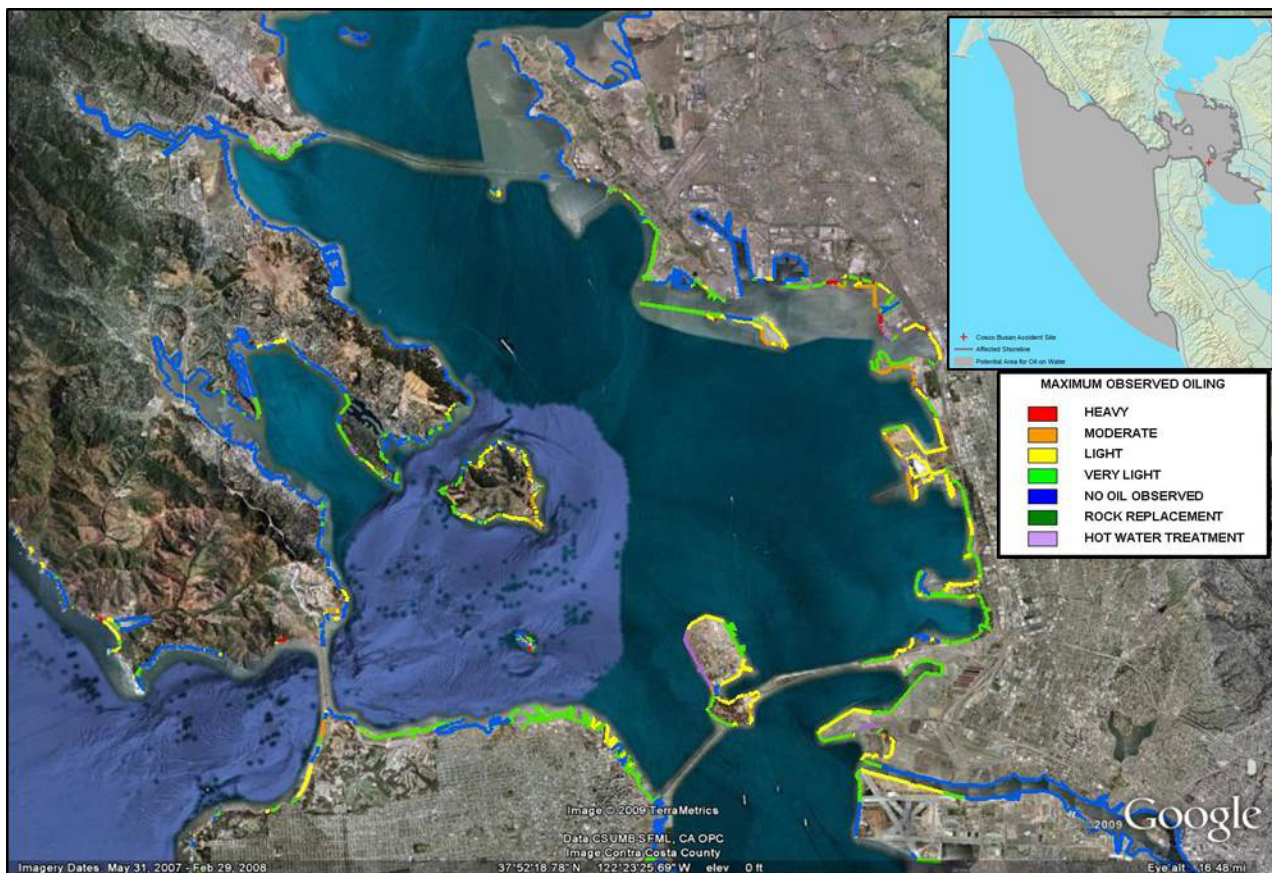

Figure 1 Maximum observed oiling along the shorelines of the central San Francisco Bay during the M/V Cosco Busan incident. Inset illustrates the M/V Cosco Busan spill zone, November 2007. Source: CBOST 2012.

Common biota that inhabit rocky shorelines within the Cosco Busan incident area include barnacles (*Chthamalus* spp.) and periwinkle snails (*Littorina* spp.) in the supralittoral zone; fucoids (*Pelvetiopsis limitata*, *Silvetia compressa*, and *Fucus distichus*, previously known as *Fucus gardneri*) and red algal turf species in the upper- to mid-intertidal; mussels (*Mytilus* spp.) in the mid-intertidal; and surfgrass (*Phyllospadix* spp.) and kelp (*Laminaria* spp.) in the low intertidal zones (Hedgpeth 1962; Silva 1979).

Although all of these species assemblages are important components of any rocky intertidal ecosystem, fucoid presence is particularly beneficial because of the habitat-forming role it plays in structuring rocky shore communities. Canopy-forming seaweeds such as *F. distichus* are crucial to understory flora and fauna since they ameliorate the effects of desiccation, temperature fluctuations, and wave stress (Bertness et al. 1999; Bulleri et al. 2002). They also serve as a food source for some gastropods (Chapman 1990; Wooton 1997), and contribute significantly more primary production than smaller turf-forming algae (Littler and Murray 1974). Altering rocky shores by damaging *F. distichus* communities is likely to have cascading negative effects on other species in the rocky intertidal zone.

During the Cosco Busan oil spill, direct impacts on *F. distichus* included hand removal of oiled rockweed during clean-up activities (Figures 2 and 3) and hot-water washing of oiled, rocky substrata. The persistent negative effects of oil spill clean-up actions on *F. distichus* have been documented at other locations (Hoff and Shigenaka 1999; Kimura and Steinbeck 1999). After the 1989 Exxon Valdez spill in Prince William Sound, Alaska, and subsequent clean-up actions, *F. distichus* was slow to recover, which in turn stimulated an interest in actively re-establishing the rockweed at damaged sites (De Vogelaere and Foster 1994; Stekoll and Deysher 1996; Driskell et al. 2001).

The Cosco Busan Damage Assessment and Restoration Plan (*http://www.darrp.noaa.gov/southwest/cosco/restore.html*) included an objective to restore 2,000 linear meters of *F. distichus* within the central San Francisco Bay.

Figure 2   Manual clean-up actions at Point Blunt, Angel Island, November 21, 2007. Photo: Dan Richards, NPS (DARP).

Figure 3   Bag of oiled *F. distichus* (including holdfasts) from cleaning activities at Point Blunt, Angel Island. Photo: Dan Richards, November 21, 2007.

However, four goals were to be accomplished before the *F. distichus* restoration was initiated):

1. Mapping the existing distribution of *F. distichus* within the central bay.

2. Quantifying the relative abundance of individual plants within established algal stands.

3. Analyzing *F. distichus* genetics in the bay to determine potential donor sites.

4. Determining the maximum percentage of *F. distichus* that is available to harvest for restoration.

The purpose of this paper is to provide information for goals 1–3. The study was designed to identify potential donor sites for *F. distichus*, with particular emphasis placed on sites with substrata (e.g., cobble, small riprap, and boulders) that could be translocated to other sites. Final identification of donor sites required the evaluation of the genetic diversity of this species within the central bay to ensure that potential donor sites are matched with appropriate fucoid translocation sites.

MATERIALS AND METHODS

Study Area

The geographic scope of this mapping effort for *F. distichus* focused on the central bay, one of the four main basins within the San Francisco Bay. The boundaries of the central bay were adopted from Schaeffer et al. (2007), which used the Richmond–San Rafael Bridge to delineate the boundary with San Pablo Bay, the Golden Gate Bridge as the western boundary, and the Oakland–Bay Bridge (including all of Yerba Buena Island) to delineate the southern boundary with south bay counties (Figure 4).
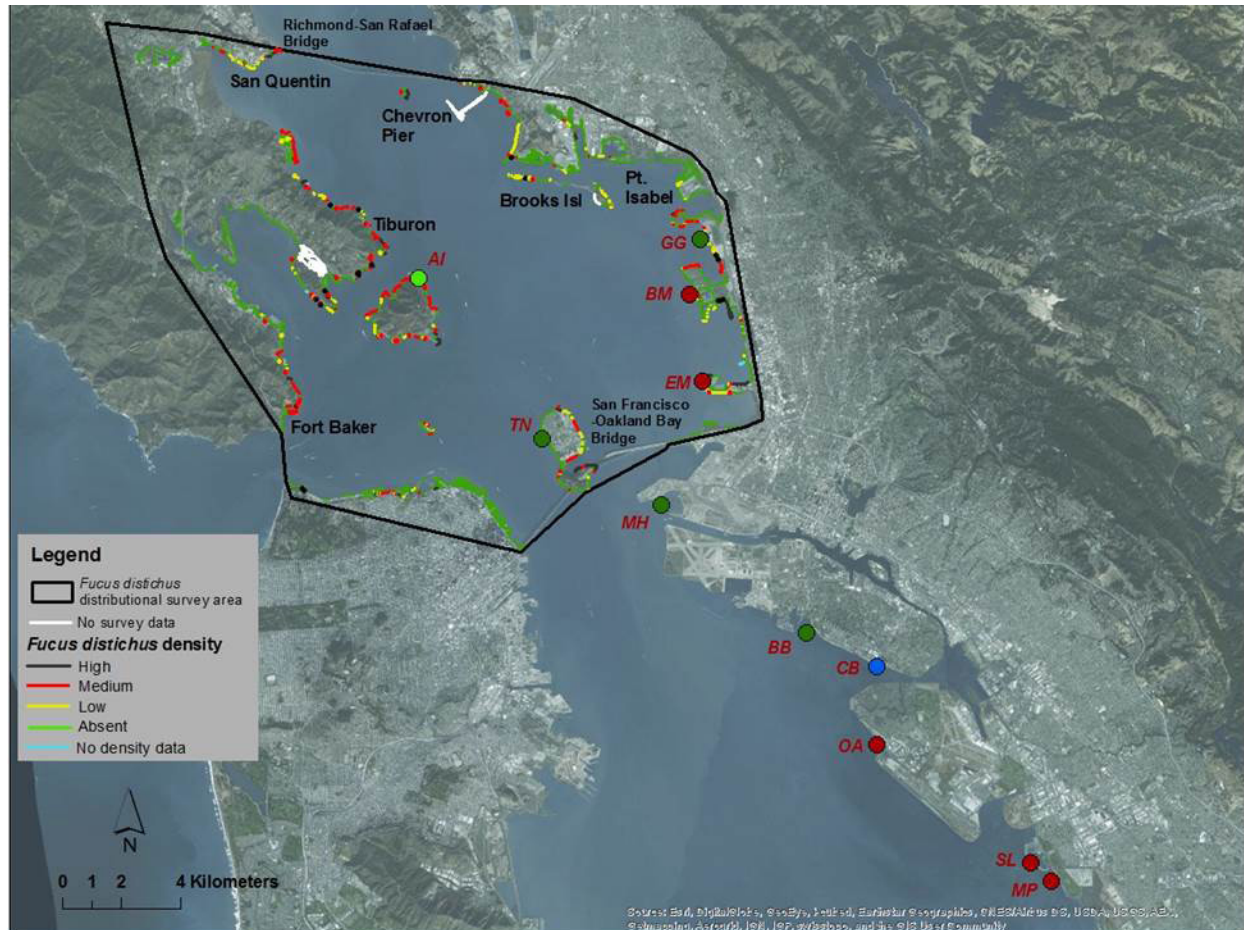

Figure 4 *F. distichus* distribution, density, and genetic relatedness within the central San Francisco Bay, 2012–2013. Note: red font indicates the locations of sites chosen for Structure analysis that depict sampling sites of *F. distichus* analyzed for microsatellite polymorphisms. Similar colors depict the same genetic group; unique colors represent *F. distichus* populations with genetic makeup distinct from the other populations sampled. Refer to Table 2 for site abbreviations. Image: ESRI, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS user community.

Field surveys focused on rocky shorelines comprising natural or artificial hard substrata. We identified sites using recent Environmental Sensitivity Index (ESI) maps developed for San Francisco Bay. ESI maps describe the entire San Francisco Bay shoreline according to substratum type and grain size, among other factors (NOAA and CDFG 1998). These linear shoreline habitats were originally mapped during over-flights conducted in January 1986 and updated based on August 1996 1:24,000 natural color vertical aerial photography (NOAA and CDFG 1998). The shoreline habitats selected for field surveys included the following habitat classifications: 1A) exposed rocky shores, 1B) exposed, solid man-made structures, 2A) exposed wave-cut platforms, 6A) gravel beaches, 6B) riprap, 8A) sheltered rocky shores, 8B) sheltered, solid man-made structures, and 8C) sheltered riprap. These habitat types totaled 151.73 km of shoreline in the central bay.

Generally, we conducted shoreline field surveys from −0.3 to +1 m mean lower low water, the approximate elevation range of *F. distichus* in the central bay, in July 2012 and June 2013. We surveyed most of the shoreline in a shallow-draft vessel or kayak, except for the urban shoreline in San Francisco, which we surveyed on foot. We did not survey small areas of habitat because of access and time constraints. The distance of shoreline not surveyed totaled 10.4 km and mainly comprised the Chevron refinery seawall/breakwater near Point Richmond and the developed shoreline of Belvedere Lagoon. We included in the survey all other locations in the central bay composed of hard substrata.

Study Species

The fucoid, *F. distichus* Linnaeus (formerly *F. gardneri* P. C. Silva), forms dense stands throughout the mid to upper intertidal gradient of protected to fully wave-exposed rocky shorelines from Alaska to the Point Conception biogeographic boundary in California, including portions of the San Francisco Bay (Abbott and Hollenberg 1976). As an occupant of the intertidal zone, *F. distichus*, like many fucoids, is highly vulnerable to oil spills. A number of experimental studies and syntheses of post-accident observations have well documented the deleterious effects of oil on fucoids (reviewed in Foster et al. 1988).

F. distichus is a hermaphroditic alga capable of self fertilization (Pollock 1970). Gametes are released from reproductive structures (conceptacles) housed within swollen thallus tips (receptacles) (Pearson and Brawley 1996). The timing of gamete release primarily occurs from late fall through early spring (Bird and McLachlan 1975) during daylight hours concurrent with low-tide aerial exposure (Pearson and Brawley 1996; Serrão et al. 1996). This reproductive strategy enhances fertilization success for *F. distichus* (Pearson and Brawley 1996; Brawley et al. 1999) and other fucoid species (Pearson and Brawley 1996; Serrão et al. 1996; Pearson et al. 1998), but results in low dispersal of gametes relative to algal species that utilize alternative strategies (Moss 1975; Hardy and Moss 1979). These traits allow reproductive isolation of distinct genetic types of *F. distichus* to be maintained even where they co-occur within small distances (Neiva et al. 2012).

Mapping Methods

We mapped the occurrence of *F. distichus* using a hand-held GPS (Trimble Geo XT and Garmin eTrex) and ArcMAP GIS software. We delineated the area occupied by the alga as either a point or a continuous line, depending on size. We mapped smaller patches of rockweed that occupied 10 m² or less of substrata as single points. We identified larger expanses by lines with endpoints at the locations where we first and last saw *F. distichus* along the shore. We determined populations of *F. distichus* were unique if they were separated by more than 10 m of unoccupied shoreline. We plotted the GPS points and lines as close to the shoreline as was physically possible based on boat access. At locations where the boat's track log did not match the shoreline position, or where we only had coordinates for segment endpoints, we digitized line segments in GIS based on digital imagery of the shoreline.

We estimated relative abundance of *F. distichus* plants from the vessel using simple cover categories determined by the following rubric: High = 75–100% cover, Medium = 25–74% cover, Low = 1–24% cover, and Present = present but no cover data. We visually estimated bed width and abundance based on the average of the entire surveyed point or line. In some cases, estimates were difficult, particularly when

5

density ranged from High to Low (or vice versa) over a given area. This particular situation resulted in an overall density assessment equivalent to Medium. In rare instances, we recorded only the presence of *F. distichus*. Whenever possible, we mapped beds of *F. distichus* with highly variable abundances separately to reveal the differences in algal cover. We photographed representative areas along the shoreline throughout the entire survey area. We recorded abundance, mean bed width or patch size, and dominant and secondary substratum type (bedrock, boulder, cobble, riprap, seawall) into the GPS and in field books. We chose substratum types based on the habitat classifications utilized by the ESI maps.

Because the frequency distribution of *F. distichus* bed widths was skewed toward narrow widths (Figure 5), we used a or generalized Lagrangian mean (GLM) to determine whether dominant substratum type resulted in differences in mean bed width of *F. distichus*. Since data were heavily skewed toward zero, we compared Gaussian, Gamma, and Inverse Gaussian distributions for best fit (lowest Akaike information criterion [AIC]) and behavior of residuals (r-project 2016)

Population Genetic Variability of *F. distichus* within San Francisco Bay

We sampled populations of *F. distichus* for genetic analysis in 2013 at 11 sites inside the San Francisco Bay (Table 4 and Figure 4). We chose sites with known high abundances of *F. distichus* and uncomplicated access across a wide geographical area of the bay. We included nearly half of the sites selected for genetic analysis in the mapping effort to determine the distribution and relative abundance of *F. distichus* within the central bay. We collected vegetative tips of *F. distichus* individuals along two linear transects (156 tips per transect), corresponding to High and Low shore, at Angel Island (AI), Berkeley Marina (BM), and Oakland Airport (OA), whereas we made collections along a single line at the remaining sites (corresponding to the middle of the intertidal band where *F. distichus* is naturally found).

At each sampling site, we excised vegetative tips from individual algae, and stored and dehydrated tissue in silica-gel crystals until DNA extraction. We extracted genomic DNA using an adaptation of the cetyltrimethylammonium bromide (CTAB) protocol

(Hoarau et al. 2007) or, for some populations, using the commercial Nucleospin 96 Plant II kit (Macherey–Nagel Duren, Germany). We assessed amplification success and scoring patterns for ca. 20 microsatellite loci previously developed for several Fucus species, and eventually selected 13 loci (for being the most polymorphic along this species range) to generate multi-locus genotypes for all individuals (see Table 1 for primer sources, sequences, and amplification details).

We peformed polymerase chain reactions (PCRs) in 10 μL total volume containing 10–20 ng of DNA template, 1× GoTaq® Flexi Buffer, 2.0 mM MgCl$_2$, 0.2 mM deoxyribonucleotide triphosphate (dNTPs) (Bioline, London), 0.3 μM of labelled forward primers (0.167 μM for L20 and L94 primers), 0.3 μM of reverse primers (0.333 μM for L20 and L94 primers), and 0.5 U of GoTaq Flexi DNA Polymerase (Promega, Madison, WI). Touchdown PCRs for F primers involved an initial denaturation step (95°C, 5 min), 25 cycles of 95°C for 30 s, a primer-specific annealing temperature (T$_a$, Table 1) for 10 s, reduced by 0.2°C for each subsequent cycle, and 72°C for 35 s, followed by ten cycles of 95°C for 30 s, T$_a$ − 5°C for 10 s, and 72°C for 35 s, finalizing with a longer extension step of 72°C for 10 minutes. Simple PCRs for L primers involved an initial denaturation step (95°C, 5 min), followed by thirty cycles of 95°C for 30 s, T$_a$ for 35 s, and 72°C for 40 s, also finalizing with an extension step of 72°C for 10 min.

We ran amplified fragments in an ABI PRISM 3130xl automated DNA sequencer (Applied Biosystems) at the Centre of Marine Sciences, University of Algarve, Portugal. We manually scored alleles in STRand software (Toonen and Hughes 2001) using the GeneScan 500 LIZ size standard (Applied Biosystems), and binned them using MsatAllele (Alberto 2009).

We calculated and tested for significance with Genetix v4 (Belkhir et al. 1996) summary statistics of genetic diversity within populations, including standardized allelic richness (Â), private alleles (PA), expected heterozygosity or gene diversity (H_E), observed heterozygosity (H_O), and inbreeding coefficients (F_{IS}). We assessed genetic structure using both population (allele frequency-based) and individual (genotype-based) approaches. We estimated pairwise F_{ST} (θ; Weir and Cockerham 1984) with Genetix. We used the software STRUCTURE 2

of Pritchard et al. (2000) to assess whether genetic groups in the data set of *F. distichus* populations were sampled within the San Francisco Bay. This algorithm estimated the number of genetic groups that minimize Hardy–Weinberg disequilibrium, and assigned a proportion of the genome of each individual to those genetic clusters. It is, therefore, an analysis that is not sensitive to rare alleles but rather represents mating patterns, grouping individuals into clusters more likely to be mating at random.

RESULTS

Distribution Study

We mapped *F. distichus* occurrence as 251 linear segments (32.03 km) and 80 patches (0.13 km) that totaled 32.16 km of linear shoreline in the central bay. Of the 151.73 km of available rocky shoreline throughout the central bay, *F. distichus* occupied about 21% of that distance. The distribution and relative abundance of *F. distichus* are provided in

Table 1 Primer sequences, PCR conditions, and original sources of the 13 microsatellite loci used to screen the populations of *F. distichus*

Locus	Primers	T_a	Cycles	Source
F19	F: AGGTTTCAACCTGCTTCTGG R: TGCTACATCCAAGAATTGCAG	60	35	Coyer et al. 2009
F49	F: TGCTGTAGAAGGCCGAAGTT R: AACGAGTTCGTCGAGTGTCC	60	35	Coyer et al. 2009
F42	F: AGTGTGACTGCCCATTAGGG R: AGACGTAACCCAGTGCTGCT	60	35	Coyer et al. 2009
F50	F: GGTGTTGCTTTTCCGAGTGT R: GGGCGTGTGTCTCTTTGTTC	60	35	Coyer et al. 2009
F47	F: CCCTTGGCAAAGAGCAAATA R: GCAGAAGGAAGGTGGATGAG	60	35	Coyer et al. 2009
F21	F: CATGTAGCGTGAAGCGTTTG R: CACGCAAACAAAACGTCAAC	60	35	Coyer et al. 2009
L94	F: TTAGGAATGGGCGGGATG R: GATTTCGTGAGGCTGGTTCA	57	30	Engel et al. 2003
L20	F: ACTCCATGCTGCGAGACTTC R: CCTCGGTGATCAGCAATCAT	55	30	Engel et al. 2003
F58	F: CGTGTTTTGTCCGTCCTTTT R: CGGAACAGATGGGAGACAAT	60	35	Coyer et al. 2009
F63	F: GGAATCGGTTGGCATTGTAG R: ACCCTTCCGACGAACAATC	60	35	unpublished
F22	F: CCGTCTACGTTTCGTTTCGT R: ATCCGAGAGACGGATAGCAA	58	35	Coyer et al. 2009
F59	F: TCGCCATATCTGTGTCAAGG R: AACAAATTGGTGCCGAGTGT	60	35	Coyer et al. 2009
F64	F: CGAGGAGTGAAGGAGAGCAG R: TTCGAAACGTAGCAAACACG	60	35	unpublished

Figure 4. *F. distichus* was mostly absent from the urbanized San Francisco shoreline within the central bay, except near Marina Green (Figure 4). The fucoid was more prevalent in the western half of the central bay, where we observed high- and medium-density bands of *F. distichus* along the shoreline of Marin County (Fort Baker–Sausalito, Angel Island, eastern Tiburon peninsula) (Figure 4).

We observed *F. distichus* growing on various types of natural and artificial hard surfaces, including bedrock, boulders, cobble, seawalls, and riprap (Figures 6–8). Riprap and bedrock-dominated shores supported the greatest abundance (measured as shoreline length) of *F. distichus* (Table 2). Seawalls and shorelines composed primarily of cobble or boulder had lower abundances of the fucoid.

Overall, *F. distichus* generally formed narrow (1–2 m wide) bands along the shoreline (Figure 9). However, bed widths varied significantly (GLM [Inverse Gaussian], $p < 0.05$, t = 6.039) across the various habitat types (Table 3). Riprap supported marginally greater cover of the alga than bedrock but a substantially greater shoreline length. The width
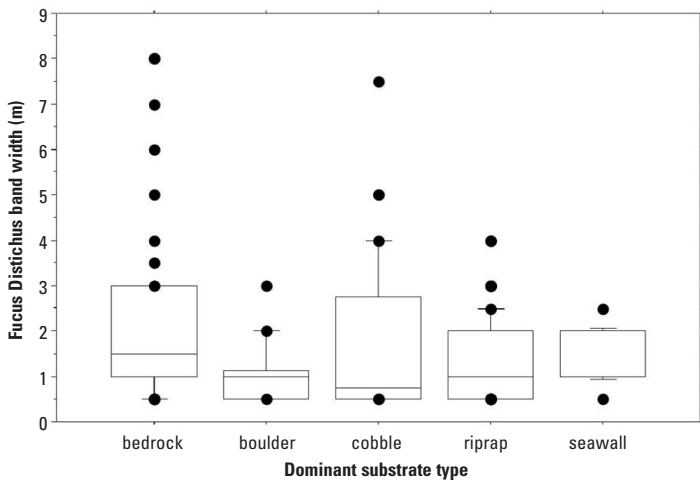

Figure 5 Box plot of *F. distichus* band widths by dominant substratum in central San Francisco Bay, 2012–2013. Horizontal lines starting from bottom to top represent the 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below the 10th percentile and above the 90th percentile lines shown as points.

Figure 6 *F. distichus* (dark brown to olive green blades) broadly distributed along bedrock bench, Alcatraz Island, San Francisco County, November 11, 2012

Figure 7 *F. distichus* at base of seawall shoreline near Marina Green, San Francisco, October 14, 2012

Figure 8 *F. distichus* (dark brown to olive green band) at base of small, riprap shoreline near Fisherman's Wharf, San Francisco, October 14, 2012
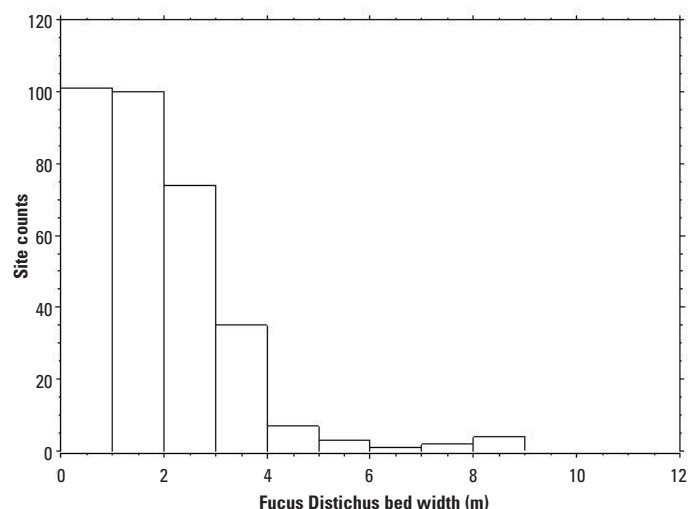

Figure 9 Frequency distribution of *F. distichus* bed widths within the central San Francisco Bay, 2012–2013 ($n = 327$)

of shoreline occupied by the fucoid was generally narrower on riprap than on bedrock shorelines (Figures 5 and 9). Bedrock habitats, which were often composed of gently sloping surfaces, had the highest mean band width of rockweed. One site, in particular, that was composed of bedrock supported a bed of *F. distichus* that measured approximately 8 m wide (Figure 5). Conversely, steep seawalls had the narrowest bed width of 2.5 m (Figure 5).

Table 2 Length (km) of shoreline occupied by *F. distichus*, classified by dominant substrate within the central bay of San Francisco, 2012–2013 (high = 75%–100%, medium = 25%–74%, low = 1%–24% cover)

Subtrate	High	Medium	Low	No data	Grand total
Bedrock	2.15	5.81	1.68	0.11	9.74
Boulder	0.01	0.16	0.85	0.00	1.01
Cobble	0.52	1.23	1.63	0.21	3.59
Riprap	2.12	7.14	7.67	0.00	16.93
Seawall	0.19	0.63	0.07	0.00	0.89
Grand total	4.99	14.96	11.89	0.32	32.16

Table 3 Area (ha) of *F. distichus* by dominant substrate within the central bay of San Francisco Bay, 2012–2013 (high = 75%–100%, medium = 25%–74%, low = 1%–24% cover, present = no cover data)

Subtrate	High	Medium	Low	Present	Grand total
Bedrock	0.77	1.09	0.21	0.01	2.08
Boulder	0.00	0.02	0.08	<0.01	0.09
Cobble	0.21	0.37	0.13	0.08	0.79
Riprap	0.50	1.28	0.61	0.00	2.39
Seawall	0.03	0.09	0.01	0.00	0.12
Grand total	1.51	2.83	1.04	0.10	5.48

Genetics Study

Allele frequencies in *F. distichus* (for the 13 selected loci) are depicted in Figure 10. When we estimated diversity indices, mean allelic richness was very low at all sites, averaging slightly over 1 allele per locust (Table 4; also see Figure 10, which depicts allele frequencies), showing almost no variation within each site. Angel Island and Emeryville Marina had the highest allelic diversities. We found private alleles (unique to a site) in populations at Angel Island (L20, L94, and F47) and Emeryville Marina (F59), but these were nevertheless present in relatively low frequencies.

Gene diversity (expected heterozygosity) was lower than expected at all sites, reflecting the usual dominance (or even fixation) of one allele at each of the loci. Even in the loci where two or more alleles were present, they did not usually appear
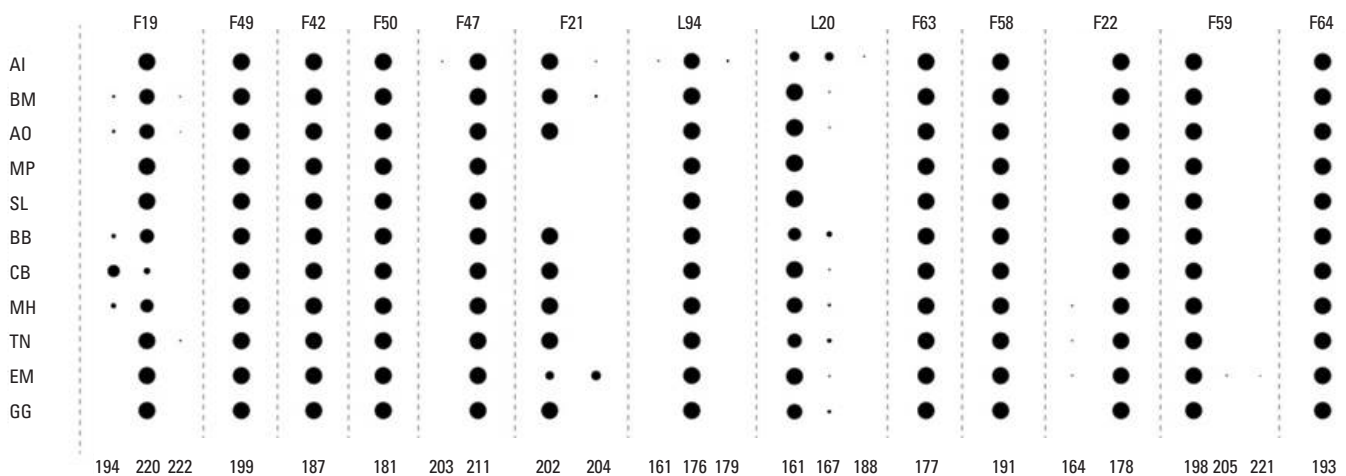
Figure 10 Microsatellite allele frequencies in each sampling site of *F. distichus*. The presence of an allele in a population is indicated by a circle with an area proportional to its frequency. Numbers on top are loci names, on bottom are allele sizes (base pairs).

Table 4 Estimates of Nei's gene diversity (H_E), observed heterozygosity (H_o), multi-locus inbreeding coefficient [F_{IS}, *$p < 0.05$ (1000 permutations)], standardized allelic richness ($\hat{A}$), for a minimum common sample size of N = 47, and average number of private alleles (PA) for each sampling site of *F. distichus*

Site	Code	Coordinates	N	Â	PA	HE	HO	FIS
Angel Island	AI	37.870765, -122.427634	155	1.30	3.078	0.052	0.003	0.941*
Berkeley Marina	BM	37.859777, -122.315521	154	1.26	0.014	0.029	0.004	0.854*
Oakland Airport	OA	37.729155, -122.24877	155	1.14	0.001	0.017	0.003	0.824*
Marina Park	MP	37.694717, -122.190633	48	1	0	0	0	—
San Leandro Marina	SL	37.696862, -122.194305	47	1	0	0	0	—
Ballena Bay	BB	37.764586, -122.286605	48	1.15	0	0.054	0	1.000*
Crown Beach	CB	37.767519, -122.279235	47	1.15	0	0.037	0.007	0.825*
Oakland Middle Harbor	MH	37.800604, -122.329398	47	1.23	0	0.044	0.010	0.777*
Treasure Island North	TN	37.830454, -122.368142	48	1.23	0.242	0.031	0.003	0.894*
Emeryville Marina	EM	37.841123, -122.315007	47	1.39	2.006	0.049	0.017	0.655*
Golden Gate Fields	GG	37.887879, -122.315991	48	1.08	0	0.017	0.007	0.624*

in the same individual. Individuals tended to occur as homozygotes more frequently than what would be expected if they crossed at random (in the Hardy–Weinberg equilibrium). The inbreeding coefficients (F_{IS}) confirmed the significant ($p < 0.05$ [1000 permutations]) deficit of heterozygotes at all sites (Table 4). This was particularly shown in loci F19, L94, and L20 (data not shown). These levels of inbreeding were very extreme (e.g., $F_{IS} = 1$ means that in all individuals sampled at that site, there was not one single heterozygote).

Despite the low allelic diversity, most pairs of sites differed significantly from each other as assessed through F_{ST} (Table 5). The structure analysis (which looks for clusters that minimize departures from random mating) of populations of *F. distichus* inside San Francisco Bay (Figure 4), distinguished unique populations at Angel Island, the Berkeley Marina, and the Emeryville Marina (Figure 4). By contrast, populations at Crown Beach and Oakland Airport clustered together, as did populations of *F. distichus* at all remaining sites (Figure 4). These results suggest that some genetic exchange occurs among sites that

Table 5 Estimates of pairwise differentiation [F_{ST} (θ)] between sites of *F. distichus*. Significant F_{ST} values (1000 permutations) are depicted in bold. Site codes as in Table 4.

	AI	BM	OA	MP	SL	BB	CB	MH	TN	EM
AI	—									
BM	0.348	—								
OA	0.359	−0.005	—							
MP	0.332	0.094	0.078	—						
SL	0.331	0.094	0.078	0	—					
BB	0.100	0.159	0.175	0.239	0.237	—				
CB	0.534	0.540	0.528	0.660	0.658	0.339	—			
MH	0.227	0.050	0.062	0.187	0.185	0.037	0.279	—		
TN	0.126	0.126	0.136	0.173	0.172	0.040	0.502	0.080	—	
EM	0.288	0.065	0.054	0.038	0.038	0.170	0.589	0.125	0.097	—
GG	0.183	0.111	0.117	0.137	0.135	0.100	0.576	0.119	0.011	0.059

cluster together, despite the predominant selfing revealed by the inbreeding coefficient levels (Table 4).

DISCUSSION

An objective of the Cosco Busan Damage Assessment and Restoration Plan involved actively restoring 2,000 linear meters of *F. distichus* within the central San Francisco Bay. Before this study, little information existed on the distribution or relative abundance of the fucoid throughout the Bay. This information is essential to inform future restoration activities involving *F. distichus*, and to determine potential donor sites.

Approximately 21% (32.16 km) of the available rocky shoreline throughout the study area was occupied by *F. distichus*. The greatest abundance of the rockweed occurred in the western portion of the central bay (Marin County), which, coincidentally, was the region in the study area that received the lowest amount of oiling observed during the Cosco Busan incident (Figure 1). In contrast, *F. distichus* was scarce or absent from the urbanized San Francisco shoreline which included several of the sites that were cleaned using high-pressure hot-water washing (Figure 1). These hot-water treatments occurred at Treasure Island (City of San Francisco), Middle Harbor Shoreline Park (City of Oakland), Berkeley Marina (City of Berkeley), Albany Bulb (City of Albany), Point Isabel (City of Richmond), Shimada Friendship

Park (City of Richmond), and Belvedere (City of Tiburon) (CBOST 2012, Appendix F). However, whether these areas supported viable assemblages of *F. distichus* before the oil spill is unknown (CBOST 2012, Appendix F).

Areas chosen for high-pressure hot-water clean-up consisted primarily of artificial hard substrata such as riprap. These locations are likely to be of high priority for future restorative actions (CBOST 2012). One possible out-planting strategy for these damaged areas may involve translocating entire rocks with mature *F. distichus* thalli into candidate restoration sites as recommended, in part, by Whitaker et al. (2010). Rocks from areas of cobble, small riprap, or boulders could potentially be translocated for restoration. But throughout the study area, cobble-dominated habitats supported relatively low abundances of the rockweed. In contrast, riprap habitats hosted the greatest quantity (measured as shoreline length) of *F. distichus* compared to all other hard subtrata types, including bedrock. Most of the riprap occupied by the study species also appeared appropriately sized for hand-carrying without special tools or equipment being needed. Thus, the availability of donor material is not likely to be a limiting factor for future restoration actions.

To our knowledge, transplantations involving rocky intertidal species for the purpose of restoration have rarely been conducted. We identified only a few case studies in the literature, two of which involved

the use of fucoids (see Stekoll and Deysher 1996; Whitaker et al. 2010). The results of both studies suggest that fucoids may be good candidates for re-establishment for ecological purposes, assuming that certain techniques are utilized and some environmental and biological factors are taken into account. For example, Whitaker et al. (2010) successfully re-established the fucoid *Silvetia compressa* at a southern California study site by transplanting larger, reproductively fertile thalli. The relocation of smaller, non-reproductive thalli resulted in significantly lower survival. Seeding was also ineffective (Whitaker et al. 2010). Stekoll and Deysher (1996) experienced similar failure using seeding methods to re-introduce *F. distichus* on Alaskan rocky shores after the Exxon Valdez oil spill, yet transplanted fertile adult thalli slightly increased recruitment. For fucoids in general, recruitment appears to be most restricted during early embryogenesis (Brawley and Johnson 1991; Brawley et al. 1999).

Another factor that should be considered before restoration is within-species genetic variability, since it is crucial to long-term population sustainability (Frankham at al. 2004). This is particularly true for a habitat-forming species such as *F. distichus*, which provides ecosystem services for a multitude of rocky intertidal organisms. Effort should be made, however, to retain natural levels of genetic variability when algae is transplanted for restoration. In the case of fucoids that have low genetic variability, transplants should be taken from donor sites that are as near the recipient sites as possible to promote populations that are genetically similar to what would have occurred previously in the damaged area.

Microsatellite genotyping revealed exceptionally low allelic richness and almost no heterozygosity in the *F. distichus* populations at all 11 sites studied in the San Francisco Bay (Figure 10). This could, hypothetically, be a result of a strong recent bottleneck or a recent founder effect in the origin of the colonization of the bay. Alternatively, extreme levels of inbreeding and limited dispersal could cause the traits of these populations. Extreme inbreeding would not be surprising since *F. distichus*, like all hermaphroditic fucoid species, is capable of self fertilization (e.g., Pollock 1970; Coleman and Brawley 2005). As indicated, *F. distichus* is a self-compatible

hermaphrodite, which provides a mechanism for gametes to self-fertilize. Additionally, the study species can typically only disperse its gametes short distances away from parent populations since water motion inhibits gamete release (Pearson and Brawley 1996; Serrao et al. 1996) and zygotes are non-motile and sticky (Moss 1975; Hardy and Moss 1979; Norton 1981). These two life history traits, shared by many fucoids including *F. distichus*, together may account for the extremely low genetic diversity results we observed here.

Low genetic diversity and high inbreeding have also been documented for other selfing hermaphroditic fucoid species (e.g., Coleman and Brawley 2005; Perrin et al. 2007). Using the rockweed *Silvetia compressa*, a species that has mating and dispersal mechanisms similar to that of *F. distichus*, Williams and DiFiori (1996) determined that heterozygosity was exceptionally low—comparable to the results of this study in which each locus was dominated by only one allele (in most cases). We encountered rare alleles only in thalli distributed near the landward edge of the study population, which suggests that reproductive isolation may be possible at remarkably small spatial scales (Williams and DiFiori 1996). This is even more striking in enclosed environments such as Yaquina Bay (Oregon). There, distinct genetic types of this same species, *F. distichus*, were found to co-occur at very small scales, and reproductive isolation was maintained in populations that occurred side by side (Neiva et al. 2012).

Genetic structure estimated by F_{ST} appeared stronger than expected for populations of *F. distichus* analyzed throughout the bay, particularly since allelic diversity was so low. However, these levels of population differentiation must be interpreted with caution. Pairwise F_{ST} values may not be a good measure of population differentiation for populations with very low intra-population diversity (Meirmans and Hedrick 2011). In such cases, F_{ST} becomes overly sensitive to even minor differences in allele frequencies between populations. F_{ST} is an index that is very sensitive to population genetic diversity levels (i.e., in low-diversity populations, F_{ST} increases with small differences; whereas in high-diversity populations, it is hard to find significant differentiation, despite many differences). Differentiation between some of the sites in the

central bay was supported by a distinct analysis (structure), although in this case the analysis is also influenced by highly inbred data. Globally, when the very low diversity and high inbreeding present all across every population in the Bay are considered, it can be inferred that the spatial trends in population differentiation have weak conservation significance.

CONCLUSIONS

Fucoid populations in central San Francisco Bay seem to reproduce mainly by selfing, maintaining distinct alleles in predominantly homozygous states within populations. We identified some unique private alleles in some populations, but with low frequencies (rare alleles). We have determined translocation of naturally-existing *F. distichus* to be the preferred method for rockweed restoration; several locations throughout the central bay may provide potential donor sites. These areas are primarily composed of small riprap with medium to high densities of *F. distichus*. Depending on the location, it will be possible to translocate entire rocks with rockweed attached into candidate restoration sites. Our genetic assessment in San Francisco Bay indicates significant geographic structure in populations of *F. distichus*, and provides a basis for selection of donor populations (for use in restoration) that would minimize mixing of genetically distinct populations. Results suggest that, for effective restoration, the choice of donor sites should be based mostly on practical criteria.

ACKNOWLEDGEMENTS

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