

Exotic Oyster Survey, Removal and Research in San Francisco Bay

Second Progress Report, covering the 2008 and 2009 field seasons

Final Report, February 12, 2010

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Summary

- *There is significant potential for the exotic oyster, **Crassostrea gigas**, to become established in San Francisco Bay, and to have substantial harmful impacts on existing organisms, on native oyster restoration efforts, and on the restoration of intertidal and subtidal habitats. The oyster removal project was launched to try and prevent the establishment of **C. gigas** in the Bay.*
- *From its inception, the project has been hampered by sporadic and uncertain funding, so that it has been possible to conduct only limited or no field work during the peak field season in most years. Current funding will end in the spring of 2010.*
- *Despite these limitations, the data show that the project has been progressively reducing the **C. gigas** population in San Francisco Bay each year. There is every reason to believe that eradication will be achieved if the project is properly funded.*
- *Accepted best practice for an eradication effort is to begin eradication efforts as soon after discovery as possible, work systematically and rapidly until all of the population that can be targeted is removed, and then monitor and follow-up as needed for several years to ensure that the population does not regrow. While some eradications may succeed without following this practice, not following it risks losing any gains that have been achieved. In the current project, eradication work began 10 days after the population was discovered, but funding has been erratic, and future funding is uncertain.*

Background: Activities through 2007

In June 2004, UC Davis student Andy Chang discovered the empty shell of a large and clearly nonnative oyster attached to a rock near the eastern end of the Dumbarton

Bridge. In late July 2006, Rena Obernolte found five large live oysters in the same area while conducting a survey for native oysters, and notified us. Our initial search yielded additional large oysters that we subsequently identified through genetic analysis as the Pacific Oyster *Crassostrea gigas*, which is native to Japan.

We consulted with the relevant agencies to determine what, if anything, should be done. Oysters produce enormous numbers of tiny drifting larvae, and a well-established population would probably be impossible to eradicate. However, although *C. gigas* had been raised in large numbers in Central California oyster farms for many decades, it had rarely reproduced here. So if the conditions in the Bay were not particularly hospitable to reproduction by these oysters, and if the current population was relatively small and limited in distribution, it might be possible to remove a large enough portion of the population to reduce the likelihood of permanent establishment.

Encouraged by several agencies, with assistance of various types from CDFG, USFWS/Don Edwards NWR, the East Bay Regional Parks District (EBRPD), Save the Bay and from students, researchers and other volunteers, and initial funding from the San Francisco Bay Joint Venture (SFBJV), we mobilized an effort to survey and collect as many of the nonnative oysters as we could during the last good low tides of the 2006 season. With the information gained, we convened a meeting of interested agencies and organizations in September 2006 (Table 1). The consensus of this group was that

Table 1. Participants in the September 11, 2006 planning meeting.

Participant	Affiliation
Rachel Barnett	California Department of Water Resources
Marcia Brockbank	San Francisco Estuary Project [1,2]
Andrew Cohen	San Francisco Estuary Institute [3]
Natalie Cosentino-Manning	National Oceanographic and Atmospheric Administration
Abe Doherty	California State Coastal Conservancy [4]
Jennifer Feinberg	San Francisco Bay Conservation and Development Commission
Julie Horenstein	California Department of Fish and Game
Mike Koslosky	Hayward Area Parks and Recreation District [2]
Susan Ma	US Army Corps of Engineers
Lia McLaughlin	US Fish and Wildlife Service
Tom Moore	California Department of Fish and Game [2]
Caitlin Sweeney	San Francisco Bay Conservation and Development Commission
Mark Taylor	East Bay Regional Park District
Claire Thorp	National Fish and Wildlife Foundation

[1] Also participated on behalf of the San Francisco Bay Regional Water Quality Control Board.

[2] Now retired.

[3] Now at the Center for Research on Aquatic Bioinvasions.

[4] Now at the California Ocean Protection Council.

Table 2. Funding awarded through January 2010.

Source	Amount	Purpose	Date Funds First Available – End Date	Balance Remaining 1/1/10
SFBJV	\$2,000	Survey/removal	Aug. 2006	\$0
SCC	\$25,000	Survey/removal	5/8/07–3/31/08	\$0
RMP	\$30,000	Survey and research	5/11/07–12/31/08	\$0
NFWF	\$46,895	Survey/removal, research, outreach	12/12/07–9/30/08	\$0
SCC	\$109,476	Survey/removal, research, outreach	8/12/08–3/31/10 [1]	\$48,936

[1] The end date is being amended to 6/30/10.

we should seek funds to continue the survey and removal effort and to investigate certain key questions. Partway through 2007 we were awarded funding from the California State Coastal Conservancy (SCC) and the Regional Monitoring Program for Trace Substances (RMP) (Table 2), and conducted an abbreviated field season. The remainder of these funds, plus additional funding received from the National Fish and Wildlife Foundation (NFWF) at the end of 2007 provided sufficient funding for a full field season in 2008.

In January 2008 we held the first official meeting of the Advisory Panel (Table 3). The Panel reviewed the work to date including the draft of the First Annual Progress Report, confirmed that the oysters posed a substantial and immediate threat to the Bay, and approved an expanded effort to survey and remove the oysters and to research questions about sources, vectors, and factors facilitating settlement. The expanded effort was expected to cost around \$150,000/year and to continue over several years. Several key pieces of information supported the decision to expand the effort:

- Preliminary results from shell isotope analyses suggested that the *C. gigas* population centered in the South Bay consisted of at least two year-classes—thus successful reproduction and settlement in the Bay was not a freak, one-time occurrence.
- We found measurable morphological differences between the South Bay population and the illegally planted Loch Lomond population, with the Loch Lomond population resembling *C. gigas* as it was generally known and the South Bay population differing from it. The cause of the morphological differences was not known, but could be due to the South Bay population being genetically distinct. This in turn would be consistent with a possible explanation for the oyster's successful settlement in Central California after many decades of failing to do so: that current settlement was due to the introduction of a population that was genetically better adapted to conditions in the region. On the other hand, the morphological differences might be due to differences in either habitat conditions or exposure to contaminants.

Table 3. Advisory Panel members (January 2008).

Member	Affiliation
Joy Albertson	US Fish and Wildlife Service
Pete Alexander	East Bay Regional Park District [1]
William Brostoff	US Army Corps of Engineers
Adrian Deponte	Hayward Area Parks and Recreation District
Abe Doherty	California State Coastal Conservancy [1,2]
Naomi Feger	SF Bay Regional Water Quality Control Board [1]
John Finger	Hog Island Oyster Company
Tom Hall	EOA Consultants [1,3]
Beth Huning	San Francisco Bay Joint Venture
Judy Kelly	San Francisco Estuary Project
John Krause	California Department of Fish and Game
Peter Lacivita	US Army Corps of Engineers [1]
Marilyn Latta	Save the Bay [4]
Susan Ma	US Army Corps of Engineers
Karen McDowell	San Francisco Estuary Project [1]
Tom Moore	California Department of Fish and Game
Frances Parchaso	US Geological Survey [1]
Korie Schaeffer	National Oceanographic and Atmospheric Administration [1]
Delmarie Snodgrass	San Leandro Marina
Caitlin Sweeney	SF Bay Conservation and Development Commission
Mark Taylor	East Bay Regional Park District [1]
Claire Thorp	National Fish and Wildlife Foundation
Tanya Veldhuizen	California Department of Water Resources
Kim Ward	State Water Resources Control Board

[1] Participated in the Jan. 14, 2008 Panel meeting.

[2] Now at the California Ocean Protection Council.

[3] Representing the South Bay POTW segment of the Regional Monitoring Program.

[4] Now at the California State Coastal Conservancy.

- The surveys found *C. gigas* to be present mainly in two locations, at Loch Lomond and along the eastern shore of the South Bay from around Dumbarton Point to the San Leandro Marina; and found no evidence of a subtidal population.
- On repeated site visits, the number of *C. gigas* that were found declined. It thus appeared that survey and removal by hand could remove most or all *C. gigas* from a site; and there was no indication of settlement of a new year-class since the effort began in summer 2006.

These factors provided additional support for trying to prevent the establishment of the oyster, and showed that the effort was making progress and had a reasonable chance of success. The meeting produced a statement of conclusions that urged CDFG to help obtain funding for an expanded effort from the Wildlife Conservation Board (WCB) and to remove *C. gigas* from an illegally planted site at Loch Lomond in Marin County. Subsequent to the meeting we also began working with Abe Doherty to obtain additional funding from SCC. The Progress Report was amended to include the meeting results and sent as a final report to all Panel members.

Overview of 2008-2009: Funding and Work Status

In 2008 we received an additional award of \$225,000 from SCC and were working on obtaining another \$225,000 from the WCB, which would have supported an expanded program at \$150,000/year for three years from 2009 through 2011. By the fall we had received the support of WCB staff and assembled the necessary paperwork, and were waiting for an opportunity to present the project to the Board. The award of the SCC funds and the expectation of funding from WCB allowed us to begin systematically planning and organizing our field work for a multi-year effort, as described below. The final approval and release of the SCC funds came in August 2008. After using up the remaining funds that we had received from other sources, we began drawing on the SCC funds in November 2008. At that time we were planning and organizing for the 2009 field season, preparing the Annual Progress Report, and planning for the annual Advisory Panel meeting.

On December 19, 2008 we received a stop-work order from SCC, because of the state budget crisis. This left us with no funding for the project, and we ceased work on all activities. WCB funding was also put on hold. In January 2009 we sent the Advisory Panel a brief update on the funding and work status in lieu of an annual report.

At times during the first half of 2009, SCC indicated that if we did some work on the project we would be able to bill for it retroactively when funding was reactivated, although they could not say when that would occur or guarantee that it would occur. We did a limited amount of field work during this period. On July 14 SCC sent us a restart notice stating that part of the funding was available but would need to be used by March 31, 2010, and that we would need to draft a new work plan to reflect these changes. By mid-August we had submitted and SCC had approved a new work plan. The total amount made available under both the initial and revised work plans (from August 2008 through March 2010) was \$109,476. It is unclear at this point when or if the remainder of the \$225,000 originally awarded by SCC will become available. WCB staff do not expect to have any funding available for new projects until at least 2011; and the WCB staff person we've mainly been working with is retiring in June 2010.

Field work in 2006-2009 and planned for Dec. 2009 to Mar. 2010

All of the field work has been conducted in the intertidal zone during low tides. As shown in Table 4, in San Francisco Bay low tides that are good for field work do not occur evenly throughout the year. The main field season runs from December through August, with virtually no good low tides in the fall. By far the best months, with the largest number of workable low tides and the lowest low tides are April through July. As the above overview of the project suggests, we were unable to conduct field work during the peak low tide periods in most years. In 2006 the oysters were not discovered until the end of July; most of the work that year was done during a single low tide series in August. In 2007, funding did not become available until mid-May; because of necessary planning and organizing, we missed all of the low tide work windows in April, May and part of June. We did have continuous funding in 2008, and were able to work the full season. We expected to have expanded funding in 2009, and began planning a full field season; but because of the stop-work order we had no funding, though we did some limited work. Funding became available again in August 2009, just after the peak field months of 2009, and was initially scheduled to end in March 2010, just before the peak field months of 2010. We are currently working with Lisa Ames at SCC to amend the work plan to allow us to do some field work during the peak period of low tides between March 31 and June 30, 2010. The seasonal variation in daytime low tides should be borne in mind when considering the work that has been completed and what could be accomplished if consistent funding were available.

Table 4. Monthly daytime low tide statistics for San Francisco Bay. These statistics, estimated from tide tables for zones with an approximately 80 minute time lag relative to the Golden Gate (e.g. around the San Mateo Bridge in the south and around Point Pinole in the north), indicate the relative availability of tides suitable for field work in different months of the year. Field work is usually planned only for days when the predicted water surface is below 2' MLLW for at least 4 daytime hours. In practice, we maximize the available work days by considering the predicted tide levels, sunrise and sunset times, and field work locations (tides occur earlier toward the mouth of the Bay and later toward the head of the estuary).

Month	Lowest daytime tide (ft MLLW)	Number of days with tide below MLLW during daylight hours
Jan 2010	-1.0'	7
Feb 2010	-0.7'	8
Mar 2010	-0.3'	7
Apr 2010	-1.3'	17
May 2010	-1.4'	20
Jun 2010	-1.6'	17
Jul 2010	-1.5'	12
Aug 2010	-0.9'	6
Sep 2010	-0.4'	1
Oct 2010	0.2'	1
Nov 2010	0.0'	3
Dec 2010	-0.6'	6

The four maps below (Figure 1) show the areas surveyed and the oysters collected in water years 2006-2009.¹ The total number collected has declined each year, as has the number collected in successive years at sites where surveys were conducted in more than one year (Table 5 and Figure 2). Although the level of effort was not always the same in each year, these results nonetheless show that the number of *C. gigas* at individual survey sites and in the Bay as a whole is being progressively reduced over time.

Table 5. Total *Crassostrea gigas* (live + dead) collected in San Francisco Bay in the 2006–2009 water years (Oct. 1-Sept. 30).

Site	2006	2007	2008	2009
Vallejo	1		3	
Sausalito				1
Alcatraz	1			
Richmond	1			
Treasure Island			2	
Oakland				5
Alameda				1
Bay Farm Island				6
San Leandro Marina		4	1	2
San Leandro Marina to Sulphur Creek	4			
Hayward Landing	46	37		11
Hayward Landing to San Mateo Bridge			18	
San Mateo Bridge (east side)			22	
Eden Landing North		2		29
Eden Landing South		55	50	5
South Bay Wreck			13	
Coyote Slough to Ideal Marsh	79	79	13	17
Ideal Marsh to Dumbarton Hwy Bridge	111	25		10
Dumbarton Highway Bridge (east side)	1	5		
Dumbarton Fishing Pier (east side)	3			
Dumbarton Hwy to RR Bridge	20	4	13	16
Dumbarton Railroad Bridge (east side)		4		
Foster City	2	2		
Cooley Landing			1	
Total	269	217	136	103

¹ In addition, Andy Chang collected a pair of attached *C. gigas* valves in 2004. We organized the collection data by water years (which run from Oct. 1 to Sept. 30) since they correspond to our field season, with the poorest working tides in September and October (see Table 4).

Figure 1. Surveyed areas and numbers of total *Crassostrea gigas* (live + dead) collected in San Francisco Bay in the 2006, 2007, 2008 and 2009 water years (Oct. 1-Sept. 30). These numbers do not include the *C. gigas* collected from the illegally planted site at Loch Lomond, or unattached *Crassostrea* valves without ligaments or soft tissue which were assumed to be remnants either from earlier commercial or experimental plantings or dried shell used by recent native oyster restoration programs.

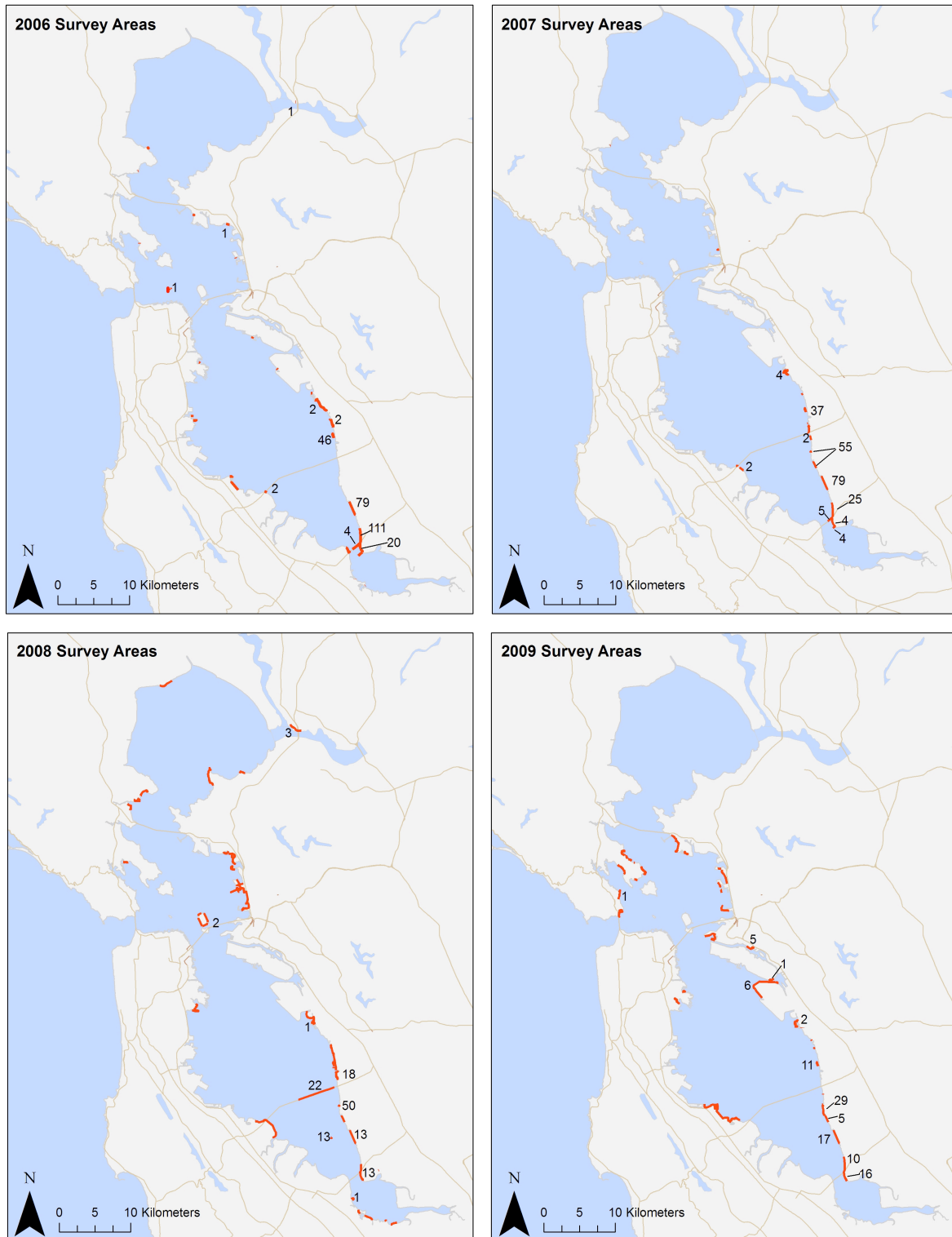
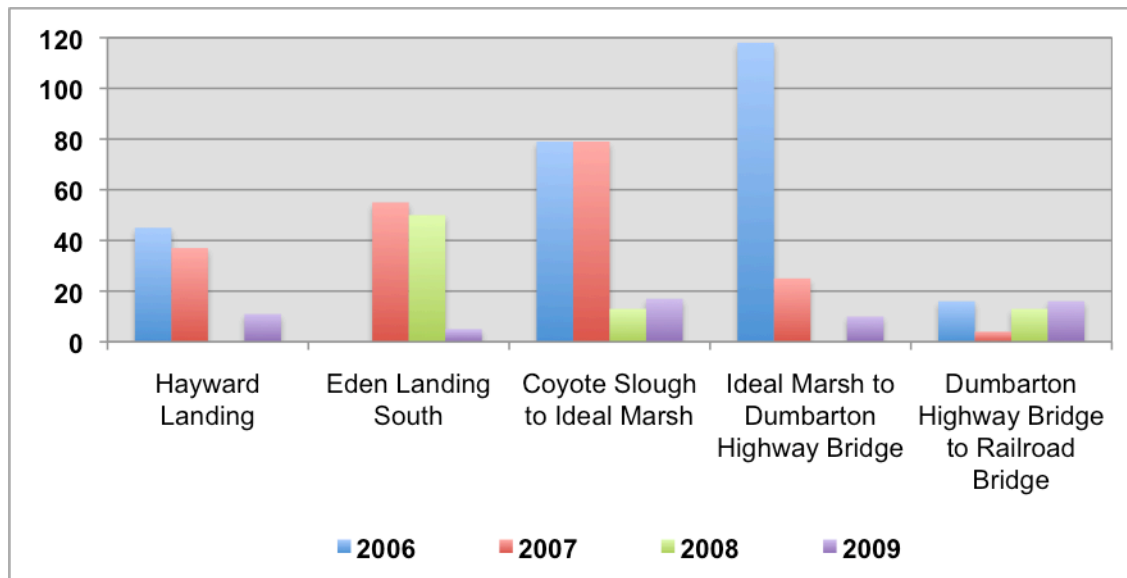


Figure 2. Numbers of total *Crassostrea gigas* (live and dead) collected at selected San Francisco Bay sites in 2006, 2007, 2008 and/or 2009 water years (Oct. 1-Sept. 30), where surveys have been conducted in more than one year. In years when no oysters were collected, no survey was conducted at the site.



In addition to these surveys, we assisted CDFG in removing *C. gigas* from a site near the Loch Lomond Marina in Marin County that had been illegally planted with Pacific Oyster spat, which apparently had been purchased from the Coast Oyster Company in 1999 (T. Moore, pers. comm.). This planting was discovered in 2006, and in January 2007 the Advisory Panel recommended that we make it a priority to work with CDFG to remove them. We collected a substantial number from the site in 2007; and in an effort organized by Tom Moore in May 2008, we joined about 20 mostly CDFG staff to remove oysters from the site. We estimated that over 1,000 oysters were taken, but that at least one additional removal should be done at the site within the next year.

In 2009, CRAB began work on a small eradication project for the Atlantic snail, *Littorina littorea*, which had been discovered in the Bay in three small populations in recent years. The project consists of intensive surveys of the sites where *L. littorea* has been found, marking off side-by-side vertical transects (high to low tide) about 1-2 m wide in upper intertidal rocky substrate, the same type of habitat where *C. gigas* has been found in the Bay, and successively searching every appropriate rock surface for a small (about 0.75-1" high) snail. Many of the individuals conducting the *Littorina* field work had also worked on the *C. gigas* surveys, so the *Littorina* work effectively also served as intensive surveys for *C. gigas* over restricted areas. In a *Littorina* survey at Dumbarton Point we found two live *C. gigas*, although we had already surveyed the site several times (though not as intensively) for *C. gigas*. We decided that it would be worthwhile to conduct intensive surveys of this type targeting *C. gigas* at selected sites where we had previously found high densities of *C. gigas*, and that these would serve as a check of the effectiveness of our regular surveys.

As mentioned above, in late 2008, with funding awarded from SCC and anticipated from WCB, we began systematic planning and for a multi-year field effort. As part of this work Alicia Gilbreath began mapping the Bay according by habitat types and the estimated difficulty of surveying for *C. gigas*, using satellite/aerial imagery. Results from this work are shown in Figures 3 and 4.

As described earlier, our current SCC work plan requires us to complete any work done with the available funding by March 31, 2010 (or June 30, 2010, if we are successful in amending the work plan), and it is not known when or if the remainder of the award made in 2008 will become available. We therefore propose to focus our field work using the current funding on (1) removing as many *C. gigas* as possible from sites where we have found them in the past and where we think there may still be significant numbers; (2) assessing the efficacy of our removal work at a few selected sites with intensive, vertical transect surveys; (3) further removal at the Illegally planted site near the Loch Lomond Marina, if this can be coordinated with CDFG; and (4) searching additional sites that are near sites where we have found *C. gigas* populations or outliers in the past, concentrating on areas that are relatively easily searched (*i.e.* “Walkable Shoreline” where permitting issues do not present an obstacle). Table 6 provides an initial list of priority sites; some that require co-ordination with or approvals by various agencies may not be feasible during this period.

Figure 3. General classification and survey status of San Francisco Bay shoreline. In this map the shoreline is classified into one of three categories based on satellite/aerial imagery and survey records: shoreline that lacks any significant hard substrate for *C. gigas* to settle on (green line, “Marsh or Beach”); shoreline with appropriate habitat for *C. gigas* which has been surveyed (blue line, “Surveyed”); and shoreline with appropriate habitat which has not been surveyed (red line, “Unsurveyed”).

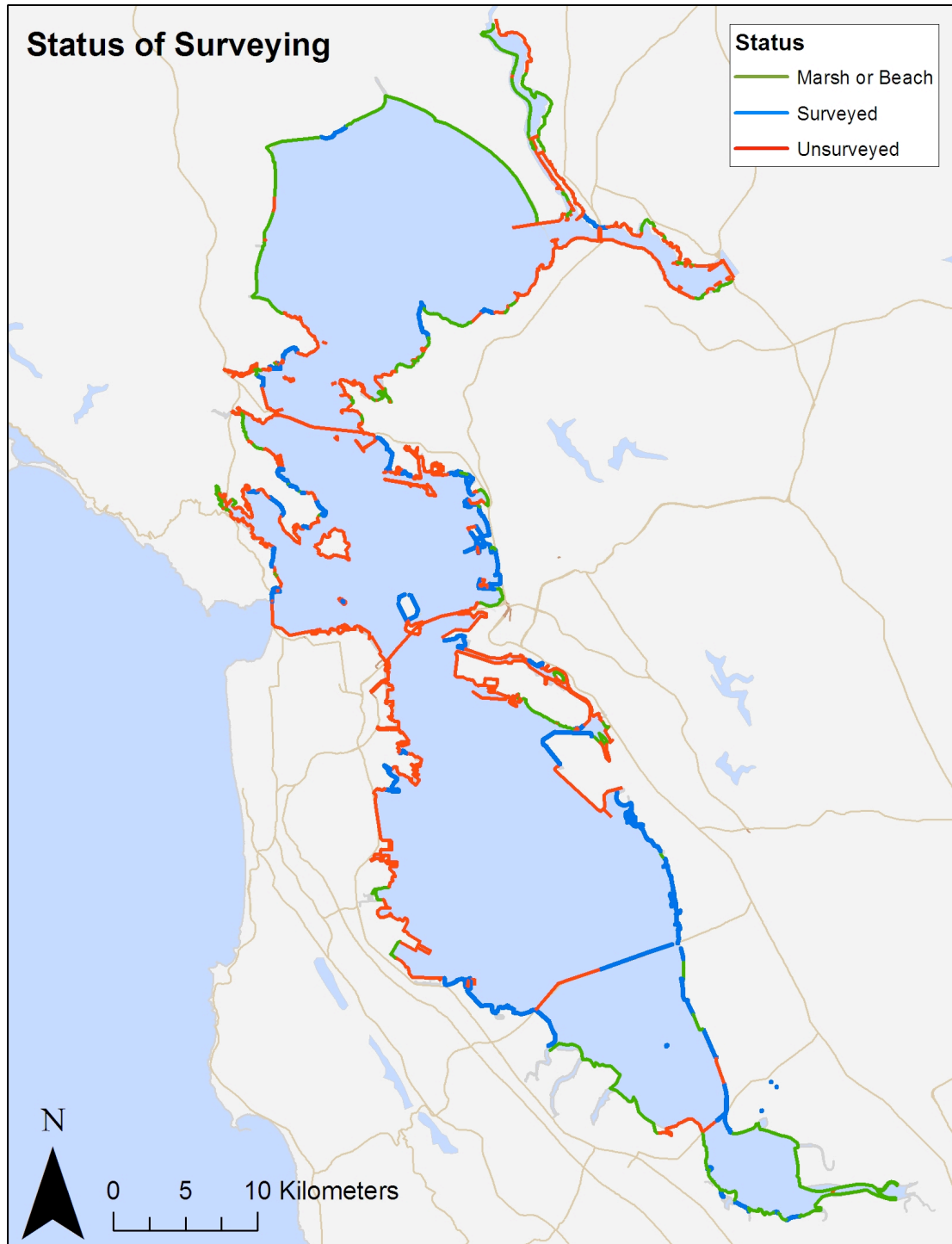


Figure 4. Classification of unsurveyed San Francisco Bay shoreline. In this map the unsurveyed shoreline (red line in Figure 3), is classified into one of three categories based on satellite/aerial imagery: shoreline with hard substrate and with some sediment surface exposed at low tide bayward of the hard substrate (teal line, “Walkable Shoreline”); shoreline with riprap or bedrock that descends directly into the water at low tide, without any exposed band of sediment bayward of it (gold line, “Difficult Shoreline”); and shoreline that has a dense concentration of artificial structures (e.g. ports, marinas, urban or industrial areas with seawalls or wharves), and structures such as bridges and long piers (purple line, “Structured Shoreline”). One site classified as “Marsh or Beach” in Figure 3—Ideal Marsh in the South Bay, which is bracketed by areas that had relatively high densities of *C. gigas* and which some imagery suggests may have appropriate habitat for *C. gigas* along parts of its bayward edge—is included in the “Walkable Shoreline” category.

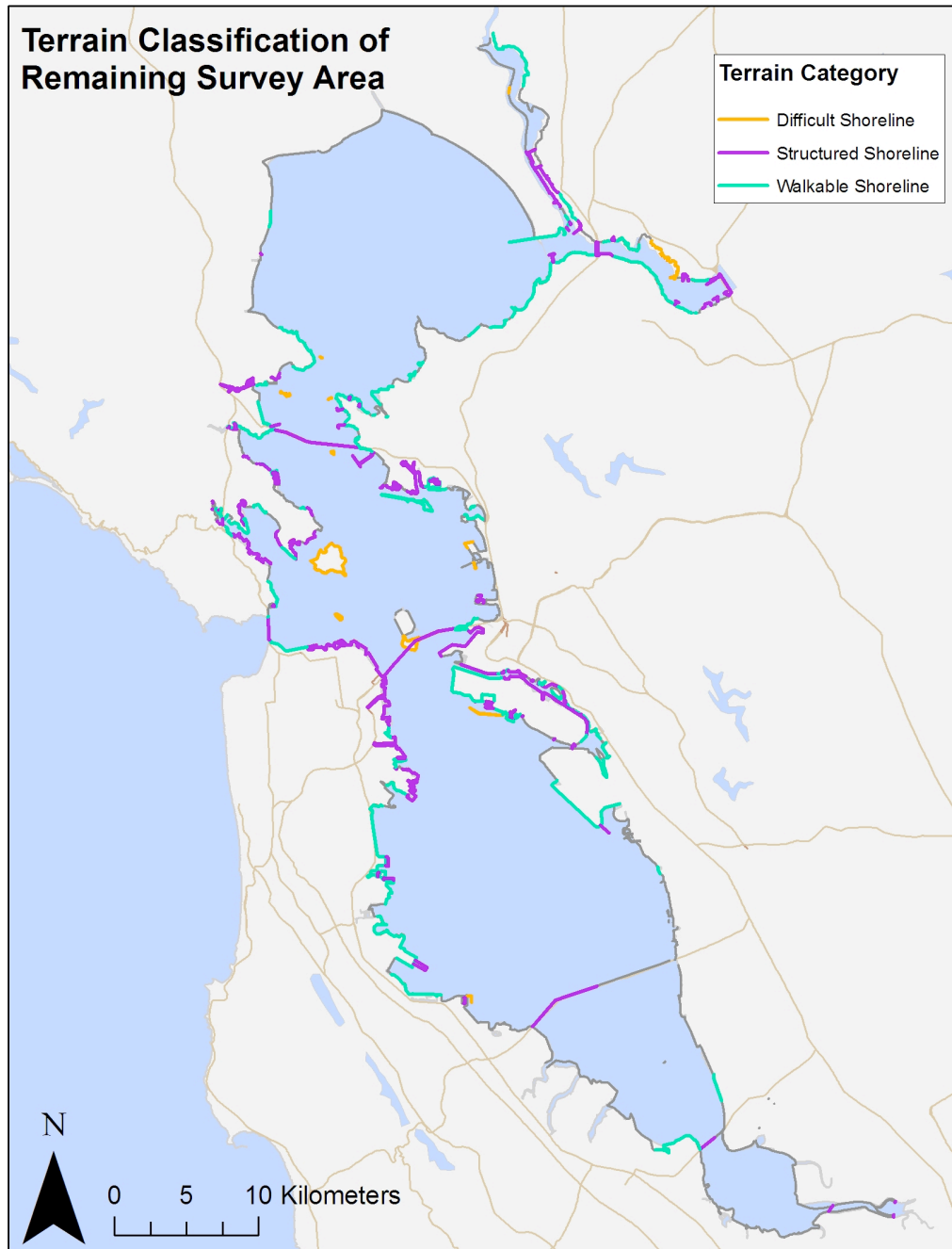


Table 6. Priority sites for *Crassostrea gigas* surveys in San Francisco Bay in Dec. 2009 to June 2010. Priority types are as described in the text on page 10.

Site	Priority Type	Comment
Vallejo and vicinity	(4)	
Loch Lomond vicinity	(4)	
Loch Lomond, site of illegal planting	(3)	If it can be arranged with CDFG.
Oakland Estuary to San Leandro Bay	(4)	
Oakland Airport perimeter	(4)	If it can be arranged with the airport.
Hayward Landing	(1),(2)	
Hayward Landing to San Mateo Bridge	(1)	
San Mateo Bridge east side	(1)	If airboat can be arranged for a good low tide.
San Mateo Bridge channel toward east side	(4)	If boat can be arranged for a good low tide.
Eden Landing North	(1),(2)	
Eden Landing South	(1),(2)	
South Bay Wreck	(1)	If it can be arranged for a good low tide.
Coyote Slough to Ideal Marsh	(1),(2)	
Bayward edge of Ideal Marsh	(4)	If timing is approved by FWS/Don Edwards.
Ideal Marsh to Dumbarton Highway Bridge	(1)	
Dumbarton Bridges channel and landward	(4)	If boat can be arranged for a good low tide.
Dumbarton Highway Bridge to RR Bridge	(1)	
Ravenswood Slough to Dumbarton Hwy Br	(4)	

Research

Research has been conducted mainly in three areas: analyses of shell sizes and morphologies to assess the presence of multiple cohorts and distinct populations; genetic analysis to identify the oysters and to try to determine their source population; and sclerochronological analysis to estimate the age of oysters at the time of death or collection and the number and dates of distinct settlement events.

We measured and recorded the height of most *C. gigas* and the length and width of some *C. gigas* collected in 2006, and the height, length and width of all *C. gigas* collected in 2007, 2008 and 2009. Multiple cohorts cannot be distinguished in the size distribution graphs based on height and estimated volume, although the appearance or greater abundance of *C. gigas* in the smallest size classes (60-80 mm in height, ≤ 100 cm³ in estimated ellipsoid volume) in the 2008 and 2009 collections suggests a possible small recruitment event in 2006 or 2007. The *C. gigas* collected in San Francisco Bay range in size from a 63 mm high *C. gigas* collected live in June 2009 to a 223 mm high *C. gigas* collected live in August 2006; and in terms of volume, they range from a 47

cm³ (estimated ellipsoid volume) *C. gigas* collected live in June 2009 to an 810 cm³ *C. gigas* collected live in May 2008.² This broad size range would seem to indicate that more than one annual cohort is present; however, there is also a very large size range in the live-collected oysters from Loch Lomond—from 34 to at least 190 mm in height and from 4 to at least 491 cm³ in estimated ellipsoid volume—and these oysters are believed by CDFG to have all grown from a single planting of 1999 spat.

The population of San Francisco Bay *C. gigas* outside of the illegally planted site at Loch Lomond differs morphologically from both the Loch Lomond population and from the general conception of *C. gigas* morphology (mainly in having a generally lower height-to-length ratio; in the draft key to the oysters in Coan and Valentich-Scott 2007, *C. gigas* was described as being "frequently twice as long as wide,").³ These morphological differences could be due to genetic differences, growth in different environmental conditions, or differences in contaminant exposures.

The initial genetic analysis, conducted by Patrick Gaffney's laboratory at the University of Delaware, sequenced mitochondrial DNA (16S and COI genes) and identified the large exotic oysters as *Crassostrea gigas*. Oysters within the size range of *Ostrea lurida* (12-44 mm high) collected from the South Bay in 2006 were confirmed to be *O. lurida*.

Further genetic work is underway to compare the genetic profile of San Francisco Bay *C. gigas* with potential source populations. A trial analysis of 31 individuals from three hatchery populations and the Loch Lomond site, which sequenced the major noncoding region (≈1,000 base pairs), revealed some haplotype differences that may be sufficient to distinguish populations. Further work with more populations and a larger number of specimens has been hampered first by staff turnover in the Gaffney lab and then by the Dec. 2008 stop-work order. However, Dr. Gaffney expects to complete work on the next planned phase of work in a few months. This will involve sequencing and analyzing a ≈0.7 kb fragment of the mitochondrial CO3 gene and five protein-coding fragments of nuclear DNA in 12 individuals from each of 8 populations (Table 6).⁴ Based on other work in *Crassostrea* species, Dr. Gaffney believes that some of these genes should have the right level of variability to distinguish among *C. gigas* populations. If this effort is successful, the two most effective genes will be sequenced from a larger set of *C. gigas* populations.

² There was also a 1,146 cm³ *C. gigas* collected dead in May 2008. The massive size of this oyster may be due to deformed shell growth due to infestation of the shell by the boring sponge *Cliona*, or possibly due to exposure to contaminants such as tributyltin.

³ We and other researchers also suspected that there were morphological differences between *Ostrea lurida* (the native Pacific Coast oyster) collected in the South Bay and *O. lurida* collected in the North Bay in 2006. These were not the same differences as those observed between the South Bay *C. gigas* and typical *C. gigas*, but might nonetheless indicate that environmental conditions or contaminant exposures were altering oyster morphology in the South Bay. However, no one has yet measured a large series of *O. lurida* from both the North and South Bay to confirm or reject the possibility of morphological differences.

⁴ Dr. Gaffney also has sequences of nuclear genes from a small number of *C. gigas* from Hokkaido and Kyushu in Japan and from Korea. These may also be used in the analysis.

Table 6. *Crassostrea gigas* populations to be sequenced in the next phase of genetic analysis.

Population	Notes
San Francisco Bay	outside of the Loch Lomond site
Loch Lomond	illegal planting
Taylor Shellfish Hatchery	in Dabob Bay, WA; obtained from the Hog Island Oyster Company
Coast Seafoods Hatchery	in Quilcene Bay, WA; obtained from the Tomales Bay Oyster Company
Whiskey Creek Oyster Farms Hatchery	in Tillamook Bay, WA and initially reared by Kuiper Mariculture in Humboldt Bay; obtained from the Hog Island Oyster Company
RMP 1999 Wet Season	archived (frozen) oysters deployed in Jan.-Apr. 1999 for bioaccumulation studies
RMP 1999 Dry Season	archived (frozen) oysters deployed in June-Sept. 1999 for bioaccumulation studies
RMP 2000 Dry Season	archived (frozen) oysters deployed in June-Sept. 2000 for bioaccumulation studies

Age determinations of oysters based on annual shell rings or ridges on oysters (often difficult or impossible to distinguish) or shell size (which is highly variable) are at best rough and unreliable. Working with David Goodwin's sclerochronology lab at Denison University, the Stable Isotope Laboratory at the University of Arizona and Peter Roopnarine at the California Academy of Sciences, we have analyzed profiles of oxygen and carbon isotope ratios along the growth transects of a set of *C. gigas* collected in San Francisco Bay, an analytical technique more often used in paleontology. These profiles showed two distinct patterns indicating two annual cohorts, the more recent one closely matching the isotope profile predicted from measured environmental parameters for a cohort that settled in 2002, and the other fitting best with a cohort that settled in 1998-2000. As reported previously, we submitted a paper on this analysis ("Forensics on the half-shell: a sclerochronological investigation of a modern biological invasion") to the journal *Palaeos*. Following peer-review we revised the paper, which we expect to resubmit shortly, adding more mensurative detail to the description of profile differences between the two cohorts and including an analysis based on the Von Bertalanffy growth function that corroborates the age of the older cohort. We are considering several possible further analyses to either corroborate or extend these results (Table 7).

Table 7. Potential analyses in the next phase of sclerochronological analysis of *Crassostrea gigas* in San Francisco Bay.

Analysis	Purpose and Comments
Smaller <i>C. gigas</i>	The first analysis involved oysters 110-223 mm in height collected in 2006. Analysis of oysters 63-80 mm in height collected alive in 2008 and 2009 would assess whether a more recent cohort is present. This would be relevant to assessing the risk of establishment, and help with the search for environmental factors contributing to successful recruitment.
Finer resolution sampling	Finer resolution sampling of stable isotopes along the later part of the growth transects of the oysters classified as the older cohort would determine the year of settlement more precisely. This would help determine the introduction vector and assess the environmental parameters of recruitment.
Other aging techniques	Alternate techniques would be used to check the ages of the oysters and possibly determine the age of the older cohort more precisely. Possible approaches include assessment of: annual growth lines on polished radial valve sections (Harding & Mann 2006); seasonal variation in magnesium concentrations determined by cathodoluminescence (Langlet et al. 2006; Cardoso et al. 2006); seasonal variation in strontium:calcium ratios (Richardson 2001); or annual growth breaks determined by etching and staining with hematoxylin and eosin (Kent 1992) or by acetate peels (Kent 1992; Richardson et al. 1993a; Richardson 2001). This would clarify the risk of establishment and could help determine the introduction vector and assess the environmental parameters of recruitment.
Loch Lomond oysters	Sclerochronological analysis of the Loch Lomond oysters would determine whether their broad size range (34-190 mm) is due to the presence of more than one cohort. This would indicate either recruitment within San Francisco Bay, or additional spat purchases not known to CDFG. This information would be relevant to risk assessment, assessing the environmental parameters of recruitment, and enforcement.
Geographic outliers	The stable isotope profiles of <i>Crassostrea</i> oysters collected live in Nov. 2005 at Alcatraz and Vallejo differ from those of the other analyzed oysters, with one indicating an age of ≈ 1.5 yr and thus settlement in the first part of 2004. Other outlying oysters would be analyzed and all would be compared to the annual/seasonal pattern of measured environmental parameters in the regions where they were collected. These may reveal other settlement patterns, which would be relevant to risk assessment and assessing the environmental parameters of recruitment.

There are several possible source populations and transport mechanisms that could have introduced the *C. gigas* population to San Francisco Bay. Genetic analysis and consideration of other factors may allow us to determine which source population and vector are the likeliest (Table 8).

Table 8. Possible sources and vectors for the San Francisco Bay *Crassostrea gigas* population.

Vector	Comments
Remnant from previous experimental plantings in the Bay	<i>C. gigas</i> were grown commercially in San Francisco Bay in 1932-1938 and there were occasional experimental plantings through the late 1970s (Carlton 1979). The most recent experimental planting was by CDFG in 1981, to assess the potential for rearing <i>Crassostrea</i> oysters in the Bay. 560 <i>C. gigas</i> and 600 shells with <i>C. gigas</i> spat were obtained from Pacific Mariculture in Elkhorn Slough, from Eureka, CA and from Washington state and deployed in racks, buckets and on stakes for 5-6 months at six sites in San Francisco Bay including San Leandro Marina, Foster City and the mouth of Redwood Creek in the South Bay (McAllister and Moore 1982). These deployments appear to be too early to account for a population first discovered in 2004 whose earliest known settlement was in 1998-2000.
Derived from illegal planting at Loch Lomond	This planting is believed to be derived from spat obtained from Coast Oyster Company in 1999 (Tom Moore, pers. comm.). Genetic analysis might match the San Francisco Bay population with the Loch Lomond population, or alternately might exclude it as source.
Derived from bioaccumulation studies in the Bay	<i>C. gigas</i> were deployed for contaminant bioaccumulation studies at various sites in San Francisco Bay, including the South Bay, by CDFG/SFRWQCB in 1991 and 1992 and by the RMP in every year from 1993-2002; and in the North Bay by CCCSD in 1991-94. The oysters were purchased from West Coast oyster farms, and were typically hung in bags from structures or buoys for 90-100 days, with typical deployments being 45 oysters at a site by CDFG/SFRWQCB, 150 oysters at a site by RMP, and 450-810 oysters at a site by CCCSD. The oysters were generally mature and capable of spawning, and based on weight changes during deployment and condition on recovery it seems likely that at least some of them did spawn during deployment. Also some of the oysters were never recovered, as when bags were destroyed or lost due to storms. In all, over 20,000 <i>C. gigas</i> appear to have been deployed in San Francisco bay over this period. The earliest settlement determined by sclerochronological analysis (1998-2000) is consistent with the period of bioaccumulation deployment (could be the first or later generation derived from a deployment in 1991-2000), and the geographic center of the invading population (southeastern San Francisco Bay) corresponds with deployments in the South Bay by CDFG/SFRWQCB and RMP. Archived oysters from RMP deployments in 1999-2000 were available and are included in the genetic analysis, which might match the San Francisco Bay population to one of these deployments. Alternately, matching the San Francisco Bay population with one of the hatcheries might support some of the deployments as possible sources along with other possible sources; matching with one of the hatcheries might exclude some of the deployments; and matching with non-hatchery genes would exclude the deployments.
Larval drift from a West Coast oyster farm	The closest oyster farms are in Drakes Estero and Tomales Bay, all of which use spat from the three West Coast hatcheries. Genetic analysis that matched the San Francisco Bay population with one of the hatcheries would support one or more of these farms as possible sources along with other possible sources; matching with one of the hatcheries might exclude some of these farms; and matching with non-hatchery genes would exclude these farms.

Table 8 continued. Possible sources and vectors for the San Francisco Bay *Crassostrea gigas* population.

Vector	Comments
Illegal planting of live oysters purchased as seafood	Live oysters purchased from food markets in the Bay Area would come from a West Coast oyster farm. Genetic analysis will not be able to distinguish oysters purchased as seafood from larval drift from an oyster farm that supplies oysters to Bay Area markets as potential sources.
Larval drift from a West Coast wild population	There are a few wild populations of <i>C. gigas</i> on the Pacific Coast that might produce larvae that could drift to San Francisco Bay. Genetic analysis might be able to distinguish these as possible sources.
Arrival as larvae in ballast water or oysters on the hulls of vessels from the West Coast	Genetic analysis would not be able to distinguish among transport mechanisms (larval drift, larvae in ballast water, oysters in hull fouling) for oysters from a particular West Coast source (whether wild or farmed), but other considerations (locations of shipping routes, ports and marinas relative to the source population, current patterns) may suggest which transport mechanism is likeliest for a given source population. It is possible that assessment of genetic variation within the source population and the San Francisco Bay population could determine a degree of “genetic bottleneck” that would help to distinguish the likely transport mechanism.
Arrival as larvae in ballast water or oysters on the hulls of vessels from Asia	Genetic analysis might be able to distinguish an Asian source from any possible West Coast sources, and with appropriate sampling might be able to pinpoint a particular Asian source population. Assessment of genetic bottleneck might suggest ballast water larvae or hull fouling as the likely transport mechanism.

Summary and Conclusions

Numerous studies have demonstrated *C. gigas*’ ability to alter habitats and impact other organisms, and in various parts of the world it is considered a harmful invading organism and has even been banned, despite its value as a food resource and marketable species (Bayne 2002; Chew 2003; Nehring 2003; Diederich *et al.* 2005; Smaal *et al.* 2005; Diederich 2006; Ruesink *et al.* 2006). Potential impacts in San Francisco Bay include interference with native oyster restoration efforts through competition for food, overgrowth, or impairment of growth with metabolites or feces (Bayne 2002; Chew 2003); similar impacts on other epibenthic species; impacts on benthic or pelagic species by consumption and reduction of phytoplankton populations and the alteration of food webs; and impacts on subtidal or intertidal habitat restoration by causing structural changes and habitat alterations. Additional impacts noted in other areas include the fouling of power plant cooling systems, making shore access difficult, and cutting hands and feet. On the other hand, there may also be positive impacts on some species or on some ecosystem functions.

The appearance of a substantial settled population in San Francisco Bay, consisting of multiple cohorts, after eight decades of virtually no *C. gigas* settlement in central California despite ample spawning opportunity (Carlton 1979; D. Alden, pers. comm.; J. Finger, pers. comm.; California Academy of Sciences’ Invertebrate Collection records),

suggests that there is now a significant risk of establishment of *C. gigas*. The introduction of a novel genetic strain better suited to central California conditions, or changed environmental conditions (e.g. Cloern *et al.* 2006, 2007) are possible explanations. It may also be relevant that the sudden establishment and spread of *C. gigas* after decades of opportunity has also been documented in other parts of the world (Diederich *et al.* 2005; Robinson *et al.* 2005).

From its inception in 2006, the oyster removal project in San Francisco Bay has been hampered by sporadic and uncertain funding, so that in most years field work has been limited or absent during part or all of the peak season for low tide work in April to July. Deadlines on the use of current funding will restrict the opportunity for such work during the peak season in 2010, and the end of that funding and the uncertainty regarding future funding prevent the systematic planning and the consistent and persistent execution that is normally essential for a successful eradication effort.

Despite these problems, the data show that the project has been progressively reducing the *C. gigas* population in San Francisco Bay. There is every reason to believe that if adequate, reliable funding is provided to properly complete the work, eradication will be achieved.

Alternately, it is also possible that by the spring of 2010, when the current funding will be depleted, that the *C. gigas* population will have been reduced to a point where it will fully die out over the following years, even if we walk away from the project and there is no further funding, monitoring or removal effort. However, such a result is uncertain. The accepted best management practice for an eradication effort is to continue the effort at an aggressive level until all of the population that can be targeted is removed or killed, monitor regularly for several years thereafter, and follow-up with additional removal as needed to be sure that the population does not regrow. Not following such practice in this case could easily lead to the resurgence and permanent establishment of *C. gigas* in San Francisco Bay.

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