# Rapid Response and Eradication Plan for the Invasive Green Alga *Caulerpa prolifera* in Newport Bay

Southern California *Caulerpa* Action Team Final May 2021 as revised July 2021<sup>1</sup>

#### BACKGROUND

In April 2021, the non-native alga *Caulerpa prolifera* was confirmed growing in the China Cove area of Newport Bay, California. The species has previously invaded seagrass and soft-bottom habitats in the Suez Canal (A-F.A. Gab-Alla 2007), the Canary Islands (Tuya et al. 2013), and Portugal (Parreira, et al. 2021), dramatically displacing native biota. Further, based on environmental impacts of other *Caulerpa* species, this alga is a potentially serious invasive species. Other species of *Caulerpa* are welldocumented as having aggressively displaced native habitats when introduced, both in California, Australia (Creese et al. 2004), and the Mediterranean Sea (Meinesz et al. 2001, Verlaque).

Allowing any species of *Caulerpa* to become established and spread within California coastal areas and embayments is likely to result in considerable economic, recreational, and biological impacts. In 2000, the seriousness and acknowledged threat from its close relative, *Caulerpa taxifolia*, prompted an effective, highly successful *C. taxifolia* eradication project in two southern California locations. This effort occurred over a period of eight years at a cost of more than seven million dollars (M&A 2006). The action and investment protected local resources and led to the recovery of critically important eelgrass habitat, and protected California's coastal ecosystems (Anderson 2005). Risk modeling for Caulerpa conducted in 2006 placed China Cove in the highest risk category for an introduction (M&A 2008a).

Based on preliminary surveys conducted in late April 2021, it is estimated that there is less than 200 square meters of seafloor infested with *C. prolifera*, distributed over a roughly 1.2-hectare infestation area within China Cove in the entrance channel area of Newport Bay (Figure 1). However, high intensity and eradication level survey and mapping efforts proximal to the infestation have not been conducted.

The *C. prolifera* occurrence varies from expansive rooted patches to un-attached, mobile clumps alone or on loose detrital algae that move with tidal currents (photos 1 and 2). The loose patches could serve to spread the infestation and may warrant different eradication treatment approaches. There are numerous thalli (strands of the alga) extending above the rooted beds that may be broken loose by human activity such as anchoring and diving, or natural disturbance, and establish new infestations (photo 3). Within the bed of attached *C. prolifera*, there is some rooted eelgrass that cannot be readily separated from the *C. prolifera* itself without risk of missing fragments of the invasive alga.



Photo 1. Largest patch of *C. prolifera*. Note remnants of eelgrass bed displaced by the alga. Photo 2. *C. prolifera* that is not rooted, growing on top of drift *Gracilaria*. Photo 3. The white/brown material are plumes of *C. prolifera* rhizoids from dislodged thalli, ready to spread.

<sup>&</sup>lt;sup>1</sup> Note that this plan may change during eradication efforts due to operational and other needs.



Figure 1. Distribution of Caulerpa prolifera in China Cove (known as of 4/21/21)

*C. prolifera* is known to grow in fine sediments, sand, and on rocky substrate. Within the Newport Bay infestation, *C. prolifera* has only been identified as growing on sandy substrate and as loose thalli, and thus the present plan does not address eradication of this alga on rocky surfaces. If the alga is found growing on rocky substrates in other areas, this plan would be amended to address those areas.

# **ACTION NEEDED**

It is presently believed the extent of the alga is limited to the China Cove area, and the scientific consensus is that immediate action to eradicate this population be undertaken. Due to the high level of recreational use in the area, the strong currents and tidal movements in the channel, and the great ease with which this species spreads by small fragments, the aim is to initiate a removal as soon as possible.

# CONTAINMENT

Disturbance of the alga by boat anchors, boat wakes, divers, swimmers, and other human activity can release even very small fragments that can drift away and start new infestations. The identified infestation area has been visually isolated by the City of Newport Beach (City) with floating buoy lines to discourage and help exclude boat and swimmer access. To provide geographic clarity, the China Cove beach area can be divided into two distinct sections (Figure 1): (1) the large beach between Dahlia Avenue and Cove Street, and (2) the small beach area at the end of Fernleaf Avenue.

At the initiation of eradication treatment, a portion of the large beach area within China Cove may also be closed to public access temporarily while large patch treatments are undertaken. This closure would be expected to require approximately one week of site occupancy to complete the initial work.

#### ERADICATION

Eradicating an invasive species is a multi-step process, with an extended element of re-survey, retreatment, and verification necessary to declare the invasive species formally eradicated. This process can be lengthy (up to several years) to address repeated peak growing seasons and resurgence of growth from previously missed occurrences. This plan is broken into two phases designed to eradicate *C. prolifera* from China Cove: Phase 1 will include increased intensity surveys, containment of the infestation, and initial removal, followed by Phase 2, which will address the extended work needed to ensure the technical designation of "Eradication" has been met. As a result, while Phase 1 action is expected to provide high confidence that the *Caulerpa* within the known infestations has been removed, further Phase 2 actions are needed to (1) ensure it does not re-establish from unknown sources and (2) follow up monitoring can detect any new occurrences, so that eradication can be achieved.

#### **Options for Eradication Methods**

There are limited options to eradicate the alga. Algae across the Caulerpa genus have similar growth and dispersal tactics, therefore examining attempts to control other Caulerpa species is helpful. With the Caulerpa taxifolia discovery in San Diego, many techniques were tested and evaluated (e.g., dredging, barriers, hand picking, various chemicals). In that case, sealing the alga under heavy benthic barrier material and pumping chlorine underneath was determined to be the best course of action for the conditions present in the area, an approach that proved effective. A similar efficacy could likely be achieved without the chlorine, provided the barriers could be maintained in place with certainty. Placing barriers in the China Cove site would be challenging due to the slope, swells, and currents, making it difficult to ensure the tarp would not dislodge and further spread the alga. Researchers attempting to control two species in the Mediterranean Sea have found physical removal to be temporarily effective at removing biomass in small occurrences, but the site had to be repeatedly revisited to remove new growth emerging from the thread-like rhizoids inevitably left behind. Similar observations were made in California with Caulerpa taxfolia. A problem with hand removal is the generation of fragments that can drift away during the process. This risk can be mitigated by providing the diver with a suction device to remove the alga. The C. prolifera patches are too large to pick without the suction support. Suctionassisted diver removal can be mobilized quickly, and the majority of biomass could be removed within a two-to-three-day period. For these reasons, the following survey and removal description is provided.

#### PHASE 1

#### Initial Localized Eradication Level Survey

Prior to removal of the alga, an intensive survey will be conducted within the China Cove infestation area. Although surveys of surrounding areas are necessary to support the eradication effort, the focus in this document is on the known infestation area. Additional surveys in surrounding areas are anticipated to support the eradication program and can be detailed in a separate work plan.

Within the China Cove infestation area, intensive eradication level surveys (100% coverage) will use vessel positioned transects and divers to systematically search the area. A tending vessel will deploy and retrieve a series of parallel survey transects placed with a high degree of precision using a real-time kinematic (RTK) global positioning system (GPS). The vessel has a motorized spool of nylon survey rope and will deploy an anchor to secure the start point of the survey line at a designated waypoint. The vessel will then back up over the intended transect position while deploying the rope from the bow. At approximate 40-meter intervals, a 5-pound weight will be attached to the transect line. Because the

position of the primary dense patch of *C. prolifera* is already known, care will be taken to deploy the survey line weights well outside the known locations of the alga. During the deployment, the vessel will track the intended transect placement to within 2 meters by observing the cross-track error on the vessel's navigation computer. After deployment of each weight, the deployed transect spool will be stopped and the vessel aligned to within 0.25-meter of cross-track error by reversing against the anchored line. Once on target, the spool can then be slowly released to allow continued placement of the transect. Once at the end of the transect, a 10-pound weight is placed on the end of the transect line. A buoyed marker will also be placed on the end of the line. A technician then holds the buoy line and slowly lowers the weighted end while the vessel operator aligns the vessel to within 0.25-meter of cross-track error while keeping the line taught. Transects will be placed parallel to one another at a spacing relative to the number of divers present and visibility.

To survey a transect, divers will be aligned perpendicular to the transect. A short length of rope with a knot tied at each diver's position will be created to assist divers in maintaining spacing during the survey. A diver spacing of no more than 1.5 meters is recommended. Thus if 4 divers are utilized, the diver rope would be 6 meters long. The lead diver will then follow the transect line approximately 0.75 meters to its side. Each subsequent diver will keep pace with the lead diver, but follow slightly behind on their transect side. This arrangement prevents the team from circling inward toward the transect line. At the end of the transect, the lead diver tugs the line to indicate that the team should pivot 180 degrees around the lead diver and then proceed along the opposite side of the transect line toward the start point. The water clarity and habitat complexity (e.g., bare bottom, eelgrass cover) dictates the speed and spacing of the survey team. All divers will be qualified and trained in *Caulerpa* and native species identification.



Photo 4. Diver spaced by a section of knotted rope during training on signals and procedures used during the survey. Photo 5. A team of divers aligning themselves at the surface and preparing to swim a laid transect.

The above procedure would be followed with transects being placed and surveyed throughout the survey area (Figure 2). All transects will be placed on 12-meter centers (assuming the above 4 diver and 1.5-meter scenario). Although transect end points can readily be controlled, intra-transect accuracy is a function of keeping the vessel on track during deployment to prevent the transect from becoming fouled on obstacles that cause the line to be placed off the intended track. Keeping the line taught during deployment helps keep the vessel on track. Any bows in the line can cause unnecessary overlap and survey gaps when surveying adjacent lines.



Example Eradication Survey Configuration For Four Diver Survey Team





All seafloor-attached *C. prolifera* encountered will be flagged near the occurrence by florescent pin flags and its position recorded using surface GPS on the tender vessel. Where divers encounter *C. prolifera* that is not attached to the bottom but rather is mobile in the detrital drift, the algae will be collected within a 3-mm or finer mesh bag carried by the diver and taken immediately to the surface where the material will be transferred to a tender vessel for upland disposal; the position of the encounter will be recorded by GPS. This methodology is consistent with research on the viable fragment size for *C. prolifera* and a desire to remove vegetation that is mobile and can drift if left unattended. An infestation map will be generated and used to systematically guide the removal strategy. The mapping will note both the attached and unattached algal distribution. These recorded positions will be used to target smaller patches and allow the site to be revisited to ensure removal. The survey will be implemented before and after the removal of the *C. prolifera* in China Cove. Additional surveys will be needed, including QA/QC survey efficacy assessments during each, to reach a point where the *C. prolifera* can be declared eradicated with some degree of certainty (Phase 2). Efficacy of the survey is measured by seeding the survey area with fake *Caulerpa* prior to survey and then assessing the return of fake *Caulerpa* as a quantitative measure of how much and what size of *Caulerpa* would likely have been found had it been present.

## Initial Localized Removal

Suction-assisted diver removal of *C. prolifera* will be the initial method of response within China Cove. This methodology is well-tested and regularly implemented in the state to remove invasive aquatic species. The China Cove area has site-specific benefits and limitations related to vehicle accessibility, proximity to the municipal sanitary sewer, storage space, and beach access. These factors will need to be taken into account when determining the specific equipment to be used, and are not fully known at this time. An adaptive approach will most certainly be needed, but the overall strategy will be diver removal to the adjacent beach. The equipment and removal techniques will be demonstrated and practiced at an adjacent non-infested area to remove native surface detritus and to remove controlled depths of sand suitable for rhizoid removal. This will allow all elements of the collection and return water system to be tested and for the eradication team to best understand and adjust methods to minimize risk of spread at the removal site.

The suction will be directed by scuba diver, to completely remove the entire thallus, including rhizoids. Several trained support scuba divers will assist by retrieving *C. prolifera* dislodged by the suction and guide the suction diver toward target areas. The removal will utilize a 3 to 6-inch hydraulic pump and be selected to prevent clogging of the discharge line and ensure that adequate suction is always maintained. The material will be pumped to the beach for separation of *C. prolifera* and solids from the water. The discharge will be handled with great caution to prevent re-introduction of the alga to the nearshore environment.



Photo 6. Diver-assisted suction removal of Eurasian watermilfoil (diver not shown). Photo 7. Floating pumps used for diver-assisted suction removal of Eurasian watermilfoil.

The discharge will be routed well above the high tide line and exit into a geotextile filter (Figure 3). The filter Apparent Opening Size (maximum pore diameter) will be less than 1 millimeter to retain most of the sand and all viable *C. prolifera* thalli and fragments. The water that filters through the filter will flow onto the beach behind a sand berm formed between the waterline and the filter. The sand berm would be approximately one meter high and wrap around all sides of the filter. This will prevent the filtered water from running directly back to the harbor, providing a second level of protection. The water will be allowed to gravity filter down through the sand.



Figure 3. Treatment Area and Process Schematic

While it is not anticipated that there will be excess pumped water volume, two contingencies will be in place to manage the water in the event that the rate of filtration through the sand berm cannot keep pace with the rate of the pump discharge. The first contingency is that the City of Newport Beach will coordinate one to two Vactor trucks to be on standby. If necessary, the Vactor trucks will remove excess water to prevent it from going around or eroding the berm and hold it until it can be allowed back behind the berm and filter through the sand. The second contingency for excess water management would be the placement of a standpipe through the berm. The pipe would have a 1 millimeter or less mesh filter bag or filter fabric placed over it in a manner that allows the water to be more rapidly filtered and returned to the harbor. Following the removal process, the contents of the de-watered filter bags will be taken to an upland disposal site. The sand berm will then be recontoured to return the beach to its original configuration immediately following the removal of the filter bag for disposal.

The algae removal will be coordinated by topside staff communicating with the dive team. This will allow the topside to stop pumps at any time when the diver is not actively removing material. This will lessen the burden on the system and prevent a loss of containment. Similarly, if the pump rate threatens to cause a loss of containment at the filter bag, the topside personnel can stop the pumps and notify the dive team prior to re-starting the pumping.

Care will be taken to minimize the removal of native marine species. The C. prolifera in China Cove has invaded and displaced native eelgrass (Zostera spp.), almost to its full exclusion in the main infestation patch (Photo 1). Therefore the impacts to eelgrass are anticipated to be primarily at the margins of the C. prolifera patch, and in the small "satellite" patches that surround the primary patch. The satellite patches principally occur on bare bottom, or have not grown to the point of displacing eelgrass. Therefore where eelgrass and C. prolifera are intermixed, there will be complete removal of the eelgrass and C. prolifera above and below the sediment. It is estimated that up to 80 square meters of very lowto low-density eelgrass could be impacted by the removal work. Removal of the C. prolifera and surrounding sediment to a depth sufficient to remove the rhizoids, currently estimated to be conservatively as deep as 10 centimeters (California Department of Fish and Wildlife has reported observations to 6 cm in preliminary sampling), will also remove benthic invertebrate species. Unnecessary removal of sediment will be avoided to limit biological impact and reduce the amount of material to be processed on shore. It is anticipated that the density of invertebrates is already diminished within the infested areas. Within the C. taxifolia infestation areas in Agua Hedionda Lagoon, the sediment invertebrate community was greatly reduced in diversity and supported only 12 percent of the invertebrate density found in adjacent healthy eelgrass beds (M&A 2008b, Appendix A). Available data on marine life in Newport Bay and reported reductions in invertebrate abundance in *Caulerpa* beds have been used to roughly estimate the potential impact to non-target species in Appendix B.

It is expected that the eradication removal will collect and export a portion of the benthic infaunal communities, but will not remove deeper burrowing organisms. The removals would not alter the substrate from the sand conditions present prior to eradication. As a result, benthic community recovery from larval recruitment and immigration of organisms from the adjacent areas is expected to be relatively rapid. Studies of recovery of benthic communities in large scale dredging projects demonstrate the process of invertebrate community recovery to be relatively rapid taking months and not years to be completed. At the present time known infestations are limited to discrete areas and as such the damage to infaunal communities from the algae as well as the subsequent eradication would be expected to be limited in scope, but could expand if the infestation areas are allowed to expand further.

Before and during the localized removal process, a suspended net may be used to block the spread of *C. prolifera* fragments. If placed before the removal effort to control the distribution of *C. prolifera*, the block net would need to surround the infestation area. This block net would be subject to significant tidal current and surge. Under this scenario, the block net would need to be tended daily to ensure it remains free of debris and does not get dragged through the infestation area. If used during the removal effort, smaller block net segments can be deployed immediately down current of the removal activity to help collect any fragments that might be mobilized during the removal effort. The use of block nets will be at the discretion of the removal team, based on effectiveness and environmental parameters at the time of the activity.

Following the suction-assisted diver removal, the infestation area will be swept again with eradication level surveys 2, 4, 8, and 12 weeks following the first removal effort. During these surveys, *C. prolifera* patches that have regrown from rhizoids or were missing in the initial removal will be removed by trained divers by hand and placed in collection bags designed to prevent loss of fragments. If no more occurrences are found after this period, the China Cove *C. prolifera* can designated as controlled, but not eradicated.

Following the initial *C. prolifera* twelve week removal effort, a surveillance plan will need to be implemented as described above, which would like involve quarterly resurveys in the infestation area.

## PHASE 2

This phase involves the determination of whether *C. prolifera* has been eradicated from the site to a high degree of certainty. The criteria for successful eradication of the *C. prolifera* infestations are 1) the containment and complete removal of *C. prolifera* at the infestation site, and 2) verified absence of *C. prolifera* from the infestation site.

The first criterion can be determined through survey of the site for regrowth over time. This can be further supported by planting sediment cores from the infestation site into laboratory aquariums and monitoring them in a controlled study for regrowth of *C. prolifera*.

The second criterion is evaluated by quantifying the confidence in the post-removal surveillance efforts. Patches of artificial *C. prolifera* are placed within the site during the regular diver surveys. Confidence in the results of each survey for live *C. prolifera* can then be quantitatively estimated based on the amount of artificial *C. prolifera* found during the surveys (M&A 2005). The results of these consecutive assessments of the surveys ultimately allow for an estimation of the Eradication Certainty: the certainty that all real *C. prolifera* existing at the treated site had been found and that eradication had been achieved.



Photos 8 & 9. Artificial *Caulerpa* placed to quantitatively assess the confidence level for each survey, key to calculation of Eradication Certainty.

Photo 10. Viable fragments of real Caulerpa as small as several millimeters (right) can be easily missed by divers.

The number of repeated surveys necessary to achieve full eradication is not known. If new patches of *C. prolifera* are detected during the follow-up surveys, they will need to be removed and the survey timeline reset. It is important to have surveys repeated annually during active growing seasons. *Caulerpa* species often die back during colder seasons, particularly when they occur in waters in the lower end of their temperature tolerance. For the purposes of this document, it has been assumed to be a two-year process, with four surveys per year, though it could be longer based on recurrences and quality assurance and control of survey efficacy findings. A Determination of Eradication Plan identifying the metrics by which the alga can be designated as eradicated will be developed and agreed upon by the Southern California Caulerpa Action Team (SCCAT) once the infestation has been removed.

This robust and scientifically defensible process of determining Eradication was developed, tested, and implemented by the SCCAT during the *Caulerpa taxifolia* Eradication Program in San Diego and Orange Counties (M&A 2006, Appendix C).

#### **Public Outreach**

Signage has been posted at the China Cove beach access points by the City of Newport Beach to inform the public about the response operations. If feasible, signage will also be placed on the seaward side of the beach and exclusion buoys will be deployed to deter entrance into the eradication site. Outreach to homeowners in the China Beach area may be undertaken via direct mailings, public workshop, and online resources. Outreach would include cautions to avoid releases of pet and aquarium contents to

the bay, either directly or by dumping into storm drains, and provide information on the threat posed by *C. prolifera* to Newport Bay and the outer adjacent coastal areas.

## **Source Identification**

This is the first known introduction of *C. prolifera* on the west coast of the United States. It would be useful to know the source of the introduction. Possibilities to pursue include intentional release of aquarium contents to the bay, inadvertent introduction through storm drain discharge of aquarium water, discharge from the marine laboratory when it was in use in prior years, or natural range expansion from Mexican waters (considered unlikely). A source identification plan will be developed at a later date; the first priority is eradicating the known occurrences of *C. prolifera*.

## Research

While the present program is focused on the direct and immediate eradication of *C. prolifera* from Newport Bay, it will be of scientific interest to collect as much information as is practical concurrent with its eradication. For this reason, data collection by biologists will occur coincident with survey and eradication efforts. Information may include morphologic and growth parameters of the species, evidence of sexual reproduction, documentation of ecological impact on benthic communities, epiphytic communities, and environmental characteristics of the infestation area. Although genetic analysis by the California Department of Food and Agriculture has provided a preliminary identification (Appendix D), additional samples should be collected and analyzed to provide additional genetic information that could help with source identification. The possibility of using environmental DNA (eDNA) techniques should be explored. It may be possible to use eDNA to rapidly detect the presence of *C. prolifera* and other *Caulerpa* species, allowing for searches of other parts of Newport Bay, offshore, and other bays and estuaries that will be costly and, in some cases, difficult to perform with divers.

The eradication program would be documented using video, still cameras, detailed notes, and GIS records so that a future retrospective may be prepared to aid in the application of information learned to future infestations.

# Organization

During the 2000-2008 *Caulerpa taxifolia* eradication, the SCCAT was formed. This team was made up of federal, state, and local governmental agencies, scientists, consultants, and local stakeholders. The SCCAT has been reactivated to respond rapidly to this infestation. The SCCAT Steering Committee is made up of representatives from NOAA Fisheries, Santa Ana Regional Water Quality Control Board, California Department of Fish and Wildlife, City of Newport Beach, and Orange County Parks. The SCCAT will direct and supervise all aspects of the response, including coordination of authorizations, securing response funding, selecting survey and removal teams, reviewing work progress and efficacy, and determining eradication point.

# Timeline

The following is an estimated containment and eradication timeline (Table 1). The estimate was initially developed immediately following broad governmental awareness of the need to eradicate. There may be delays in implementation related to identifying and securing funds, securing authorizations and permits, and contracting with a response field team. While these delays may push out the timing of Phase 1, once the removal is under way the element durations will be the same. Phase 2 monitoring is only an estimate. The true schedule will be dictated by recurrences of the alga, diving conditions, measured survey efficacy, and the Determination of Eradication Plan.

Table 1. Estimated containment and eradication timeline with specified work elements. Note that exact timeline may vary depending on survey results.

WORK ELEMENT	EST. TIMING	NOTE
Re-activation of SCCAT, structure determination, sub-	April 20-April 28	-
committee assembly		
Containment/public exclusion measures/site signage	April 22-May 31	City of Newport Beach
Authorization/permit acquisition	April 30-May 15	Assume 1-2 weeks
Phase 1 Eradication	-	-
<ul> <li>Eradication level survey</li> </ul>	June 2021	2 days
<ul> <li>Initial removal equipment mobilization</li> </ul>	June 2021	-
– Initial removal effort	July 2021	3-5 days
– Post-removal resurvey and removal of missed algae	July 2021	2-3 days
<ul> <li>– 2-week post removal re-survey and response</li> </ul>	August 2021	2 days
<ul> <li>– 4-week post removal re-survey and response</li> </ul>	September 2021	2 days
<ul> <li>– 8-week post removal re-survey and response</li> </ul>	October 2021	2 days
<ul> <li>– 12-week post removal re-survey and response</li> </ul>	November 2021	2 days
Phase 2 Eradication	-	-
<ul> <li>– Re-survey w/ survey efficacy QA/QC</li> </ul>	Winter 2022	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Spring 2022	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Fall 2022	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Winter 2023	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Spring 2023	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Fall 2023	2 days
<ul> <li>– re-survey w/ survey efficacy QA/QC</li> </ul>	Winter 2024	2 days
<ul> <li>re-survey w/ survey efficacy QA/QC</li> </ul>	Spring 2024	2 days
Eradication Declaration	Unknown at this	Dependent on survey
	time	results

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## APPENDICES

## Appendices are available upon request.

Appendix A. The effect of the 2000 *Caulerpa taxifolia* infestation on invertebrate infauna and epifauna at Agua Hedionda lagoon, Carlsbad, California.

Appendix B. Estimate of non-target species removed by the *Caulerpa prolifera* proposed eradication

Appendix C. Final report on the eradication of the invasive seaweed *Caulerpa taxifolia* from Agua Hedionda Lagoon and Huntington Harbour, California.

Appendix D. California Department of Food and Agriculture Pest and Damage Record (genetic identification) of China Cove Sample.