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[1]

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 well-documented 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.

[1] 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, re-treatment, 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. Suction-assisted 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.

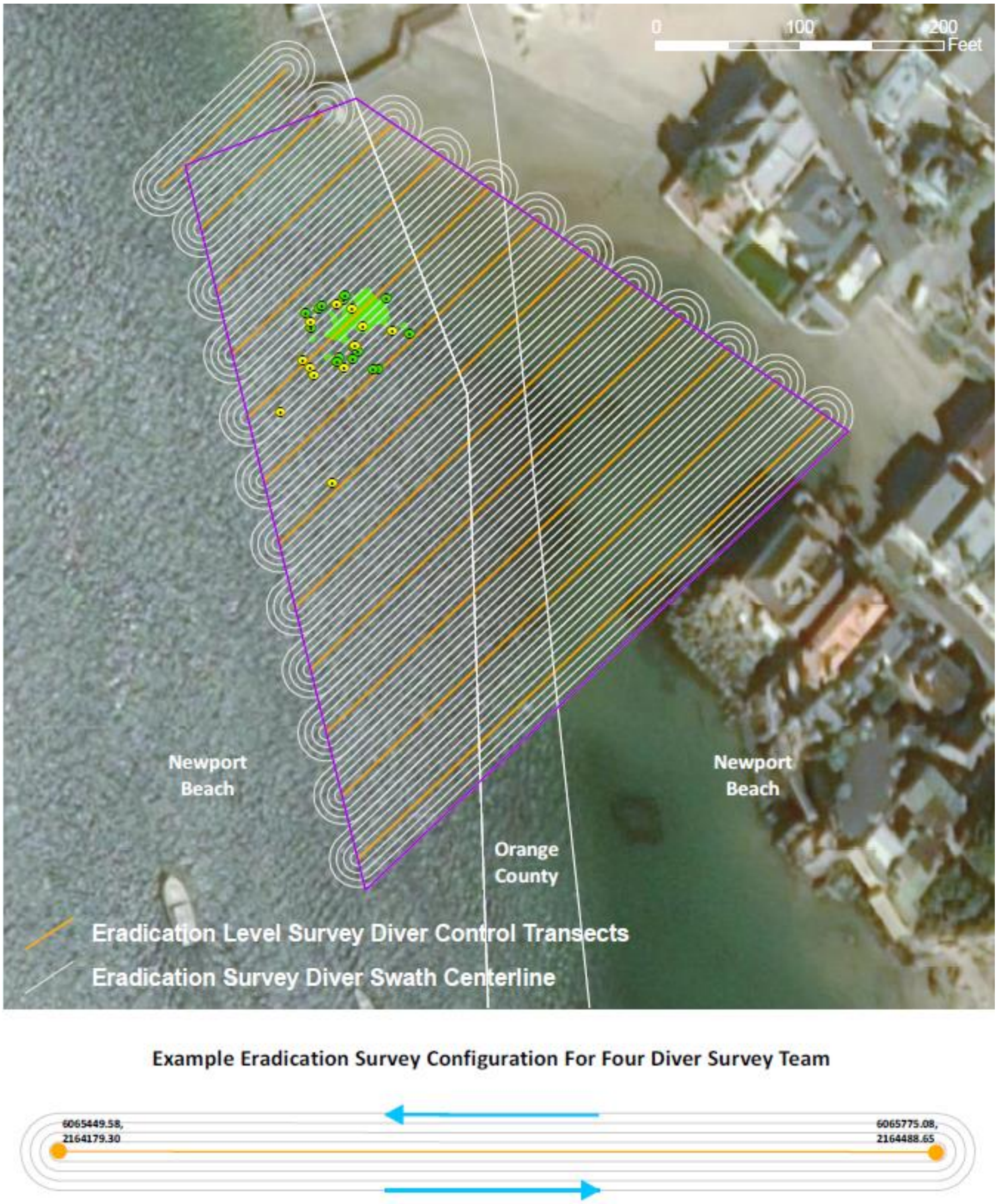

Figure 2. Example survey design for eradication level survey

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.

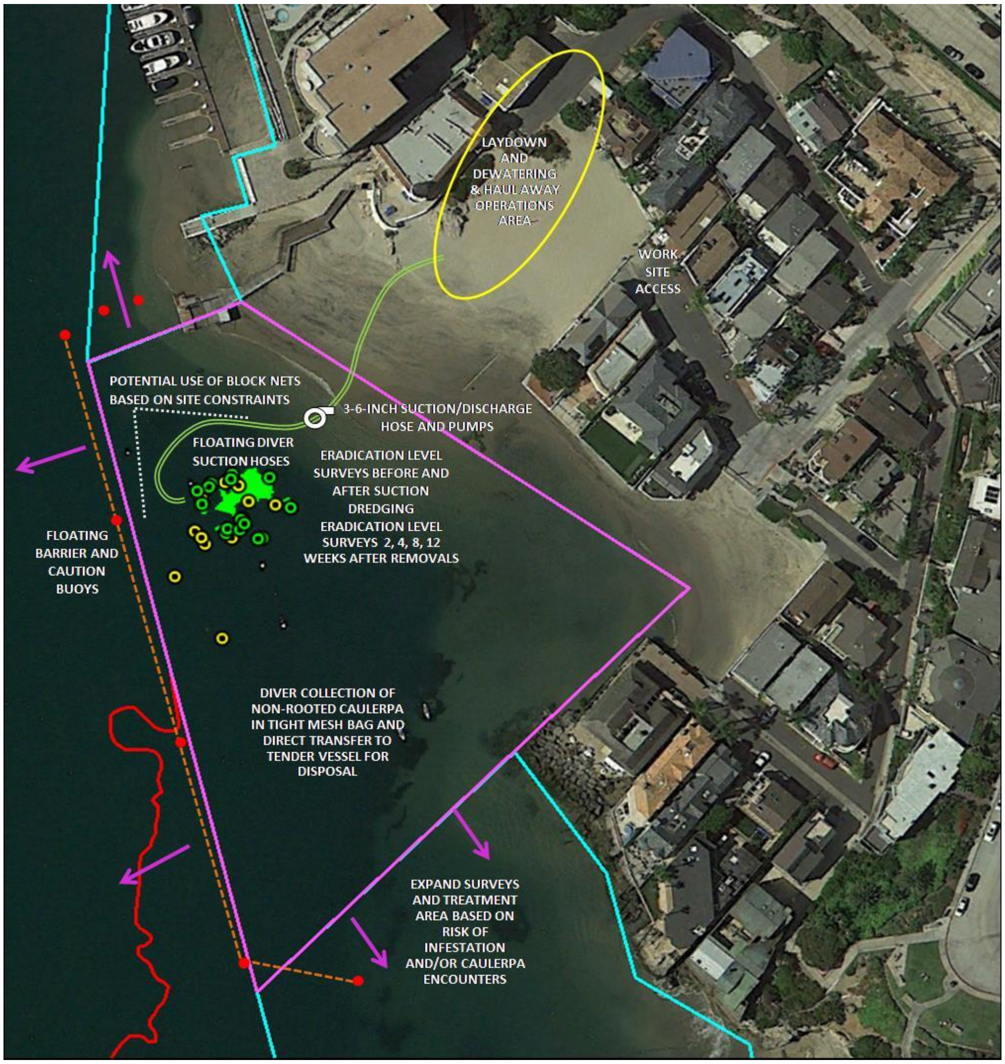

Figure 3. Treatment Area and Process Schematic

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 low- to 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 onshore. 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-committee assembly	April 20-April 28	-
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	-	-
– Eradication level survey	June 2021	2 days
– Initial removal equipment mobilization	June 2021	-
– Initial removal effort	July 2021	3-5 days
– Post-removal resurvey and removal of missed algae	July 2021	2-3 days
– 2-week post removal re-survey and response	August 2021	2 days
– 4-week post removal re-survey and response	September 2021	2 days
– 8-week post removal re-survey and response	October 2021	2 days
– 12-week post removal re-survey and response	November 2021	2 days
Phase 2 Eradication	-	-
– Re-survey w/ survey efficacy QA/QC	Winter 2022	2 days
– re-survey w/ survey efficacy QA/QC	Spring 2022	2 days
– re-survey w/ survey efficacy QA/QC	Fall 2022	2 days
– re-survey w/ survey efficacy QA/QC	Winter 2023	2 days
– re-survey w/ survey efficacy QA/QC	Spring 2023	2 days
– re-survey w/ survey efficacy QA/QC	Fall 2023	2 days
– re-survey w/ survey efficacy QA/QC	Winter 2024	2 days
– re-survey w/ survey efficacy QA/QC	Spring 2024	2 days
Eradication Declaration	Unknown at this time	Dependent on survey results

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APPENDICES

Appendices are available upon request.

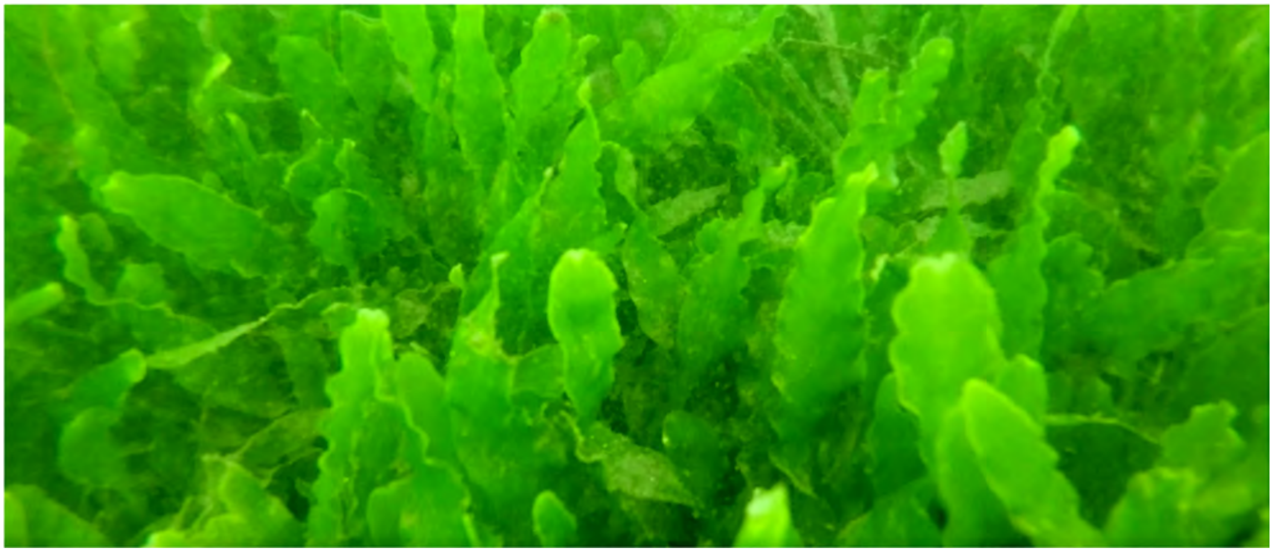
Appendix A. The effect of the 2000 *Caulerpa taxifolia* infestation on invertebrate infauna and epifauna at Agua Hedionda lagoon, Carlsbad, California.

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.

SURVEY DESIGN FOR
NEWPORT BAY *CAULERPA PROLIFERA* ERADICATION SURVEYS



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June 2022

GOAL

The overall goal of this work is to support eradication of *Caulerpa prolifera* from Newport Bay through providing strategic survey at eradication level and high intensity levels as directed by the Southern California Caulerpa Action Team (SCCAT). It is anticipated that survey will be conducted at the known locations of *Caulerpa prolifera* at China Cove and Collins Isle, Newport Bay as well as within high priority habitat areas that are deemed to be at risk of infestation based on the prior documentation of *Caulerpa* distribution and predicted patterns of spread. The results of the survey effort will inform whether additional eradication and invasive species management responses are necessary within the nearshore ecosystem. This survey effort is an element of a broader eradication program documented in the Rapid Response and Eradication Plan for the Invasive Green Alga *Caulerpa prolifera* in Newport Bay (Southern California *Caulerpa* Action Team (SCCAT) 2021) and supplemental Adaptive Management Memorandum #1 (November 2, 2021) and Adaptive Management Memorandum #2 (May 10, 2022).

This present effort includes funding for 32 acres of eradication level survey, or 64 acres of high intensity level survey, or a combination of the two survey levels. The effort is to be distributed based on guidance from the SCCAT and USFWS Project Officer considering areas of highest risk for infestation, areas likely to be surveyed by others, and survey needs to sustain present removal efforts within known infestation loci.

INTRODUCTION

In April 2021, the non-native alga *Caulerpa prolifera* was confirmed growing in the China Cove area of Newport Bay, California. The species has 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. It is suspected that *C. prolifera* can spread in both soft and hard-bottom habitats as well as within quiescent and more energetic coastal environments. This is based on observations of natural populations within the species native range, common problems with the species in aquariums overgrowing rocky substrates, and observations of congener species such as *C. taxifolia, C. racemosa*. Further, based on environmental impacts of other *Caulerpa* species, this alga is potentially a serious invasive species. Other species of *Caulerpa* are well-documented as having aggressively displaced native habitats when introduced, both in California, Australia (Creese et al. 2004), and Mediterranean waters (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. The seriousness and acknowledged threat from its close relative, *Caulerpa taxifolia,* prompted an effective, highly successful *C. taxifolia* eradication program in two southern California locations over a period of eight years at a cost of over seven million dollars. This effort was undertaken by the Southern California *Caulerpa* Action Team (SCCAT), a partnership of federal, state, and local agencies and private parties assembled to eradicate this marine invasive (Merkel & Associates 2006). The quick action and investment protected California's coastal ecosystems from the threat of invasion and led to the subsequent recovery of critically important eelgrass habitat that had already been lost due to the expansion of Caulerpa (Anderson 2005).

Based on preliminary surveys conducted in late April 2021, it is estimated that *C. prolifera* is affecting roughly 1.2-hectare of seafloor within China Cove in the entrance channel area of Newport Bay (Figure 1). 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 (SCCAT 2021). The distribution pattern noted during eradication level (100% bottom coverage) surveys revealed a central large patch and scattered smaller patches extending outward from the main patch (Merkel & Associates 2021). This is a pattern that was typical of that seen during prior infestations within other California systems by *C. taxifolia*. However, during completion of the initial infestation removal actions undertaken between July 5 and 14 it was noted that considerable fragmentation of the alga had occurred between the surveys completed from June 16-18, and the commencement of removals just two weeks later. It is likely that fragmentation at the present time is due to warming waters triggering a natural process for vegetative spread by fragmentation. Also notable during the removal process was the find of a two-frond patch attached and growing at a depth of -22.5 feet MLLW. The degree of fragmentation and depth range to which algae occurred was notable.

Within its native range, as well as in hobbyist aquariums, *Caulerpa prolifera* can spread asexually by fragments and sexually through the release of reproductive spores. The *C. prolifera* in China Cove was documented as well established as long ago as October 2020 and therefore it was suspected that the alga may have already spread to other locations. On March 25, 2022, a second infestation locus was identified at Collins Isle located approximately 2.5 km from China Cove at the western tip of Balboa Island. The detection of additional patches was not unexpected, but it was somewhat of a surprise that the next detection was so distant from the initially detected infestation site. Both known infestations are presently being treated.

There is a present and urgent need to conduct additional work to accomplish the following:

1) Complete follow-up survey within the treated locations at China Cove and Collins Isle as part of the Phase 1 removal efforts while additional funding to complete the removal at China Cove is being secured.

2) Conduct *Caulerpa prolifera* surveys in high-risk areas within Newport Bay. Most specifically, but not exclusively this need is urgently focused between the two known infestation locations at China Cove and Collins Isle.

3) Integrate existing survey coverage and strategically respond to identified survey gaps, while capitalizing on survey coverage completed by others.

The USFWS funded survey efforts contribute towards achieving the first and second of these needs.

SURVEY DESIGN

The surveys to be conducted under the present effort are intended to be responsive to direction of the SCCAT and USFWS Project Officer based on developing actionable information and survey needs as the eradication program progresses. As such, it is expected that survey locations may change over time. Further, while survey needs within Newport Bay are extensive, funding under the present contract is finite and must be strategically applied under the eradication program. As such, this survey design provides for ability to perform a minimum of 32 acres of eradication level surveys or a minimum of 64 acres of high intensity surveys, or an intermediate sized area (i.e., between 32 and 64 acres) based on allocating the same total effort among a combination of survey intensities. To be specific, the survey levels for Caulerpa surveys are defined under the Caulerpa Control Protocol (SCCAT 2021) as follows:

1) *Surveillance Level – General survey coverage providing a systematic subsampling of the entire APE during which at least 20% of the bottom is inspected and widespread occurrences of Caulerpa would be expected to be identified if present. Surveys may be accomplished using diver transects, remote cameras, and acoustic surveys with visual ground truthing. Other proposed methods may be approved on a case-by-case basis by NOAA Fisheries and CDFW.*
2) *High Intensity Level– More intensive survey using a systematic sub-sampling of the entire APE during which at least 50% of the bottom is inspected. Surveys may be accomplished using diver or remote camera transects. Other proposed methods may be approved on a case-by-case basis by NOAA Fisheries and CDFW.*
3) *Eradication Level – This is the most intensive survey using a systematic and comprehensive survey of the entire APE during which 100% of the bottom is inspected. Surveys must be accomplished using divers moving at a rate appropriate to the site conditions to ensure that all areas are comprehensively searched irrespective of site conditions which may complicate surveys. Other proposed methods may be approved on a case-by-case basis by NOAA Fisheries and CDFW.*

Caulerpa Control Protocol (SCCAT, Version 5 – October 20, 2021)

Given the scale of the areas that are considered to be at risk to infestation and the finite resources available to complete the investigations, the first element of work is to develop a strategic distribution of survey effort based on potential risk of infestation by *Caulerpa prolifera*. The second element is to define the survey methods to be applied in completing the work. Finally, the third element is to transmit the findings of the effort into the broader eradication efforts under the direction of the SCCAT and USFWS Project Officer.

To support survey effort distribution, Lower Newport Bay has been subdivided into non-uniform polygons defined by logical breaks that will allow easier management of survey effort and data collected through various sources (Figure 1). Specific inputs to the segmentation effort included: federal channel boundaries and reaches, RGP 54 boundaries, street ends and public access points, proposed project boundaries, and present known infestation survey areas. In total the 858-acre Lower Newport Bay has been segmented into 281 individual polygons that range in size from 0.34 acres to 36.80 acres, with an average area of 3.06 acres. The smaller polygons are located along the shoreline margins and are defined by street ends and designated shoreline access points and the boundaries of the RGP54 operational limits that generally extends from the intertidal zone to just beyond the bayward ends of existing docks. Larger polygons are defined in the more open areas of the bay with the largest polygons being surveyed by the Army Corps of Engineers in association with planned dredging.

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Lower Newport Bay *Caulerpa* Survey Segmentation

SCCAT Newport Bay *Caulerpa prolifera* Eradication Program

Figure 1

SURVEY METHODS

- ***Eradication Level Surveys***

This is the most intensive survey approach outlined under the Caulerpa Control Protocols and provides for a systematic and comprehensive survey of the entire APE during which 100% of the bottom is inspected. Surveys must be accomplished using divers moving at a rate appropriate to the site conditions to ensure that all areas are comprehensively searched irrespective of site conditions which may complicate surveys.

Surveys will be conducted by a tending vessel deploying and retrieving a series of parallel survey transects placed with a high degree of precision using a real-time kinematic (RTK) global positioning system (GPS) and navigational software to control line placement. 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. 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, multiple SCUBA divers (3 to 7) 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. Variable diver spacing will be used depending upon field conditions considering water clarity, bottom topology, and presence or absence of visual obstructions such as eelgrass. The separation between diver centerlines will not exceed 2 meters (clear water, unobstructed bottom) and will not be less than 1 meter (turbid water, dense eelgrass or other visual obstructions). Speed of survey will vary between approximately 0.2 and 1.2 knots.

For each survey, a lead diver will then follow the transect line offset to one side of the line. Each subsequent diver will keep pace with the lead diver but follow slightly behind the diver to 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. They are all certified Caulerpa survey divers through the SCCAT testing program administered by National Marine Fisheries Service and California Department of Fish and Wildlife.

Transects are laid in a parallel alignment to each other and divers swim up one side of the transect and then down the other as a group as shown in Photos 1 and 2 as well as Figure 2 from the China Cove known infestation area.

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.

All seafloor-attached *C. prolifera* encountered will be flagged near the occurrence by florescent pin flags and its position recorded using GPS. 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.

Any detected *Caulerpa* will be recorded and reported to the USFWS Project Officer and SCCAT within 24 hours and the needs for follow-up removal identified. Recorded positions will be used to target smaller patches and allow the site to be revisited to ensure removal. Treatment of encountered *Caulerpa* is not included in this survey effort and would be addressed through separate contracting avenues.

- ***High Intensity Level Surveys***

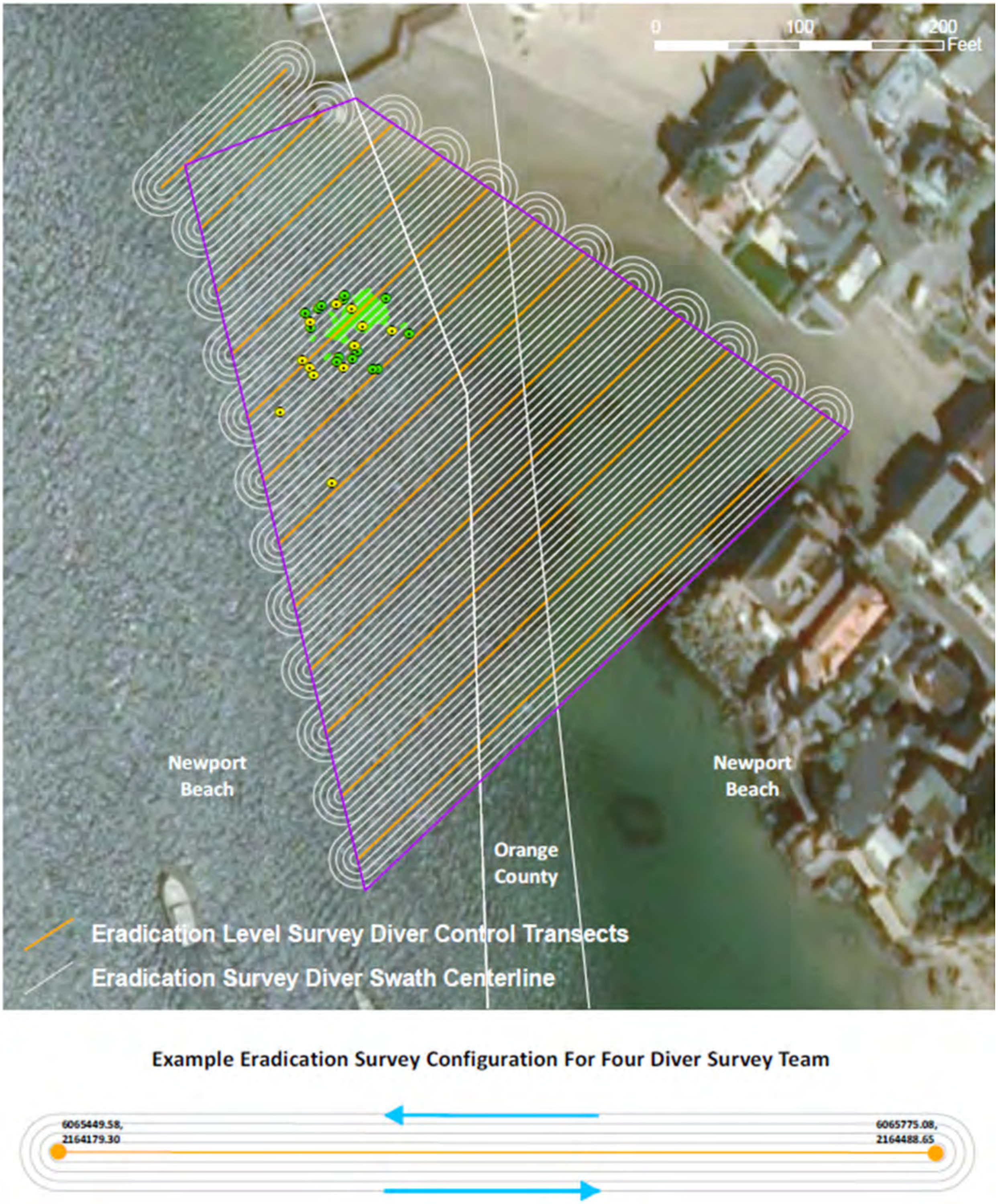
High intensity surveys call for inspection of at least 50% of the bottom and can be accomplished using diver or remote camera transects. For the present survey effort, it is the intent to principally conduct these surveys using divers following a similar systematic transect methodology outlined for eradication level surveys above. However, divers would be spaced between 2 and 4 meters apart on lines depending on conditions.

In open bottom conditions with relatively uniform depths, surveys may be conducted by vessel towed video camera arrays that are monitored in real time by *Caulerpa* surveyors monitoring video displays. The survey vessel will navigate along pre-established tracklines with spacing set to ensure that the number of cameras and spacing between cameras allows viewing of a minimum of 50% of the bayfloor. Typically, the vessel positioned by RTK GPS navigation software travels at a rate of 0.5 to 2 knots as dictated by horizontal visibility and thus the distance forward of the cameras that the bottom may be viewed.

- ***Survey Reporting***

Tracking of survey completion and results will be communicated to the USFWS Project Officer and SCCAT during regular eradication program SCCAT Steering Committee meetings using the bay segment graphic as a visual tool. A final survey report will be prepared at the end of the contract period identifying locations and areas of survey along with the survey level associated with each survey. The report would include digital data deliverables including PDF versions of the report and graphics, as well as ArcGIS files suitable to support the eradication effort.

Figure 2. Example survey design for eradication level survey



IDENTIFICATION OF HIGH RISK AND HIGH PRIORITY SURVEY AREAS

The lack of information regarding the origin of the initial infestation of Newport Bay by *Caulerpa prolifera* generates a degree of difficulty in focusing surveillance efforts under this program. As noted above, it is not known if the *Caulerpa* infestation in China Cove is the location of the initial infestation, or a secondary infestation. What is known is that the China Cove infestation has been present since at least October 2020 when the infestation was captured by a videographer, Lance Milbrand. However, the species was not identified until spring 2021 (SCCAT 2021). Video made available by Milbrand Cinema and observations made by Merkel & Associates and Marine Taxonomic Services staff have demonstrated that the infestation area has previously and is presently shedding mobile fragments that have been termed "rollers" in the eradication effort vernacular. This is based on the propensity of fragments to be loosely wrapped up with other drift algal wrack, generally *Gracilaria*, that has been observed rolling across the bay floor in the tidal currents near the known infestation location. The March 25, 2022, identification of the second infestation locus at Collins Isle was located approximately 2.5 km from China Cove and thus creates a logical reach within which to concentrate survey effort. However, there are on-going needs focused on surveys around the known infestation locations as well. For this reason, it is critical to balance survey needs against the available resources to complete the work in a prioritized manner.

Because the timeline and origin of the present infestation is unknown it is helpful to consider a conceptual model of potential pathways for *Caulerpa* spread as a means of supporting the prioritization of survey areas (Figure 3). This approach also assists in illuminating the potential strength of infestation pathways and alternative pathways.

The spread pathways model includes some basic assumptions. The first is that the infestation is due to a discrete introduction. In other words, the assumption is that the infestation identified is from a single discharge event. This is most likely due to a tropical marine aquarium release based on the widespread presence of *C. prolifera* in the aquarium trade, the lack of alternative uses for the species in California, and the regular practice of aquarium water replacement by natural sea water exchanges. Further, prior work on eradication of *C. taxifolia* suggested this avenue for initial establishment in both Agua Hedionda Lagoon and Huntington Harbour (Merkel & Associates 2006).

As a working hypothesis, it is assumed that the infestation in China Cove is the initial infestation. This is a reasonable assumption for many reasons. First the infestation site has high visitor volume and easy public accessibility to water for aquarium water exchanges. Second, the local area has a high concentration of aquariums in homes near the water based on investigations conducted during the surveillance efforts associated with the 2000-2006 infestation of *C. taxifolia*. Third, there is a potential pathway for release from an institutional aquarium seawater system at China Cove, although *C. prolifera* is not known to have been kept at the adjacent aquarium. Finally, in previous modeling conducted to assess risk of infestation by *Caulerpa taxifolia*, China Cove ranked as the highest infestation risk area within Newport Bay (Merkel & Associates 2006). If China Cove is the point of the initial infestation, then the distribution of any secondary infestation would be most likely found in proximity to China Cove and distributed along a dispersal gradient radiating away from the site based on characteristics of the operative transport mechanisms (Figure 3). This conceptual spread model was developed previously to support NOAA's National Marine Fisheries Service funded targeted survey efforts for high-risk areas in nearshore environments (Merkel & Associates 2021). While an alternative and less likely scenario is that the infestation at China Cove is a secondary infection. Under such circumstance, it would be important to examine areas both down dispersal gradient from China Cove as well as up gradient where the initial infestation loci may occur. However, absent more data, it is best to focus on the more tangible model

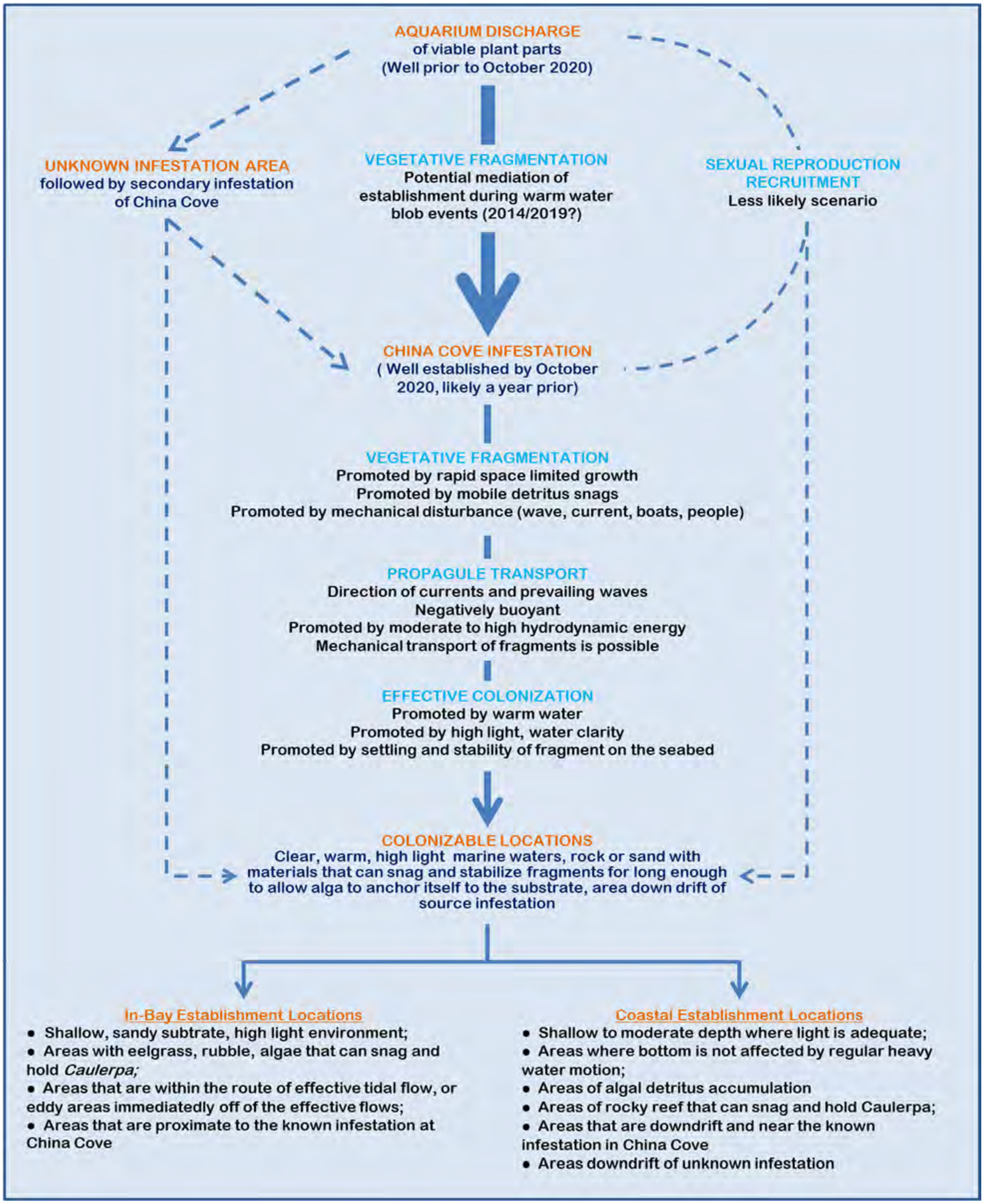


Figure 3. Conceptual model for the spread of Caulerpa prolifera from China Cove infestation

that assumes China Cove is the initial infection epicenter and Collins Isle is a secondary infection derived from spread from China Cove. This provides guidance to focus survey efforts between the known locations along the probable pathway of transport between known loci.

An additional assumption is that in the present infestation, *C. prolifera* is spread primarily by vegetative fragmentation. Under such a spread mechanism, areas that are at highest risk to additional colonization would be those supporting suitable habitat conditions to support *Caulerpa*, both inside and outside of the bay, that are within the path of transport of currents that pass through the known infestation area. Areas of high risk should be considered areas where tidally transported, negatively buoyant algal wrack is moved by tidal currents. Areas where tidal eddies would be expected such as at the confluence of channels, or after constrictions in the bay are of particular interest, as are areas of current velocity drop, or areas where material in transit may be captured and sequestered from the active transport. Areas where the channel rapidly widens and thus velocities drop, or areas where the roughness dramatically increases, such as within eelgrass beds. As mobile *Caulerpa* has been regularly observed to be associated with "rollers" mediated by balls of *Gracilaria*, the distribution of *Gracilaria* wrack accumulation is of particular interest.

Surveys have been and are presently being conducted within Lower Newport Bay within various segments with funding provided by the Army Corps of Engineers, State Water Resources Control Board, City of Newport Beach, and private parties pursuing regulated in-water work. Prior surveys have been funded by the U.S. Fish & Wildlife Service, National Marine Fisheries Service (Figure 4). While it is anticipated that future surveys will also be undertaken by an organized volunteer effort guided by California Department of Fish & Wildlife and including Orange County Coastkeepers as well as Reef Check. The distribution of prior surveys and the anticipated distribution of future survey work is useful in guiding the distribution of surveys under the current effort by principally targeting areas that are not otherwise going to be surveyed, or which have been surveyed recently.

RECOMMENDATIONS FOR TARGETED SURVEYS

Using the conceptual model presented in Figure 3 for in-bay spread combined with data from known infestation distribution and planned and completed surveys it is possible to develop a working target for completion of surveys under this effort. As additional data becomes available, the model may be adjusted.

By identifying the location of known infestation loci along with areas that have been surveyed previously and areas that are planned to be surveyed, and then adjusting the degree of confidence in survey coverage based on age of survey and initial intensity of survey, it is possible to produce a map of *Caulerpa* presence and likely absence based on available information. From this effort it is then possible to identify areas of highest risk of occurrence of *Caulerpa* based on expected infection pathway between the two known sites and presence of potential factors that would favor establishment of *Caulerpa* along the pathway such as eelgrass, null zones, or eddies in tidal circulation. These have been plotted as a heat map across segmentation cells (Figure 5). This preliminary risk map can be improved with the addition of hydrodynamic, eelgrass, and transport data, as well as any further *Caulerpa* finds. As a second step, focused Caulerpa survey data (Figure 4) were overlain to identify gaps in the surveys, difference in survey intensity, and diminishing confidence with survey age. Note that survey commitments that have not yet been fulfilled have been added to this layer as they are expected to occur and thus surveys of these areas would not warrant expenditures from the present funding.

This process has resulted in the identification of high priority sites as well as lesser, but still important areas to be surveyed. By dividing the strength of the infestation risk by the strength of survey history it is possible to develop a survey prioritization map wherein the areas of highest infestation risk and lowest level of survey confidence or coverage are merged (Figure 6).

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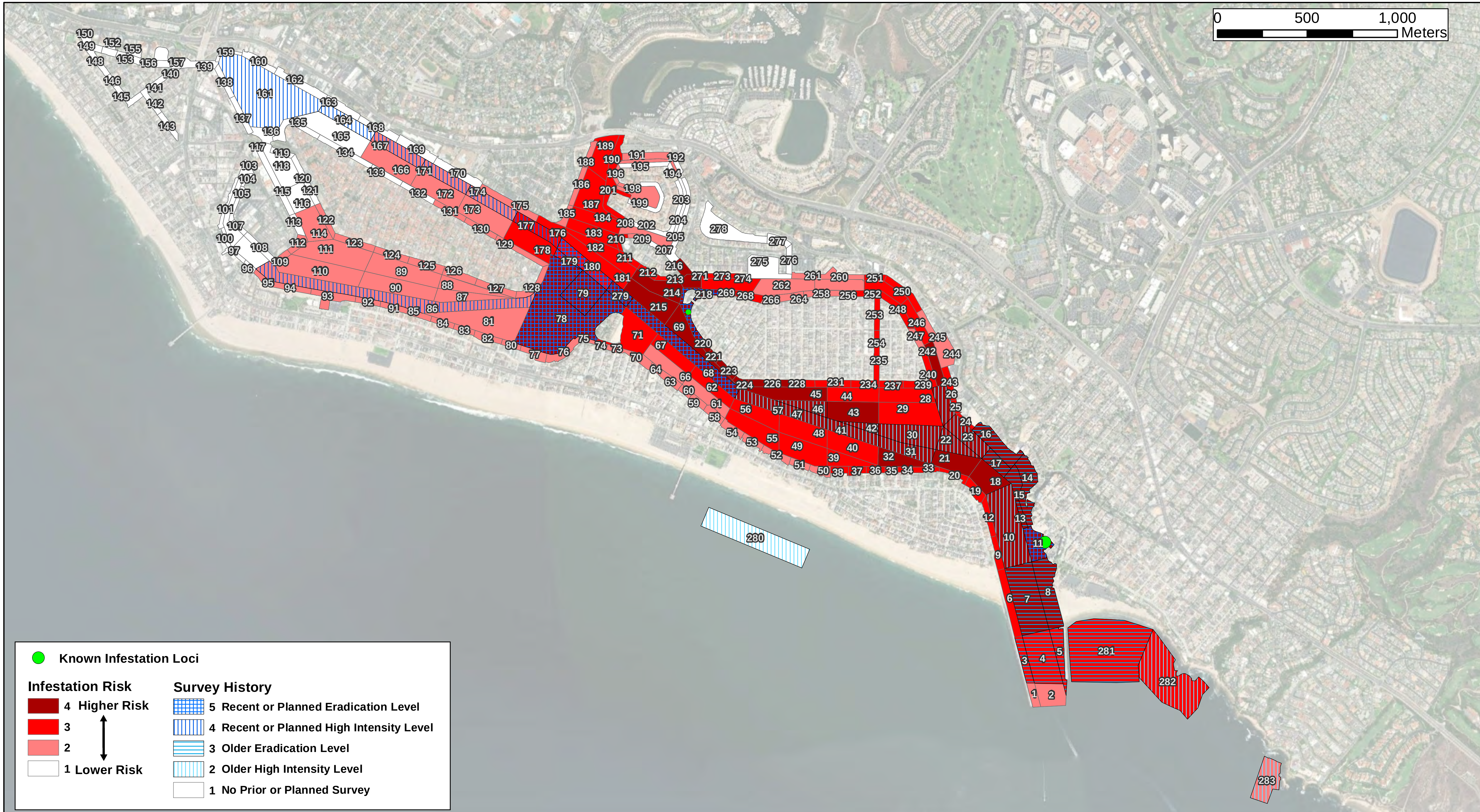
Lower Newport Bay *Caulerpa* Survey History

SCCAT Newport Bay *Caulerpa prolifera* Eradication Program

Figure 4



Lower Newport Bay *Caulerpa* Survey Segmentation

SCCAT Newport Bay *Caulerpa prolifera* Eradication Program

Figure 5

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Lower Newport Bay *Caulerpa* Survey Prioritization

SCCAT Newport Bay *Caulerpa prolifera* Eradication Program

Figure 6

The segments identified as highest survey priority are those with the highest potential for infestations and no prior or planned survey effort. These are identified in Figure 6 and summarized in Table 1 along with the area of each segment included.

It is recommended that this table be used as a starting point for conducting surveys for Caulerpa with surveys being conducted at an eradication level, commencing with segments closest to existing known infestation and working outward. The prioritized sites cannot be fully covered at the eradication level, however it is believed that this level of survey is warranted under the assumption that surveys are principally anticipated to yield small fragment or colony finds rather than more expansive colonies that would likely be detected with a 50 percent coverage High Intensity survey. Further, it is suggested that the larger segments that required tighter survey control capabilities be targeted under this contract, but the other high priority segments that are located adjacent to shorelines or in other areas where physical features such as docks and marina be used to guide supporting volunteer efforts. In this manner the segments on the Table 1 list can be systematically and efficiently covered by survey. This table is derived from the full segmentation table (Appendix A).

Table 1. Recommended Priority Ranking for Surveys

Segment	Infestation Risk	Survey History	Priority Score	Acres
18	4	1	4	5.7
21	4	1	4	6.0
32	4	1	4	4.7
43	4	1	4	7.9
45	4	1	4	7.4
69	4	1	4	6.3
214	4	1	4	5.9
215	4	1	4	7.7
216	4	1	4	1.5
217	4	1	4	1.7
220	4	1	4	0.5
221	4	1	4	0.6
222	4	1	4	0.6
223	4	1	4	0.5
224	4	1	4	0.8
225	4	1	4	1.1
226	4	1	4	0.7
227	4	1	4	0.7
242	4	1	4	3.1
271	4	1	4	0.3
TOTAL				**63.6**

In addition to the priority survey areas identified in Table 1, the present funds have been used on two separate occasions to support even higher priority survey needs directly associated with the two known infestation loci at China Cove and Collins Isle. Immediately after the discovery of the Collins Isle infestation, funding was utilized to survey areas around the known extent of the infestation including segments 218, 219, and 269 for a survey extent of 4.2 acres at an eradication survey level. This excludes

0.4 acre of segment 218 that was surveyed by funding provided by the private homeowner completing permit required surveys in conformance with the *Caulerpa* Control Protocols. As a result of this survey these segments dropped in priority and thus do not occur on Table 1.

At China Cove, the eradication program calls for recurrent systematic surveys to support Caulerpa removal from this known infestation area. In May 2022 funding for this effort that was provided by the State Water Resources Control Board ran out pending a request for expanded funding being considered by the Board. This new funding was authorized in June 2022 with expanded funds coming from the State Board and the City of Newport Beach. In order to bridge the temporal funding gap and stay on track with the eradication activities at China Cove. USFWS funding was applied to survey 3.7 acres of the infestation area at an eradication survey level. As this area is already subject to continuing funded surveys and *Caulerpa* removals, it also does not occur in the Table 1 highest priority ranking for the present funds due to the recent allocation of additional funding by the state and city.

The two infestation loci actions have expended 7.9 acres of the eradication level survey allocated under this contract effort.

ADAPTIVE MANAGEMENT

Eradication efforts are fluid and subject to continuously obtained data from a variety of sources. These sources may include the removal activity follow-up surveillance within known infestation areas, discovery of new infestation loci, information on sexual reproduction from genetic sampling of infestation or environmental DNA (e DNA) detection in other areas, to name a few. Data collected contributes to understanding the potential pathways of spread and may result in changing surveillance area priorities. As a result, this plan is intended to be adaptive to new information and allow for diversion of any remaining survey area capacity in response to new information. Such diversion of survey effort would be at the direction of the USFWS Project Officer in coordination with the SCCAT.

To respond effectively to shifting priorities, it is intended that addenda memorandums would be attached to this work plan to document survey status, and planned survey actions. This approach would allow for rapid updates to the plan of action without requiring major changes to this plan itself. To establish this process the first recommended survey actions are summarized in Appendix B (Addendum 1).

Each Addendum provides a summary of areas surveyed at either eradication level or high intensity and recommendations for subsequent allocation of survey efforts.

SURVEY DATA MANAGEMENT AND INTEGRATION

Collected survey data will be managed within an ESRI ArcView GIS database structures. Survey data from the present surveys will be provided in a summary report describing methods, findings, any issues encountered and recommendations. The report will include survey area graphics and all graphic deliverables, and the report will be provided electronically in PDF version and spatial data will be provided in a ESRI shapefile format. It is anticipated that these data, along with data from other sources will be integrated into a broader eradication program database to present information in a comprehensive fashion. The funding of this data management effort is not included in the work scope for the present survey effort.

PROGRAM SCHEDULE

The survey program would ideally extend from spring 2022 to spring 2023. This would allow funding to be utilized to provide continued surveillance of areas considered to be high risk, while extending funding availability to be applied as may be required to fill gaps in funding at known infestation locations should this be required. A survey schedule for the proposed work effort is outlined in Table 2.

Table 2. Schedule of performance and task deliverables

Deliverable	Description	Form	Period of Work
1	Survey Design	Electronic written format to USFWS and SCCAT	May – Jun 2022
2	USFWS and SCCAT review, incorporation of comments	USFWS/SCCAT teleconference participation / electronic email	Jul 6, 2022
3	Final survey design	Electronic written format to USFWS	July 16, 2022
4	Surveys Conducted	SCCAT Meeting Updates	May 2022 – Jun 2023
5	*Caulerpa* observations – location description and geographic coordinates	Electronic written format to USFWS	Within 24 hours of *Caulerpa* finds
6	Survey results documentation report	Electronic written format and spatial data to USFWS	June 2023

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APPENDIX A

LOWER NEWPORT BAY SEGMENTATATION SCORING AND AREAS

LOWER NEWPORT BAY SEGMENTATATION SCORING AND AREAS

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
1	2	1	2.0	1.4
2	2	1	2.0	4.8
3	3	3	1.0	2.8
4	3	3	1.0	11.1
5	3	3	1.0	3.6
6	3	1	3.0	4.1
7	4	3	1.3	14.6
8	4	3	1.3	9.0
9	3	1	3.0	1.7
10	4	2	2.0	16.3
11	4	5	0.8	4.5
12	3	1	3.0	1.7
13	4	2	2.0	1.0
14	4	3	1.3	6.0
15	4	3	1.3	2.5
16	4	3	1.3	5.7
17	4	3	1.3	2.8
18	4	1	4.0	5.7
19	3	1	3.0	1.7
20	3	1	3.0	1.0
21	4	1	4.0	6.0
22	4	2	2.0	4.4
23	4	2	2.0	5.9
24	3	1	3.0	0.6
25	3	1	3.0	0.5
26	3	1	3.0	0.8
27	4	2	2.0	4.1
28	3	1	3.0	6.0
29	3	1	3.0	10.4
30	4	2	2.0	8.7
31	4	2	2.0	5.4
32	4	1	4.0	4.7
33	3	1	3.0	1.1
34	3	1	3.0	0.6
35	3	1	3.0	0.6
36	3	1	3.0	0.9
37	3	1	3.0	1.1
38	3	1	3.0	1.0
39	3	1	3.0	3.7
40	3	1	3.0	7.3
41	4	2	2.0	4.5
42	4	2	2.0	1.7
43	4	1	4.0	7.9
44	3	1	3.0	5.6

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
45	4	1	4.0	7.4
46	4	2	2.0	3.3
47	4	2	2.0	4.2
48	3	1	3.0	6.9
49	3	1	3.0	7.7
50	2	1	2.0	1.4
51	2	1	2.0	1.1
52	2	1	2.0	1.4
53	2	1	2.0	1.3
54	2	1	2.0	1.4
55	3	1	3.0	7.2
56	3	1	3.0	5.5
57	4	2	2.0	3.7
58	2	1	2.0	1.3
59	2	1	2.0	1.0
60	2	1	2.0	0.7
61	2	1	2.0	4.5
62	3	1	3.0	4.4
63	2	1	2.0	1.4
64	2	1	2.0	0.6
65	2	1	2.0	0.9
66	2	1	2.0	4.6
67	3	1	3.0	4.6
68	4	4	1.0	12.0
69	4	1	4.0	6.3
70	2	1	2.0	0.6
71	3	1	3.0	8.6
72	2	1	2.0	0.5
73	2	1	2.0	0.5
74	2	1	2.0	0.5
75	2	1	2.0	0.5
76	2	1	2.0	2.3
77	2	1	2.0	1.5
78	4	5	0.8	36.8
79	4	5	0.8	8.1
80	2	1	2.0	0.8
81	2	1	2.0	14.3
82	2	1	2.0	1.4
83	2	1	2.0	0.9
84	2	1	2.0	1.0
85	2	1	2.0	1.1
86	2	4	0.5	22.2
87	2	1	2.0	4.4
88	2	1	2.0	4.3
89	2	1	2.0	9.9

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
90	2	1	2.0	8.7
91	2	1	2.0	1.2
92	2	1	2.0	1.1
93	2	1	2.0	3.1
94	2	1	2.0	1.8
95	2	1	2.0	0.8
96	1	1	1.0	0.5
97	1	1	1.0	0.6
98	1	1	1.0	4.1
99	1	1	1.0	2.6
100	1	1	1.0	0.9
101	1	1	1.0	0.7
102	1	1	1.0	0.6
103	1	1	1.0	2.2
104	1	1	1.0	0.6
105	1	1	1.0	0.5
106	1	1	1.0	0.7
107	1	1	1.0	0.6
108	1	1	1.0	1.7
109	2	1	2.0	0.9
110	2	1	2.0	11.5
111	2	1	2.0	11.3
112	2	1	2.0	1.3
113	1	1	1.0	1.2
114	2	1	2.0	5.9
115	1	1	1.0	1.2
116	1	1	1.0	4.3
117	1	1	1.0	2.4
118	1	1	1.0	6.4
119	1	1	1.0	1.0
120	1	1	1.0	1.1
121	1	1	1.0	1.2
122	2	1	2.0	1.5
123	2	1	2.0	2.1
124	2	1	2.0	1.8
125	2	1	2.0	2.2
126	2	1	2.0	2.1
127	2	1	2.0	2.1
128	2	1	2.0	2.1
129	2	1	2.0	2.3
130	2	1	2.0	1.5
131	2	1	2.0	1.5
132	1	1	1.0	1.6
133	1	1	1.0	1.4
134	1	1	1.0	2.5

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
135	1	1	1.0	2.9
136	1	1	1.0	2.2
137	1	1	1.0	1.7
138	1	1	1.0	1.4
139	1	1	1.0	2.2
140	1	1	1.0	0.5
141	1	1	1.0	0.8
142	1	1	1.0	1.5
143	1	1	1.0	1.3
144	1	1	1.0	1.0
145	1	1	1.0	0.6
146	1	1	1.0	0.5
147	1	1	1.0	0.8
148	1	1	1.0	0.6
149	1	1	1.0	0.9
150	1	1	1.0	0.9
151	1	1	1.0	0.8
152	1	1	1.0	0.8
153	1	1	1.0	0.5
154	1	1	1.0	0.8
155	1	1	1.0	1.0
156	1	1	1.0	0.6
157	1	1	1.0	1.5
158	1	1	1.0	1.3
159	1	1	1.0	0.7
160	1	1	1.0	1.8
161	1	4	0.3	31.8
162	1	1	1.0	3.3
163	1	1	1.0	2.3
164	1	4	0.3	5.0
165	1	1	1.0	10.2
166	2	1	2.0	8.4
167	2	4	0.5	4.8
168	1	1	1.0	2.1
169	1	1	1.0	2.9
170	1	1	1.0	3.2
171	2	4	0.5	4.4
172	2	1	2.0	7.7
173	2	1	2.0	7.9
174	2	4	0.5	4.5
175	2	1	2.0	3.3
176	3	1	3.0	1.9
177	3	4	0.8	4.4
178	3	1	3.0	7.6
179	3	5	0.6	1.0

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
180	3	5	0.6	5.6
181	3	1	3.0	6.8
182	3	1	3.0	6.3
183	3	1	3.0	5.0
184	3	1	3.0	5.0
185	2	1	2.0	1.1
186	2	1	2.0	1.6
187	3	1	3.0	7.8
188	2	1	2.0	1.1
189	3	1	3.0	5.8
190	3	1	3.0	1.6
191	2	1	2.0	2.7
192	1	1	1.0	0.6
193	1	1	1.0	1.6
194	1	1	1.0	0.5
195	1	1	1.0	1.7
196	2	1	2.0	0.6
197	3	1	3.0	1.9
198	2	1	2.0	0.6
199	1	1	1.0	2.6
200	2	1	2.0	3.8
201	3	1	3.0	2.1
202	1	1	1.0	1.8
203	1	1	1.0	1.8
204	1	1	1.0	1.5
205	1	1	1.0	2.1
206	1	1	1.0	1.3
207	1	1	1.0	0.9
208	2	1	2.0	3.7
209	2	1	2.0	0.7
210	3	1	3.0	0.7
211	3	1	3.0	1.1
212	3	1	3.0	1.0
213	3	1	3.0	0.7
214	4	1	4.0	5.9
215	4	1	4.0	7.7
216	4	1	4.0	1.5
217	4	1	4.0	1.7
218	4	5	0.8	3.4
219	4	5	0.8	0.6
220	4	1	4.0	0.5
221	4	1	4.0	0.6
222	4	1	4.0	0.6
223	4	1	4.0	0.5
224	4	1	4.0	0.8

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
225	4	1	4.0	1.1
226	4	1	4.0	0.7
227	4	1	4.0	0.7
228	3	1	3.0	0.7
229	3	1	3.0	0.7
230	3	1	3.0	0.7
231	3	1	3.0	0.6
232	3	1	3.0	0.7
233	3	1	3.0	0.7
234	3	1	3.0	0.6
235	3	1	3.0	1.3
236	3	1	3.0	0.7
237	3	1	3.0	0.7
238	3	1	3.0	0.7
239	3	1	3.0	0.8
240	3	1	3.0	0.7
241	3	1	3.0	1.0
242	4	1	4.0	3.1
243	3	1	3.0	0.8
244	2	1	2.0	3.7
245	2	1	2.0	1.6
246	3	1	3.0	3.4
247	2	1	2.0	1.3
248	3	1	3.0	2.2
249	3	1	3.0	3.3
250	3	1	3.0	2.4
251	3	1	3.0	1.0
252	3	1	3.0	0.8
253	3	1	3.0	1.4
254	3	1	3.0	1.1
255	3	1	3.0	0.6
256	2	1	2.0	0.6
257	2	1	2.0	0.6
258	2	1	2.0	0.5
259	2	1	2.0	4.1
260	2	1	2.0	2.2
261	2	1	2.0	1.1
262	2	1	2.0	6.5
263	2	1	2.0	0.6
264	2	1	2.0	0.6
265	2	1	2.0	0.7
266	2	1	2.0	1.0
267	3	1	3.0	0.8
268	3	1	3.0	0.7
269	3	1	3.0	0.6

Segment #	Infestation Risk	Survey History	Priority Score	Area (acres)
270	3	1	3.0	5.1
271	4	1	4.0	0.3
272	3	1	3.0	0.5
273	3	1	3.0	0.5
274	3	1	3.0	0.6
275	1	1	1.0	6.0
276	1	1	1.0	1.2
277	1	1	1.0	1.5
278	1	1	1.0	7.9
279	4	5	0.8	2.2
280	1	2	0.5	17.0
281	3	3	1.0	13.3
282	3	2	1.5	34.8
283	2	2	1.0	7.0

APPENDIX B

SURVEY STATUS AND RECOMMENDATION ADDENDA

Merkel & Associates, Inc.
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San Diego CA • San Rafael CA • Arcata CA • Nehalem OR • Shelton WA

SURVEY DESIGN FOR NEWPORT BAY *CAULERPA PROLIFERA* ERADICATION SURVEYS

ADDENDUM 1

June 25, 2022

From: Keith Merkel

To: Will Miller, USFWS Project Officer

CC: SCCAT Steering Committee

This addendum to the Survey Design for Newport Bay *Caulerpa prolifera* Eradication Surveys is to accomplish the following:

1) Document completed eradication level surveys over 7.9 acres of bay bottom at Collins Isle and China Cove. These surveys were conducted in direct support of the infestation response at these locations when alternative funding for efforts was not available in these locations. Because these surveys were completed ahead of the screening effort undertaken for the work plan, the surveys were considered in the ranking, thus lowering the priority for these locations under the present contract;
2) Recommend eradication level surveys be performed at bay segments 69, 18, and 21. These segments, like many others score highest on the survey prioritization, but have been identified as priorities here due to their lack of prior survey adjacency to the known infestation loci, and location within the anticipated route of spread between the two known infestation loci;
3) Recommend guiding volunteer survey efforts towards bay segments 220-228 as a priority. Segments 229-240 are the next priority for volunteer survey. These areas are highly accessible off the street ends or by boat and are well defined spatially due to the presence of multiple bounding docks and other features. As a result, they lend themselves t easy survey and reporting using aerial image field maps and limited GPS coordinates. Because they are located mostly along the shoreline margins, they support a mixture of no eelgrass and abundant eelgrass and will required variable amounts of time to complete. However, each segment is a small enough element to be completed by a small crew in a few hours.

Summary Status Table

Segments	Acreage	Survey Level	Status	Approved	Remaining*
218, 219, 269	4.2	Eradication	Completed 4/8/22		27.8 ac. erad level
11	3.7	Eradication	Completed 5/26/22		24.1 ac. erad level
69, 21, 18	18.0	Eradication	Recommended 6/25		
TOTAL	**25.9**	**Eradication**			

*Contract has available 32 acres of eradication level or 64 acres of high intensity level survey that can be used.

ERADICATION OF *CAULERPA PROLIFERA* IN NEWPORT BAY, CALIFORNIA

California Department of Food and Agriculture
***Caulerpa* Eradication Services Agreement**
Task 1. Survey Plan

Prepared for:

California Department of Food and Agriculture
Plant Health and Pests Protection Services
Agreement #24-0042
David Pegos, CDFA
Nicole Lucas, CDFA

and

Southern California *Caulerpa* Action Team (SCCAT)

Prepared by:

Keith Merkel, Merkel & Associates, Inc.
Rachel Woodfield, Merkel & Associates, Inc.

September 20, 2024

ERADICATION OF *CAULERPA PROLIFERA* IN NEWPORT BAY, CALIFORNIA

California Department of Food and Agriculture
***Caulerpa* Eradication Services Agreement**

Task 1 Survey Plan

September 2024

INTRODUCTION

In April 2021, the invasive non-native alga *Caulerpa prolifera* was confirmed growing in the China Cove area of Newport Bay, California. The Southern California *Caulerpa* Action Team (SCCAT), a partnership of federal, state, and local agencies and private parties, assembled to respond to this marine invasive and developed a Rapid Response and Eradication Plan for the Invasive Green Alga *Caulerpa prolifera* in Newport Bay (SCCAT 2021b). Implementation of this plan has been underway since July 2021, conducted by Merkel & Associates, Inc. (M&A) and MTS, Ltd, under direction of SCCAT. The first phase of the eradication program involved the mapping and initial removal of *C. prolifera* from China Cove. It was completed in September 2023 after three consecutive surveys were conducted with no new finds of *C. prolifera* in the infestation area (SCCAT 2023). The first phase was funded primarily by the State Water Resources Control Board Cleanup and Abatement Fund.

This agreement with the California Department of Food and Agriculture, Plant Health and Pest Protection Services (CDFA/PHPPS) provides funding to continue implementation of the Eradication Plan through systematic inspection and response in the known infestation area in China Cove, to identify and survey other high-risk areas in Newport Bay, and to conduct outreach to prevent future introductions of *Caulerpa* into California's waterways.

The first task of this agreement is to conduct quarterly dive surveys in known previously infested areas in Newport Bay to confirm the sites continue to be free of *C. prolifera* and/or remove any *C. prolifera* finds. The following is the plan for the survey work. This plan is a deliverable for the agreement with CDFA/PHPPS (Agreement #24-0042).

TASK 1 WORK PLAN

The methods of survey and treatment conducted under Task 1 of the agreement will be consistent with the Eradication Plan and two subsequent adaptive management memoranda that have been issued to the plan (SCCAT May 2021, September 2021, and May 2022).

Surveys

Two locations will be surveyed under Task 1: China Cove and Collins Isle in Newport Bay (Figure 1). China Cove will be surveyed quarterly by *Caulerpa* Certified Divers from M&A and MTS using a combination of two methods. The core of the infestation site will be surveyed by examining a fixed grid deployed in the center of China Cove. This method was developed in response to the need for divers to work at their own pace to examine the seafloor for small occurrences of *C. prolifera* hidden in dense eelgrass (*Zostera marina*) beds of similar color. The grid is broken into cells, and each will be surveyed at eradication level intensity (100% coverage). The survey timing of each grid cell is highly dependent upon the extent of eelgrass present within the cell. Dense and tall eelgrass greatly hinders the rate of progress of the surveys, while relatively unvegetated cells can be surveyed more quickly. Surveys will target slack or neap tide conditions with little current, allowing eelgrass to stand upright making it less difficult to search the areas below the eelgrass canopy and thus improving search efficiencies.

The remainder of the infestation area will be surveyed at the eradication-level surveys using vessel-positioned transects and divers. 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 each 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 weight will be attached to the transect line as it is deployed to the bottom and then anchored at the end of the transect and marked with a buoy. Transects will be placed parallel to one another at a spacing relative to underwater visibility and the number of divers present.

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 assist divers in maintaining spacing during the survey (Figure 2). Diver spacing will be adjusted depending on the horizontal visibility at the time of the survey and will never exceed 1.5 meters. The lead diver will follow the transect line with each subsequent diver keeping pace with the lead diver but following 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 will tug the line to indicate that the team needs to 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) will dictate the speed and spacing of the survey team.

Figure 1. Location of two sites where *Caulerpa prolifera* has been discovered in Newport Bay.

Figure 1. Divers spaced by knotted rope during training on survey procedures (left), and a team of divers aligning in preparation to swim a laid transect (right).

The above procedure is illustrated in Figure 1. Transects will be placed and surveyed throughout the infestation area, around the survey grid.

Any 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 1-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 proper disposal and the position of the encounter recorded by GPS.

Collins Isle is located in central Newport Bay (Figure 1). The small infestation found there in May 2022 was addressed through hand removal and subsequent placement of barriers to ensure any below-ground material did not survive. Once the removal was determined to be successful, the site was dredged following additional follow-up inspections required by the project permits. Although extensive surveys of the surrounding area were performed in 2022, SCCAT feels the infestation site and surrounding area should be examined again under this task. The area will be surveyed by laid lines as described above at a frequency to be determined by SCCAT following completion of the first survey.

The proposed schedule for the Task 1 surveys is provided in Table 1 at the end of this plan document.

Efficacy Assessment

This phase of the eradication included in this agreement involves the determination of when *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) the verified absence of *C. prolifera* from the infestation site.

The first criterion can be met through survey of the site for regrowth over time. It 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*.

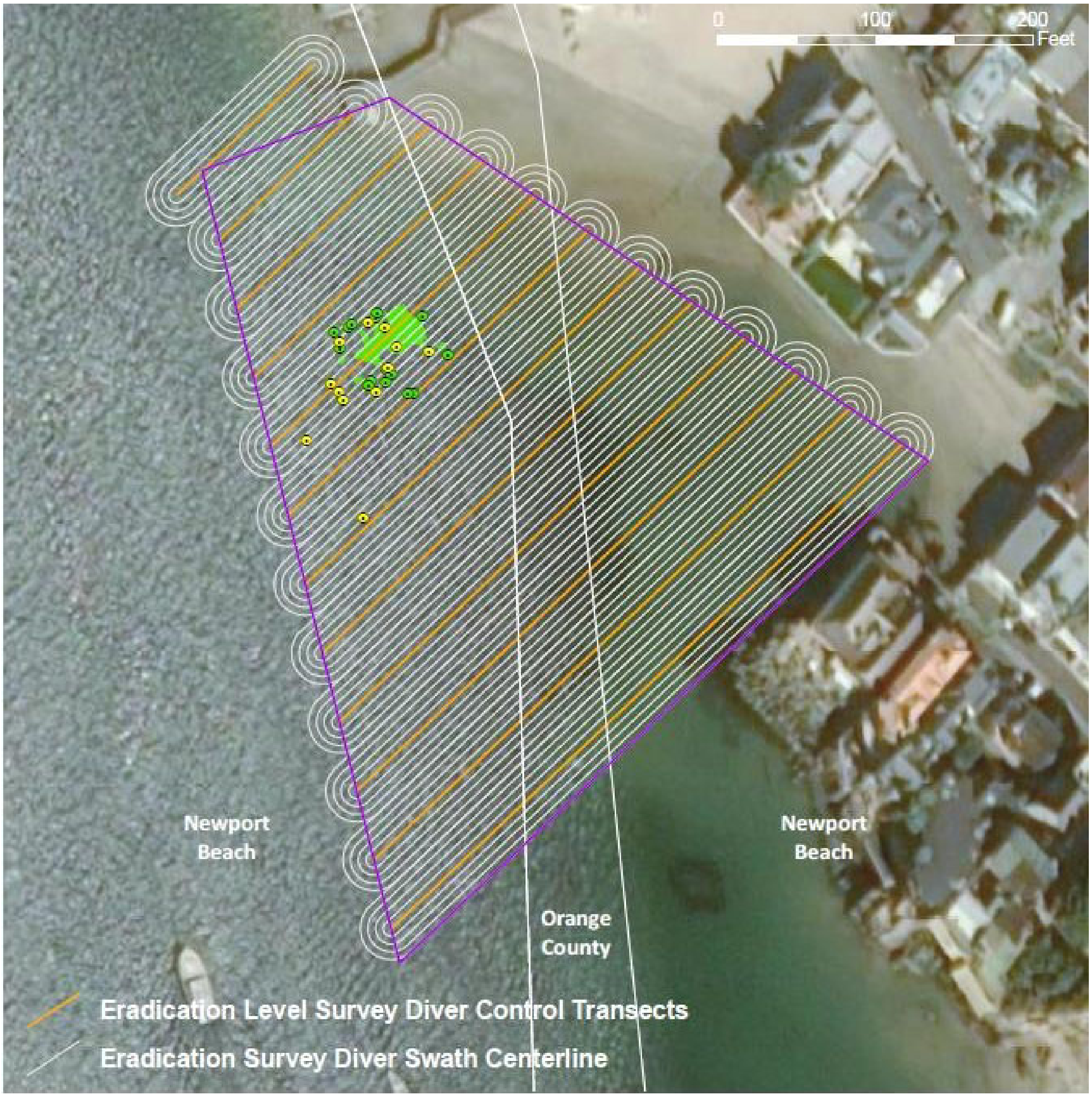
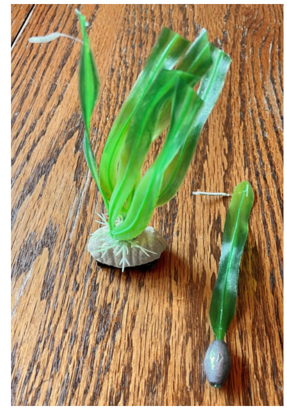


Figure 3. Diagram showing the seafloor coverage by divers using the laid transect method.



***6-frond and 1-frond artificial* Caulerpa.**

The second criterion will be evaluated by quantifying the confidence in the surveillance efforts through an efficacy assessment. Prior to each survey, patches of tagged artificial *C. prolifera* will be placed within the China Cove infestation site. A total of twenty 1-frond plants and twenty 6-frond plants will be deployed, split evenly between the grid and laid-line survey areas.

The find rate by the survey divers will be recorded and confidence in the results of each survey for live *C. prolifera* will 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 will ultimately allow for the estimation of the Eradication Certainty: the probabilistic certainty that all real *C. prolifera* existing at the infestation site has been found and that eradication has been achieved.

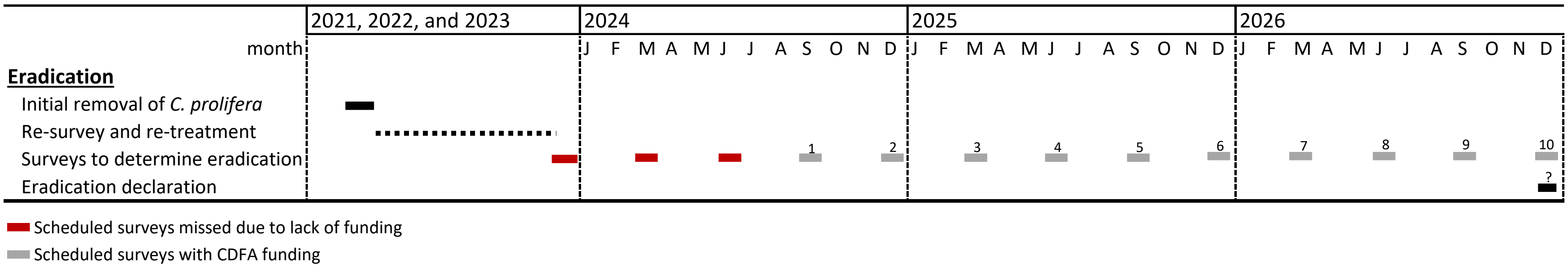
Quarterly Reporting

Following each quarterly survey a standardized report will be prepared using the Caulerpa Control Protocol Survey Form, along with GIS mapping of areas covered by each survey. The reported data will include dates of survey, survey efficacy, and any detections. The results will also be presented at regularly scheduled meetings of SCCAT.

Reporting of Finds

Any finds of *C. prolifera* made during the quarterly surveys of the infestation area will be reported to the CDFA point of contact and the SCCAT Steering Committee within 24 hours of the completion of the survey via email.

Table 1. Proposed schedule for Task 1 surveys.



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