FINAL REPORT ON **ERADICATION OF THE INVASIVE SEAWEED *CAULERPA TAXIFOLIA* FROM AGUA HEDIONDA LAGOON AND HUNTINGTON HARBOUR, CALIFORNIA**

May 2006

Prepared by:
Rachel Woodfield, Merkel & Associates, Inc.
Keith Merkel, Merkel & Associates, Inc.

Prepared for:

Steering Committee of the Southern California *Caulerpa* Action Team

- California Regional Water Quality Control Board – San Diego Region
- California Regional Water Quality Control Board – Santa Ana Region
- California Department of Fish and Game
- NOAA National Marine Fisheries Service
- U.S. Department of Agriculture – Agricultural Research Service

TABLE OF CONTENTS

APPENDICES

FINAL REPORT ON **ERADICATION OF THE INVASIVE SEAWEED *CAULERPA TAXIFOLIA* FROM AGUA HEDIONDA LAGOON AND HUNTINGTON HARBOUR, CALIFORNIA**

EXECUTIVE SUMMARY

In summer 2000, the first known Western Hemisphere infestations of the invasive strain of the tropical marine alga, *Caulerpa taxifolia*, were discovered in Agua Hedionda Lagoon, Carlsbad, California and in Huntington Harbour, Huntington Beach, California. Commonly used in saltwater aquarium systems, earlier releases of *C. taxifolia* into coastal European and Australian waters have resulted in the establishment of extensive dense carpets of the seaweed, smothering diverse natural communities and dramatically reducing biodiversity by displacing native seaweeds and animals. Based on the aggressive nature of this species and the displacement of native marine resources observed upon its discovery in California, it was recognized that the infestations posed a major threat to coastal ecosystems, and recreational and commercial uses dependent upon coastal resources.

Following the discovery in Carlsbad, a team of resource and regulatory agencies, marine biologists, and stakeholders were brought together under the name of the Southern California *Caulerpa* Action Team (SCCAT). The SCCAT Steering Committee is comprised of representatives from the San Diego Regional Water Quality Control Board, California Department of Fish and Game, National Oceanic and Atmospheric Administration National Marine Fisheries Service (Chair), Santa Ana Regional Water Quality Control Board, and U.S. Department of Agriculture-Agricultural Research Service. The goals of the SCCAT are the eradication of the known infestations of *C. taxifolia*, and the prevention and detection of new infestations through outreach and surveillance. Eradication efforts have been ongoing since June 2000 at a cost of over $7,000,000.

The criteria for successful eradication of the *C. taxifolia* infestations are 1) the containment and lethal treatment of *C. taxifolia* at the infestation site, and 2) verified absence of *C. taxifolia* from the infestation site. Treatment efforts consisted of covering *C. taxifolia* with heavy black PVC tarps under which chlorine was either injected as sodium hyopchlorite, or placed as a solid, pelleted formulation, which provided full containment of *C. taxifolia* while minimizing the water quality impacts of the treatment on the surrounding waters. The containment and treatment efforts lasted approximately two years, with divers undertaking intensive surveillance concurrently to search for remaining *C. taxifolia*. *Caulerpa taxifolia* was last detected in Agua Hedionda Lagoon in September 2002 and in Huntington Harbour in November 2002. No *C. taxifolia* has been discovered at either site during intensive, systematic surveillance conducted through December 2005.

Evaluations of the treatment effectiveness have been performed both in the laboratory and at both infestation sites. The laboratory experiments involved the collection of previously infested sediment from under the treatment tarps. The sediment cores were planted into laboratory aquariums and monitored in a controlled study for regrowth of *C. taxifolia*. No *C. taxifolia* grew from any of the treated cores. Additionally, removal of portions of the treatment tarps at the infestation sites resulted in no regrowth of *C. taxifolia* after four years of monitoring. These data indicate that the treatment approach used was lethal to *C. taxifolia* and that Eradication Criterion 1 has been met at both sites.

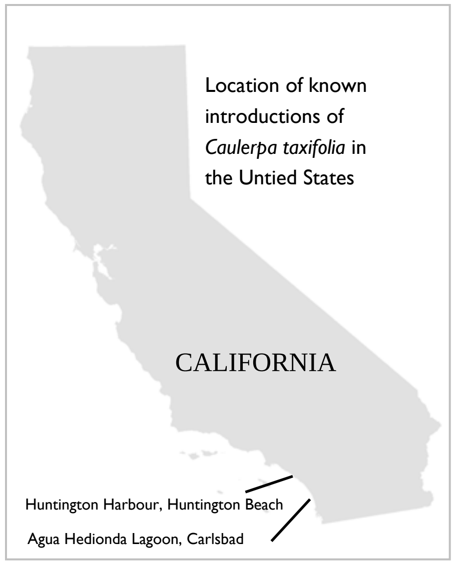
The second criterion was evaluated by quantifying the confidence in the surveillance efforts at both infestation sites. Patches of artificial *Caulerpa* were placed within each of the two sites during the regular diver surveys. Confidence in the results of each survey for live *C. taxifolia* could then be quantitatively estimated based on the amount of artificial *Caulerpa* found during the surveys. The results of these consecutive assessments of the

surveys ultimately allowed for an estimation of the eradication certainty, the certainty that all real *C. taxifolia* existing at the two sites had been found and that eradication had been achieved. The assessments determined that there is a 97.71% certainty that eradication has been achieved at Agua Hedionda Lagoon, assuming the worst conditions, and a 99.86% certainty if the average conditions are assumed. There is a 99.99% certainty that eradication has been achieved at Huntington Harbour under all conditions. These results indicate that Criterion 2 has also been met at both sites with a high degree of certainty.

Based on the results of the work performed, SCCAT believes that the criteria necessary to verify and document eradication of *Caulerpa taxifolia* at Agua Hedionda Lagoon and Huntington Harbour have been met.

INTRODUCTION

The highly invasive aquarium strain of the tropical marine alga *Caulerpa taxifolia* was discovered in Agua Hedionda Lagoon, Carlsbad, California in June 2000 and later confirmed in July 2000 at a second site in Huntington Harbour, Orange County. These discoveries represented the first known infestations of this strain within the Western Hemisphere (Jousson et al. 2000). Based on the aggressive nature of *Caulerpa taxifolia* and the displacement of native marine resources observed upon its discovery in California, it was recognized that the infestations potentially posed a major threat to coastal ecosystems, and recreational and commercial uses dependent upon coastal resources.



Caulerpa is a group of seaweeds that occurs naturally in tropical seas worldwide. Prized for their beauty and ability to uptake excess nutrients, many species of *Caulerpa* are widely used in saltwater aquarium systems. A cold-tolerant and fast-growing strain of the species *C. taxifolia*, referred to as the aquarium strain (or Mediterranean strain), is known for its tendency to rapidly overtake aquariums with its prolific growth. Careless disposal of *C. taxifolia* removed from unwanted or overgrown aquariums has led to infestations in the wild.

***C. taxifolia* displacing native seagrasses**

In 1984, this strain was believed to be accidentally released from the Oceanographic Museum of Monaco into the Mediterranean Sea, where it formed a small patch that rapidly grew into a large bed. Spread by small fragments transported primarily by boat anchors and fishing gear, one assessment estimates that *C. taxifolia* now blankets over 13,000 hectares (32,000 acres/50 square miles) of seafloor off six Mediterranean countries (Meinesz et al. 2001). Extensive dense carpets of *C. taxifolia* have smothered diverse natural communities and dramatically reduced biodiversity by displacing native seaweeds and animals. Fisheries and tourism have suffered as a result, and in some cases boating restrictions have been instituted to slow the spread of the species. *Caulerpa taxifolia* infestations have also resulted from its release into Australian waters. Because *Caulerpa* species contain toxins that make them unpalatable to many grazing animals that feed on seaweeds, prolific growth and spread of *C. taxifolia* is not constrained by typical, native herbivores. At the time of its discovery in California, no technique had been demonstrated to effectively eradicate similarly sized *C. taxifolia* infestations.

Following the discovery of the infestation of *C. taxifolia* in Carlsbad, a team of resource managers, marine resource and pest control scientists, resource and regulatory agencies, marine biologists, land-owners and environmental stakeholder representatives were brought together under the name of the Southern California *Caulerpa* Action Team (SCCAT). The SCCAT Steering Committee is comprised of representatives from the San Diego Regional Water Quality Control Board, California Department of Fish and Game (CDFG), National Oceanic and Atmospheric Administration National Marine Fisheries Service (Chair), Santa Ana Regional Water Quality Control Board, and U.S. Department of Agriculture-Agricultural Research Service. The goals of the SCCAT are the eradication of the known infestations of *C. taxifolia*, and the prevention and detection of new infestations in southern California through public outreach and surveillance.

The criteria established through SCCAT's Technical Advisory Committee for successful eradication of the *C. taxifolia* infestations are 1) the containment and lethal treatment of *C. taxifolia* at the infestation site, and 2) the verified absence of *C. taxifolia* from the infestation site. This document provides a history of the infestation discoveries, a summary of the results of the rapid response efforts in the context of the eradication criteria, and the rationale for recommending that *C. taxifolia* be declared eradicated from the two known infestation sites.

CALIFORNIA INFESTATIONS

Genetic analysis confirmed the *C. taxifolia* at the two California infestation sites to be the aquarium strain, a genetic match to the alga infesting Mediterranean and Australian waters (Jousson et al. 2000). However, the Agua Hedionda Lagoon and Huntington Harbour infestations are believed to have arisen from separate introductions. The resulting infestations were distinctly different due to the physical dissimilarity of the two sites. Due to the geographic separation of the infestations, the unique nature of each infestation, and differences in treatment techniques and survey results between sites, the two infestation sites are addressed separately in this document.

AGUA HEDIONDA LAGOON

Agua Hedionda Lagoon is a 100.6-hectare (248-acre), fully tidal, coastal lagoon. Activities within the lagoon include boating, fishing, and jet-skiing. The lagoon also serves as a source of cooling water for a power generating station. *Caulerpa taxifolia* was discovered in Agua Hedionda Lagoon by SCUBA divers monitoring the status of an eelgrass restoration program. When discovered, the infestation consisted of numerous dense patches, located primarily where native eelgrass beds had previously existed. Lagoon-wide surveys found additional *C. taxifolia* concentrated in five areas further east in the lagoon. In total, approximately 0.13 hectare of *C. taxifolia* was distributed over a 42.3-hectare (104.6-acre) area of the eastern basin. The western-most occurrence was 1.2 km (0.7 mile) from the outlet of the lagoon to the Pacific Ocean. *Caulerpa taxifolia* was likely released from a home aquarium into the lagoon either directly or though one of the storm drains that empties into the lagoon.

HUNTINGTON HARBOUR

In Huntington Harbour, the *C. taxifolia* infestation was located primarily in two shallow artificial ponds connected to the northern portion of the harbor. The enclosed ponds are surrounded by upscale homes and not accessible by boat, and are known dumping locations for both wild and aquarium biota. The *C. taxifolia* growth pattern in Huntington Harbour was much different from Agua Hedionda Lagoon, with the alga typically growing as hundreds of small, scattered plants. Because there were few distinct patches with discrete boundaries, quantification of the area covered by each occurrence of *C. taxifolia* at the site was not possible. All occurrences of *C. taxifolia* were distributed over a 1.1-hectare (2.6-acre) area. The infestation was located 4.4 km (2.7 miles) from the open ocean.

TREATMENT APPROACH

A review of previous international attempts to eradicate *C. taxifolia* revealed no effective treatment approaches for large-scale infestations, due to issues such as incomplete treatment or dispersal of algal fragments. Therefore, simultaneous with initial survey efforts and formation of SCCAT, containment and treatment investigations were initiated through the testing of hand picking, dredging, tarping, and herbicide application approaches, both in the field and laboratory. Application of chlorine bleach proved the most promising treatment, due to the lethal effect on *C. taxifolia*, readily detectable effect (visual bleaching), ease of application, low cost, and non-toxic residuals. The tendency of *C. taxifolia* to easily fragment and grow into new plants from even very small fragments dictated that the *C. taxifolia* should be treated in-place, with minimal disturbance.

In order to provide full containment of patches and fragments of *C. taxifolia* during treatment, while minimizing the impacts of chlorine application to surrounding waters, a tarping approach was adopted. This approach is outlined in the *Revised Eradication Plan for Caulerpa taxifolia in California* (Merkel & Associates, 2001). Divers covered patches of *C. taxifolia* with black PVC tarps that were sealed to the bottom with rebar and sandbags. Initially, tarps placed over large patches of *C. taxifolia* in Agua Hedionda Lagoon were fitted with ports, and liquid chlorine (sodium hypochlorite) was pumped under the tarps from storage tanks on shore. In later treatments, and in Huntington Harbour, solid chlorine tablets (trichloroisocyanuric acid) replaced the liquid treatment for safety and ease of application in areas of reduced biomass.

All eradication activities conducted at each site were regulated through U.S. Army Corps of Engineers Nationwide Permit Number 25 and Regional General Permit No. 64, a California Coastal Commission Consistency Determination, a Research Authorization and Special Local Need Registration issued by the California Department of Pesticide Regulation, and a Notice of Exemption (CEQA). Due to the ease with which *C. taxifolia* can be spread and for the safety of the divers conducting the work, active treatment areas were closed to public access until all patches were covered and treated.

AGUA HEDIONDA LAGOON

At Agua Hedionda Lagoon, treatment activities were intensive and ongoing from June 2000 to September 2001. Hundreds of patches of *C. taxifolia* were tarped and treated; the largest patch was estimated to have a biomass in excess of 18 metric tons (20 tons) of the alga. Following the initial treatment efforts, the focus shifted to intensive surveillance for residual, undetected patches of *Caulerpa*. Finding the small patches of *C. taxifolia* proved challenging in the murky water and dense eelgrass. After testing a variety of survey methods including aerial surveys, remote cameras, and laser line-scan, it became clear that closely spaced divers moving systematically at a measured pace along the bottom resulted in the most effective search.

In September 2001 a quarterly survey program was initiated lagoon-wide, covering the west, central, and east basins of Agua Hedionda Lagoon. Surveys used a team of divers swimming along transect lines deployed by a small boat using differential GPS. The divers used a guide-line to maintain a 1-meter diver spacing, and varied their swimming speed based on visibility and density of eelgrass. Each full survey of the lagoon involved swimming more than 800 kilometers (500 miles) over the course of two to three months. When *C. taxifolia* was detected, its dimensions were assessed and recorded and location recorded by dGPS. Each patch detected was contained and treated within 24 hours of its discovery.

The amount of *C. taxifolia* found was reduced with each consecutive survey, with the coverage in fall 2001, winter 2001, spring 2002, and summer 2002 measuring 33.6 m^2, 2.7 m^2, 0.5 m^2, and 0.4 m^2, respectively. The last patch of *C. taxifolia* was found on September 11, 2002. Intensive surveillance continued, however due to water temperatures unfavorable to *C. taxifolia* growth and to conserve limited fiscal resources, the winter 2002 and spring 2003 survey efforts were not lagoon-wide, but rather limited to areas previously known to support *C. taxifolia*. Lagoon-wide surveys resumed in summer 2003, with two full surveys being conducted each year in summer and fall. No additional *C. taxifolia* has been detected in seven surveys, conducted over the course of three years through December 2005 (Merkel & Associates 2006a, Appendix A).

HUNTINGTON HARBOUR

Treatment activities in Huntington Harbour began in October 2000 following intensive mapping efforts. Tarps were secured to the bottom, with solid chlorine tablets placed underneath to treat the widely distributed *C. taxifolia*. Following the completion of the initial treatment in February 2001, follow-up surveys to detect and treat remaining *C. taxifolia* were initiated using techniques similar to those developed at Agua Hedionda Lagoon. Due to the small size of the area to be examined, surveys could be completed in three days, allowing for numerous repeated surveys to be conducted with the funding available. Additional tarps were added, as needed, to treat additional patches when discovered, with most treatment activities being completed by summer 2001. There were fewer, and generally smaller, patches of *C. taxifolia* found in each subsequent survey. The total sizes of patches found in fall 2001, winter 2001, spring 2002, summer 2002, and fall 2002 measured 9.9 m^2, 1.2 m^2, 1.3 m^2, 0.5 m^2, and 0.5 m^2, respectively. The last patch of *C. taxifolia* was found and treated on November 19, 2002. Quarterly surveys continued for the next three years. No *C. taxifolia* has been detected in thirteen additional surveys, conducted over three years through December 2005.

Although initial surveillance indicated that the infestation was restricted to a small area in the northern portion of Huntington Harbour, two harbor-wide surveys of the remaining areas were conducted by teams of divers spaced one meter apart. Full surveys of the entire harbor were conducted in 2001 and again in 2005. *Caulerpa taxifolia* was not detected elsewhere in the harbor.

QUALITY CONTROL AND QUALITY ASSURANCE

ASSESSMENT OF TREATMENT TECHNIQUE

In order to assess the effectiveness of the treatment technique used in the eradication program, cores of sediment that had been infested by *C. taxifolia* were removed from underneath the treatment tarps at Agua Hedionda Lagoon in 2001 and 2002. The cores were planted into laboratory aquaria and monitored in a controlled study for regrowth of *C. taxifolia*. No *C. taxifolia* grew from any of the treated cores, suggesting that the treatment methodologies used at both Agua Hedionda Lagoon and Huntington Harbour had a lethal effect on *C. taxifolia* (Anderson et al. 2005, Appendix C). In addition, subsequent laboratory testing with small-scale replicated treatments showed that chlorine could kill *C. taxifolia* (Williams and Schroeder 2004).

An assessment of the treatment methodology was also conducted in the field during 2001 and 2002. Portions of the tarps were removed after treatment, and the exposed plots of bottom were carefully monitored for regrowth of *C. taxifolia* and recovery of native species. The plots were monitored through December 2005 with no regrowth of *C. taxifolia* observed in any of the study plots (Woodfield 2002a, Merkel & Associates 2006c). These results again indicate that the treatment approach used at Agua Hedionda Lagoon and Huntington Harbour had a lethal effect on *C. taxifolia*. Collected data also reflect the significant regrowth and recolonization of native species that has occurred within the plots.

The effectiveness of the treatment approach was also supported by the eventual and continued absence of *C. taxifolia* from Agua Hedionda Lagoon and Huntington Harbour as repeated surveys of the treated areas failed to detect persisting occurrences.

ASSESSMENT OF SURVEY TECHNIQUE

As fewer and smaller occurrences of *C. taxifolia* were being discovered during the eradication effort, a program was developed to assess the survey dive team's ability to locate *C. taxifolia* in the often murky water of the infestation sites. Patches of artificial *Caulerpa* of various small sizes were randomly placed at Agua Hedionda Lagoon and Huntington Harbour during each of the regular surveys to test the dive team. Confidence in the results of each survey for live *C. taxifolia* could then be quantitatively estimated based on the proportionate amount of known quantities of artificial *Caulerpa* found by the divers. The results of the consecutive assessments of the efficacy of the surveys ultimately allowed for an estimation of the eradication certainty that all real *C. taxifolia* existing at the two sites had been found and that eradication had been achieved.

Artificial *Caulerpa* used in diver survey assessments.

Agua Hedionda Lagoon

Based on the results of survey efficacy assessments conducted since fall 2002, there is a 97.71% certainty that eradication has been achieved at Agua Hedionda Lagoon, assuming the worst level of detection during each survey, and a 99.86% certainty if the average detection levels are assumed (Merkel & Associates 2006d, Appendix D).

The survey efficacy assessment also determined that the smallest patch size of artificial *Caulerpa* that could be found with greater than 99% efficiency was 1-m wide; smaller sizes were found with less frequency. Based on the growth rates of the aquarium strain of *C. taxifolia* measured at Agua Hedionda Lagoon and other sites, it is assumed that any undetected patches of *C. taxifolia* would have grown to at least 1 meter in diameter within two years (Woodfield 2002b, Meinesz et al. 1995). Seven repeated surveys of Agua Hedionda Lagoon were conducted over more than a three-year period with no *C. taxifolia* detected.

On February 16, 2006, California Department of Fish and Game conducted an independent diver survey for *C. taxifolia* within locations that had been most highly infested at Agua Hedionda Lagoon. Their divers detected no *C. taxifolia* during the survey (CDFG 2006, Appendix E).

Huntington Harbour

The results of the survey efficacy assessments conducted in Huntington Harbour since summer 2003 indicate that there is a 99.99% eradication certainty at the Huntington Harbour infestation site, when calculated using either worst detection levels (such as the smallest patch in the lowest visibility) or average survey efficacy assessment detection levels (all sizes in all visibility levels) (Merkel & Associates 2006d, Appendix D).

On February 17, 2006, the California Department of Fish and Game conducted an independent diver survey for *C. taxifolia* at Huntington Harbour. Their divers detected no *C. taxifolia* during the survey (CDFG 2006, Appendix E).

CRITERIA FOR DETERMINATION OF ERADICATION

SCCAT has set two criteria to be met to achieve the stated goal of eradicating *C. taxifolia* from Agua Hedionda Lagoon and Huntington Harbour. The criteria for each site are:

Criterion 1) The containment and lethal treatment of *C. taxifolia* at the infestation site
Criterion 2) The verified absence of *C. taxifolia* from the infestation site

The degree to which these criteria have been met under the current program is discussed below in the context of evaluations of treatment approach, the biology of *C. taxifolia,* and the measured capabilities of the survey team.

CRITERION 1

Prior sections of this report have reviewed the evidence that the treatment method applied was lethal to *C. taxifolia* at both infestation sites. The laboratory assessment of the viability of *C. taxifolia* in sediment from treated areas found no evidence of *C. taxifolia* regrowth. Field assessments of study plots that had been infested with *C. taxifolia*, treated, and then later exposed to the open water found no evidence of *C. taxifolia* regrowth after more than three and a half years of monitoring at Agua Hedionda Lagoon and for over four and a half years in Huntington Harbour. Based on these studies, there is a very high degree of certainty that Criterion 1 has been met.

CRITERION 2

Intensive, systematic surveys of the infestation sites, over three years since the last detection of *C. taxifolia,* found no persisting *C. taxifolia*. Recognizing that the remaining occurrences were likely to be very small in size, the quality control procedure described above was implemented to quantify the ability of the survey team to detect small *C. taxifolia* in the challenging environmental conditions present at the infestation areas. The results of these assessments were used to estimate an eradication certainty at Agua Hedionda Lagoon of greater than 99% based on the average conditions, or greater than 97% based on worst-case conditions. An estimate of well over 99% eradication certainty can be made at Huntington Harbour under all conditions.
These estimates of eradication certainty are conservative, recognizing that while the survey efficacy assessment studied the probability of detecting a fixed number of objects of a static size (artificial *Caulerpa*), live *C. taxifolia* is biologically active and capable of expanding to larger sizes over time and multiplying in number. Additionally, *C. taxifolia* was not randomly distributed over the infestation sites, but rather clustered in certain areas, which allowed for more intensive scrutiny focused in these areas. As noted above, the estimated time for a small fragment of *C. taxifolia* to grow to a size large enough to be found every time (one meter wide) was two growing seasons. Given that intensive surveillance was continued through three full growing seasons at both infestation sites without detection of *C. taxifolia*, there is a very high probability that *C. taxifolia* is no longer present at these sites and that Criterion 2 has been met.

ERADICATION RECOMMENDATIONS

The two criteria for eradication have been met at Agua Hedionda Lagoon. It is therefore recommended that *Caulerpa taxifolia* be declared eradicated from Agua Hedionda Lagoon.

The two criteria for eradication have been met at Huntington Harbour. It is therefore recommended that *Caulerpa taxifolia* be declared eradicated from Huntington Harbour.

ADDITIONAL RECOMMENDATIONS

The SCCAT Steering Committee has identified additional recommendations based on their experience of responding to *C. taxifolia* in California. First, it is critical that awareness of the threat posed by many species of *Caulerpa* be maintained after the eradication of the two known infestations. The need to detect new infestations of *Caulerpa*, to identify potential sources or pathways of *Caulerpa* movement, and to prevent new introductions of *Caulerpa* into California waters requires an effective outreach and education program. The Southern California *Caulerpa* Outreach and Education Program currently underway is one mechanism to address this need and it is recommended that similar efforts be initiated and maintained in other coastal regions of the state.

Additionally, it is recommended that legislative efforts to prevent new introductions of *Caulerpa* species to the coastal waters of California be continued. Legislation banning the transport, sale, and possession of nine potentially invasive species of *Caulerpa*, including *C. taxifolia*, was enacted in the State of California in September 2001 (AB 1334). However, many resource managers, invasive species experts, and SCCAT believe that due to the difficulty associated with distinguishing various species of *Caulerpa*, a ban on the entire *Caulerpa* genus is necessary and critical to controlling the importation, distribution, or release of invasive *Caulerpa* species in California.

It is also recommended that surveillance for as-yet undetected infestations of *C. taxifolia* be continued in California. Although a multi-year surveillance program is currently under way in southern California, ongoing surveillance is necessary because the threat of new introductions is ever-present. Additionally, the tolerance of low temperatures exhibited by the aquarium strain of *C. taxifolia* suggests that all coastal waters of California could be threatened by invasion, although southern California waters are at the greatest risk. Surveys north of the present northern survey boundary of Santa Barbara are warranted and should be undertaken. It must be noted that it is high likely that any future detection of *C. taxifolia* in Agua Hedionda Lagoon or Huntington Harbour would be the result of a reintroduction.

Finally, although a unique combination of private and public funding was assembled to respond to the first two *C. taxifolia* invasions, it is unlikely that a repeat effort could be similarly funded today. Without immediate action, new invasions could continue to grow and spread to a point where eradication is not possible. It is therefore recommended that a permanent rapid response fund be established for an effective and immediate response to newly discovered infestations of *Caulerpa* or other marine invasives, and for increased support of enforcement of existing state laws banning marine invasive species.

ONGOING ERADICATION PROGRAM WORK

Although it is recommended that the determination be made that eradication of *C. taxifolia* has been achieved at both known sites, there are several elements of the program that have been ongoing and will continue into 2007. Remaining work elements of the program include implementation of the Outreach and Education Program. This program targets groups such as saltwater aquarists, SCUBA divers, harbor masters, anglers, public aquariums, environmental groups, and governmental agencies. The goal of this program is to prevent new, and detect potentially existing, infestations of *C. taxifolia* in California. Successful achievement of this goal will require distribution of informational materials, presentations to clubs and associations, development of Internet resources, and placement of articles and advertisements in target publications.

A major remaining work element is the removal of treatment materials from the two infestation sites. The tarps were left in place to this point to discourage regrowth of any material that may have not been treated by the chlorine. Based on the studies outlined above, removal of the treatment tarps will not result in regrowth of *C. taxifolia*. Removal of the tarps will facilitate a full recovery of the flora and fauna present prior to the introduction of *C. taxifolia*. If *C. taxifolia* is detected at the infestation sites in the future, it will be the result of new introductions.

Although success has been achieved in Agua Hedionda Lagoon and Huntington Harbour, the possibility of other introductions or infestations is ever-present. Surveillance for *Caulerpa* species is currently ongoing throughout southern California through the Southern California *Caulerpa* Surveillance Program. Preliminary low-intensity, broad-scale surveys have been performed throughout southern California water bodies. These surveys have provided data that is currently being analyzed to determine potential pathways for introduction and habitats most likely to support *Caulerpa* species. The results of these analyses are guiding the scope of future high-intensity surveillance focused on high-risk areas. The program has the added benefit of allowing surveillance for other invasive marine species concurrent with the search for *Caulerpa*. To date, no other infestations of *Caulerpa* have been detected. It is projected that funding for this surveillance program will end in early 2007.

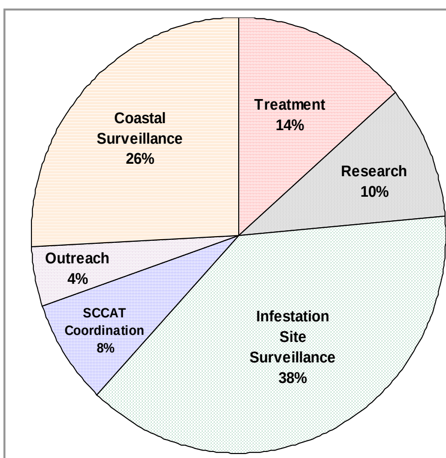
LESSONS LEARNED

Despite tremendous efforts and contributions to the body of knowledge on *C. taxifolia*, a successful eradication of an infestation of the aquarium strain of *C. taxifolia* has never been achieved before now. Reflection on the process results in a long list of lessons learned by the ground-breaking effort, that can be summarized briefly with a list of the critical elements of a successful eradication response: 1) rapid response; 2) assumption and belief that eradication is possible; 3) incorporation of the lessons from other responses; 4) adequate funding to maintain a sustained, persistent response; 5) intensive, repeated surveys over an extended period of time after the last discovery; 6) quantitative evaluation of the effectiveness of survey and treatment elements; 7) identification and engagement of stakeholders; 8) adaptation and adjustment to approach in response to financial circumstances, stakeholder response, or independent review. There is wide agreement that the key elements of the *C. taxifolia* eradication effort were the quick recognition of the threat posed by *C. taxifolia*, prompt organization of a response team, effective acquisition of funding, and an immediate field response to assess the extent of the infestation and develop a response plan.

Unfortunately, new introductions of *C. taxifolia* may occur in California's waters because despite restrictions, *C. taxifolia* is still available in other states and continues to be used in California. It is critical that the lessons learned from this eradication effort be recognized in order to respond appropriately and effectively when the next infestation is discovered. Hopefully outreach and education efforts will help prevent new introductions and existing infestations will be detected quickly while there is still time to respond.

FUNDING AND ACKNOWLEDGEMENTS

The Southern California *Caulerpa taxifolia* Eradication Program has received funds from many sources since it began in 2000. Although expenses included program research and development, treatment of *C. taxifolia*, surveillance and mapping, outreach and education, and a variety of data management and reporting tasks, the vast majority of the funds expended were for the intensive surveillance work. The work elements conducted from June 2000 to December 2005, leading to the recommendation to declare eradication made above, were directly funded by grants and allocations totaling approximately $3,340,000 at Agua Hedionda Lagoon and $660,000 at Huntington Harbour. However, these figures do not capture extensive additional research, outreach, scientific reviews, surveillance of other sites in southern California, or all remaining work elements including removal of treatment tarps and eradication program reporting. The total of all allocations for response to the discovery of *C. taxifolia* infestations in California covering all elements listed above will be approximately $7,700,000 upon work completion in 2007 (Appendix F).



Distribution of all *C. taxifolia* response funds by activity (2000-2007)

This funding was provided by the State Water Resources Control Board (through Cleanup and Abatement Account funds, Proposition 13 grants, and an EPA Clean Water Act Section 319h grant), California Coastal Conservancy (Southern California Wetlands Recovery Project grant), California Department of Fish and Game, NOAA National Marine Fisheries Service, Cabrillo Power LLC, U.S. Fish and Wildlife Service, California Department of Parks and Recreation, the National Fish and Wildlife Foundation, the FishAmerica Foundation, the NOAA Community-Based Restoration Program, and the Agua Hedionda Lagoon Foundation.

In acknowledging the participation and fiscal contributions to the *C. taxifolia* eradication efforts it is important to note that the obligation to respond to an invasive marine species does not fall squarely on any one entity. The willingness and cooperation of numerous public agencies and private organizations was essential and not always obligatory. This fact alone makes it relatively remarkable that so much support was garnered so widely from agencies, environmental groups, industry, elected officials, and affected municipalities and communities.

Additional costs of eradication not accounted for above include the tremendous contributions of time and knowledge from all active and historical SCCAT participants, including the California Department of Fish and Game, National Oceanic and Atmospheric Administration National Marine Fisheries Service, the San Diego and Santa Ana Regional Water Quality Control Boards, U.S. Department of Agriculture-Agricultural Research Service, UC Davis, University of California Cooperative Extension, the City of Carlsbad, Agua Hedionda Lagoon Foundation, Cabrillo Power I LLC, M-Rep, Hofman Planning, and the staff of Merkel & Associates.

Finally, the generous and willing contribution of the collective knowledge, experience, and encouragement of our colleagues battling *C. taxifolia* in Australia, France, and Croatia is gratefully acknowledged and much appreciated.

The successes of the Caulerpa taxifolia eradication program are gratefully dedicated to Greig Peters, of the San Diego Regional Water Quality Control Board, who was among the first to recognize the immediate threat posed by Caulerpa taxifolia and was instrumental in mobilizing an immediate response. We regret that he passed away before the successful completion of the project, but his dedicated contributions will long be remembered.

REFERENCES

Anderson, L.W. J., W. Tan, R. Woodfield, R. Mooney, K. Merkel. 2005. Use of Sediment Bioassays to Verify Efficacy of Caulerpa taxifolia Eradication Treatments. J. Aquat. Plant Manage. 43: 1-9.

Anderson, L.W.J. 2005. California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. Biological Invasions 7:1003-1016.

California Department of Fish and Game. 2006. CDFG OSPR Memo on *Caulerpa taxifolia* diver surveys. Report prepared for CDFG.

Jousson, O., Pawlowski, J, L. Zaninetti, F. W. Zechman, F. Dini, G. Di Guiseppe, R. Woodfield, A. Millar, and A. Meinesz. 2000. Invasive alga reaches California. Nature 408:157-158.

Meinesz A., Belsher T, Thibaut T, Antolic B, Ben Mustapha K, Boudouresque C-F, Chiaverini D, Cinelli F, Cottalorda J-M, Dejellouli A, El Abed A, Orestano C, Grau AM, Ivesa L, Jaklin A, Langar H, Massuti-Pascual E, Peirano, A, Tunesi L, Vaugelas J de, Zavodnik N, Zuljevic A. 2001. The introduced alga *Caulerpa taxifolia* continues to spread in the Mediterranean. Biological Invasions 3:201-210.

Merkel & Associates, Inc. 2006a. Fifth Year Status Report – Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates, Inc. 2006b. Eradication and Surveillance of *Caulerpa taxifolia* within Huntington Harbour, Huntington Beach, California, Status Report. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates, Inc. 2006c. Southern California *Caulerpa taxifolia* Eradication Program- *Caulerpa taxifolia* Treatment Efficacy Assessment at Agua Hedionda Lagoon. Report prepared for Southern California *Caulerpa* Action Team. In preparation.

Merkel & Associates, Inc. 2006d. Southern California *Caulerpa taxifolia* Eradication Program- *Caulerpa taxifolia* Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates, Inc. 2001. Revised eradication plan for *Caulerpa taxifolia* in California. Prepared for Southern California *Caulerpa* Action Team.

Williams, S.L. and S. Schroeder. 2004. Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. Marine Ecol. Prog. Ser. 272: 69-76.

Woodfield, R. 2002a. Tarp Removal from Treated *Caulerpa taxifolia* in the Huntington Harbour Infestation. In *In Progress Review: Ancillary Data and Observations from* Caulerpa taxifolia *Eradication Efforts at Agua Hedionda Lagoon and Huntington Harbour*. Prepared for Southern California *Caulerpa* Action Team.

Woodfield, R. 2002b. Investigation of Eradication Treatments of Caulerpa taxifolia in Agua Hedionda. In *In Progress Review: Ancillary Data and Observations* from Caulerpa taxifolia *Eradication Efforts at Agua Hedionda Lagoon and Huntington Harbour*. Prepared for Southern California *Caulerpa* Action Team.

Woodfield, R. and Mooney, R. 2002. Field Response to Infestations of *Caulerpa taxifolia* in Southern California. California and the World Ocean 2002 Proceedings.

Funding for this project has been provided in part by the U.S. EPA pursuant to Assistance Agreement No. C9-9768201-0 and any amendments thereto which has been awarded to the State Water Resources Control Board (SWRCB) for the implementation of California's Nonpoint Source Pollution Control Program. The contents of this document do not necessarily reflect the views and policies of the USEPA or the SWRCB, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

APPENDIX A
Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon
Fifth Year Status Report

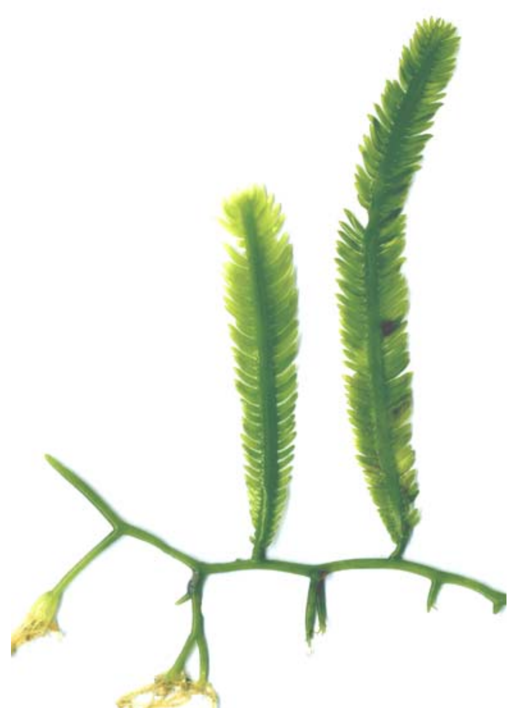
Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California Fifth Year Status Report

January to December 2005

Prepared for:

Steering Committee of the Southern California *Caulerpa* Action Team

- California Regional Water Quality Control Board – San Diego Region
- California Regional Water Quality Control Board – Santa Ana Region
- California Department of Fish and Game
- National Marine Fisheries Service
- U.S. Department of Agriculture – Agricultural Research Service



Prepared by:

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

March 2006

TABLE OF CONTENTS

FIGURES

Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California Fifth Year Status Report

January to December 2005

EXECUTIVE SUMMARY

On June 12, 2000 the first known infestation in the Western Hemisphere of the invasive strain of the tropical marine alga, *Caulerpa taxifolia*, was discovered in Agua Hedionda Lagoon, in Carlsbad, California. This document reports the results of the fifth year of the eradication program undertaken by the Southern California *Caulerpa* Action Team (SCCAT). Merkel & Associates has been contracted to conduct the eradication under the oversight of the SCCAT, a broad-based task force assembled from federal and state resource and regulatory agencies, exotic species experts, and marine resource scientists.

During the fifth year, survey work involved two full surveys of the entire lagoon, conducted in summer and fall 2005. No *C. taxifolia* was found in the lagoon, marking the completion of three and a half years of surveys with none being found. *Caulerpa taxifolia* was last detected on September 11, 2002 (Year 2).

The SCCAT Technical Subcommittee has established two criteria for successful eradication of the *C. taxifolia* infestation at Agua Hedionda Lagoon: 1) the containment and lethal treatment of *C. taxifolia* at the infestation site, and 2) the demonstrated absence of *C. taxifolia* from the infestation site.

Treatment efficacy assessments performed in both the field and laboratory indicate that the treatment approach used was lethal to *C. taxifolia* and that Criterion 1 has been met at Agua Hedionda Lagoon.

The second criterion is addressed by the intensive, long-term surveillance for undetected *C. taxifolia* in the lagoon. Seven full surveys of the entire lagoon have been conducted over the course of three years with no *C. taxifolia* detected, with two additional surveys that focused only on previously infested areas also detecting no *C. taxifolia* (conducted in winter 2002 and spring 2003). The confidence in these survey results was quantified by the Survey Efficacy Assessment Program, involving the placement of patches of artificial *Caulerpa* during each of the surveys. The results of the consecutive assessments of the surveys ultimately allowed for the estimation of the eradication certainty: the certainty that all real *C. taxifolia* existing at Agua Hedionda Lagoon has been found and eradication achieved. The assessments determined that there is a 97.71% certainty that eradication has been achieved at Agua Hedionda Lagoon, assuming the worst conditions (small patch size and low visibility), and a 99.86% certainty if the average conditions are assumed. These results indicate that Criterion 2 has also been met at Agua Hedionda Lagoon.

A recommendation that eradication be declared at Agua Hedionda Lagoon is in preparation and will be submitted to the California Department of Fish and Game by the SCCAT Steering Committee for consideration. The final determination on the status of the eradication will be made by the California Department of Fish and Game after reviewing the collected data.

INTRODUCTION

The highly invasive Mediterranean strain of the tropical marine alga *Caulerpa taxifolia* was discovered in Agua Hedionda Lagoon, Carlsbad, California in June 2000 (Figure 1). Its discovery represented the first known occurrence of this strain within the Western Hemisphere and is believed to pose a major threat to coastal ecosystems and recreational and commercial uses dependent upon coastal resources. While the species was also identified at a second site in California (Huntington Harbour, Orange County), the Agua Hedionda Lagoon infestation is the larger of the two known infestations. It is likely that *C. taxifolia* had been in the lagoon for at least four years prior to its discovery. It is not known whether other infestations also exist elsewhere in the United States. The continued availability and use of this species by saltwater aquarists is cause for concern.

In the United States, the Mediterranean strain of *Caulerpa taxifolia* has been banned from importation and interstate commerce since 1999 through the Federal Noxious Weed Act. Legislation banning the transport, sale, and possession of nine potentially invasive species of *Caulerpa*, including *C. taxifolia*, was enacted in the State of California in September 2001. Earlier in 2001, the City of San Diego adopted an ordinance with similar restrictions applicable to the entire genus of *Caulerpa*.

Since the discovery of *C. taxifolia* in Agua Hedionda Lagoon in June 2000, eradication, surveillance, public outreach efforts, eradication research, and legislative efforts have been initiated and are on-going. The primary goal of the Southern California *Caulerpa* Action Team (SCCAT), which is made up of resource managers, marine resource and pest control scientists, permitting agencies, marine biological consultants, land-owners and environmental stakeholder representatives, has been the eradication of the known infestations.

From the date of discovery (June 2000) until the end of the Summer 2001 survey, the eradication effort at Agua Hedionda Lagoon primarily involved the treatment of all detected *C. taxifolia*. The amount of *C. taxifolia* present in the lagoon at the time of discovery was estimated to be 1,047 m^2 (Merkel & Associates, 2001a). By the end of the second year of eradication efforts at Agua Hedionda Lagoon the amount of *C. taxifolia* discovered had been reduced to 0.4 m^2 (Merkel & Associates, 2003). The last discovery of *C. taxifolia* at Agua Hedionda Lagoon was on September 11, 2002. The location of all *C. taxifolia* discovered since the beginning of the eradication effort is indicated in Figure 1.

During the third year, there was no *C. taxifolia* detected over the course of four lagoon-wide surveys (Merkel & Associates, 2004). In response to concerns that funding for future surveillance could be exhausted prior to completion of the eradication program, SCCAT adopted a survey plan for the fourth year that involved conducting surveys only in the high growth seasons of summer and fall. No *C. taxifolia* was detected during the fourth year surveys, conducted in summer and fall.

A similar schedule was maintained in the fifth year of the program. This document provides a synopsis of the fifth year's efforts and costs, and reports on the status of the lagoon and the progress toward the final goal of full eradication of *Caulerpa taxifolia* from Agua Hedionda Lagoon. Please refer to the Year 1, Year 2, Year 3, and Year 4 reports for details on survey and treatment actions completed during those years.

Caulerpa taxifolia discovered since June 2000
Agua Hedionda Lagoon, Carlsbad, CA
Last find: September 2002

Figure
1

Merkel & Associates, Inc.

FIFTH YEAR SURVEILLANCE - JANUARY TO DECEMBER 2005

Following the intensive summer 2001 survey and treatment season, a systematic quarterly survey program was undertaken to search for additional patches of *C. taxifolia*. During the second year of the program surveys were conducted lagoon-wide, covering the west, central, and east basin of Agua Hedionda Lagoon. During the third year, surveys were still conducted quarterly, however the winter and spring surveys were reduced to focused surveys of high-risk areas in the lagoon. During the fourth and fifth year, surveys were only conducted during summer and fall surveys. These surveys covered all basins of the lagoon.

METHODS

All surveys during the fifth year used the laid-transect line method. This method employs the use of SCUBA divers swimming along transects lines deployed by a small boat using differential GPS. The divers use a guide-line to maintain their spacing at 1 meter apart, and vary their swimming speed based on visibility and density of eelgrass. Having tested a variety of other survey methods, including towed divers, towed cameras, and laser line scan, it appears that the most effective approach to conducting intensive surveys that can locate very small patches of *C. taxifolia*, even within dense eelgrass beds, is the current method employed. This survey intensity is defined as an eradication level survey in which divers are used to make visual searches to ensure 100% viewing of the study area (NMFS, 2002).

Survey staff were trained and prepared to respond to new discoveries. If *C. taxifolia* were to be found by divers, its location would be recorded by dGPS and the patch assessed by a biologist. The dimensions and, if possible, the number of fronds, number and length of thalli, and typical frond lengths would be recorded for each patch located. The patch would be marked by colored pin-flags to be left in place during treatment in order to relocate the treated *C. taxifolia* at a later date if necessary for efficacy investigations. The *C. taxifolia* would be contained within 24 hours with a PVC tarp and treated with solid chlorine pucks, as outlined in the Revised Eradication Plan for *Caulerpa taxifolia* in California (Merkel & Associates, 2001b).

During each survey, assessments were made of the efficacy of the survey methodology. This was achieved through the placement of synthetic *Caulerpa* in the lagoon during the survey. These efficacy trials were conducted twice during each survey, once in Snug Harbor, which is relatively clear and typically supports dense eelgrass, and one further east off of Bristol Cove, in an area with only sparse eelgrass and often poor visibility. The amount of plants found by the team was analyzed based on water clarity, plant size, and density of eelgrass in the survey area. Extraordinary rain events during the winter of 2004-2005 and subsequent red tides in summer 2005 are believed to have significantly reduced the amount eelgrass habitat in Agua Hedionda Lagoon. Thus, the summer and fall 2005 efficacy trials were performed with all patches of synthetic *Caulerpa* being placed on bare bottom.

By testing the team's ability to find synthetic patches of *Caulerpa*, confidence in the results of each survey could be quantitatively estimated. The survey efficacy assessments addressed the questions: (1) what are the relationships between important environmental variables and survey efficacy, and (2) how do the estimates of survey efficacy during each survey event translate to eradication certainty? A full discussion of this efficacy program is detailed in *Caulerpa taxifolia Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour* (M&A 2006).

RESULTS

The results of the surveys conducted at Agua Hedionda Lagoon during the fifth year of the eradication program are discussed below. Surveys were limited to summer and fall months when conditions for the growth of *C. taxifolia* were most favorable.

Summer 2005

The Summer 2005 survey effort was conducted from June 10 to October 6, 2005. The completion of the summer survey was delayed during July and again in August when survey work had to be temporarily suspended due to the extremely heavy red tide that persisted along the coast of southern California that summer.

All basins of the lagoon were surveyed using the laid line methodology with divers at 1-meter spacing (Figure 2). No *C. taxifolia* was found in the lagoon during this survey.

A survey efficacy trial was conducted during this period in Snug Harbor and off of Bristol Cove. In Snug Harbor, 66% of the synthetic *Caulerpa* placed in the study area was found by the survey team. Off of Bristol Cove, visibility was commonly very low, resulting in 52% of the synthetic *Caulerpa* being found.

A second element of the efficacy assessment included the placement of larger synthetic patches of *Caulerpa* in the survey area, sized 0.3m, 0.5m, and 1m wide, in order to determine the minimum sized plant that would be detected 100% of the time. The results for these larger patches at Snug Harbor were 60%, 90%, and 100%, respectively. The results for 0.3-m, 0.5-m, and 1-m wide patches at Bristol Cover were 70%, 90%, and 100%, respectively, suggesting that the 1-m patch was the smallest patch size that would always be detected, regardless of environmental conditions.

Fall 2005

The Fall 2005 survey effort was conducted from October 7 to December 22, 2005. All basins of the lagoon were surveyed using the laid line methodology with divers at 1-meter spacing (Figure 2). No *C. taxifolia* was found in the lagoon during this survey.

A survey efficacy trial was conducted during this period in Snug Harbor and off of Bristol Cove. In Snug Harbor, the survey team found 58% of the synthetic *Caulerpa* placed in the study area. Off of Bristol Cove, 48% of the synthetic *Caulerpa* was found.

The results for the 0.3-m, 0.5-m, and 1-m wide patches at Snug Harbor were 80%, 60%, and 90%, respectively. The results for 0.3-m, 0.5-m, and 1-m wide patches at Bristol Cover were 80%, 100%, and 100%, respectively. These results represent the only time a 1-m patch was not detected by the survey team. Over the entire length of the survey efficacy assessment program, out of ninety 1-m patches that were placed, eighty-nine were detected. Based on this very high find rate of 99%, and the high find rate for the 0.5-m patches (87%), it is still assumed that a 1-m patch of *C. taxifolia* would always be detected, regardless of environmental conditions.

Transects surveyed for *Caulerpa taxifolia* during Year 5 of the eradication effort
January - December 2005
Agua Hedionda Lagoon, Carlsbad, CA

Figure
2

Merkel & Associates, Inc.

TREATMENT EFFICACY

An assessment investigating the efficacy of the treatment methodology of tarping and chlorinating was initiated in April 2002. Round openings were cut into selected tarps of various ages and monitored for regrowth of *C. taxifolia*. Since that time no regrowth of *C. taxifolia* has been observed in any of the study plots. The monitoring for both *C. taxifolia* regrowth and for recovery of native species to the exposed bottom continued in the fifth year in April and October 2005. The data collected over three and a half years indicate that the treatment methodology that was used in the eradication effort was effective. They also suggest that once the treatment tarps are removed there will be a recovery of eelgrass and invertebrate infauna. A separate report on the treatment efficacy and biotic recovery will be prepared following the completion of the study in 2006.

ERADICATION STATUS

The completion of the Fall 2005 survey marked the third year of surveys of Agua Hedionda Lagoon with no *C. taxifolia* found. It was estimated that 1,047 m^2 of *C. taxifolia* was present lagoon-wide at the start of the eradication effort in summer 2000. This amount declined steadily throughout the eradication effort, with only 0.4 m^2 found lagoon-wide in summer 2002. No *C. taxifolia* has been detected since that date.

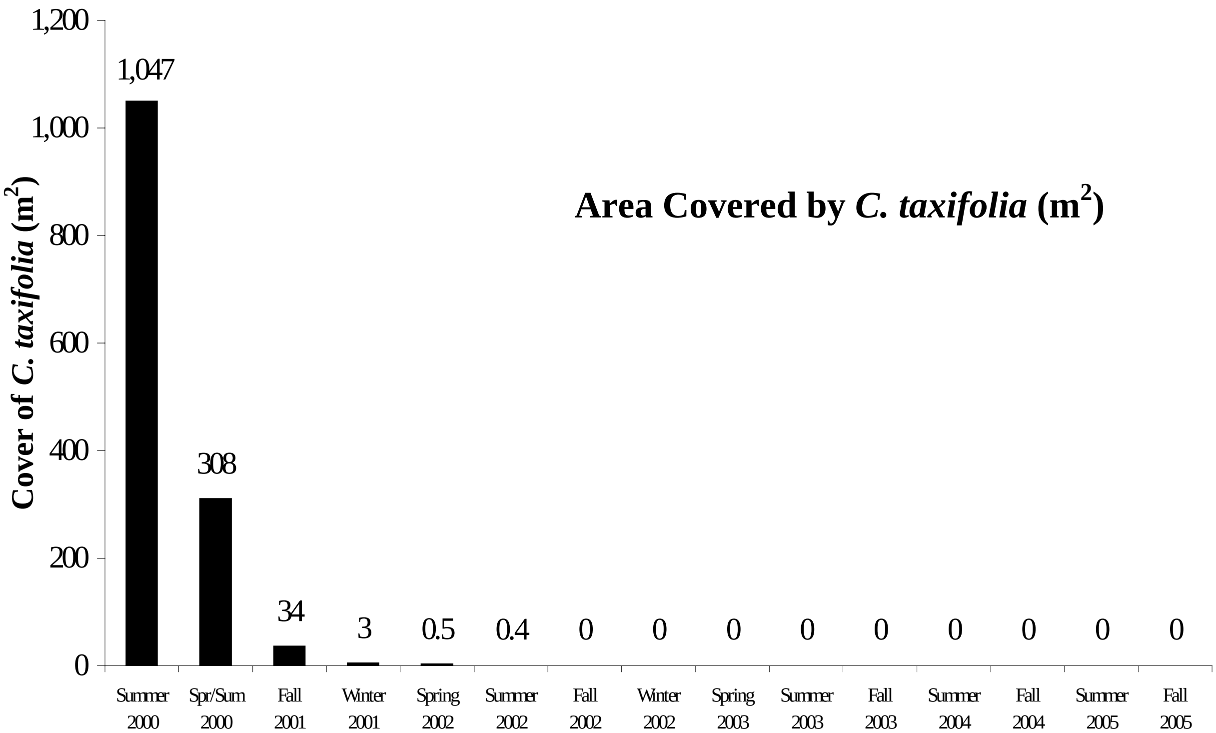


Figure 3. Areal coverage of *Caulerpa taxifolia* in Agua Hedionda Lagoon in square meters.

The SCCAT Technical Subcommittee has established two criteria for successful eradication of the *C. taxifolia* infestation at Agua Hedionda Lagoon: 1) the containment and lethal treatment of *C. taxifolia* at the infestation site, and 2) the demonstrated absence of *C. taxifolia* from the infestation site.

The treatment efficacy assessment mentioned above, in addition to evaluations of the treatment efficacy that have been performed in the laboratory, indicate that the treatment approach used was lethal to *C. taxifolia* and that Criterion 1 has been met at Agua Hedionda Lagoon.

The second criterion is addressed by the intensive, long-term surveillance for undetected *C. taxifolia* in the lagoon. Seven full surveys of the entire lagoon have been conducted with no *C. taxifolia* detected, with two additional surveys that focused only on previously infested areas also detecting no *C. taxifolia* (conducted in winter 2002 and spring 2003). To illustrate the exhaustive coverage of the survey work, all transects that have been surveyed by divers since the last discovery of *C. taxifolia* in September 2002 are presented in Figure 4.

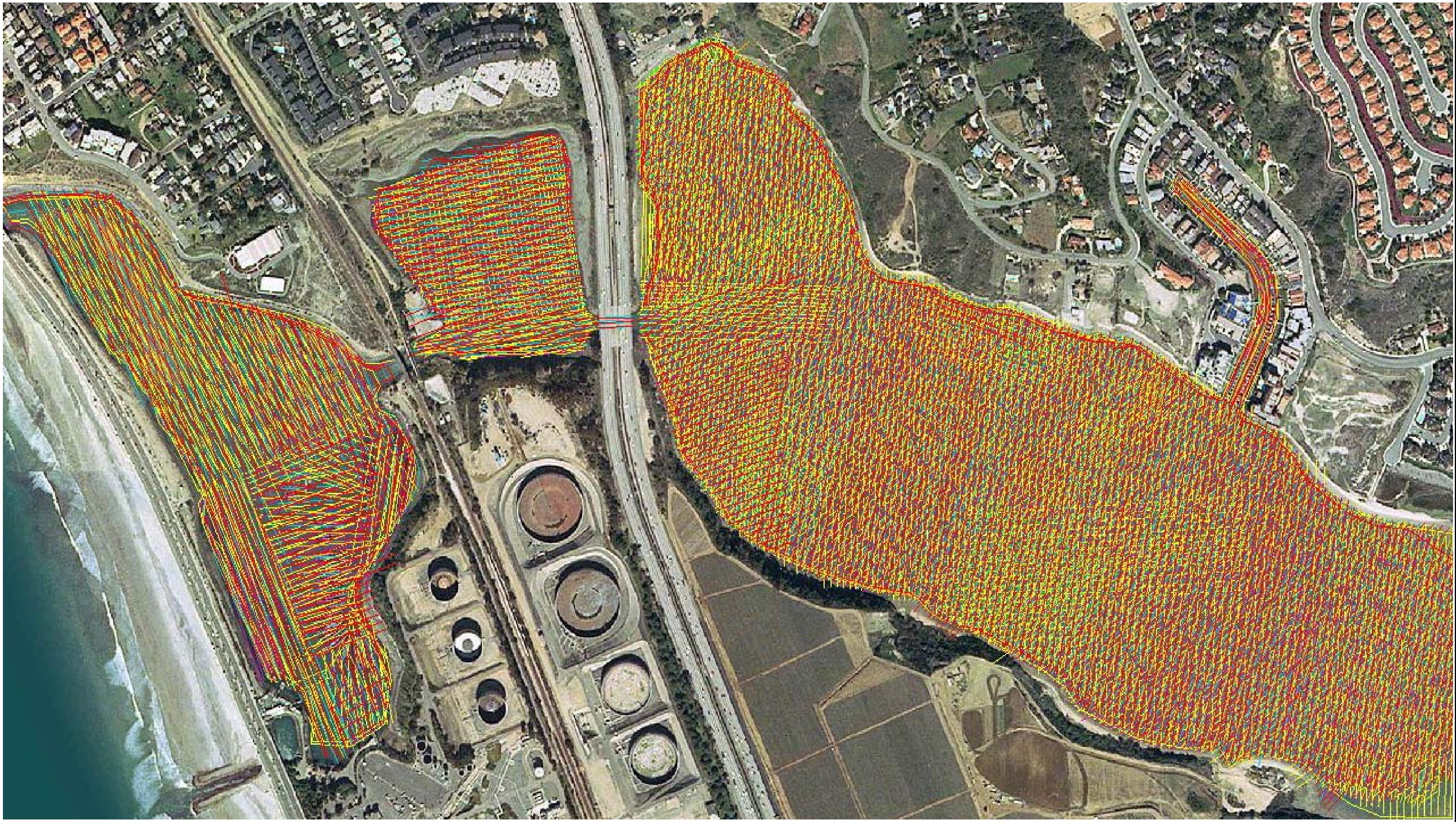
The evaluation of our confidence in these survey results was quantified by the Survey Efficacy Assessment Program mentioned above, involving the placement of patches of artificial *Caulerpa* during each of the surveys. Confidence in the results of each survey for live *C. taxifolia* was quantitatively estimated based on the amount of artificial *Caulerpa* found by the survey divers. The results of the consecutive assessments of the surveys ultimately allowed for an the estimation of the eradication certainty, the certainty that all real *C. taxifolia* existing at the two sites had been found and that eradication had been achieved. The assessments determined that there is a 97.71% certainty that eradication has been achieved at Agua Hedionda Lagoon, assuming the worst conditions (small patch size and low visibility), and a 99.86% certainty if the average conditions are assumed. These results indicate that Criterion 2 has also been met at both sites with a high degree of certainty.

A recommendation that eradication be declared at Agua Hedionda Lagoon is in preparation and will be submitted to the California Department of Fish and Game by the SCCAT Steering Committee for consideration. The final determination on the status of the eradication will be made by the California Department of Fish and Game after reviewing the collected data.

ONGOING ERADICATION PROGRAM WORK

Although it is recommended that *C. taxifolia* be declared eradicated from Agua Hedionda Lagoon, there are remaining work elements that will be undertaken through the end of 2007. A major remaining task is the removal of treatment materials from the lagoon. The tarps were left in place to this point to discourage regrowth of any material that may have not been treated by the chlorine. Based on the studies outlined above, removal of the treatment tarps will not result in regrowth of *C. taxifolia*. Removal of the tarps will facilitate a full recovery of the flora and fauna present prior to the introduction of *C. taxifolia*. This work will be conducted in late summer and fall 2006.

Although success has been achieved in Agua Hedionda Lagoon, the threat of a repeated introduction is ever-present. Funds have been set aside to conduct one full survey of the lagoon in late 2007 in order to detect any new infestations that may have developed from new introductions.



Transects surveyed for *Caulerpa taxifolia* since the last detection in September 2002
Fall 2002, Winter 2002, Spring 2003, Summer 2003, Fall 2003, Summer 2004, Fall 2004, Summer 2005 and Fall 2005
Agua Hedionda Lagoon, Carlsbad, CA

Figure 4

COORDINATION WITH LAGOON USERS

During the fifth year of the eradication program, activities on the lagoon continued to be coordinated through the Interim Management Plan (Plan), a document drafted and adopted by the SCCAT, the Agua Hedionda Lagoon User Representatives, and the City of Carlsbad. This plan partitioned the lagoon into management units and established safety guidelines for both the eradication crew and recreational users of the lagoon. To coordinate the activities of all users, informational signage at access points around the lagoon was posted with regular activity updates, and a recorded phone message with schedule updates was maintained. This Plan allowed the survey work to be conducted more safely and efficiently than before the adoption of the plan. The City of Carlsbad and SCCAT review the Plan annually to assess its efficacy and consider modifications.

The first version of the Plan originally adopted in June 2002 included the following restrictions on the lagoon relating to the control of *C. taxifolia*: a ban on anchoring and fishing throughout the east basin, a prohibition of wake height by boats in excess of 0.3 m (12 inches) when measured from the undisturbed water surface to the top of crest, and continued exclusion of all unauthorized vessels from most of Snug Harbor, the most infested area of the lagoon.

These restrictions were regularly reviewed by SCCAT in the context of the progress of eradication efforts. In November of 2002, SCCAT recommended the re-opening of the eastern portion of the east basin to fishing, given that after two years of survey, no *C. taxifolia* had been found there. The following year, in May 2003, SCCAT also recommended that the previously closed area in Snug Harbor be opened to passive use vessels (non-motorized vessels). In fall of 2003, SCCAT further recommended that Snug Harbor be returned to its original use as the operational area for the vessels of Carlsbad Watersports, located in Snug Harbor. The Carlsbad City Council approved and adopted each of these changes, which were implemented throughout the fourth year.

The plan was extended for an additional year in April 2005, with a new expiration of June 30, 2006. The only remaining restriction was the prohibition of anchoring in the east basin, the limitation of fishing to the passive use area, and the closure of zones to facilitate eradication activities. It is anticipated that zone closures will be needed to carry out the additional work elements outlined above in 2006 and 2007. SCCAT will continue to revisit the Plan annually with the goal of eventually recommending the complete return to pre-*C. taxifolia* uses.

ERADICATION COSTS

During the fifth year of the eradication program at Agua Hedionda Lagoon, Merkel & Associates performed many tasks, including SCCAT coordination and presentations, outreach, surveillance and mapping, collection and management of data relating to efficacy of treatment and survey efforts, reporting, and a variety of other tasks, as assigned. The vast majority of the funds expended were for the surveillance work. During the fifth year (January to December 2005), approximately $524,000 was expended on the above-described work. This funding was provided by the State Water Resources Control Board (through an EPA 319h Water Quality Implementation Project grant), the California Coastal Conservancy (through a Southern California Wetlands Recovery Project grant), and the Agua Hedionda Lagoon Foundation. Since June 2000, eradication efforts at Agua Hedionda Lagoon have cost approximately $3.34 million.

Additional costs of eradication not accounted for above include the contributions of all active SCCAT members including the California Department of Fish and Game, National Marine Fisheries Service, the San Diego and Santa Ana Regional Water Quality Control Boards, U.S. Department of Agriculture, UC Davis, Agua Hedionda Lagoon Foundation, and Cabrillo Power I LLC.

Funding for this project has been provided in part by the U.S. EPA pursuant to Assistance Agreement No. C9-9768201-0 and any amendments thereto which has been awarded to the State Water Resources Control Board (SWRCB) for the implementation of California's Nonpoint Source Pollution Control Program. The contents of this document do not necessarily reflect the views and policies of the USEPA or the SWRCB, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

Merkel & Associates, Inc. 2006. Southern California *Caulerpa taxifolia* Eradication Program-*Caulerpa taxifolia* Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2005. Fourth Year Status Report – Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2004. Third Year Status Report – Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2003. Second Year Status Report – Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2001a. One Year Status Report – Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2001b. Revised eradication plan for *Caulerpa taxifolia* in California. Prepared for Southern California *Caulerpa* Action Team.

National Marine Fisheries Services. 2001. *Caulerpa* Control Protocol.

APPENDIX B

Eradication and Surveillance of *Caulerpa taxifolia* within Huntington Harbour

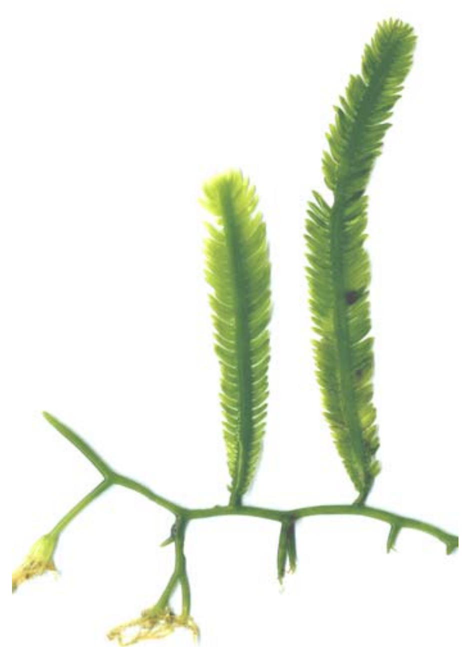
Status Report

Eradication and Surveillance of *Caulerpa taxifolia* within Huntington Harbour, Huntington Beach, California
Status Report

Prepared for:

Steering Committee of the Southern California *Caulerpa* Action Team

- California Regional Water Quality Control Board – San Diego Region
- California Regional Water Quality Control Board – Santa Ana Region
- California Department of Fish and Game
- National Marine Fisheries Service
- U.S. Department of Agriculture –Agricultural Research Service



Prepared by:

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

May 2006

TABLE OF CONTENTS

FIGURES

Eradication and Surveillance of *Caulerpa taxifolia* within Huntington Harbour, Huntington Beach, California Status Report

May 2006

INTRODUCTION

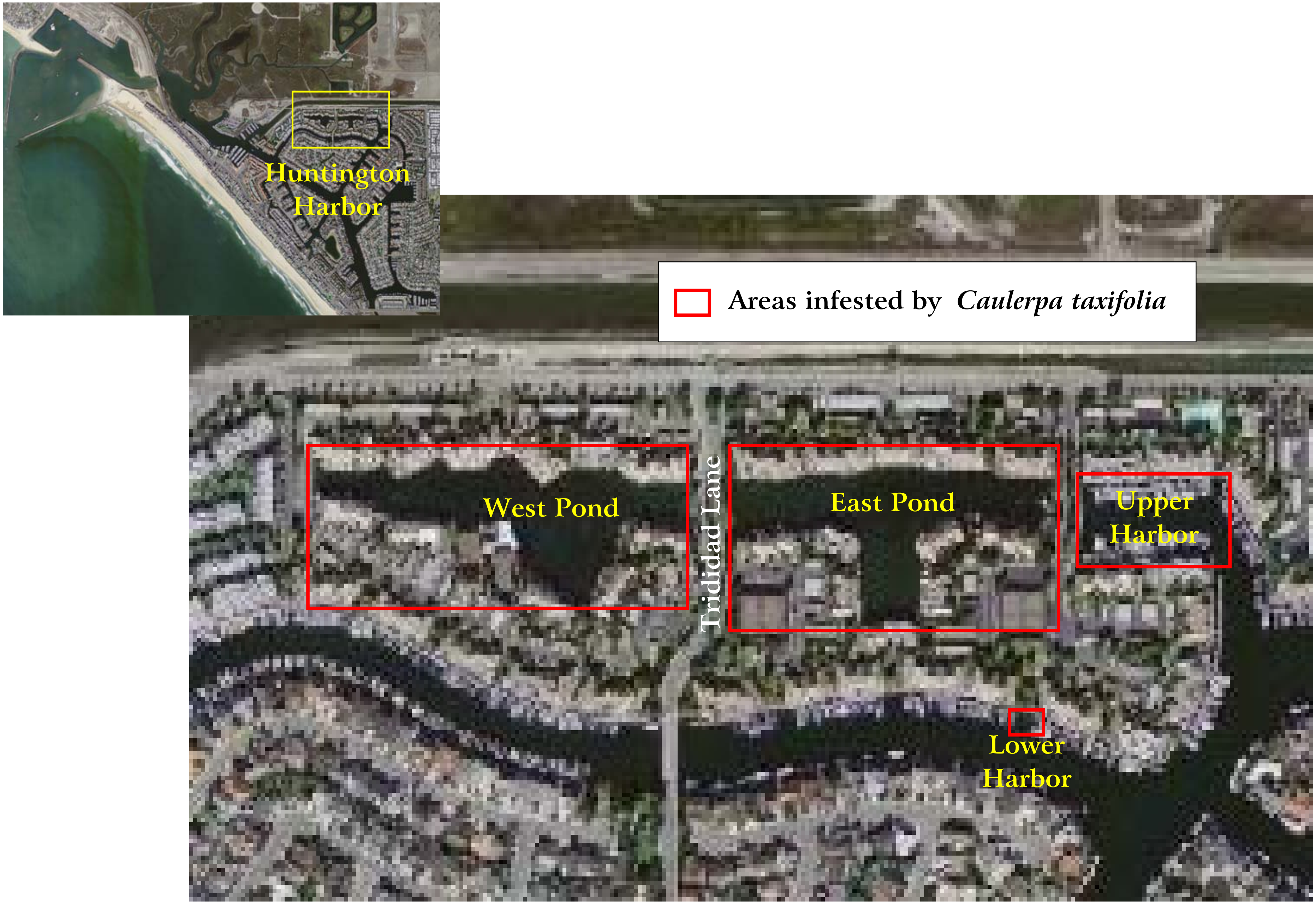
In 1984, an invasive, aquarium-raised strain of the seaweed *Caulerpa taxifolia* was first noted growing as a small patch in the Mediterranean Sea. Although the native strain occurs naturally in tropical waters, an aquarium strain of *C. taxifolia* had been cultivated for use in saltwater aquariums, becoming notably cold-tolerant and easy to cultivate. The small Mediterranean patch likely resulted from the disposal of aquarium water into the sea. Due to the rapid and aggressive growth habits of this strain of *C. taxifolia*, the patch of seaweed quickly spread and grew to form extensive meadows that displaced expanses of native habitat in much of the coastal northwestern Mediterranean, covering as much as 13,000 hectares (32,000 acres) of seafloor by 2001 (Meinesz et al. 2001). The aquarium strain of *C. taxifolia* has also been released into several water bodies in Australia in recent years.

In June 2000, this seaweed was discovered growing in Agua Hedionda Lagoon in Carlsbad in San Diego County, where it was quickly spreading to cover large areas, displacing the native seagrass beds found there. Because the invasive strain of this seaweed does not exist naturally in the wild in the United States, it is believed that the seaweed must have been released from a saltwater aquarium. The resulting press coverage of the infestation brought to light a second infestation in the SeaGate Lagoons of Huntington Harbour in Huntington Beach in Orange County. This infestation is also believed to have resulted from the release of saltwater aquarium material into the lagoons. *Caulerpa taxifolia* was found not only in both SeaGate Lagoons, but also in two locations in the main harbor, immediately adjacent to drains that empty lagoon water into the harbor. Genetic studies have determined both California infestations to be identical to the strain invading the Mediterranean Sea (Jousson et al. 2000).

Since the discovery of *C. taxifolia* in California in June 2000, eradication, surveillance, public outreach efforts, eradication research, and legislative efforts have been initiated and are on-going. The primary goal of the Southern California *Caulerpa* Action Team (SCCAT), which is made up of resource managers, marine resource and pest control scientists, permitting agencies, marine biological consultants, land-owners and environmental stakeholder representatives, has been the eradication of the two known infestations: Huntington Harbour and Agua Hedionda Lagoon.

INFESTATION AREAS

The Huntington Harbour *C. taxifolia* infestations were discovered in the northern portion of the harbor, primarily in the water bodies known as SeaGate Lagoons. The "lagoons" consist of two man-made ponds located on Trinidad Lane in the City of Huntington Beach (Figure 1). The ponds contain harbor water, but are non-tidal. Water level is maintained by pumps that fill the east pond with water from the harbor, which drains into the west pond, and then back out into Huntington Harbour. The ponds are enclosed on all sides by concrete bulkheads, with residential condominium patios forming the "banks" of the ponds. The ponds range from three to six feet



Caulerpa taxifolia **infestation areas in Huntington Harbour - 2000**
Huntington Beach, CA

Figure
1

Merkel & Associates, Inc.

in depth, with an approximately one-foot thick soft sediment layer over harder sediment beneath. There are no boats in the ponds with the exception of several pedal-boats.

The pumping mechanisms and grating at all drains generally restrict the immigration of large fish into the ponds from the outer harbor, suggesting that the majority of the larger fish know to live in the ponds were intentionally placed there. This has been confirmed by reports from numerous local residents who regard many of these animals as pets or claim to have released marine life into the ponds themselves.

In addition, *C. taxifolia* was discovered growing at two locations where outflow pipes drain the east pond back into the harbor. These sites are referred to at the north and south harbor sites (Figure 1). These areas reach a maximum depth of approximately -17 ft Mean Lower Low Water (MLLW).

The infestation of *C. taxifolia* at the Huntington Harbour sites was distinctly different than that observed in Agua Hedionda Lagoon. Rather than growing in discrete, dense patches, the infestation generally consisted of hundreds of small plants scattered over a wide area, making measurement of the actual areal coverage of *C. taxifolia* on the bottom difficult. In many cases, fragments of *C. taxifolia* were drifting loose on the bottom, further spread and fragmented by benthic species of fish (primarily round stingrays [*Urobatis halleri]* and various species of goby). A measurement was therefore made of the area of bottom "affected", including all rooted *C. taxifolia*, drifting fragments, and bare areas in between. The actual biomass of *C. taxifolia* discovered in Huntington Harbour was considerably less than that observed at Agua Hedionda Lagoon, and distributed over a much smaller area. The estimation of the total affected area in Huntington Harbour at the time of first assessment in Fall/Winter 2000 was 7,890m^2, distributed over a 1.1-hectare (2.6-acre) area in both ponds and the affected open harbor areas.

Detailed discussions of the extent of the infestation in each area, the treatment response, and the results of follow-up surveys are presented in the following sections.

East Pond

The most extensive infestation of *C. taxifolia* was discovered growing in the east pond. Due to the scattered distribution of the plants and fragments, the eradication response focused on treating the entire affected area. A treatment approach involving the placement of tarps over the affected areas had been effectively developed for use at the Agua Hedionda Lagoon infestation site, as outlined in the *Revised Eradication Plan for <u>Caulerpa taxifolia</u> in California* (Merkel & Associates, 2001). This approach was also applied at the Huntington Harbour infestation site, with treatment efforts consisting of covering *C. taxifolia* with heavy black PVC tarps under which a solid, pelleted chlorine formulation was placed, which provided full containment of *C. taxifolia* while minimizing the water quality impacts of the treatment on the surrounding waters. All work was conducted by a team of SCUBA divers. In order to contain the *C. taxifolia* and small fragments scattered over an estimated 6,704m^2 of the east pond, treatment tarps were placed over much of the western and southern portion of the pond and secured with sandbags and rebar (Figure 2). Initial placement of treatment tarps in the east pond was conducted from October 2000 to February 2001 (fall/winter 2000).

Infested/tarped area fall/winter 2000

Infested/tarped area summer 2002

Infested/tarped area summer 2001

Infested/tarped area fall 2002

Infested/tarped area fall 2001

Path of underground pond drain

Locations of *Caulerpa taxifolia* infestation and treatment in Huntington Harbour
Huntington Beach, CA

Figure 2

Merkel & Associates, Inc.

Following the completion of initial survey and treatment efforts, a systematic quarterly survey program was undertaken to search for additional patches of *C. taxifolia*. The transect methodology employed was designed with the goal of surveying 100% of the infested area. A lead SCUBA diver followed a transect line with three to six additional divers, spaced 1 meter apart, positioned next to the lead diver, perpendicular to the transect line. Each diver scanned the bottom area within 0.5 m to either side of their line of travel. If a diver encountered *C. taxifolia*, the rest of the crew was signaled to stop, and the position marked with a float and recorded with a differential global positioning system (dGPS)(±50cm accuracy). In the east pond, the transects were established by the lead diver, who used the bulkhead walls as a guide. The outer diver in the line dragged a blunt object through the mud at the outside of the search area. The created 'drag-line' was then used to establish the transect line of the next survey route. This method was followed until the entire pond had been surveyed. Later in the program, the transect lines were placed by a boat using dGPS.

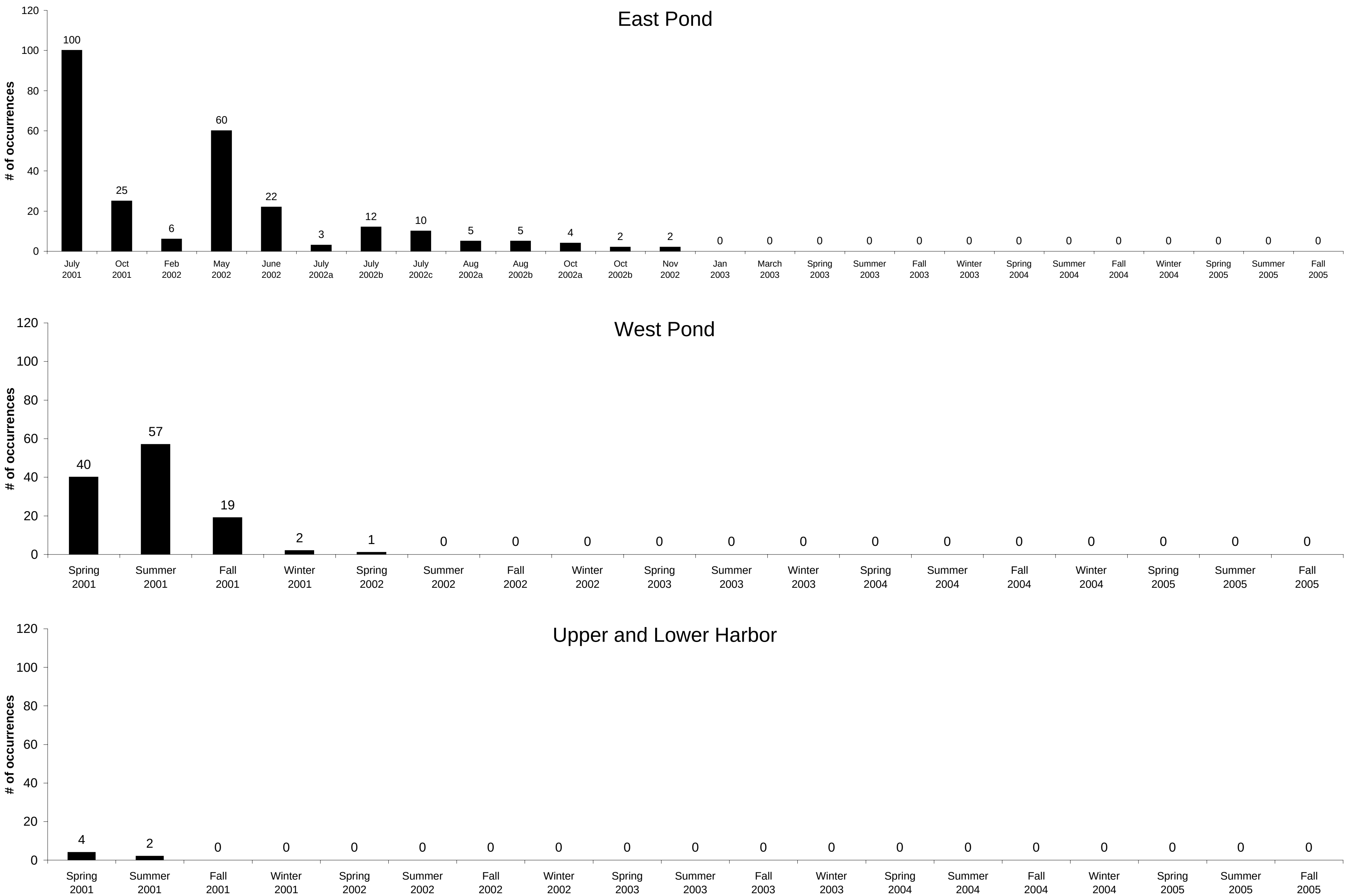
As the water temperature rose in spring and summer 2001 following the initial treatment, additional patches of *C. taxifolia* emerged from the sediment in the east pond and additional tarps were placed to treat them (Figure 2). Follow-up surveys were repeated regularly to search for additional patches that may have arisen or grown to a larger, more detectable size. Observations by the survey team suggested that the burrowing and bolting behavior of the large population of round stingrays and gobies that occupied the east pond may have facilitated the spread of small fragments of *C. taxifolia*. Additionally, chronic high turbidity in the east pond severely limited visibility for the SCUBA survey team, increasing the possibility that small patches or fragments of *C. taxifolia* would go undetected until they grew to a larger size. As a result, surveys were conducted more frequently than quarterly during 2001 and 2002, with any detected occurrences quickly removed or tarped.

All treatment tarps were left in place to discourage regrowth of any material that may have not been treated by the chlorine. A thick layer of sediment quickly settled onto the tarps, in effect providing additional substrate for the establishment of fragments of *C. taxifolia*. Initially these fragments were collected by hand as they were found, however *C. taxifolia* often regrew in these areas, suggesting that rhizoid material (root-like structures of *C. taxifolia*) remaining in the sediment was leading to regrowth. Therefore an approach of placing new tarps on top of existing tarps, where necessary, was adopted in summer 2002. This proved extremely effective at preventing new growth and few additional occurrences were found subsequently. Surveys in the east pond were therefore reduced to quarterly intervals starting in spring 2003, when it appeared that significant strides had been made toward controlling *C. taxifolia* in the east pond.

The progress of the eradication effort was tracked by the number of new plants or fragments detected during a given survey. Figure 3 presents the number of occurrences found during each survey. By fall 2002, intensive surveys were finding only a few small plants remaining, with the last occurrence detected on November 19, 2002. Follow-up surveys continued over the next three years. There have been thirteen additional surveys of the east pond since that time, with no *C. taxifolia* detected. The final survey (fall 2005) was conducted in December 2005.

West Pond

The west pond receives water from the east pond through a culvert under Trinidad Lane (Figure 2). The infestation of *C. taxifolia* in the west pond may have resulted the drifting of fragments



Number of new occurrences of *Caulerpa taxifolia* detected in follow-up surveys
Infestation Areas: East Pond, West Pond, Upper and Lower Harbor
2001-2005

Figure 3

Merkel & Associates, Inc.

from the east to the west pond. Likely due to reduced turbidity (allowing increased light penetration) and to a lower abundance of benthic fish species, *C. taxifolia* in the west pond was distributed in more discrete patches. This made mapping and treatment less problematic than in the east pond. The infestation was detected in eight areas affecting approximately 1,094 m^2 of bottom within the west pond, which were tarped over a period extending from February to April 2001 (winter 2000)(Figure 2).

The follow-up monitoring program described above was initiated in the west pond spring 2001, using the same methodology. As anticipated, new occurrences were detected as the water temperature increased, and two additional areas in the west pond were tarped in August 2001 (Figure 2). Additional quarterly surveys found a small number of new occurrences over the next three quarters, generally associated with the edge of an existing tarp and likely the result of a fragment or buried plant material that had not been contained under the original tarp. These occurrences were treated with additional small tarps and chlorine.

The last occurrence of *C. taxifolia* was detected in the west pond in May 2002 (Figure 3). Quarterly surveys continued over the next three years. Thirteen additional consecutive quarterly surveys of the west pond have failed to detect any persisting or new *C. taxifolia.* The final survey (fall 2005) was conducted in December 2005.

Upper and Lower Harbor

It was initially believed that *C. taxifolia* was restricted to the east and west ponds (SeaGate Lagoons). However, an October 2000 investigation of areas of the main harbor “upstream” of the east pond, as well as portions of the main harbor where water drained from the east and west ponds, revealed two additional areas of infestation described below (Figure 1). There is a skimmer located in the east pond that drains to the two harbor locations found to be infested with *C. taxifolia* (Figure 2). It is possible that water containing fragments of *C. taxifolia* passed out of the east pond into the harbor through this skimmer. It is also possible that the infestation originated in the upper harbor area and was then pumped up into the east pond, then spread to the west pond. The exact origin and pathways of spread throughout the Huntington Harbour site will likely never be determined.

The lower harbor infestation site is located immediately at the outflow of an underground drain that conveys skimmed water from the east pond to the lower harbor site (Figure 1). A small patch of *C. taxifolia* (~0.1m^2) was discovered in October 2000, growing approximately 2 meters from the drain outlet, at a depth of approximately -6 ft MLLW. A single treatment tarp and chlorine was immediately placed over the patch.

A much larger area of infestation was also discovered in October 2000 at the upper harbor site, which is much deeper than the ponds (up to -17 ft MLLW) (Figure 1). The infestation was sparsely distributed, growing as small plants, with few fragments noted. Treatment of the detected *C. taxifolia* began immediately, with the placement of treatment tarps and smaller treatment tubs and chlorine over an affected area of approximately 93m^2. The intial treatment was completed by November 2000 (Figure 2).

Follow-up surveys of the infested upper and lower harbor sites were initiated in spring 2001. *Caulerpa taxifolia* was never again detected at the lower harbor site (Figure 3). Additional occurrences were detected at the upper harbor site in two follow-up surveys and were promptly treated. The last occurrence of *C. taxifolia* was detected in the harbor in July 2001. Seventeen additional consecutive quarterly surveys have failed to detect any persisting or new *C. taxifolia* in the infested harbor areas. The final survey (fall 2005) was conducted in December 2005.

REMAINDER OF HUNTINGTON HARBOUR

Although initial surveillance conducted in 2000 indicated that the infestation was restricted to the east and west ponds (SeaGate Lagoons) and the small areas in the northern portion of Huntington Harbour discussed above, two harbor-wide surveys of the remaining areas of the entirety of Huntington Harbour were conducted by teams of SCUBA divers. These full surveys of the entire harbor were conducted in 2001 and again in 2005. *Caulerpa taxifolia* was not detected elsewhere in the harbor during these surveys.

ERADICATION STATUS

The completion of the Fall 2005 survey marked the third year of surveys of the Huntington Harbour infestations sites with no *C. taxifolia* found. None has been detected since November 2002.

A program to assess the survey dive team's ability to locate *C. taxifolia* in the often murky water of the infestation sites was developed at Agua Hedionda Lagoon. Patches of artificial *Caulerpa* of various small sizes were randomly placed at Agua Hedionda Lagoon during each of the regular follow-up surveys to test the dive team. Confidence in the results of each survey for live *C. taxifolia* could then be quantitatively estimated based on the proportionate amount of known quantities of artificial *Caulerpa* found by the divers. Based on the tremendously useful data collected by this program at Agua Hedionda Lagoon, a similar program was implemented at the Huntington Harbour infestations sites in summer 2003 and continued through the last quarterly survey in fall 2005.

The survey efficacy assessment trials at Huntington Harbour resulted in a combined return of 400 out of 500 (80%) patches of synthetic *Caulerpa* over the course of seventeen trials. Individual trial results ranged from 68% to 87% success at detecting the synthetic *Caulerpa*. A full discussion of this efficacy assessment program is detailed in *Caulerpa taxifolia Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour* (M&A 2006). The results of the consecutive assessments of the efficacy of the surveys ultimately allowed for an estimation of the eradication certainty that all real *C. taxifolia* existing in the Huntington Harbour infestation areas had been found. This estimation was calculated as of December 2005 to be 99.86% (M&A 2006).

A recommendation that *C. taxifolia* be declared eradicated from Huntington Harbour is in preparation and will be submitted to the California Department of Fish and Game by the SCCAT Steering Committee for consideration. The final determination on the status of the eradication will be made by the California Department of Fish and Game after reviewing the collected data.

ONGOING ERADICATION PROGRAM WORK

Although it is recommended that *C. taxifolia* be declared eradicated from Huntington Harbour, there are remaining work elements that will be undertaken through the end of 2007. A major remaining task is the removal of treatment materials from the infested areas. The tarps were left in place to this point to discourage regrowth of any material that may have not been treated by the chlorine. This removal work will likely be conducted in late summer and fall 2006.

Although success has been achieved in Huntington Harbour, the threat of a repeated introduction is ever-present. Funds have been set aside to conduct one full survey of the east and west ponds and affected harbor areas in late 2007 in order to detect any new infestations that may have established from new introductions.

ERADICATION COSTS

The funding for the *C. taxifolia* eradication program at Huntington Harbour has been provided by the Santa Ana Regional Water Quality Control Board. Since August 2000, eradication efforts at the Huntington Harbour infestation sites have cost approximately $660,000. Additional funding was provided for the harbor-wide surveys in 2001 and 2005 by the California Department of Fish and Game, NOAA's National Marine Fisheries Service, and by the State Water Resources Control Board (Proposition 13 Watershed Protection Program Grant).

Additional costs of eradication not accounted for above include the contributions of all active SCCAT members including the California Department of Fish and Game, NOAA's National Marine Fisheries Service, the San Diego and Santa Ana Regional Water Quality Control Boards, and U.S. Department of Agriculture- Agricultural Research Service.

REFERENCES

Jousson, O., Pawlowski, J, L. Zaninetti, F. W. Zechman, F. Dini, G. Di Guiseppe, R. Woodfield, A. Millar, and A. Meinesz. 2000. Invasive alga reaches California. Nature 408:157-158.

Meinesz A., Belsher T, Thibaut T, Antolic B, Ben Mustapha K, Boudouresque C-F, Chiaverini D, Cinelli F, Cottalorda J-M, Dejellouli A, El Abed A, Orestano C, Grau AM, Ivesa L, Jaklin A, Langar H, Massuti-Pascual E, Peirano, A, Tunesi L, Vaugelas J de, Zavodnik N, Zuljevic A. 2001. The introduced alga *Caulerpa taxifolia* continues to spread in the Mediterranean. Biological Invasions 3:201-210.

Merkel & Associates, Inc. 2006. Southern California *Caulerpa taxifolia* Eradication Program-*Caulerpa taxifolia* Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour. Report prepared for Southern California *Caulerpa* Action Team.

Merkel & Associates. 2001. Revised eradication plan for *Caulerpa taxifolia* in California. Prepared for Southern California *Caulerpa* Action Team.

APPENDIX C

Use of Sediment Bioassays to Verify Efficacy of *Caulerpa taxifolia* Eradication Treatments

J. Aquat. Plant Manage. 43: 1-9

Use of Sediment Bioassays to Verify Efficacy of *Caulerpa taxifolia* Eradication Treatments

LARS W. J. ANDERSON[1], WAILUUN TAN[1], RACHEL WOODFIELD[2], ROBERT MOONEY[2] AND KEITH MERKEL[2]

ABSTRACT

Infestations of the marine macrophytic alga *Caulerpa taxifolia* were discovered in Agua Hedionda Lagoon, California in 2000. Rapid response actions included containment under pvc tarps coupled with injection of liquid sodium hypochlorite. To assess the efficacy of these treatments, replicated sediment cores were removed from representative treated sites and transferred to grow-out facilities. Similar cores from uninfested (control) sediments were removed, inoculated with viable explants of *C. taxifolia* and placed in grow-out facilities. Results from two sampling periods (1 year, 2 years post-treatment) showed that no viable *C. taxifolia* emerged in cores, and that inoculated "control" sediments supported normal growth. Eelgrass (*Zostera marina* L.) seedlings emerged from native seed-banks in "treated" cores, which also supported growth of some invertebrates (annelid worms and hydroids). This study provided essential verification of *C. taxifolia* eradication efforts, and demonstrates the feasibility of incorporating quality control/quality assurance components in rapid response actions. Results of this study also suggest that seeds of eelgrass are viable for at least two years.

Key words: Zostera marina, rapid response, invasive species, chlorine.

INTRODUCTION

Invasive, nonnative species cause a range of negative environmental and economic impacts, and are a continuing threat to aquatic ecosystems (Sakai et al. 2001). Recent estimates of economic costs of exotic aquatic invasive species in the US alone range from $1 to $2 billion annually (Rockwell 2003). Furthermore, the direct costs of controlling these types of infestation increase dramatically once they have begun to spread from established, pioneer populations to larger areas (Mullin et al. 2000, Rejmanek and Pitcairn 2002). Thus, effective rapid response to incipient populations not only prevents further spread, but also greatly reduces long-term costs and ecological damage (Western Regional Panel 2003, FICMNEW 2003).

When *Caulerpa taxifolia,* an invasive non-native marine macrophytic alga, was found in a California coastal lagoon (Agua Hedionda Lagoon) in 2000, a rapid response plan was implemented and field treatments were begun less than three weeks after the discovery (Anderson 2001, Anderson and Keppner 2001, Jousson et al. 2001, Anderson 2002, Anderson 2004, Williams and Groscholz 2002) (Figure 1). This immediate action was prompted by the history of detrimental impacts of *C. taxifolia* in Mediterranean coastal waters, where it has spread, unchecked, to about 13,000 ha encompassing the shorelines of six countries since 1984 (Meinesz 1999, 2002, Meinesz et al. 2001). Observed negative impacts of dense *C. taxifolia* colonies include displacement of native seagrasses and other benthic organisms, reduction in fish and invertebrate diversity, and degradation of aesthetic values (Meinesz 2002). In addition, the Mediterranean strain of *C. taxifolia* had been placed on the Federal Noxious Weed list in 1999 as an initial step in preventing its introduction into US coastal waters. Therefore, this alga posed a serious threat to vast areas of California coastal ecosystems due to temperature regimes and habitats similar to those in the highly invaded Mediterranean area (Anderson 2004).

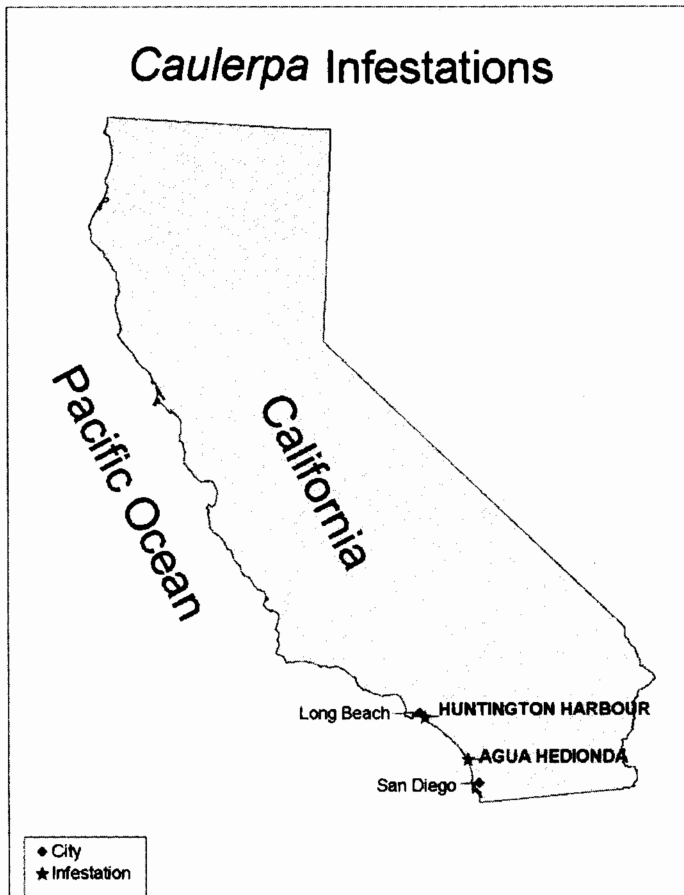


Figure 1. Map showing two *Caulerpa taxifolia* infestations in California.

[1]USDA-Agricultural Research Service, Exotic and Invasive Weed Research Laboratory, Davis, CA 95616.

[2]Merkel and Associates, Inc., 5434 Ruffin Ave., San Diego, CA. Received for publication December 22, 2004 and in revised form March 24, 2005.

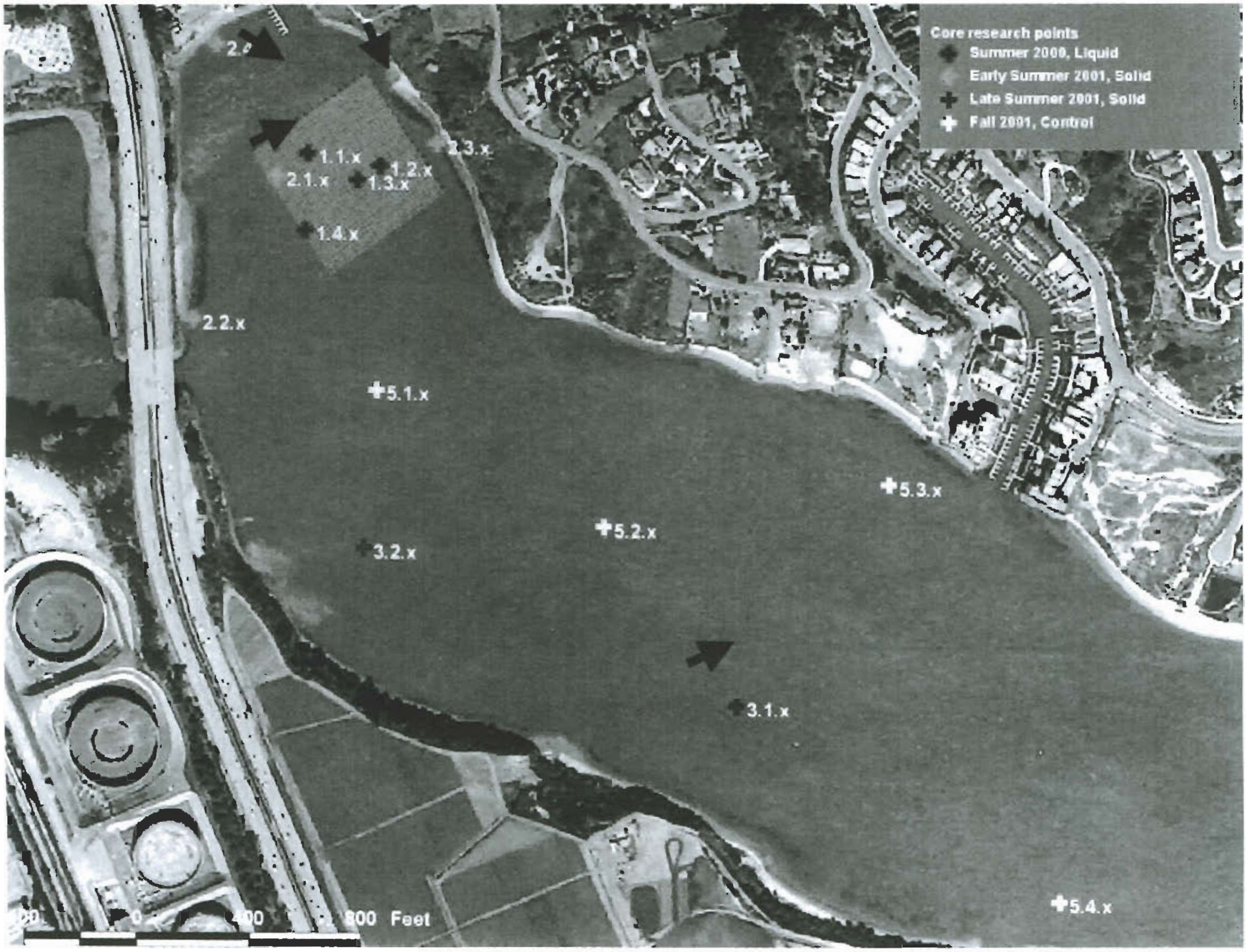


Figure 2. Map showing locations in the inner basin at Agua Hedionda Lagoon (Carlsbad, CA) where core samples were taken to assess efficacy of eradication treatments in December 2001 Legend indicates treatment date and type of treatment. Arrows show locations where second set of cores were taken in August, 2002.

As part of the rapid response actions, colonies of *C. taxifolia* were covered with 20-mil polyvinyl chloride (pvc) sheets beneath which liquid sodium hypochlorite was injected (Merkel et al. 2001). Beginning in July 2000, and continuing to late summer of 2001, *C. taxifolia* colonies of various sizes and densities were covered with pvc tarps and liquid chlorine (sodium hypochlorite) was injected to begin the eradication of the alga. Twenty large and densely populated colonies were treated, along with some smaller colonies discovered in 2001. In 2001, solid chlorine tablets (tricloro-s-triazinetrione) were used instead of liquid sodium hypochlorite. *In situ* visual observations of above-sediment thalli showed that within 2 to 7 days, photosynthetic pigments were thoroughly bleached (R. Woodfield, pers. obs.). No re-growth from these bleached thalli was observed after they were placed in small aquaria that were filled with natural Agua Hedionda Lagoon seawater. However, since *C. taxifolia* produces rhizoids that are anchored on and into sediments and since these rhizoids may be protected from the topical exposure to chlorine, it was essential to determine if treatments completely destroyed the potential for regrowth from these structures or possibly from small fragments of thallus. This paper describes the rationale, methods, and results of two replicated efficacy-verification assessments conducted on sediment removed from several tarped and treated areas in Agua Hedionda Lagoon, California.

MATERIALS AND METHODS

Selection of Treated Colonies

Specific tarped *C. taxifolia* colonies were selected from which sediment core samples were removed in December 2001 and August 2002. The criteria used for sampling were: 1) areas that had contained the largest, and initially most dense colonies were identified by number, and placed into a common "pool" for each of the four treatment times (seasons) during 2000 and

Figure 3. Example of *C. taxifolia* explants used to inoculate "control" cores removed from untreated areas in Agua Hedionda.

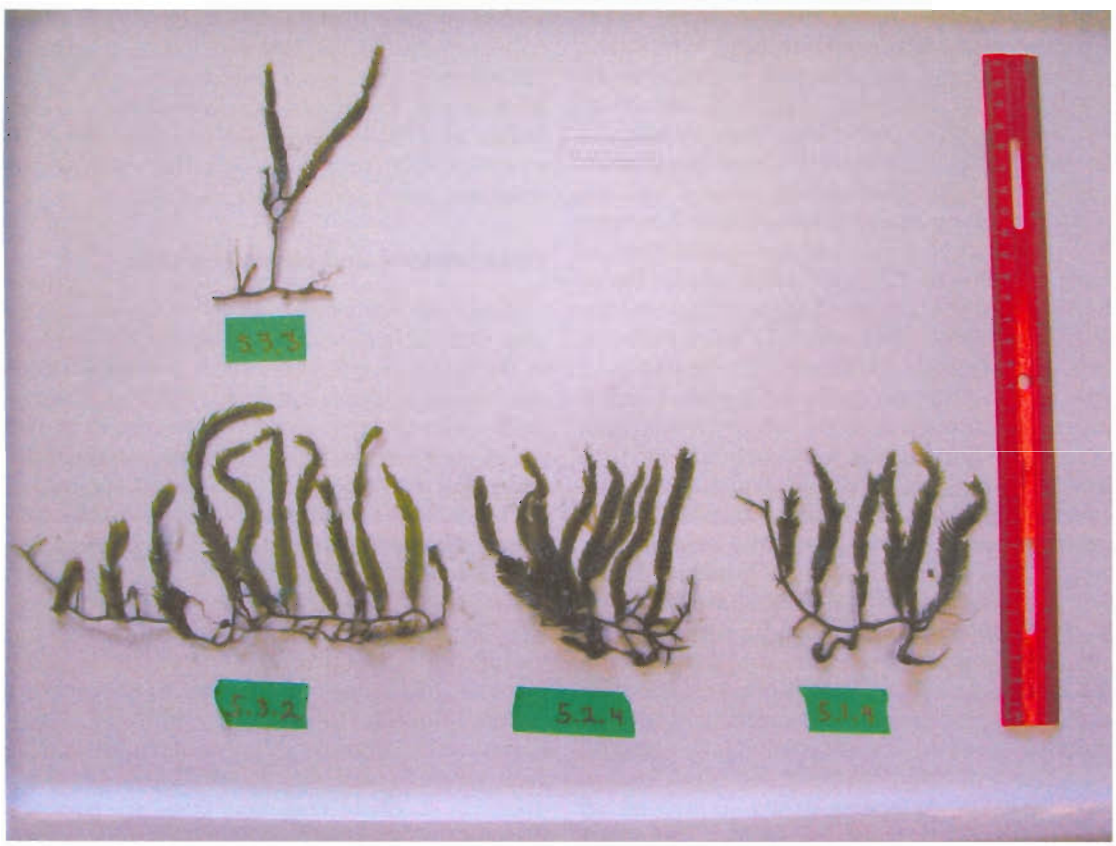


Figure 4. Examples of *C. taxifolia* 76 days after inoculation into "control" cores. Numbers indicate specific cores.

2001. The rationale for this was that areas that had contained dense colonies offered the highest probability of "capturing" areas that had the most mature and well-established growth of *C. taxifolia*, and 2) from the total pool of treated areas, three colonies were randomly selected for each treatment time period (i.e., for each season of treatment). Thus, 12 tarped colonies were selected. Figure 2 shows sampling sites for the December, 2001, and August, 2002 assessment.

Sediment Core Sampling

On December 13 and 14, 2001, intact sediments were removed by scuba divers using 10 cm diameter by 45 cm long pvc cylinders (one per sample) to a depth of 20 cm. Each sample was retained within its cylinder and labeled in permanent ink with sample date and a code that could later identify the colony and its treatment date. Labels did not indicate treatment date or type of treatment so that subsequent laboratory observations were "blind". The pvc sampler was fitted at the upper end with a flat cap, which had a 2-cm diameter hole that allowed water to escape as the sampler was being inserted into the sediment. After inserting the core sampler with the bottom uncapped and the top hole un-covered, it was gently rotated and tilted to facilitate capping the bottom opening. The sampler hole was then sealed with a rubber stopper and the core was transferred to a cooler at the surface. During the transfer to the surface, divers kept the core samples vertical to minimize mixing and shifting of the sediments. Water depth where cores were removed ranged from 2 to 3.5 m MLLW (mean lower lowest water). A total of four cores per colony-site were removed: two from the center of the tarped area and two from each of two edges within 15 cm of the outer pvc "cage" that supported the tarp. Within the largest and most densely populated area that was treated, 20 cores were taken from four locations (Figure 2, see cross-hatched square). Based upon the extent and density of *C. taxifolia* at this site, it was probably the oldest population and may have been the original infestation since it was offshore from a storm drain. Uninfested "control" sites were also sampled and these sediments were used for inoculation of *C. taxifolia* explants. A total of 72 cores were taken. These sampling sites represented the following types of treatments: 1) liquid sodium hypochlorite (16 cores); 2) solid chlorine tablets (16 cores); 3) tarp-only (8 cores); 4) controls (16 cores). Sediment cores in their pvc tubes were sealed within the coolers with ice and transported via vehicle from Agua Hedionda Lagoon to the United States Department of Agriculture- Agricultural Research (USDA-ARS) Aquatic Weed Facility on the University of California, Davis campus. During transport, sediment cores in their pvc tubes were kept in their original orientation. This insured the least possible disturbance of the cores. Transit time was approximately 11 hours.

On August 8, 2002, scuba divers removed a second set of 20 cores from an additional set of colonies in Agua Hedionda. In three sites that had been treated, five cores were removed. Five cores were also removed from an additional control site (Figure 2). Cores were transported as before and controls were inoculated on August 9, 2002. All cores (inoculated control cores, cores from beneath tarps in treated areas, and those areas tarped but not exposed to chlorine) were placed in grow-out conditions described below.

Assessment of Regrowth from Core Sediments

Cores were placed in a complete-randomized block design in a walk-in growth chamber; three cores were placed in each of 24 pvc pails (20-liter). Pails with cores were kept at 18 C (+/-2 C) under fluorescent lamps producing ca. 250 µmol m^{-2} sec^{-1} cool-white fluorescent light on a L:D 14:10 photoperiod. Pails were filled with artificial seawater ("Instant Ocean"®), and one-half of the volume of each pail was replenished with freshly made seawater at 14-day intervals. Each pail was aerated with a 1.2 cm diameter aeration stone supplied by a common air pump. Temperature in two of the pails was monitored with max/min thermometers.

To confirm that the grow-out conditions provided would facilitate growth of the target alga, "control" sediments (i.e., sediments never having had populations of *C. taxifolia*) were removed from Agua Hedionda Lagoon using the same methods as described above and were inoculated with live "explants" of *C. taxifolia* that had been cultured from the original population in the lagoon (Figure 3). In the first set of core samplings (December, 2001) six replicate cores were used from each site. On Dec. 15, 2001, two types of *C. taxifolia* explants were inserted 2 to 3 cm deep into sediments as follows: (1) stolon plus single frond and (2) stolon with rhizoid only (no frond attached). These cores were maintained identically, but in separate pails, to the cores removed from tarped/treated areas. In the second core sampling (August 2002), five cores from untreated areas were inoculated with fronds having attached stolons and rhizoids. Due to poor growth in four of these cores, control cores #1-4 were re-inoculated on November 1, 2002, and observed at 3, 12, and 24 days after start of the assay. Grow-out conditions were as follows: (1) temperature: 20.4-19.3 C; (2) Salinity: 29.4 ppt (+/-0.9 ppt); (3) light was cool-white fluorescent overhead supplemented with incandescent bulbs; (4)L:D 14:10 at 230 µmol m^{-2} sec^{-1}; and (5)aeration was provided for 6 hours at 6- hour intervals (one 1.2-cm diameter stone per 20-liter container).

Observations and Measurements

Any evidence of viable regrowth of *C. taxifolia* would indicate that the treatments did not kill rhizoids, stolons, or other parts that may have been within the sediments. Each core was visibly inspected weekly for the presence of emerging thalli (treated cores) or any other plant growth. For the first set of core samples taken in December 2001, all cores were harvested 76 days after the start of the assay as follows: (1) each core was removed and, following the final visible observation, sieved through 0.5-mm mesh; and (2) any pieces of plants were collected, measured, and photographed. Cores sampled on August 8, 2002, were harvested 108 days after start of the assay. Salinity, pH, and dissolved oxygen were monitored periodically. Over the course of the growth period, the following observations were recorded: length of fronds, number of fronds emerging, presence of other plants, and presence of animals. At the terminations of the assay period, cores were carefully disassembled and sediment material carefully removed from the top-down and sieved through 2-mm mesh screen to determine if any pieces of *C. taxifolia* or other plants were present.

Estimates of biomass production of inoculated *C. taxifolia* during the grow out period were made by applying a conversion factor to convert weekly incremental increases in thallus length to fresh weight. This factor was determined by sampling seven replicate 1-cm samples of thalli and determining their fresh weight. The total (cumulative) elongation rate was then converted to estimate fresh weight per day. We used linear regression analysis (Statview) to estimate growth rates (fresh weight increase) during the 78 day grow-out period for the first set of core samples that were initiated from explants that had one frond and a subtending stolon.

RESULTS AND DISCUSSION

December 13, and 14, 2001 Core Sampling

No growth of *Caulerpa taxifolia* was observed in any of the cores removed from the tarped and treated areas at 76 days after start of the assay. Furthermore, no *C. taxifolia* tissues were observed in the sieved cores on November 25, 2002. However, production and elongation of inoculated *C. taxifolia* in the control cores was excellent (Figures 4-6). Figures 6, 7, and 8 show that the growing conditions led to rapid proliferation and increase in estimated biomass of explants containing fronds. Explants without fronds did not emerge as well as those that had their subtending stolon and rhizoids attached and only one core produced new growth (Figure 6). No intact tissue of *C. taxifolia* was recovered from the other cores at the time of harvest. Seedlings of eelgrass (*Zostera marina* L.) emerged from nearly all of the cores that had been removed from the previously treated sites (Figures 9 and 10). This was not expected since some of the sites had been covered for more than two years and also because of the possibility that residual chlorine had affected seed viability. Sprouted seedlings were first observed 20 days after planting (DAP), and more seedlings sprouted by the end of the grow-out period (Figures 9-11). The lack of emergence of eelgrass from control cores in the August sampling was probably due the absence of eelgrass stands in the areas where the control cores were removed (Merkel and Associates 2003; R. Mooney, pers. communication).

In addition to eelgrass, several invertebrate species were observed or recovered during the sieving of the cores from both treated and some control cores 76 days after start of the assay. These included hydroids, bivalves, and annelid worms (data not shown).

August 8, 2002 Core Sampling

There was no evidence of *C. taxifolia* growth in any of the cores from treated areas. Growth of inoculated *C. taxifolia* in control cores was neither as robust nor as frequent compared to the December 2001 cores. By 24 days after re-inoculating control cores, frond length in two of the cores had increased from 11 cm to 18 cm. Fronds in the other two cores remained green

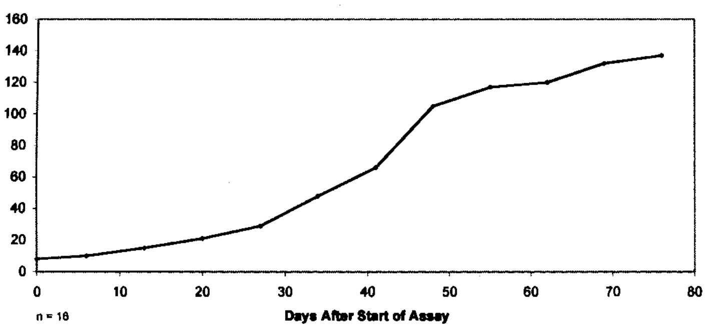


Figure 5. Growth of *C. taxifolia* in inoculated "control" cores removed from Agua Hedionda August, 2001. Data are cumulative numbers of all fronds produced in all cores.

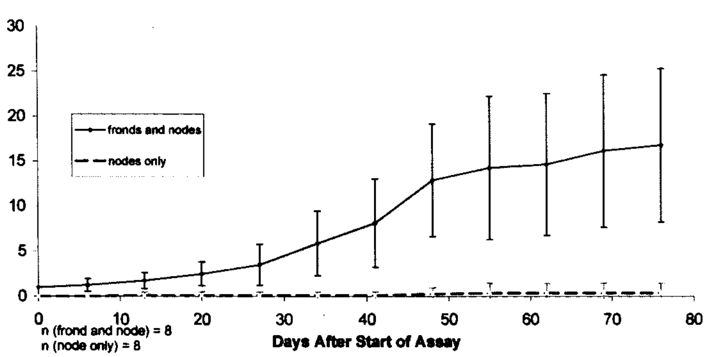


Figure 6. Number (mean +/- SD) of fronds produced in each core originally inoculated with *C. taxifolia* explants containing a frond+ node (solid line) or nodes only (dashed line).

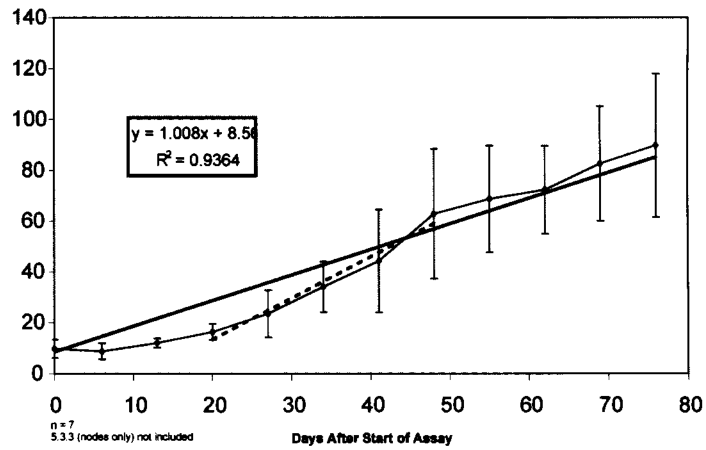


Figure 7. Growth of *C. taxifolia* (frond length), mean+/- SD per inoculated control core.

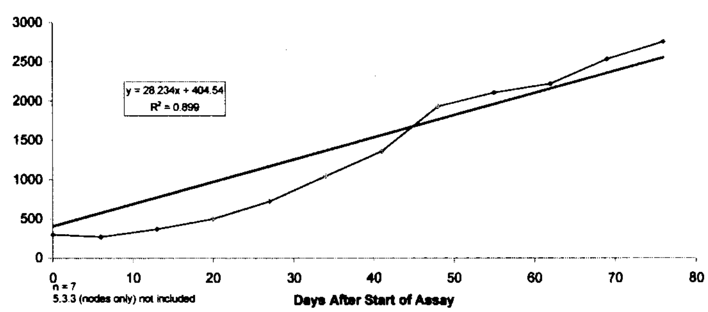


Figure 8. Estimated production (fresh wt. /day) of *C. taxifolia* inoculated in control cores. Estimates were generated from conversion of frond length to fresh weight based on data from 7 fronds.

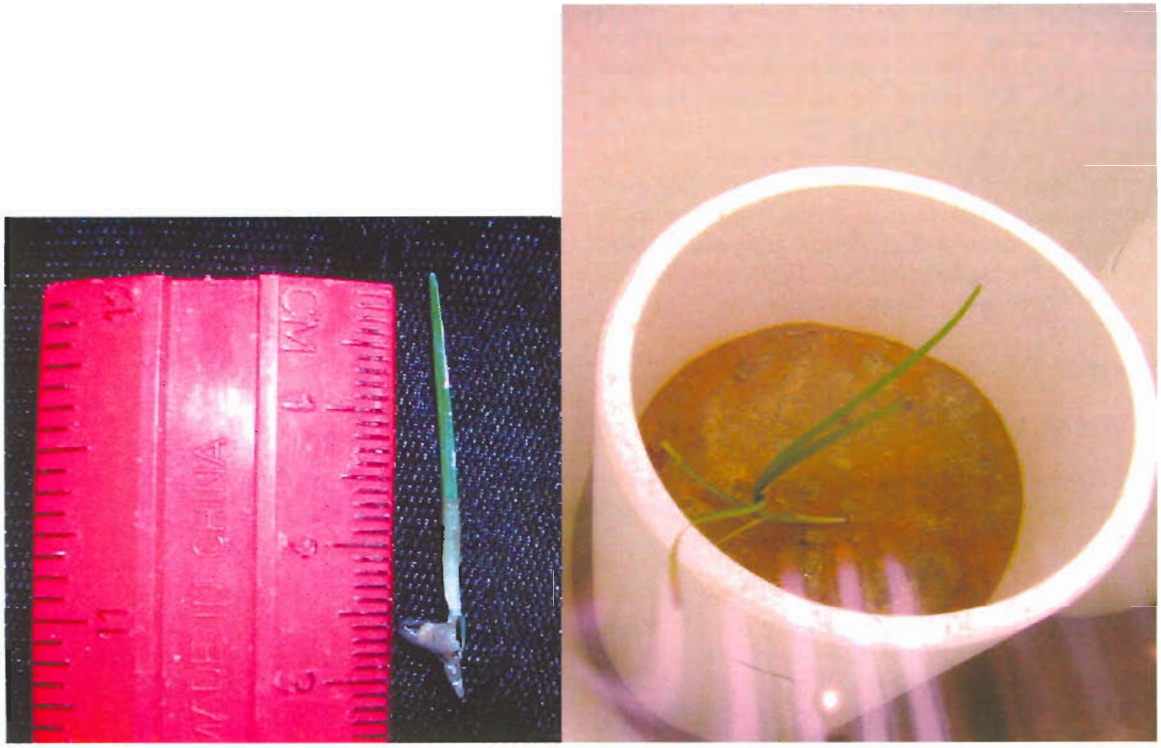


Figure 9. Examples (left) of sprouting eelgrass seedling at harvest 76 days after start of assay (left), and seedlings that had emerged 20 days after the start of grow out (right).

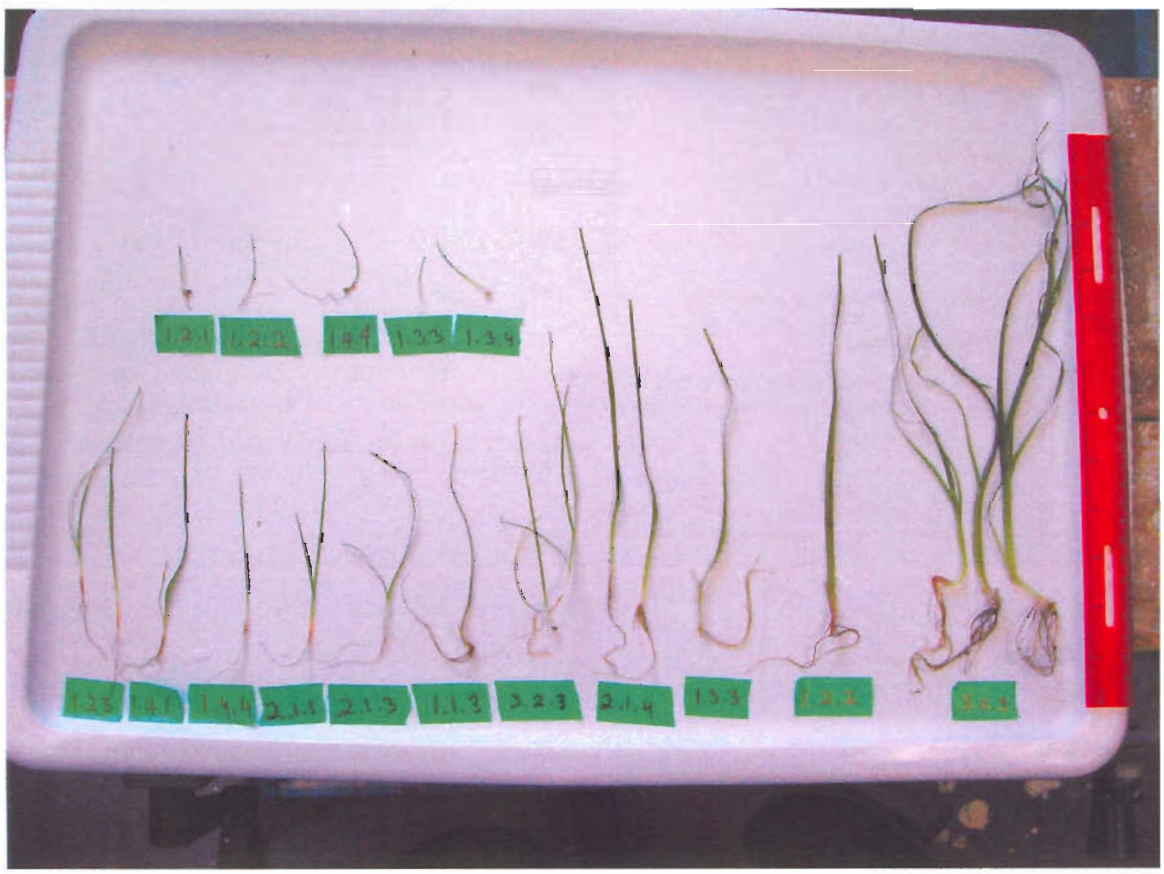


Figure 10. Examples of sprouted eelgrass seedlings that had emerged from cores removed from *C. taxifolia* treatment tarps 76 days after start of assay in grow-out conditions. Each number represents a single core.

and viable, but did not elongate. As in the December core samples, several eelgrass seedlings emerged from the "treatment" cores by the end of the growout period (Figure 12). Various invertebrates also emerged during the 108-day grow-out period as in the December 2001 cores (data not shown). During this grow out assessment, salinity, DO, and pH remained stable throughout the 108-day period (data not shown).

Thus, assessments of cores from both sampling periods consistently showed that treatments were effective. No *C. taxifolia* emerged from any of the cores from any of the treated sites 76 and 108 days after transfer to grow-out conditions from the December 2001 and August 2002 sampling times, respectively. At no time were any signs of viable *C. taxifolia* observed in any of the cores taken from treated sites, including one site that had been tarped but had not been injected with chlorine. *Caulerpa taxifolia* explants grew well in all inoculated "control" cores in the December, 2001 sampling. Only one core produced re-growth in initial inoculations of the controls from the August sampling. However, re-inoculation of four control cores from the August sampling produced growth up to 24 DAP, at which time all cores were disassembled and sieved (data not shown). Filamentous algae appeared in two cores. At the time of harvest, no visible parts of *C. taxifolia* were found in the sieved "treated" cores 76 or 108 days after the start of the assay.

The growth rate (frond elongation) observed in the inoculated "control" cores, from 1- to 2 cm per day (Figure 6), is similar to that reported by Meinesz (1999) and Piazzi et al. (2001). These plants produced approximately one new frond per day during the maximum growth period, between 30 and 60 days after start of the assay (Figure 7). The decline in rate of new frond production may have been due to limited space within the pvc columns or to lack of nutrients. However, these results show that the conditions used here were adequate for the inocula to achieve typical growth rates.

Various invertebrates [annelids, hydroids (coelenterates), gastropods, bivalves] were present in the treated and control cores from both sampling dates. Since these are typical infauna species and may not have been directly exposed to the chlorine applications, their presence is not surprising. Alternatively, it may be that the annelids and mollusks entered from beneath the tarped areas after the treatments were made.

Seedlings of eelgrass emerged from the control cores for the August assessments. This is understandable since populations of eelgrass were in this area and were never covered or treated. However, the emergence of eelgrass seedlings from several covered and "treated" cores in both sampling dates was somewhat unexpected. Several studies on *Z. marina* indicate that most seeds sprout within a few months following their production and that there is little, if any, multi-year seed-bank carryover (Churchill 1983, Harrison 1993). The processes that determine seed longevity and survival in this species are poorly understood, even though restoration efforts, including "seeding," have received a great deal of attention (Moore et al. 1993, Wyllie-Echeverria and Fonseca 2003, Orth et al. 2003). Our results demonstrate that eelgrass, which had been established, but was invaded by *C. taxifolia* colonies, had contributed seed (before being tarped) capable of germinating two years later. Possibly, the containment and treatments reduced potential predation on the seed or altered the physico-chemical environment (e.g., sustaining anoxic conditions), which extended survivorship. We found one to three seedlings per core (78.5 cm^2), which corresponds to about 125 to 400 seedlings/m^2. This is in the range reported for early seedling emergence in the Gulf of California (Meling-Lopez and Ibarra-Obando1999), but lower than the seed bank reported for a population in The Netherlands, e.g., 1,000 to 7,000/m^2, depending upon tidal elevations and month (Harrison, 1993). Although this initial density seems high, Harrison (1993) noted that actual seedling production was much less and that percent of viable seed declined sharply resulting in only ca. 100 to 600 genets/m^2, which is within the range we observed. Also, an earlier study in The Netherlands reported much lower estimates of seed bank density of ca. 200/m^2 for *Z. marina* (Hootsmans 1987). High levels of seed, ca. 1,000/m^2 were also reported by Reusch (2002) and Orth et al. (2000). All these values are far lower than those reported by Meling-Lopez and Ibarra-Obando (1999) for seed abundance within the eelgrass canopy in the Gulf of California (ca. 42,000 to 100,000/m^2). Although the sediment cores sampled in Agua Hedionda represent only a small part of the total eelgrass community, the emergence of seedlings suggest that there is recruitment potential, even in areas treated and tarped for over two years. However, the survivorship of emerging seedlings *in situ* would have to be assessed in a separate study.

These results show that replicate core samples from within tarped and treated areas that are placed in appropriate "grow-out" conditions can provide a useful assessment of treatment efficacy. Taken together, the observations that inoculated *C. taxifolia* grew well in several "control" cores, coupled with the emergence of both invertebrates and seedling eelgrass, indicate that this system provided adequate conditions for growth of macrophytic algae, vascular plants, and some invertebrates. Due to variability in sediment characteristics and diversity of flora and fauna contained within each core, the inclusion of several replicate cores per treatment site would be essential in other eradication projects as well.

The advantage of this approach to assessing efficacy in eradication projects compared to simply removing containment tarps after treatment was that it afforded security against potential dispersal of viable propagules (i.e., fragments) of *C. taxifolia* if these had survived the treatments. It also provided quality assurance and quality control since grow-out conditions could be maintained and replicated. Though there was certainly inherent variability introduced by using natural (non-infested) sediments for the "controls" this was probably outweighed by the greater similarity of these substrates to infested sites. There were also some disadvantages with this approach since the sediments were removed from their normal environment, and, thus they were not subjected to field (*in situ*) variability such as diurnal temperature and light fluctuations, tidal flows, and interactions with adjacent benthic organisms. However, we believe this method provided a reasonable and prudent approach, with the least likely risk of releasing potentially live propagules.

The absence of viable *C. taxifolia* propagules in sediments that were contained and treated over two years prior to core samplings is encouraging and suggests that this treatment approach has been effective in stopping further growth of the alga. Furthermore, subsequent laboratory studies on the re-

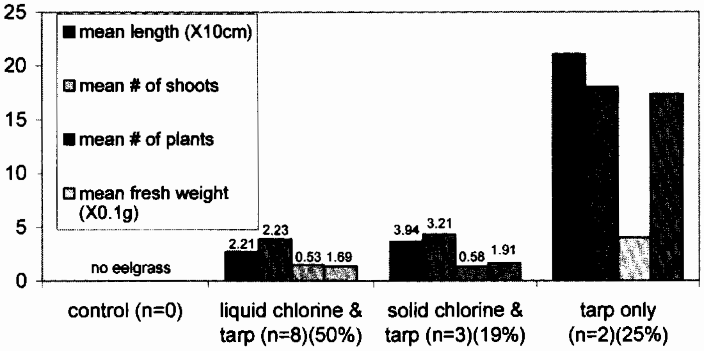


Figure 11. Seedling eelgrass production in control cores and cores removed from areas treated to eradicate *C. taxifolia* in Agua Hedionda Lagoon in December, 2002. Numbers above bars = standard deviations. Number in parentheses = percent of total cores sampled that produced eelgrass seedlings.

ponses of *C. taxifolia* to chlorine from bleach showed that short duration exposures of 0.5 to 125 ppm Cl^- concentration can kill 4-mm explants (Williams and Shroeder 2003, 2004). However, since the explants used in their laboratory studies were not established, anchored plants, longer duration exposures may be required to kill clones *in situ* that have well-developed, attached rhizoids and stolons. Also, as these authors note, inactivation of Cl^- in the presence dissolved or attached organic material may necessitate longer exposures at high concentrations. These limitations suggest that the treatment efficacy observed in Agua Hedionda, may have been facilitated greatly by the use of containment tarps, which prolonged contact time and reduced dilution. In fact, since November, 2002, no new colonies of *C. taxifolia* have been detected in Agua Hedionda Lagoon following extensive twice-yearly surveillance by scuba divers (SCCAT, 2004).

Results of additional field surveillance through 2005 will be used to determine the overall success of this project in eradicating *C. taxifolia*. However, the development of new approaches to early detection and rapid response, including assessments of treatments, remains extremely important given the continuing risks from this species and other exotic macrophytic marine algae such as *Undaria pinnatifida* (Komatsu et al. 2003, National Invasive Species Council 2003, Verlaque et al. 2004, Miller 2004, Casas et al. 2004). Moreover, the widespread availability of *Caulerpa* spp. and a plethora of other non-native aquatic organisms obtainable via the aquarium trade (including internet commerce) continue to jeopardize aquatic ecosystems (Frisch 2003).

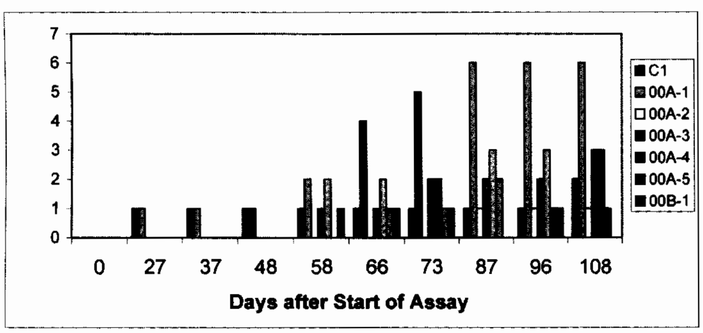


Figure 12. Eelgrass emergence from cores removed from Agua Hedionda in August, 2002 (C = controls cores; A, B from areas tarped and treated with chlorine in June, 2000). Bars represent each core that produced eelgrass seedlings.

ACKNOWLEDGMENTS

We thank Dr. Doreen Gee and Mr. Aaron O'Callaghan for their technical assistance. Funding for this project was provided in part by the California Department of Fish and Game. The Southern California Caulerpa Action Team (SCCAT) Technical Committee assisted in reviewing protocols for sampling and grow-out. We also appreciate the constructive suggestions made by anonymous reviewers of this paper.

LITERATURE CITED

Anderson, L. W. J. 2004. California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. Biol. Invasions (in press).

Anderson, L. W. J. 2002. *Caulerpa taxifolia* in the United States: Rapid response and eradication program. Proc. Internatl. *Caulerpa taxifolia* Conf. San Diego, CA, California Sea Grant Publication No. T-047.

Anderson, L. W. J. 2001. *Caulerpa taxifolia*: New marine algal invader in U.S. Waters. Aquatic Invaders 12:1-6

Anderson, L. W. J. and S. Keppner 2001. *Caulerpa taxifolia*: marine algal invader provokes quick response in U.S. waters. Aquatic Nuisance Spec. Digest 2:13-23.

Casas, G., R. Scrosati and M. L. Piriz. 2004. The invasive kelp *Undaria pinnatifida* (Phaeophyceae, Laminariales) reduces native seaweed diversity in Nuevo Gulf (Patagonia, Argentina). Biol. Invasions 6:411-416.

Churchill, A. C. 1983. Field studies on seed germination and seedling development in *Zostera marina* L. Aquatic Botany 16:21-29.

FICMNEW (Federal Interagency Committee for the Management of Noxious and Exotic Weeds). 2003. A national early detection and rapid response system for invasive plants in the United States: Conceptual Design. 24 pp. (http://ficmnew.fws.gov)

Frisch, S. 2003. Taxonomic diversity, geographic distribution, and commercial availability of aquarium-traded species of *Caulerpa* (Chlorophyta, Caulerpaceae) in southern California, USA. Master's Thesis, California State University, Fullerton.

Harrison. P. G. 1993. Variations in demography of *Zostera marina* and *Z. noltii* on an intertidal gradient. Aquatic Bot. 45: 63-77.

Hootsmanns, M. J. M., J. E. Vermaat and W. Van Vierssen. 1987. Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem. Aquatic Botany 28: 275-285.

Jousson, O., J. Pawlowski, L. Zaninetti, F. W. Zechman, F. Dini, G. Di Guiseppe, R. Woodfield, A. Millar and A. Meinesz. 2001. Invasive alga reaches California. Nature 408:175-158.

Komatsu, T., T. Ishikawa, N. Yamaguchi, Y. Hori, and H. Ohba. 2003. But next time?: unsuccessful establishment of the Mediterranean strain of the green seaweed *Caulerpa taxifolia* in the Sea of Japan. Biol. Invasions 5:275-277.

Meinesz, A. 1999. Killer Algae. Univ. Chicago Press, 360 pp. (Translated by Daniel Simberloff).

Meinesz, A. 2002. Killer Algae, 2nd Edition. Univ. Chicago Press, (Translated by Daniel Simberloff).

Meinesz, A., T. Belsher, T. Thibaut, B. Antolic, K. B. Mustapha, C-F Boudoresque, D. Chiaverini, F. Cinelli, J-M Cottalorda, A. Djellouli, A. El Abed, C. Orestano, A. M. Grou, L. Ivesa, A. Jaklin, H. Langar, E. Eassuti-Pascual, A. Perirano, L. Tenesi, J. de Vaugelas, N. Zavodnic and A. Juljevic. 2001. The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. Biol. Invasions 3:201-210.

Merkel and Associates. 2003. Eradication and surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California. Second Year Report (Fall 2001 through Summer 2002) to Southern California Caulerpa Action Team (SSCCAT), 15 pp.

Meling-Lopez, A. E. and S. E. Ibarra-Obando. 1999. Annual life cycles of two *Zostera marina* L. populations in the Gulf of California: contrasts in seasonality and reproductive effort. Aquatic Botany 65:59-69.

Miller, K. A. 2004. California's non-native seaweeds. Fremotia 32:100-15.

Moore, K. A., R. J. Orth and J. F. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. Aquatic Botany 45:79-91.

Mullin, B. H., L. W. J. Anderson, J. M. Ditomaso, R. E. Eplee and K. D. Getsinger. 2000. Invasive Plant Species. Council for Agricultural Science and Technology (CAST) Issue Paper No. 13, 18 pp.

National Invasive Species Council. 2003. Guidelines for establishment and evaluation of invasive species early detection and rapid response systems I. 16 pp. National Invasive Species Council, Washington, DC.

Orth, R. J., J. R. Fishman, M. C. Harwell and S. R. Marion. 2003. Seed-density effects on germination and initial seedling establishment in eelgrass *Zostera marina* in the Chesapeake Bay region. Mar. Ecol. Prog. Ser. 250:71-79.

Piazzi, L., D.Blata, G. Ceccherelli and F. Cinelli. 2001. Comparative study on the growth of the two co-occurring introduced green algae *Caulerpa taxifolia* and *Caulerpa racemosa* along the Tuscan coast (Italy, western Mediterranean). Cryptogami, Algol. 22:459-466.

Rejmanek, M. and M. J. Pitcairn. 2002. When is eradication of exotic pest plants a realistic goal? pp. 249-253. *In:* C. R. Veitch and M. N. Clout (eds.) Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group, Glaqnd, Switzerland and Cambridge, UK.

Reusch, T. B. H. 2002. Microsatellites reveal high population connectivity in eelgrass (*Zostera marina*) in two contrasting coastal areas. Limnol. Oceanogr. 47:78-85.

Rockwell, H. W. 2003. Summary of a survey of literature on the economic impact of aquatic weeds. Report to the Aquatic Ecosystem Research Foundation. 18 pp.

SCCAT 2004. Southern California Caulerpa Action Team. Status of Eradication for Agua Hedionda. Report to SCCAT on Status of Surveillance.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Hoen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson and S. G. Weller. 2001. The population biology of invasive species. Ann. Rev. Ecol. and Syst. 32:305-332.

Verlaque, M., J. Alfonso-Carrillo, M. C. Gil-Rodgriquez, C. Durand, C. F. Boudouresque and Y. LeParko. 2004. Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindraceae* (Bryopsidales, Chlorophyta) reaches the Canary Islands (NE Atlantic). Biol. Invasions 6:269-281.

Western Regional Panel on Invasvive Aquatic Weeds. 2003. Draft Rapid Response Plan. Website: http://answest.fws.gov

Williams, S. L. and S. Schroeder. 2003. Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. Final Report to California Dept. of Fish and Game. 25 pp.

Williams, L. S. and S. L. Schroeder. 2004. Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. Marine Ecol. Prog. Series 272:69-76.

Williams, S. and E. D. Groscholz. 2002. Preliminary reports from the *Caulerpa taxifolia* invasion in southern California. Marine Ecol. Progr. Ser. 233:307-310.

Wylie-Escheverria, S. and M. Fonseca. 2003. Eelgrass (*Zostera marina* L.) research in San Francisco Bay, California from 1920 to the present. Literature Review: Natl. Centers for Coastal Ocean Science (NCCOS) 20 pp.

APPENDIX D

Caulerpa taxifolia Survey Efficacy Assessment at Agua Hedionda Lagoon and Huntington Harbour

M&A #00-088-01

SOUTHERN CALIFORNIA *CAULERPA TAXIFOLIA* ERADICATION PROGRAM

Caulerpa taxifolia Survey Efficacy Assessment

at Agua Hedionda Lagoon and Huntington Harbour

Prepared for:

Steering Committee of the Southern California *Caulerpa* Action Team

- California Regional Water Quality Control Board – San Diego Region
- California Regional Water Quality Control Board – Santa Ana Region
- California Department of Fish and Game
- NOAA National Marine Fisheries Service
- U.S. Department of Agriculture – Agricultural Research Service

Prepared by:

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

SUMMARY

As part of the Southern California *Caulerpa taxifolia* Eradication Program, an assessment program was developed to determine the efficacy of SCUBA diver surveys for the invasive seaweed *Caulerpa taxifolia* at the two infestation sites in Agua Hedionda Lagoon and Huntington Harbour, California. The survey efficacy assessment program was developed to address the questions: (1) what are the relationships between important environmental variables and survey efficacy, and (2) how do the estimates of survey efficacy during each survey event translate to eradication certainty? To answer these questions, synthetic *Caulerpa* was placed within the two infestation sites during the course of regular survey efforts. The small synthetic *Caulerpa* patches were similar to patches of real *C. taxifolia* that were found during the second year of the eradication effort and were useful for assessing the survey team's ability to detect small patches remaining at the infestation sites.

After three years of trials, the answer to the first question is variable. In moderate or favorable underwater visibility conditions, the size of the patch and the presence of other vegetation significantly affects the divers' ability to find patches of *C. taxifolia.* When visibility is poor, these factors do not have a significant affect; divers are likely to detect a small patch of *C. taxifolia*, regardless of its size, only if it falls directly in their line of sight.

Based on the results of the survey efficacy assessment, there is a 97.71% certainty that eradication has been achieved at Agua Hedionda Lagoon, assuming the worst conditions. There is a 99.99% eradication certainty at the Huntington Harbour infestation site.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SOUTHERN CALIFORNIA *CAULERPA TAXIFOLIA* ERADICATION PROGRAM

Caulerpa taxifolia Survey Efficacy Assessment

at Agua Hedionda Lagoon and Huntington Harbour

INTRODUCTION

Caulerpa taxifolia is a coenocytic macroalgae common to soft- and hard-bottom habitats in tropical and subtropical seas. Introduction into the Mediterranean Sea in the 1980s proved the presence of a strain capable of surviving winter temperatures ranging from 11-13^{o} C (Meinesz and Hesse 1991). This "aquarium strain" of *C. taxifolia* has proven capable of rapid range expansion due to asexual reproduction (Ceccherelli and Piazzi 2001; Ceccherelli and Cinelli 1999; Smith and Walters 1999; De Vaugelas et al. 1997) and possesses chemical defenses against native temperate herbivores (Mozzachiodi et al. 2001; Bourdouresque et al. 1996; Pesando et al. 1996). These traits have made the aquarium strain of *C. taxifolia* a highly-invasive exotic that now threatens ecosystems in eight countries on four continents (ICC 2002).

The aquarium strain of *C. taxifolia* was discovered as an escaped exotic in California in June 2000 when it was identified at Agua Hedionda Lagoon in Carlsbad. The first confirmed occurrence of this invasive species in California caused considerable alarm. The resulting press coverage of the issue led to the confirmation of a second infestation of *C. taxifolia* later in 2000 in Huntington Harbour, Huntington Beach (75 miles north of the Carlsbad occurrence). Genetic studies determined that both California infestations were a clone of the strain introduced to the Mediterranean Sea (Jousson et al. 2000).

An eradication program was initiated in June 2000 and is ongoing (Merkel & Associates 2006). Eradication efforts at the California sites involved repeated systematic surveys of the seafloor by a team of SCUBA divers. Any *C. taxifolia* found during a survey event was mapped and treated within forty-eight hours. As the eradication effort proceeded, the biomass and average patch size of discovered *C. taxifolia* patches was reduced. After December 2001, no single patch of discovered *C. taxifolia* was larger than 1 m^2. The last discovery of any *C. taxifolia* at Agua Hedionda and Huntington Harbour was in September 2002 and November 2002, respectively. Repeated surveys to search for *C. taxifolia,* with none found, continued until December 2005.

The purpose of this study, conducted during the regular surveys for *C. taxifolia*, was to assess the survey team's ability to locate synthetic *Caulerpa* patches at scales that were biologically meaningful during the latter portions of the eradication components of the program. The team was being tested for their ability to find relatively small patches and to determine the smallest patch size that could be detected regardless of survey conditions. Small synthetic *Caulerpa* patches were similar to patches of *C. taxifolia* that were found during the second year of treatment activities, and represented what would be expected of any recolonizing fragments. The assessment program evolved as the eradication program progressed, with various refinements and expansion of the scope of the assessment is reflected in the methods described below.

By testing the team's ability to detect synthetic patches of *Caulerpa*, our confidence in the results of each survey can be quantitatively estimated. The survey efficacy assessment addresses the questions: (1) what are the relationships between important environmental variables and survey efficacy, and (2) how do the estimates of survey efficacy during each survey event translate to eradication certainty? The data are discussed in terms of survey efficacy under different environmental conditions and the relevance of these results to the ultimate success of the eradication effort.

METHODS AND MATERIALS

STUDY SITES

Agua Hedionda Lagoon

Agua Hedionda Lagoon is a tidal lagoon consisting of three basins connected by narrow channels (Figure 1). The west basin opens to the Pacific via two channels that pass under Pacific Coast Highway. This basin is 20.4 hectares and connects to the 8.1-hectare central basin under a railroad overpass. The east basin receives tidal flow through a channel running under the interstate highway (I-5 Freeway). The east basin is the largest basin (72.1 hectares) and is the only basin in which *C. taxifolia* was found during this eradication effort. All three basins typically support healthy populations of eelgrass (*Zostera marina*).

Huntington Harbour

The Huntington Harbour site is composed primarily of two ponds located on the north side of Huntington Harbour, in the City of Huntington Beach (Figure 2). The ponds are man-made and non-tidal. Water level is maintained by pumps that fill the east pond (1.5 hectares), which drains into the west pond (1.5 hectares), and then into the harbor. The ponds are enclosed on all sides by concrete bulkheads and surrounded by residential condominiums. *Caulerpa taxifolia* has been found in both ponds, as well as in a portion (0.6 hectare) of the adjacent harbor.

SURVEYS

As part of the eradication effort, two procedures were used to survey for *C. taxifolia*. At Agua Hedionda Lagoon, a transect methodology was employed. The survey team used a boat and differential global positioning system (±50cm accuracy) to place parallel transect lines on the lagoon bottom throughout all basins of the lagoon. A lead SCUBA diver followed the transect line and set the pace of the survey based on water clarity. Three to six additional divers were positioned next to the lead diver, perpendicular to the transect line. Diver spacing was maintained by holding a spacing line with small marker loops positioned at 1-m intervals. Each diver was assigned a loop and held the line at this point. The divers followed the pace set by the lead diver and kept the spacing line taught and perpendicular to the transect line. The transect lines were laid such that each diver was responsible for surveying a 1-m swath of bottom. While surveying, each diver scanned the bottom area within 0.5 m to either side of their line of travel. If a diver encountered *C. taxifolia*, the rest of the crew was signaled to stop, and the position marked with a float.

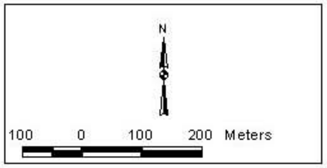
At the Huntington Harbour site, the same transect methodology was used, with a slight variation used in the early surveys of the eradication program. The method used the same spacing rope with divers aligned at 1-m spacing. In contrast to Agua Hedionda Lagoon, divers set the search pattern based on the pond boundaries. With this method, divers lined up with a lead diver who used the bulkhead walls as a guide. The outer diver in the line dragged a blunt object through the mud at the outside of the search area. The created 'drag-line' was then used to establish the transect line of the next survey route. This method was followed until the entire lagoon bottom had been surveyed. For the purposes of efficacy reporting, the two methods employed at Huntington Harbour are not distinguished, as they are believed to be comparable means of obtaining 100% survey coverage.

N
100 0 100 200 Meters

Agua Hedionda Lagoon Survey Efficacy Assessment
Study Locations

Figure 1

Merkel & Associates, Inc.



Huntington Harbour Survey Efficacy Assessment
Study Locations

Figure 2

SURVEY EFFICACY ASSESSMENT

The survey team's ability to detect small patches of *C. taxifolia* during regular surveys of the study sites was tested with the aid of synthetic *Caulerpa*. Synthetic *C. mexicana* (SeaGarden™ by Aquarium Systems model SG29) was used to imitate the presence of *C. taxifolia* in the field. The synthetic *Caulerpa* is manufactured as two 32-cm stolons with thirteen 13.5-cm fronds spaced 1.5-1.9 cm apart and was readily cut and arranged to form six patch size treatments for the test. The treatments were: 1-frond, on approximately 6 cm of stolon; 5-frond, on approximately 8 cm of stolon; and 10-frond, on approximately 25 cm of stolon (referred to in this document as the small patch-size treatments) (Figure 3). The small patch-size treatments were weighted with 30-g fishing weights attached with monofilament. Each unit was tagged with a uniquely numbered Floy t-bar monofilament fish tag (Floy Tag™, model FD-94).

Later in the study, larger patch sizes were added to the study design at Agua Hedionda Lagoon to determine the smallest patch size that could be detected 100% of the time, regardless of the location or environmental conditions. The additional treatments were: 0.3-m diameter patch, 0.5-m diameter patch, and 1-m diameter patch (referred to in this document as the large patch-size treatments) (Figure 3). The 0.3-m, 0.5-m and 1-m patch size units were made from circular re-bar rings with hardware cloth attached as a substrate for affixing the synthetic *Caulerpa*. Each unit was also tagged with a uniquely numbered monofilament fish tag.

OTHER STUDY VARIABLES

Agua Hedionda Lagoon

At Agua Hedionda Lagoon, the survey team's ability to detect the synthetic *C. taxifolia* was tested at two locations, within two habitat types (eelgrass and bare bottom), and with the six patch-size treatments. The two locations were Snug Harbor, and east Agua Hedionda Lagoon (Figure 1). The two locations are distinct in terms of their visibility regimes. Conditions at Snug Harbor typically allow approximately 0.5-1.0-m underwater visibility. This visibility regime is termed moderate because the divers can easily see within their 1-m swaths, but can barely see each other. The visibility regime at east Agua Hedionda Lagoon is typically poor with estimated visibilities between 0.2 and 0.5 m. This location's visibility regime is termed poor because divers can, at best, see only their 1-m swath and often see less than their swath. During the course of eradication program surveys for *C. taxifolia*, eight trials of the assessment were performed at the Snug Harbor location between summer 2002 and fall 2005. Six trials were performed at the east Agua Hedionda Lagoon location between fall 2002 and fall 2005. The large patch size trials were not initiated at Agua Hedionda Lagoon until summer 2003, with six trials including the large patch-size treatments at the Snug Harbor location and five trials including the large patch-size treatments at the east Agua Hedionda Lagoon location.

During each trial, the three small patch-size treatments were placed randomly within the respective survey locations. Ten of each patch-size treatment were placed in both habitat types (eelgrass and bare bottom) and at both survey locations (Snug Harbor and east Agua Hedionda Lagoon). Thus, sixty patches were placed at each location during each survey efficacy assessment trial. Extraordinary rain events during the winter of 2004-2005 and subsequent red tides in summer 2005 are believed to have significantly reduced the amount eelgrass habitat in Agua Hedionda Lagoon. Thus, the summer and fall 2005 efficacy trials were performed with all patches of synthetic *Caulerpa* being placed on bare bottom.

Photograph of synthetic *Caulerpa* patches used in the survey efficacy assessment.
From left 1.0-m diameter, 0.5-m diameter, 0.3-m diameter,
10-frond, 5-frond, and 1-frond patches.

Figure 3

The three large patch-size treatments were placed similarly to the small patch-size treatments. However, the large patch-size treatments were never placed relative to the habitat variable. During each trial, ten of each of the large patch sizes were placed randomly at each location, with no discrimination between eelgrass or bare bottom. The experimental variables and associated levels within each variable are summarized in Figure 4.

The survey efficacy assessment trials were performed by the survey team during regular *C. taxifolia* surveys as described above. If a diver detected any of the synthetic *Caulerpa* patches while surveying, the diver would return them to surface-based survey personnel (or mark the larger patches for later retrieval). The tag numbers from retrieved patches were recorded so that the resulting data could be coded with the original habitat type where the patches were placed. These patches were considered "detected". Patches that were not detected by the survey team were considered "un-detected".

Huntington Harbour

At Huntington Harbour, survey efficacy was assessed at two locations, with the three small patch-size treatments (1-frond, 5-frond, and 10-frond). The two locations were the east and west ponds (Figure 2). The two locations were similar in their visibility regime and were both classified as having good visibility. Estimated visibility generally ranged from 1 to 2 m during the assessment. With this visibility regime, the survey divers could clearly see within their 1-m swath and could easily see each other. Habitat type was not distinguished at Huntington Harbour because the ponds are generally similar throughout, with a mixture of foliose red algae and filamentous green algae distributed over mud bottom. Huntington Harbour survey efficacy-assessment trials were performed between summer 2003 and fall 2005 during regular quarterly *Caulerpa* surveys. Nine trials were performed in the west pond and eight in the east pond.

During each trial, ten of each patch size treatment were placed in each of the two ponds for a total of thirty patches per pond. The large patch sizes were not tested at Huntington Harbour. The experimental variables and associated levels within each variable are summarized in Figure 5.

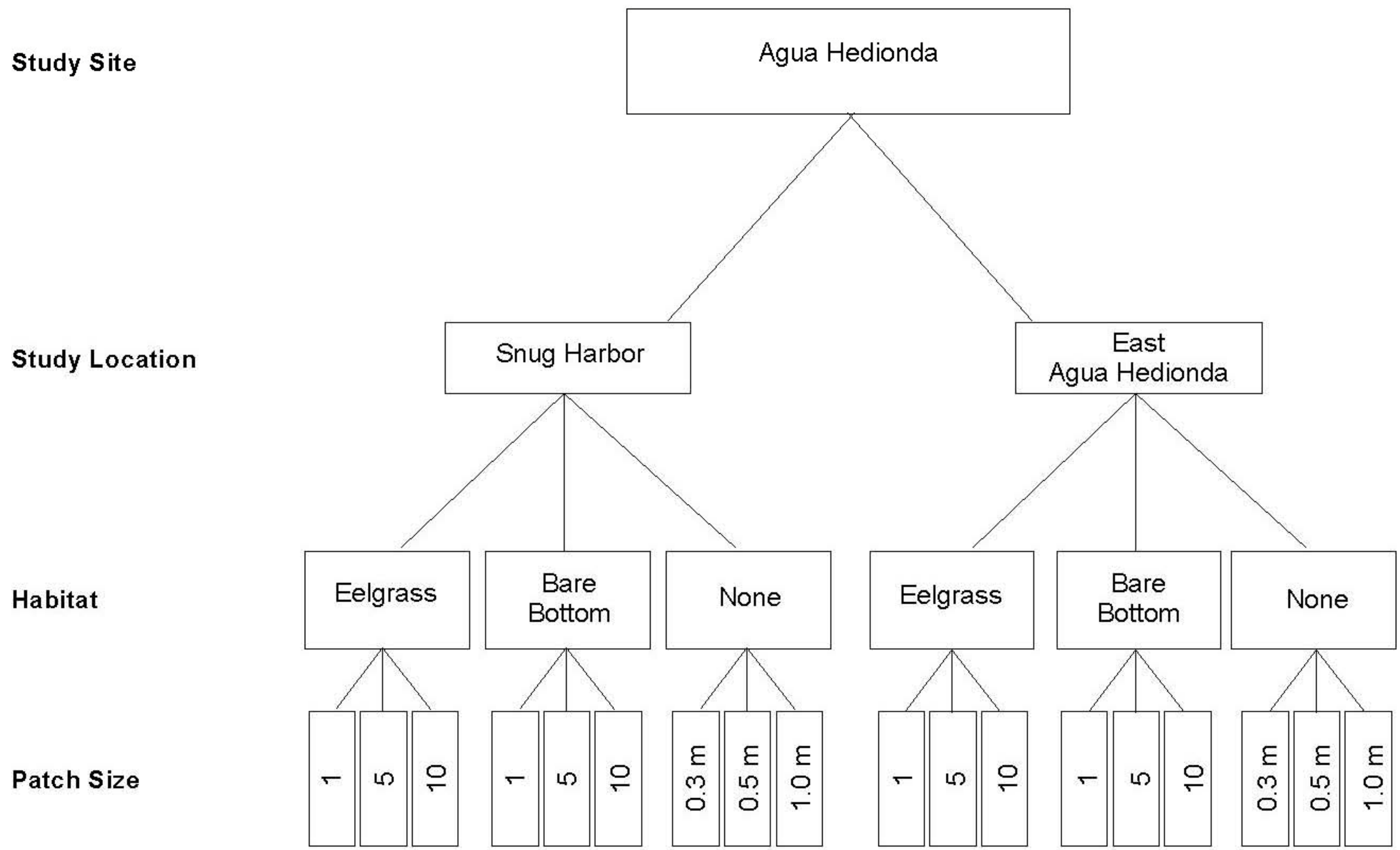
The survey efficacy-assessment trials were performed by the survey team during the quarterly *C. taxifolia* surveys as described above. If a diver detected any of the synthetic *Caulerpa* patches while surveying, the diver would return them to surface-based survey personnel. The tag numbers from retrieved patches were recorded so that the resulting data could be coded with any variables associated with the placement of the patch. These patches were considered "detected". Patches that were not detected by the survey team were considered "undetected".

DATA ANALYSIS

Environmental Variables

The proportionate return of synthetic *Caulerpa* patches (percent detected and undetected) was used to evaluate survey efficacy as it related to environmental conditions. The return proportions were organized by the variables: trial site, trial locations within sites, habitat type, and patch size. Multiple Chi-square contingency tables used to determine if dependencies existed between the proportion of *detected* synthetic *Caulerpa* patches and the above variables.

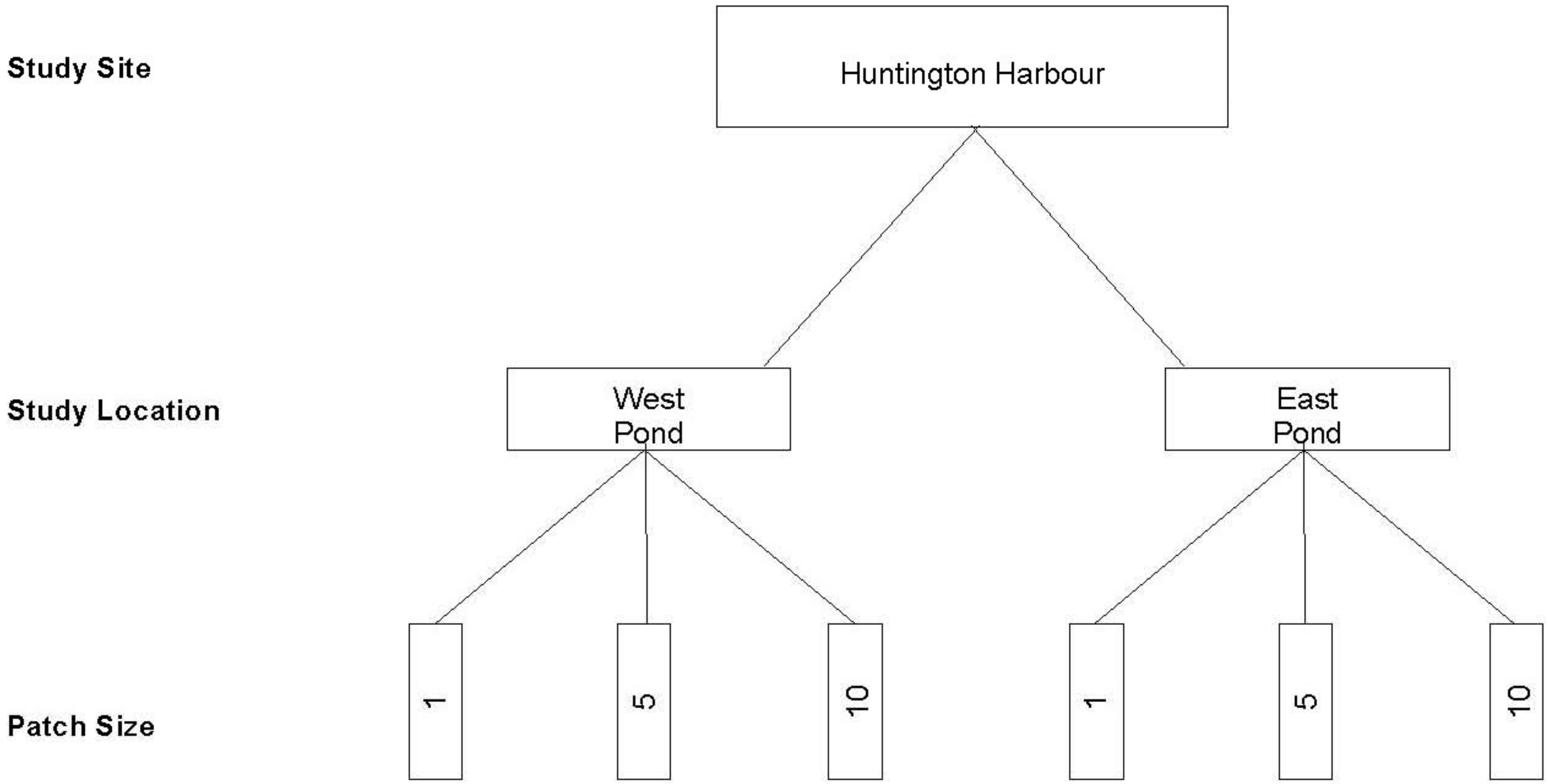
00-088-01



Summary of variables and associated levels for the Agua Hedionda Study site. Variable names are on the left with the associated levels in boxes.

Figure 4

00-088-01



Summary of variables and associated levels for the Huntington Harbour Study site. Variable names are on the left with the associated levels in boxes.

Figure 5

Higher order contingency analyses were first used to test for dependencies among multiple variables. For example, at Agua Hedionda Lagoon a 2x2x2x3 contingency table was used to determine if dependencies existed between the proportion detected (2 levels: detected and undetected), trial location (2 levels: Snug Harbor, and east Agua Hedionda Lagoon), habitat (2 levels: eelgrass and bare bottom), and patch size (3 levels: 1-frond, 5-frond, and 10-frond). If dependencies were noted among variables, lower-order contingency table analyses were performed beginning with the highest variable. If the proportion of synthetic *Caulerpa* detected were dependent on the levels of a variable, the subsequent analyses were performed within each level of the significant variable. For example, at Agua Hedionda Lagoon a 2x2 contingency table was used to test for dependency between the proportion of synthetic *Caulerpa* detected and trial location. Because trial location was significant, subsequent analyses were performed only within each trial location level.

An a priori alpha probability of 0.05 was chosen for all statistical tests. Yate's continuity correction factor was applied where appropriate for 2x2 contingency table analyses (refer to Zar 1999). For ease of interpretation, data are presented as the percentage of synthetic *Caulerpa* detected within a given set of survey conditions (variables)

Eradication Certainty

The above analysis determined the role that various environmental variables played in influencing the effectiveness of each survey. The next phase of the analysis began after the last discovery of real *C. taxifolia* at Agua Hedionda Lagoon was in September 2002. For each survey efficacy assessment trial conducted after that date, the analysis of patch size, visibility, and habitat variables was then used to determine a total percentage of synthetic *Caulerpa* detected for each survey. To test the assumption that no additional *C. taxifolia* remained in the lagoon, this figure was assumed to represent the percent of real *C. taxifolia* that would have been found during each survey. This value was calculated in two ways.

The first calculation assumed that all detection data collected during a given survey efficacy assessment trial (in moderate and poor visibility, on bare bottom and in eelgrass, and all small patch sizes pooled together) reflected the actual conditions experienced by the survey team as they moved through the lagoon during the course of the entire survey that season. Therefore the final percentage was the total number of synthetic *Caulerpa* patches detected divided by the total number placed in the lagoon during that quarter. This figure accounted for both the favorable and unfavorable conditions that existed in various areas of the lagoon during the survey.

The alternate calculation sought to find the "worst-case" value and assumed that the most unfavorable conditions existed throughout the survey. This calculation determined the set of variables with the least *detected* synthetic *Caulerpa* and selected the lowest value from that set, based on the results of the dependency assessment described above. When determining the lowest level of detection at Snug Harbor the data were examined in relation to the habitat and patch size variables, while at east Agua Hedionda Lagoon the data were pooled among the variables before comparison. The data were compared in this manner because the habitat and patch size variables were significant determinants of the proportion of synthetic *Caulerpa* detected by divers at Snug Harbor but were not significant at east Agua Hedionda Lagoon. In all cases, the lowest percent detection resulted from either the east Agua Hedionda Lagoon trial or from the 1-frond patches placed in eelgrass habitat at Snug Harbor (refer to results section). Both the average-case and worst-case figures are reported and discussed in the context of eradication certainty.

RESULTS

AGUA HEDIONDA LAGOON

Environmental Variables

The survey efficacy assessment trials resulted in a combined return of 732 of 1,107 (66%) synthetic *Caulerpa* patches over all trials (Table 1 through Table 3). Analysis of all variables by a 2x2x2x3 multidimensional contingency table indicates significant dependency among the proportion of detected synthetic *Caulerpa*, study location, habitat, and patch size (χ^2=77.46 $_{(\upsilon=18)}$; P<0.001). Results of the detection assessments where large patches sizes were used (0.3-m, 0.5-m, and 1.0-m) were excluded from this analysis because they were not grouped by the habitat variable. Analyses of the large patch size treatment levels are included in the appropriate analyses below.

Comparison of all variables within Agua Hedionda Lagoon shows that the proportion of detected synthetic *Caulerpa*, habitat, and patch size (both small and large) were mutually dependent for the Snug Harbor location (χ^2=31.14 $_{(\upsilon=7)}$; P<0.001). There were no dependencies among the study variables within the east Agua Hedionda study location. (χ^2=12.91 $_{(\upsilon=7)}$; P<0.07). The study locations themselves were significantly different with regard to the proportion of synthetic *Caulerpa* detected (χ^2=22.39 $_{(\upsilon=1)}$; P<0.001) with more synthetic *Caulerpa* detected at Snug Harbor (72%) than at east Agua Hedionda Lagoon (58%).

Analysis of the habitat and patch size variables within Snug Harbor revealed significant dependencies between each of the variables and the proportion of detected synthetic *Caulerpa*. More synthetic *Caulerpa* was detected in bare bottom habitats (72%) compared to eelgrass vegetated bottom (57%) (χ^2=11.02 $_{(\upsilon=1)}$; P<0.001). The ability to detect *Caulerpa* increased with increasing patch size for all treatment levels (χ^2=51.68 $_{(\upsilon=5)}$; P<0.001). The proportion of detected synthetic *Caulerpa* within the patch size variable was 55%, 67%, 78%, 82%, 86%, and 98% for the 1-frond, 5-frond, 10-frond, 03-m, 0.5-m, and 1-m treatments, respectively. Additionally, there were significant dependencies among the patch sizes within the small patch size classes (χ^2=18.19 $_{(\upsilon=2)}$; P<0.001), the large patch size classes (χ^2=7.27 $_{(\upsilon=2)}$; P<0.03), and between the small and large patch size classes (χ^2=27.87 $_{(\upsilon=1)}$; P<0.001).

Analysis of patch size for the east Agua Hedionda Lagoon data revealed significant dependencies between the proportion of detected synthetic *Caulerpa* and patch size within the large patch sizes (χ^2=15.77 $_{(\upsilon=2)}$; P<0.001) and between the small and large patch sizes (χ^2=55.68 $_{(\upsilon=1)}$; P<0.001). The proportion of synthetic *Caulerpa* detected at the east Agua Hedionda locations for the patch size variable was 42%, 43%, 57%, 70%, 87%, and 100% for the 1-frond, 5-frond, 10-frond, 0.3-m, 0.5-m, and 1-m treatments, respectively.

The addition of the large patch sizes to the survey efficacy assessments was intended to assist in determining the smallest patch size that could be found by the survey team 100% of the time, in all environmental regimes. All 1-m patches were found during all trials until the last trial, when one 1-m patch was missed. Out of ninety 1-m patches deployed, eighty-nine were detected (99%).

The data in Table 1 to 3 represent the combined results of all survey efficacy assessment trials at Agua Hedionda Lagoon.

Table 1. Numbers of small synthetic *Caulerpa* patches detected by survey divers during the Snug Harbor trials.

	Bare Bottom				Eelgrass Vegetated Bottom				
Patch Size	1-fr	5-fr	10-fr	Subtotals	1-fr	5-fr	10-fr	Subtotals	Totals
Detected	61	75	80	216	27	32	44	103	319
Undetected	39	25	20	84	33	28	16	77	161
Totals	100	100	100	300	60	60	60	180	480

Table 2. Numbers of small synthetic *Caulerpa* patches detected by survey divers during the east Agua Hedionda Lagoon trials.

	Bare Bottom				Eelgrass Vegetated Bottom				
Patch Size	1-fr	5-fr	10-fr	Subtotals	1-fr	5-fr	10-fr	Subtotals	Totals
Detected	38	36	49	123	8	11	14	33	156
Undetected	42	44	31	117	22	19	16	57	174
Totals	80	80	80	240	30	30	30	90	330

Table 3. Numbers of large synthetic *Caulerpa* patches detected by survey divers during the Snug Harbor and east Agua Hedionda trials.

	Snug Harbor				East Agua Hedionda				
Patch Size	0.3 m	0.5 m	1 m	Subtotals	0.3 m	0.5 m	1 m	Subtotals	Totals
Detected	49	43	49	141	35	41	40	116	257
Undetected	11	7	1	19	15	6	0	21	40
Totals	60	50	50	160	50	47	40	137	297

Eradication Certainty

Figure 6 presents the results of the individual survey efficacy assessment trials that were conducted after the last discovery of *C. taxifolia* at Agua Hedionda Lagoon (all surveys after September 2002) and used to calculate eradication certainty. Both the pooled (average-case) and worst-case results are presented each quarter. For example, during the survey efficacy assessment trial of the summer 2003 survey, 62% of all small synthetic *Caulerpa* were detected. Within that trial, the lowest level of detection, 30%, was in Snug Harbor, among the 1-frond patches, placed in eelgrass. The results presented in Figure 6 are further discussed below when assessing the degree of certainty that eradication has been achieved at Agua Hedionda Lagoon.

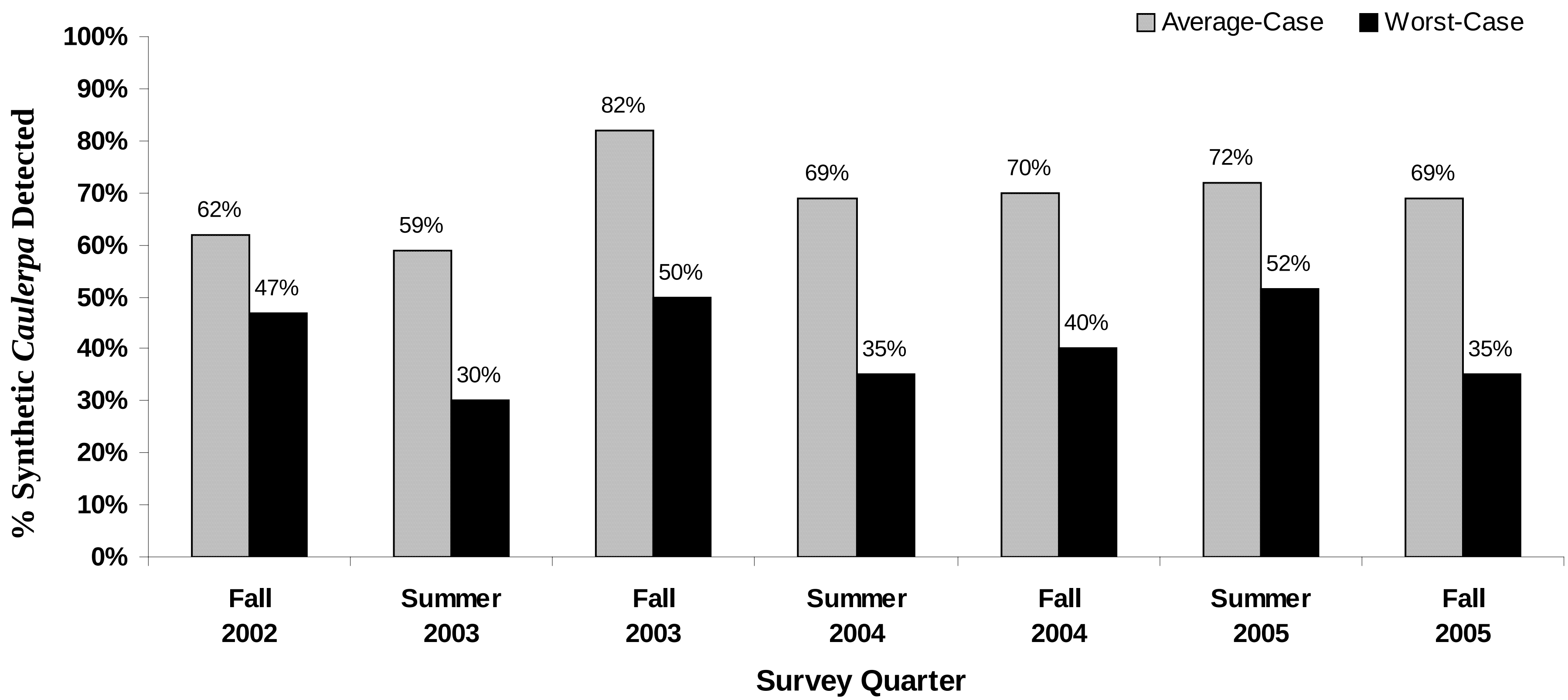
HUNTINGTON HARBOUR

Environmental Variables

The survey efficacy assessment trials at Huntington Harbour resulted in a combined return of 400 out of 500 (80%) patches of synthetic *Caulerpa* (Table 4). Analysis of all study variables at Huntington Harbour with a 2x2x3 multidimensional contingency table showed that the proportion of detected synthetic *Caulerpa*, study location, and patch size were mutually independent (χ^2=5.05 $_{(\upsilon=7)}$; P=0.65).

Table 4. Numbers of synthetic *Caulerpa* patches detected by survey divers at Huntington Harbour.

	East Pond				West Pond				
Patch Size	1-fr	5-fr	10-fr	Subtotals	1-fr	5-fr	10-fr	Subtotals	Totals
Detected	52	64	68	184	68	76	72	216	400
Undetected	16	18	12	46	22	14	18	54	100
Totals	68	82	80	230	90	90	90	270	500



Percent synthetic *Caulerpa* detected during each survey conducted after the last detection of *C. taxifolia* at Agua Hedionda Lagoon (Summer 2002). Calculated as both average-case and worst-case.

Figure 6

Eradication Certainty

Figure 7 presents the results of the individual survey efficacy assessment trials that were conducted after the last discovery of *C. taxifolia* at Huntington Harbour (after November 2002) and used to calculate eradication certainty. The first trial was conducted in summer 2003, with regular quarterly trials not initiated until winter 2003. The analysis above found the amount of synthetic *Caulerpa* detected to be independent of environmental variables; therefore there was no statistical basis for determining a worst-case result. Data from both ponds and all patch sizes were pooled to arrive at a single percentage of synthetic *Caulerpa* detected. These results will be discussed below when assessing the degree of certainty that eradication has been achieved at Huntington Harbour.

DISCUSSION

The survey efficacy assessment trials were performed using the same SCUBA diver survey protocol that was employed to survey for *Caulerpa taxifolia* at Agua Hedionda Lagoon and Huntington Harbour and therefore offer an assessment of the effectiveness of the surveys conducted over the course of the eradication effort. Additionally, having estimates of survey efficacy for each survey event means a final estimate of eradication certainty can be made for each of the infestation sites. Addressing the two questions posed in the introduction, the experimental results are discussed below for each of the infestation sites with emphasis on factors that influenced the survey team's ability to detect patches of synthetic *Caulerpa.* Secondly, a calculation of eradication certainty is made for Agua Hedionda Lagoon and Huntington Harbour.

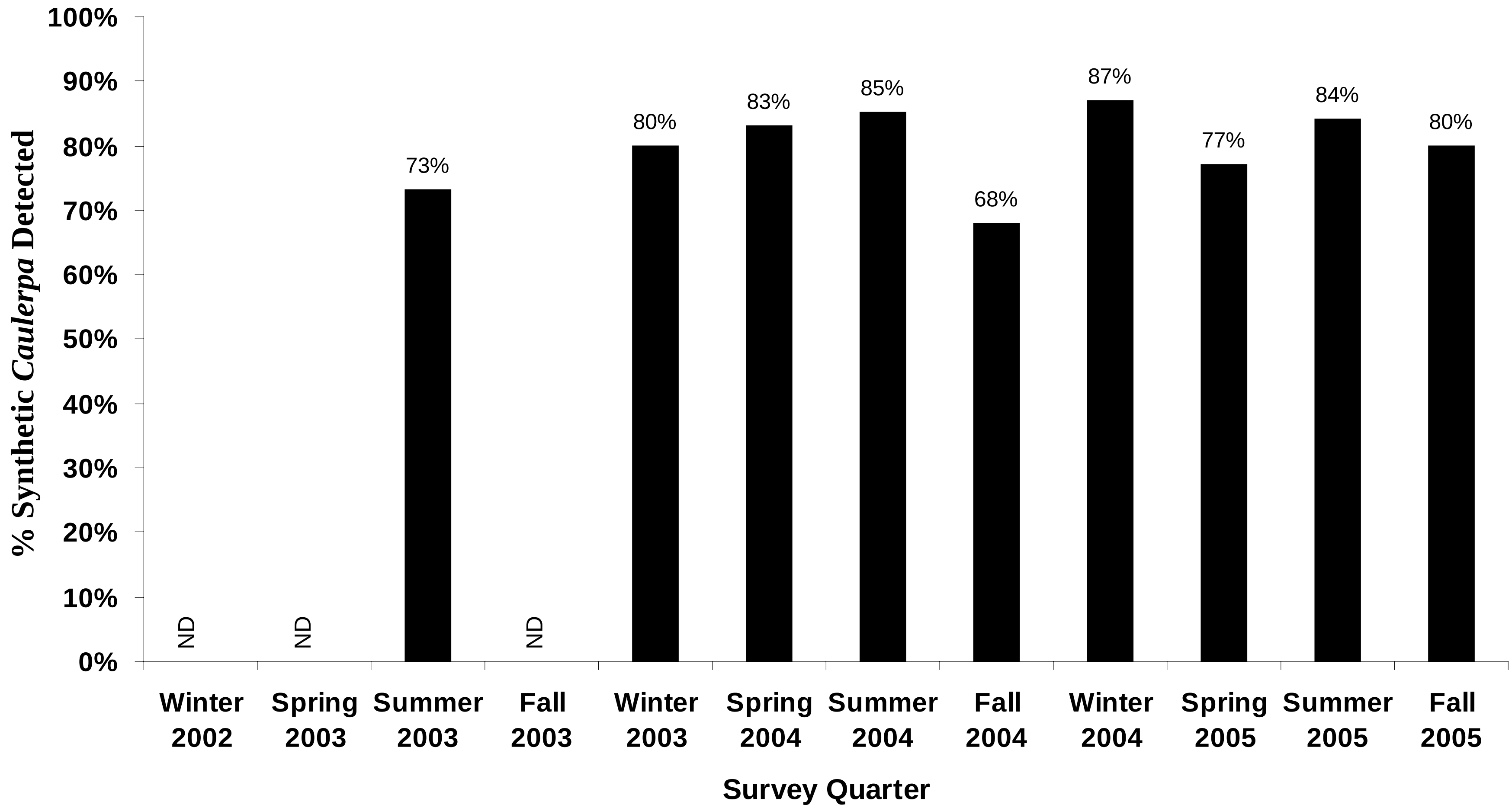
AGUA HEDIONDA LAGOON

Environmental Variables

The results suggest that the survey team's ability to find the synthetic *Caulerpa* was heavily influenced by the trial location. A greater proportion of synthetic *Caulerpa* was detected from the Snug Harbor location than from the east Agua Hedionda location (72% and 58%, respectively). Water clarity, and therefore visibility conditions for diving, seems to be the primary factor accounting for differences in survey efficacy between locations. Although other site factors may play a role, only water clarity was taken into consideration for the selection of trial locations (see above). The similarity of approaches across locations and use of the same survey team minimizes the influence of alternative factors on the assessment. The decreased proportion of synthetic *Caulerpa* detected at the east Agua Hedionda Lagoon location corresponds with the regularly observed deterioration of water clarity moving eastward through the lagoon.

Additionally, the lack of significant dependencies between the habitat type and among the synthetic, small patch size within the east Agua Hedionda location is also attributed to water clarity. It is likely that the poor visibility regime in this portion of Agua Hedionda Lagoon overwhelms the significance of the other factors. In other words, the visibility is so poor that peripheral visibility is nearly eliminated and divers are only likely to find synthetic *Caulerpa* if it lies directly within their line of sight. Thus, when the small patch sizes were used, habitat type becomes less relevant.

In contrast, at the Snug Harbor location the habitat and patch size variables were relevant to the team's ability to find synthetic *Caulerpa*. At this location, the moderate visibility meant that divers had a greater ability to detect synthetic *Caulerpa* patches that were within their 1-m swath. Thus, under this visibility regime, the patch size and habitat variables set the limits for the team's ability to find the synthetic *Caulerpa*. A small patch size was simply easier for a diver to miss while visually scanning back and forth. A small patch near the limits of a diver's visibility may not reflect enough light to draw attention to it. Regarding the habitat factor, eelgrass impedes divers' ability to detect synthetic *Caulerpa* for numerous reasons. Eelgrass is an obvious visual obstruction, physically



Percent synthetic *Caulerpa* detected during each survey conducted after the last detection of *C. taxifolia* at Huntington Harbour (Fall 2002). ND refers to no collected efficacy data during a given survey.

Figure 7

blocking a diver's horizontal line of sight. Moreover, eelgrass blades have fine sediments settled on them. These sediments are easily re-suspended as divers swim by. As a result, when divers work for a significant time period in a particular area, visibility may become locally diminished by the suspended sediment. Finally, eelgrass and attached fauna are an irritant to divers. Eelgrass-associated spirorbid worms (*Spirorbis* spp.) and stinging anemones (*Bunodeopsis* spp.) abrade and sting, respectively, as blades of eelgrass brush the faces of passing divers. This irritation causes divers to occasionally shake their heads or perform other maneuvers that may cause them to miss a small synthetic *Caulerpa* patch.

Eradication Certainty

Assessing the efficacy of each survey at Agua Hedionda Lagoon after the last discovered patch of *C. taxifolia* allows the estimation of the Eradication Certainty (EC), the certainty that all real *C. taxifolia* existing in the lagoon has been found and that eradication has been achieved. Eradication certainty is equal to 1 minus the multiplication of the proportion of undetected synthetic *Caulerpa* patches during each survey. This is expressed mathematically as:

$$EC = 100\% \cdot (1 - (P_1 \cdot P_2 \cdot P_3 \cdot \ldots P_n))$$

where EC is the final percent eradication certainty and P_1 through P_n are the proportions of undetected synthetic *Caulerpa* during each of the efficacy trials.

To adequately approximate the actual eradication certainty using data from the survey efficacy assessment trials, two assumptions must be made. First, given that the synthetic *Caulerpa* does not grow, it would have to be assumed that real *C. taxifolia* would not grow beyond the patch sizes replicated in the trials. Second, real *C. taxifolia* must be assumed to be incapable of lying dormant within the sediment for extended periods of time (e.g. one year).

The first assumption is not problematic from a management perspective because it only works to make any estimates of eradication more conservative. In other words, *C. taxifolia* does, in fact, grow and so patches not discovered during one survey would presumably become easier to find during subsequent surveys as they grow to a larger size. The second assumption is more problematic because there is some evidence that fragments of *C. taxifolia* may remain viable under sediments. Although it has been well documented that small fragments of *C. taxifolia* can grow into new patches (Ceccherelli and Piazzi 2001; Smith and Walters 1999), it is unknown how long buried fragments remain viable. To offset the potential implications of this assumption, eradication program surveys were spread temporally (over 3 years) such that the likelihood of viable material existing, but not expanding to a detectable size, was minimized.

Estimating eradication certainty at Agua Hedionda Lagoon is complicated by the fact that the lagoon is a single connected system with heterogeneous physical conditions and proportions of detected synthetic *Caulerpa*. Thus, two calculations are offered; one uses the pooled proportion of small synthetic *Caulerpa* patches detected during each trial (average-case), and the other uses the worst-case for each survey as presented in Figure 6.

The eradication certainty using the pooled detection data for the small patch sizes (average-case) during each survey is 99.86%. This value uses return data for each efficacy trial during surveys performed after the last find of *Caulerpa* at Agua Hedionda Lagoon (September 2002). The calculation does not take into account supplemental surveys that were performed in winter 2002-2003 and spring 2003, when additional transects were surveyed over the sites treated for *Caulerpa*. Inclusion of these extra survey efforts would increase the eradication certainty. These extra surveys are not included in the calculations because they were not lagoon-wide and therefore their actual contribution to eradication certainty is not easily definable.

The eradication certainty using the worst-case data during each of the surveys since the last detection of live of C. *taxifolia* is 97.71%. This calculation uses the lowest return rate within each survey season from either the Snug Harbor or east Agua Hedionda Lagoon trial data.

To address the reality that undetected patches of real, living *C. taxifolia* would grow and expand in size over time, the large patch sizes were incorporated into the survey efficacy assessment trials to determine the smallest patch size that would be detected 100% of the time. Once that size was determined, the length of time it would take for a patch of *C. taxifolia* to expand to that size could be identified. During the survey efficacy assessment trials, every 1-m patch size was detected except for one (89 out of 90). Based on the growth rates of *C. taxifolia* measured at the infestation sites, it is assumed that any undetected patches of *C. taxifolia* would have grown to at least 1 meter in diameter within three years. Seven repeated surveys of Agua Hedionda Lagoon were conducted over more than a three-year period without detection of *C. taxifolia*.

HUNTINGTON HARBOUR

Environmental Variables

The survey efficacy assessment trial results at Huntington Harbour found no influence by any of the study variables on the amount of synthetic *Caulerpa* detected. The visibility regime at Huntington Harbour would be considered moderate to good relative to Agua Hedionda Lagoon. Survey divers at Huntington Harbour could typically see one another and could sometimes even see 3 to 5 divers down the line. Under these conditions, the survey divers could scan well out in front of their line of travel. This gave them time to scan back and forth within their swath of responsibility before passing over a given point. Although conditions were typically observed to be better in the west pond than the east pond, the lower visibility in the east pond was not sufficient to significantly impact the teams' ability to detect patches of synthetic *Caulerpa*.

Eradication Certainty

Calculation of eradication certainty at Huntington Harbour is less complicated than at Agua Hedionda Lagoon. The lack of statistical dependencies within the efficacy data means that the pooled data from each trail can be used for the entire Huntington Harbour study site. Applying the same equation for eradication certainty as used for Agua Hedionda Lagoon results in an eradication certainty of greater than 99.99%.

Eradication certainty at Huntington Harbour is exceptional for two reasons. First, the typically good visibility at this site resulted in a high percentage of detection of synthetic *Caulerpa* (Table 4). Second, because of the smaller size of the Huntington Harbour site, resources were available to survey during each quarter (at Agua Hedionda Lagoon, later surveys were limited to the summer and fall quarters). Since the last discovery of *C. taxifolia* in Huntington Harbour in November 2002, there have been twelve additional surveys conducted with no *C. taxifolia* detected.

CONCLUSION

The calculations of eradication certainty based on the survey efficacy assessment indicate that it is highly probable that *C. taxifolia* has been successfully eradicated from Agua Hedionda Lagoon, with a final certainty of 99.86% (average-case) and 97.71% (worst-case). Although this study has focused only on lagoon-wide surveys conducted after the last detection of *C. taxifolia* in September 2002, more than six other surveys of the infestation areas were conducted both before and after the last find that were not accounted for in the calculations for the reasons discussed above. However these additional surveys add to the confidence that untreated *C. taxifolia* would have been detected and increase the certainty of eradication.

The calculation of 99.99% eradication certainty based on the survey efficacy assessment at Huntington Harbour indicates that it is highly probable that *C. taxifolia* has been successfully eradicated from the known infestation areas at Huntington Harbour.

ACKNOWLEDGEMENTS

The survey efficacy assessment trials were funded as part of the Southern California *Caulerpa taxifolia* Eradication Program, under the guidance of the Southern California *Caulerpa* Action Team, by Cabrillo Power I LLC, Santa Ana Regional Water Quality Control Board, San Diego Regional Water Quality Control Board, California Department of Fish and Game, National Marine Fisheries Service, State Water Resources Control Board (through an EPA 319h Water Quality Implementation Project grant), California Coastal Conservancy (through a Southern California Wetlands Recovery Project grant), Agua Hedionda Lagoon Foundation, and National Fish and Wildlife Foundation.

Funding for this project has been provided in part by the U.S. EPA pursuant to Assistance Agreement No. C9-9768201-0 and any amendments thereto which has been awarded to the State Water Resources Control Board (SWRCB) for the implementation of California's Nonpoint Source Pollution Control Program. The contents of this document do not necessarily reflect the views and policies of the USEPA or the SWRCB, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

LITERATURE CITED

Bourdouresque, C., Lemee, R. Mari, X. Meinesz, A. 1996. The invasive alga *Caulerpa taxifolia* is not a suitable diet for the sea urchin *Paracentrotus lividus*. Aquatic Botany, 53, 245-250.

Ceccherelli, G., Cinelli, F. 1999. A pilot study of nutrient enriched sediments in a *Cymodocea nodosa* bed invaded by the introduced alga *Caulerpa taxifolia*. Botanica Marina, 42, 409-417.

Ceccherelli, G., Piazzi, L. 2001. Dispersal of *Caulerpa racemosa* fragments in the Mediterranean: lack of detachment time effect on establishment. Botanica Marina, 44, 209-213.

De Vaugelas, J., Meinesz, A., Coquillard, P., Hill, D. 1997. A computer simulation to evaluate the impact of *Caulerpa taxifolia* on Mediterranean biodiversity. Vie et Milieu, 47, 397-400.

[ICC] International *Caulerpa taxifolia* Conference. 2002. Conference proceedings. January 31 February 1, 2002. San Diego, California.

Jousson, O., Pawlowski, J., Zninetti, L., Zechman, F., Dini, F., Di Guiseppe, G., Woodfield, R., Millar, A., Meinesz, A. 2000. Invasive alga reaches California. Nature, 408, 157-158.

Meinesz, A., Hesse, B. 1991. Introduction of the tropical alga *Caulerpa taxifolia* and its invasion of the northwestern Mediterranean (French). Oceanologica Acta, 14, 415-426.

Mozzachiodi, R., Scuri, R., Roberto, M., Brunelli, M. 2001. Caulerpenyne, a toxin from the seaweed *Caulerpa taxifolia*, depresses after hyperpolarization in invertebrate neurons. Neuroscience, 107, 519-526.

Merkel & Associates. 2006. Five-year status report on the *Caulerpa taxifolia* eradication program at Agua Hedionda Lagoon. Prepared for the Southern California *Caulerpa* Action Team.

Pesando, D., Lemee, R., Ferrua, C., Amade, P., Girard, J. 1996. Effects of caulerpenyne, the major toxin from *Caulerpa taxifolia* on mechanisms related to sea urchin egg cleavage. Aquatic Toxicology, 35, 139-155.

Smith, C., Walters, L. 1999. Fragmentation as a strategy for *Caulerpa* species: fates of fragments and implications for management of an invasive weed. Marine Ecology, 20, 307-319.

Woodfield, R., Mooney, R. 2002. Field response to infestations of *Caulerpa taxifolia* in Southern California. *In*, California and the World Ocean '02: revisiting and revising California's ocean agenda (Conference proceedings). October 27 – 30, 2002. Santa Barbara, California.

APPENDIX E
California Department Fish and Game
Eradication Site Inspection Report

State of California The Resources Agency

M e m o r a n d u m

To : Bill Paznokas **Date**: 27 February 2006

From : **Department of Fish and Game**: Robin D Lewis, OSPR

Subject : *Caulerpa taxifolia* diver surveys

On 16 and 17 February 2006 several Department of Fish and Game divers conducted ground truth surveys for evidence of the presence of *Caulerpa taxifolia* in Aqua Hedionda lagoon and Huntington Harbor, respectively. This report provides a summary of our dive conditions, activities and findings. All of the participating divers have successfully completed the *Caulerpa* spp. identification certification exam.

Survey #1

During 16 February Game Wardens Sean Moe and Christian Corbo, and I examined the bottom of Agua Hedionda lagoon in an effort to locate *C. taxifolia* that may be persisting following several years of eradication effort. We conducted a systematic search effort with your assistance in directing the search locations, and assistance from employees of Merkel and Associates, Inc. in placement of bottom-transect guide lines. From approximately 1030 hrs to 1230 hrs we examined the bottom of the north-east segment of Aqua Hedionda lagoon (east of I-5) along six individual transect lines, averaging approximately 475 feet in length, in a 3x3 perpendicular grid pattern (Photograph 1). Horizontal bottom visibility varied from 3 to 6 feet, but averaged about 5 feet. Divers swam abreast utilizing a perpendicular guide-line to maintain a spacing of about 6 feet while transiting individual transects. The survey team examined approximately 80,000 square feet of lagoon bottom.

The survey time was selected to take advantage of a favorable tidal condition of + 4.4 ft high tide at about 1015 hrs (negating lagoon tidal lag). Overall operational conditions were very good for this activity.

The team could find no evidence of the presence of *C. taxifolia*. We did encounter numerous areas of bottom covered by plastic tarp weighted down by sand bags, all covered by a thin layer of fine silt which varied from 0.5 to 1 inch in thickness. We observed several patches of newly developing eelgrass (*Zostera marina*) in varying degrees of growth suggesting new recruitment and likely to represent re-development of this grass species to the lagoon.

Survey #2

On 17 February three Game Wardens (Sean Moe, Christian Corbo and Paul Hamdorf) and I were assisted by Robert Mooney from Merkel, Inc in examining three discrete locations in the Huntington Harbor area. The four Fish and Game divers conducted searches of two ponds and a single channel of Huntington Harbor between approximately 0830 hrs to 1030 hrs. The tide on this day was predicted to be +3.8 ft at about 1100 hrs, although a substantial tidal lag time was expected at this area of the harbor which likely delayed the tidal change in the specific search areas. Diving conditions were very good for this activity on this day.

We began our survey at the westerly end of the westerly pond inside the Huntington Harbor condominium complex off Edinger Blvd. (Photograph 2). In this pond we utilized a guide-line that spaced divers approximately 6 feet apart along a guide-line length of about 18 feet. The dive team guided off of the south shore slope-toe following the shoreline contour from end to end. Visibility in this pond varied from 3 to 6 feet, but averaged about 5 feet providing a search area band width of about 34 ft (+/-). The length of pond searched was about 1,200 ft, producing a search area of about 41,000 sq. ft in this pond.

In the second pond divers swam independent transects from the west to east ends, and were distributed across the width of the pond. Visibility in this pond may have been slightly better than the first pond, perhaps 6 feet on average, providing a collective search band width of approximately 48 feet. Each diver made a slight foray into the mid-pond south basin, but it was only partially searched. Estimating a length of pond bottom search of about 1,300 ft, we examined approximately 62,400 sq. ft of pond bottom.

The divers then entered the back channel of Huntington Harbor where *C. taxifolia* had previously been found. The team again dispersed across the width of the channel and swam a west to east course along the bottom. Visibility in the channel was remarkably good at approximately 8 feet, providing a collective search band width of about 64 feet. Utilizing an approximate channel length of 400 ft, we searched about 25,600 sq. ft of the channel bottom.

The dive team found no evidence of *C. taxifolia* in any of the three areas searched. Numerous locations of past eradication effort were evident in these three areas as divers located various sand bags, silt covered tarps, and inverted containment devices which had been described to us in advance by Mr. Mooney.

Respectfully submitted,

Robin D. Lewis
Senior Environmental Scientist
California Department of Fish and Game
Office of Spill Prevention and Response
(858) 467 4215

APPENDIX F

Summary of Funding

Southern California *Caulerpa taxifolia* Eradication Program

Summary of Funding for the Southern California *Caulerpa taxifolia* Eradication Program and Coastal Surveillance

SOURCE		AMOUNT	MAJOR WORK ELEMENTS
Agua Hedionda Lagoon Foundation		$ 5,000	Outreach and Education Materials
Cabrillo Power I LLC		$ 123,000	Survey and Treatment at Agua Hedionda Lagoon
Cabrillo Power I LLC		$ 19,000	Grant Acquisition
Cabrillo Power I LLC		$ 84,000	Grant Acquisition
Cabrillo Power I LLC		$ 55,500	Survey at Agua Hedionda Lagoon
California Department of Fish and Game	Section 27	$ 250,000	Survey and Treatment at Agua Hedionda Lagoon/ Coastal Surveillance
California Department of Fish and Game	Section 27	$ 265,000	Treatment Research
California Department of Fish and Game	Section 27	$ 50,000	Outreach and Education Materials
California Department of Parks and Recreation		$ 15,000	Scientific Review of Eradication Program
FishAmerica Foundation	Grant to Agua Hedionda Lagoon Foundation	$ 30,000	Survey at Agua Hedionda Lagoon
National Fish and Wildlife Foundation	Grant to Agua Hedionda Lagoon Foundation	$ 95,000	Survey at Agua Hedionda Lagoon and Outreach and Education
NOAA Community-Based Restoration Program	Grant to Agua Hedionda Lagoon Foundation	$ 50,000	Survey at Agua Hedionda Lagoon
NOAA National Marine Fisheries Service	Invasives Species Program	$ 198,899	Survey and Treatment at Agua Hedionda Lagoon/ Coastal Surveillance
NOAA National Marine Fisheries Service	Invasives Species Program	$ 99,000	Survey at Agua Hedionda Lagoon
NOAA National Marine Fisheries Service/USFWS	Invasive Species Program/Bays and Estuaries Program	$ 95,693	Survey and Treatment at Agua Hedionda Lagoon
San Diego RWQCB	Cleanup and Abatement Fund	$ 100,000	Survey and Treatment at Agua Hedionda Lagoon
San Diego RWQCB	Cleanup and Abatement Fund	$ 600,000	Survey and Treatment at Agua Hedionda Lagoon
San Diego RWQCB	Cleanup and Abatement Funds to the City of Carlsbad	$ 700,000	Survey at Agua Hedionda Lagoon
Santa Ana RWQCB	Cleanup and Abatement Fund	$ 100,000	Survey and Treatment at Huntington Harbour
Santa Ana RWQCB	Cleanup and Abatement Fund	$ 600,000	Survey and Treatment at Huntington Harbour
Southern California Wetlands Recovery Project	Grant to Agua Hedionda Lagoon Foundation	$ 1,300,000	Survey at Agua Hedionda Lagoon and Outreach and Education
US Environmental Protection Agency/SWRCB	319h Grant to Agua Hedionda Lagoon Foundation	$ 500,000	Survey at Agua Hedionda Lagoon and Outreach and Education
US Environmental Protection Agency/SWRCB	Prop 13 Grant to Agua Hedionda Lagoon Foundation	$ 2,211,000	Surveillance in other southern California waterbodies
US Fish and Wildlife Service	Bays and Estuaries Program	$ 118,000	Survey and Treatment at Agua Hedionda Lagoon
US Fish and Wildlife Service	Aquatic Nuisance Species Task Force	$ 40,000	Scientific Review of Eradication Program
	TOTAL	**$ 7,704,092**	