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

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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.

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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

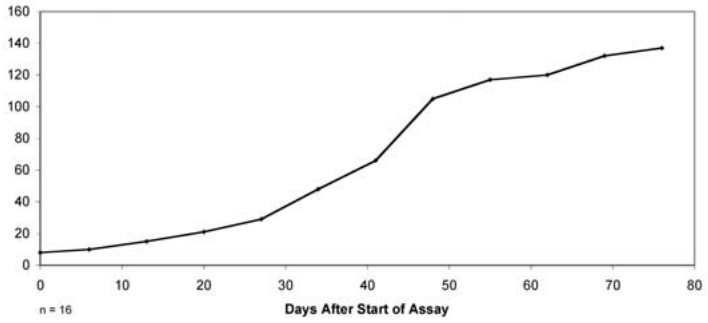

Figure 5. Growth of *C. taxifolia* in inoculated "control" cores removed from Agua Hedionda August, 2001. Data are cumulative numbers of all fronds produced in all cores.

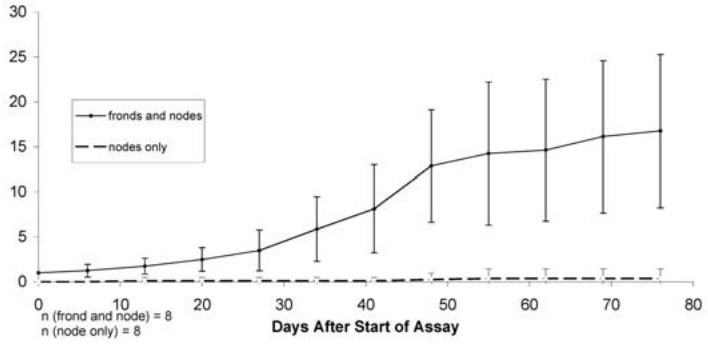

Figure 6. Number (mean +/- SD) of fronds produced in each core originally inoculated with *C. taxifolia* explants containing a frond+ node (solid line) or nodes only (dashed line).

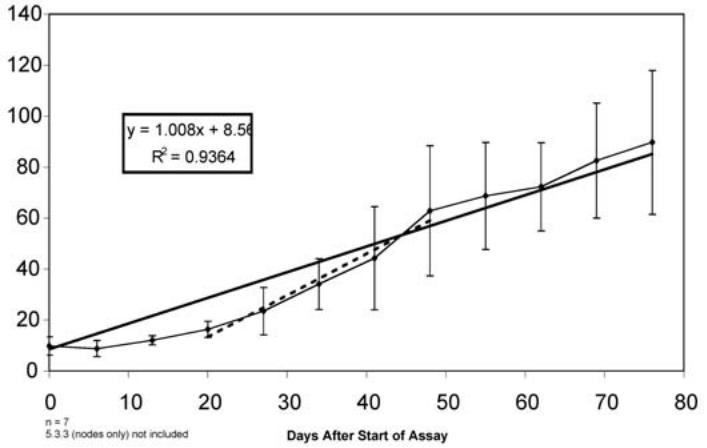

Figure 7. Growth of *C. taxifolia* (frond length), mean+/- SD per inoculated control core.

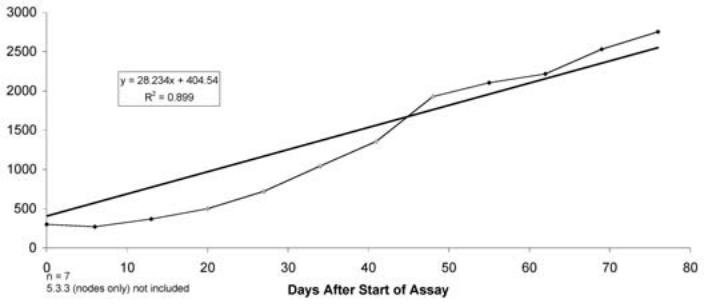

Figure 8. Estimated production (fresh wt. /day) of *C. taxifolia* inoculated in control cores. Estimates were generated from conversion of frond length to fresh weight based on data from 7 fronds.

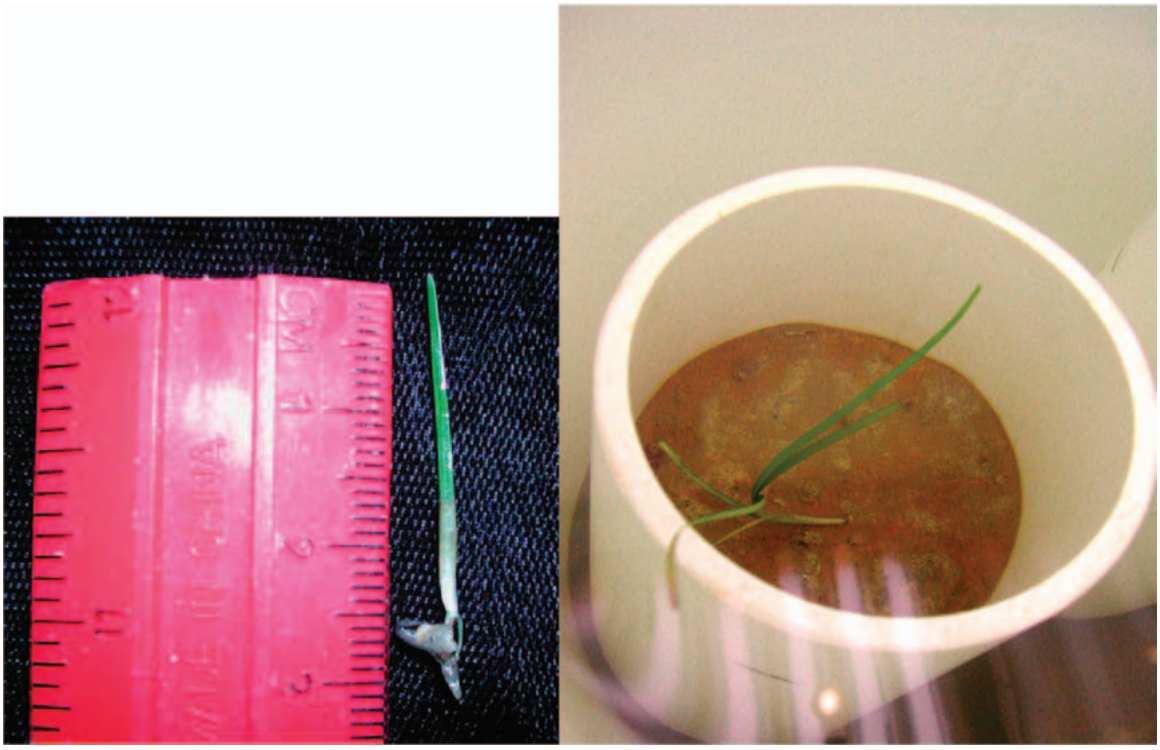

Figure 9. Examples (left) of sprouting eelgrass seedling at harvest 76 days after start of assay (left), and seedlings that had emerged 20 days after the start of grow out (right).

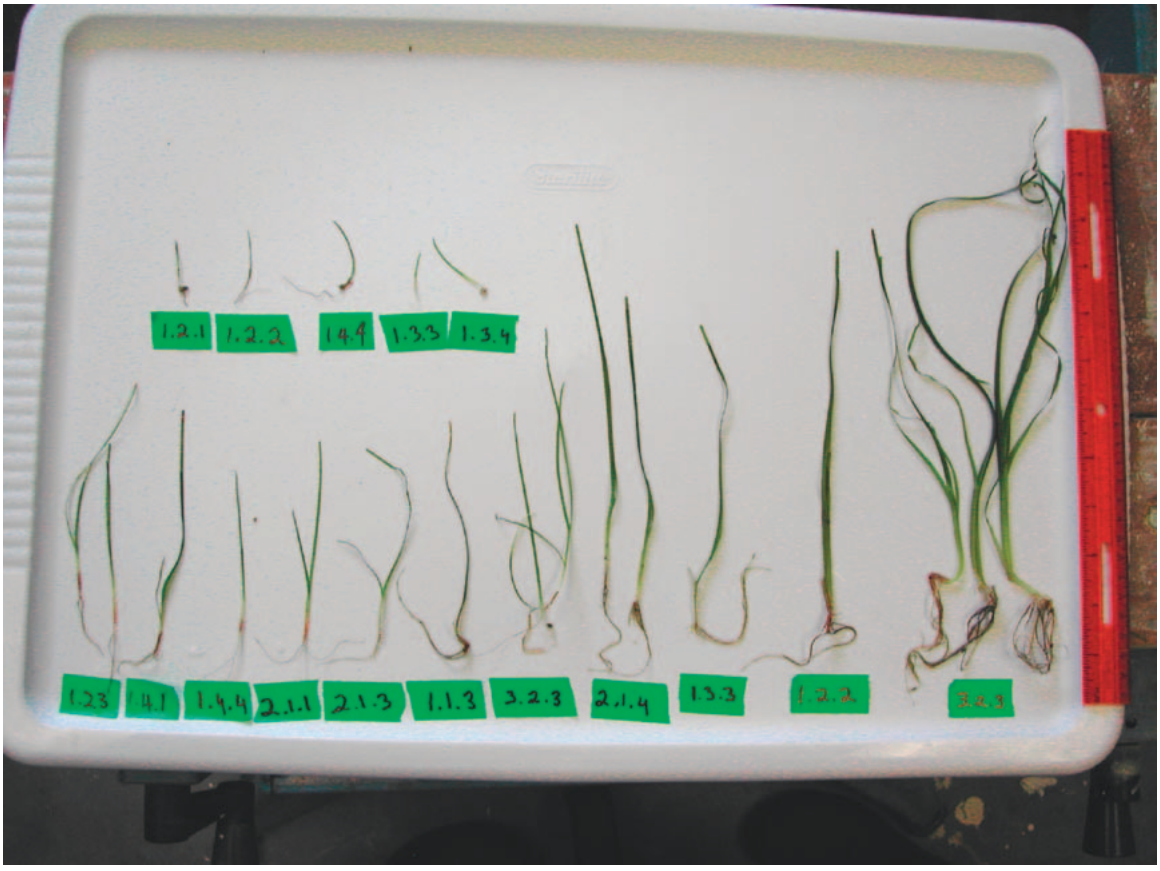

Figure 10. Examples of sprouted eelgrass seedlings that had emerged from cores removed from *C. taxifolia* treatment tarps 76 days after start of assay in grow-out conditions. Each number represents a single core.

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-

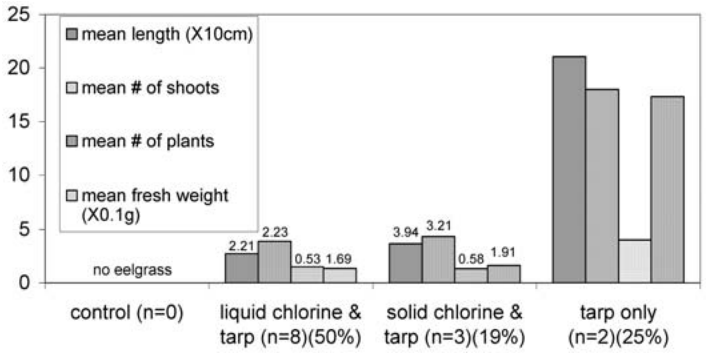

Figure 11. Seedling eelgrass production in control cores and cores removed from areas treated to eradicate *C. taxifolia* in Agua Hedionda Lagoon in December, 2002. Numbers above bars = standard deviations. Number in parentheses = percent of total cores sampled that produced eelgrass seedlings.

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).

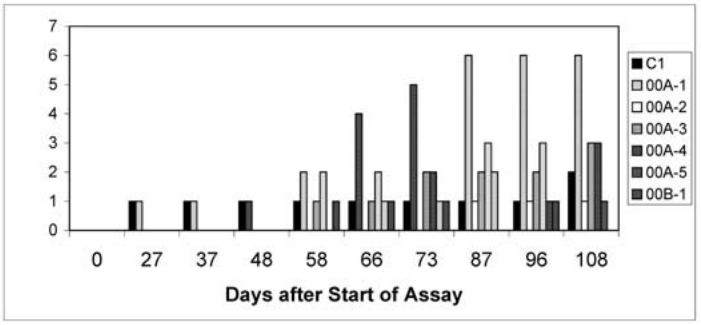

Figure 12. Eelgrass emergence from cores removed from Agua Hedionda in August, 2002 (C = controls cores; A, B from areas tarped and treated with chlorine in June, 2000). Bars represent each core that produced eelgrass seedlings.

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