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Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach

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ABSTRACT: We investigated the fate of fragments of the invasive seaweed *Caulerpa taxifolia* from southern California, USA, after exposure to chlorine (10, 15, 50, 125 ppm Cl^- at 20 to 23°C and 10 to 11°C) or temperature shock (7 to 10°C, 72°C). Chlorine bleach is currently being used to eradicate *C. taxifolia* in southern California. At temperatures favorable to growth, ~70% of the fragments survived at chlorine concentrations below 50 ppm; 1 fragment survived at 50 ppm, and none survived at 125 ppm. Within 2 wk, many of the surviving fragments regenerated. After 4 mo of cold treatments, even *C. taxifolia* not receiving chlorine treatments failed to regrow, despite unusual chloroplast migration into belowground tissues. Re-establishment of a favorable temperature regime did not result in regrowth over a 3 mo period. Acclimation of *C. taxifolia* to cold waters did not improve its survival. When exposed to 72°C seawater for 60 and 120 min, all fragments but one died within 2 wk. Despite presumably being from a single clone, *C. taxifolia* exhibited a highly variable response to treatments. Based on these results, chlorine concentrations in eradication treatments should be maintained at 125 ppm for at least 30 min in both the water column and in the sediments to reach stolons and rhizoids. Fragments of *C. taxifolia* are unlikely to survive or grow at ambient temperatures (8 to 10°C) off the open coast of northern California.

KEY WORDS: *Caulerpa taxifolia* · Invasive seaweed · Eradication · California coast

INTRODUCTION

Caulerpa taxifolia is listed as one of the top 100 invasive species on earth (International Union for the Conservation of Nature, available at: www.issg.org/booklet.pdf). Since it was first discovered in a non-native habitat on the Mediterranean coast off Monaco in 1984, the seaweed has continued to expand its range (Meinesz et al. 2001) and is now considered a major threat to native marine biodiversity (Académie des Sciences 1997). In June 2000, the Mediterranean invasive strain of *C. taxifolia* was identified at 2 sites in southern California (Jousson et al. 2000) as well as in Australia (Schaffelke et al. 2002).

Eradication of invasive species depends on early detection and rapid implementation of effective methods (Myers et al. 2000). In southern California, federal, state, and local governmental agencies began an immediate eradication program (Anderson & Keppner 2001). At the time, hardly any papers on *Caulerpa taxifolia* eradication had been published in peer-reviewed journals (but see Uchimura et al. 2000). Most of the information consisted of anecdotes, personal communications, and symposium papers (e.g. Gravez et al. 2001, Millar 2002). In preliminary tests with herbicides used to kill freshwater nuisance algae and angiosperms, *C. taxifolia* failed to respond to any treatment except chlorine bleach (sodium hypochlorite) (Anderson 2002). Bleach was adopted as the eradication treatment, and was injected in liquid form under opaque tarps placed over infested areas at one site (Agua Hedionda) and placed in solid form at another (Huntington Harbour) (Anderson 2002). More recently, Thake et al. (2003) reported that ionic aluminum might be a selective inhibitor of *C. taxifolia*.

Because restricted access to eradication sites prevented field experiments (Williams & Grosholz 2002), we conducted laboratory experiments to test the

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efficacy of chlorine bleach in killing fragments of *Caulerpa taxifolia*. The objectives of our study were (1) to determine how much bleach is necessary to kill fragments of *C. taxifolia* under different temperatures, and (2) to provide recommendations on the field eradication treatment protocol and development of quarantine facilities for future research. *C. taxifolia*, a subtropical/tropical species, might be expected to survive bleach treatments better at warmer temperatures favorable to its growth than at colder ones. Growth-optimal temperatures provide a best-case scenario for withstanding bleaching, and thus support conservative recommendations for treatment, but are not realistic for colder waters off the California coast and elsewhere. Although temperature data are limited from the invasion sites in southern California, they range from 23.8°C in August to 12.7°C in January (K. Merkel & R. Woodfield, Merkel & Associates, pers. comm.). We also tested whether hot water can kill *C. taxifolia*, as this would be a cheaper alternative treatment than bleach, with perhaps fewer environmental impacts. Although heating natural waters in invasion sites to lethal temperatures would be difficult, heating the effluent from research and public aquaria quarantine systems would be feasible. Our results also provide insight into whether *C. taxifolia* could grow in the perennial cold waters offshore of northern California.

MATERIALS AND METHODS

We obtained *Caulerpa taxifolia* from environmental consultants Merkel & Associates (San Diego), who were performing the field eradication and had obtained stock from Huntington Harbour, California, which they maintained in static indoor aquaria. *C. taxifolia* was kept in a permitted quarantined greenhouse at Bodega Marine Laboratory (BML). Upon transfer to BML, we planted thalli, which consist of upright photosynthetic fronds, stolons, and rhizoids, in aquaria (77 l each) in ~7 cm of commercially available siliceous Monterey, California beach sand and filled with artificial seawater. Fresh *C. taxifolia* was obtained from the original culture before each experiment.

Response to bleach at optimal temperatures. We treated *Caulerpa taxifolia* with bleach and monitored its response (see below) at temperatures optimal for growth (23 to 25°C, Komatsu et al. 1997). In February 2002, *C. taxifolia* was treated with 4 concentrations of Cl^- (0.02, 0.03, 0.10, and 0.25% bleach in seawater; 10, 15, 50, and 125 ppm Cl^-, respectively) at 4 different exposure times (30, 60, 90, and 120 min). The concentration of chlorine was measured using standard wastewater protocols before and after exposures (American Public Health Association 1992). We cut 1 and 4 cm fragments from the frond apical tips (n = 10 each per concentration-exposure treatment). The mean fresh mass for all 1 and 4 cm fragments used in this and experiments to follow was 0.024 g ± 0.010 SD and 0.120 g ± 0.040 SD, respectively. Before being placed in 18 l of bleach-water solution, wounds were allowed to heal for 40 to 120 min, to enable wound plug formation and recovery of photosynthesis (Dawes & Rhamstine 1967, Jacobs 1970, Dawes & Goddard 1978, Gayol et al. 1995). After exposure, we removed the fragments, rinsed them with artificial seawater, and planted each fragment upright with its cut end in sand in a 50 ml glass beaker. Controls were treated the same way for 120 min, but were not exposed to bleach (1 cm: n = 20, 4 cm: n = 25). Beakers were placed randomly in aerated aquaria filled with artificial seawater and held under natural light (see 'Results'), and their position was changed weekly. Water temperature was maintained by a recirculating freshwater bath surrounding the aquaria. Temperature was monitored daily with a thermometer and logged continuously from March 7 (Stowaway® TidbiTs®, Onset Computer Corporation). Light was monitored with a cosine sensor (Li-1400, Li-Cor) placed at the top of the aquaria and instantaneous values averaged over 10 min periods were logged. Salinity was measured weekly with a refractometer and adjusted to 34 to 36 ppt with distilled water if necessary. The salinity of natural seawater at the BML ranged from 32.0 to 33.8 ppt during the course of the experiments. Salinity during August and September at Agua Hedionda ranged from 33.8 to 34.9 and 28.0 to 34.6 in January at Huntington Harbour (K. Merkel & R. Woodfield pers. comm.). Salinity in stock aquaria reached 38 to 40 ppt with apparently no ill effect on *C. taxifolia* (R. Woodfield pers. comm.). The pH was monitored periodically to ensure that carbon was not limiting, and seawater was replaced if pH deviated outside the range 8.1 to 8.6, or every other week if not.

We monitored the fate (growth, death) of the treated fragments and controls daily for 3 wk, then weekly for a total of 77 d. Death was scored when the fragment was totally colorless and disintegrating; if any green color remained, it was scored as alive. The initiation of a new stolon or frond was scored as growth. To better follow the fate of the fragments, we kept supplementary records on whether the fragments were colorless but intact or distintegrating or disintegrated to the point where no tissue remained, the proportion of the fragment that was green, which part was green (e.g. central axis, base of frond, stolon, pinnules of frond), and the intensity of the green color (bright vs faint), but these data are not presented here. In this and the experiment to follow, frequencies of fragments exposed to chlorine that died or regrew were tested against controls using Fisher's exact test (SYSTAT 1999, SPSS).

Response to bleach at colder temperatures. In April 2002, we tested whether bleach-treated *Caulerpa taxifolia* could survive at water temperatures typical of those found along the open coast in northern California near BML (available at: www.bml2.ucdavis.edu/envdata/historical/query.asp). We exposed *C. taxifolia* to bleach solutions in an identical manner to the first experiment (described above) but used concentrations of 0.03, 0.05 and 0.10% bleach (15, 25, 50 ppm Cl^-, respectively) and 4 cm fragments only (n = 12 per concentration-exposure treatment), based on results from the first experiment. Controls were not exposed to bleach (n = 12). Fragments were immediately planted in sand-filled beakers and placed in aquaria maintained at 10°C. We had no controls for the cold treatment, but our stock culture was growing well and the first experiment was still underway in which thalli surviving bleach treatment exhibited new growth.

We monitored the color, thallus integrity, and growth of the fragments the following day, then weekly for 3 wk. Following observations in Week 4, we examined the below-sediment portion of the fragment by carefully removing thalli and inspecting them under dissecting and compound microscopes monthly over 3 mo. Thalli were immediately replanted after observations. All thalli were treated similarly.

By August (123 d after treatment initiation), the remaining thalli did not appear to be alive, but as a final check, the water temperature was increased gradually from 10 to 20–23°C at a rate of 0.2°C d^{-1} to assess whether *Caulerpa taxifolia* could regenerate under temperatures favorable to growth. The remaining buried portions (n = 91) were examined after 1 and 3 mo.

Cold acclimation. Although *Caulerpa taxifolia* might not be able to withstand an immediate cold shock, for example after an accidental release from a quarantine facility or an eradication site in warmer bay waters, it could potentially survive if preceded by a period of acclimation to cooler waters.

In July 2002, 160 individual fronds attached to stolons were planted among 5 aquaria (38 l) filled with sand and maintained at 23°C. Temperature was reduced by 0.35°C d^{-1} to 10°C over 37 d. Fronds were exposed to 7 to 11°C for 48 d, during which time we counted the number of fronds that were entirely green and healthy-looking weekly, until all fronds in the acclimation treatments appeared dead. At this time (August 29, 2002), we examined the buried portion. The temperature in the aquaria was then increased to 19 to 23°C over the course of 9 d, followed by maintenance at 19 to 23°C for 34 d (beginning October 9, 2002) to determine if *Caulerpa taxifolia* could recover from the prolonged exposure to cold. Aquaria (n = 5) holding control fronds were maintained between 19 and 23°C. We re-examined the buried portion on November 20, 2002, when the experiment was terminated.

Heat shock. The objective of this experiment was to assess whether exposure to hot seawater would kill *Caulerpa taxifolia*. Fragments (4 cm) were exposed in 2 l beakers to 72°C seawater for 1 (n = 10) and 2 h (n = 10). Control fragments (n = 10) were exposed to 26°C. After treatment, fragments were anchored in sand-filled beakers as described above, and placed in aquaria maintained at 19 to 23°C. We monitored the fragments daily for 1 wk, then again at the end of Week 2, then examined the buried portions every 4 d over 2 wk.

RESULTS

Ambient light reaching the aquaria in the greenhouse was sufficient for algal photosynthesis on most days except during thick fog. For 7.5 h each day, irradiance exceeded 300 µmol photon m^{-2} s^{-1}, the photosynthetic saturation irradiance (I_k, Chisholm & Jaubert 1997), based on daily light records averaged between February and November. For several hours at midday, the average irradiance was 1000 µmol photon m^{-2} s^{-1}.

The temperature was fairly constant in the warm aquaria, averaging 22.6°C ± 2.02 SD (n = 1130 loggings every 30 min), and in the cold aquaria, averaging 9.8°C ± 1.9 (n = 2916 loggings). During foggy periods in the fall, temperature in the warm aquaria dropped to 19°C.

Most of the initial chlorine concentration was maintained over the duration of the treatments due to the large chlorine reservoir relative to the low biomass of tissue. At 50 and 125 ppm, at least 92% of the initial chlorine remained after 120 min. At 10 ppm, 75 (120 min) to 87% (30 min) remained.

Response to chlorine at 20 to 23°C

All fragments were killed by 125 ppm Cl^- (Fig. 1). At 50 ppm, only 1 of 40 fragments survived (in the 60 min treatment after 77 d). Survival was variable at lower concentrations, but ≥30% of fragments were dead by Day 77. In treatments that killed 40% or less of the fragments, e.g. 10 ppm for 90 min, this proportion was not significantly different from the outcome in controls ($p > 0.156$). In some cases, death was not evident until 14 d after treatment. Initially, the dead fragments maintained their structural integrity despite losing color. Of the surviving fragments, 50 to 100% produced new blades or stolons, depending on the treatment, after at least 2 wk (Fig. 2). All 4 cm control fragments survived and grew. The growth of surviving bleached fragments did not differ from the growth of controls ($p > 0.100$). Neither treated nor control

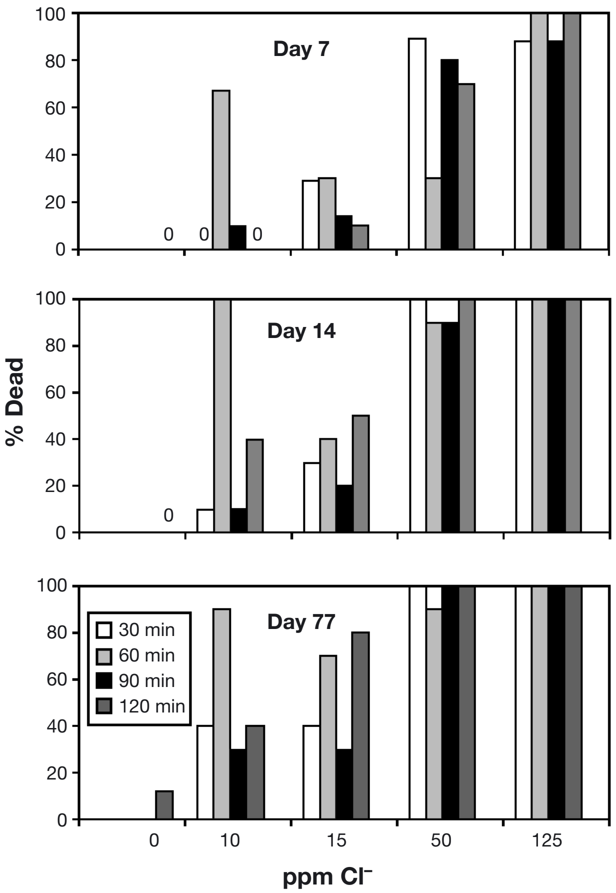

Fig. 1. *Caulerpa taxifolia*. Percentage of 4 cm fragments killed by bleach at 20 to 23°C (n = 10 treated fragments, n = 25 control fragments). A treatment result of ≥40% dead fragments was significantly different from the outcome in controls (Day 77, $p < 0.0016$). Values of zero noted on *x*-axis

1 cm fragments survived well, even when they were allowed to recover for 9 h after wounding before the experimental treatment in a supplementary experiment.

Response to chlorine and cold shock

At ambient seawater temperatures (10 to 11°C) along the open northern California coast near Bodega Bay, all bleach-treated fragments appeared white within 30 min of the treatments. Control thalli not exposed to chlorine responded identically to the treated fragments, and the effect was attributable to the cold shock.

After 4 wk of no change in the white appearance of the aboveground controls and treated fragments, 87% of the treated fragments and 83% of the controls were green immediately below the surface of the sediments (Figs. 3 & 4). This result was very surprising because *Caulerpa* spp. in their native habitats typically shuttle chloroplasts away from buried sections of the coenocytic thallus (Williams et al. 1985). Over time, most fragments lost pigmentation both above- and belowground, but still maintained their structural integrity. No fragment produced new tissue, even when the temperature was increased to 20–23°C, including fragments that had been green belowground.

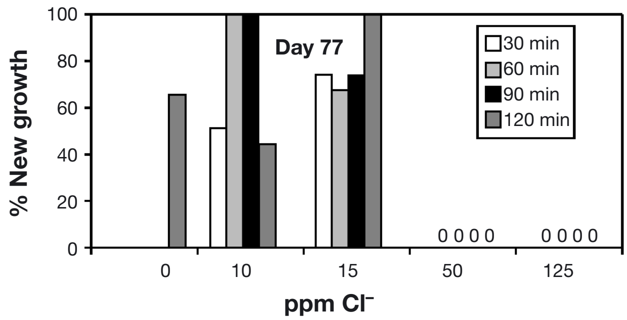

Fig. 2. *Caulerpa taxifolia*. Percentage of surviving 4 cm fragments that grew at 20 to 23°C. Values of zero noted on *x*-axis

Cold acclimation response

The number of healthy-appearing green fronds decreased steadily as the water temperature was low-

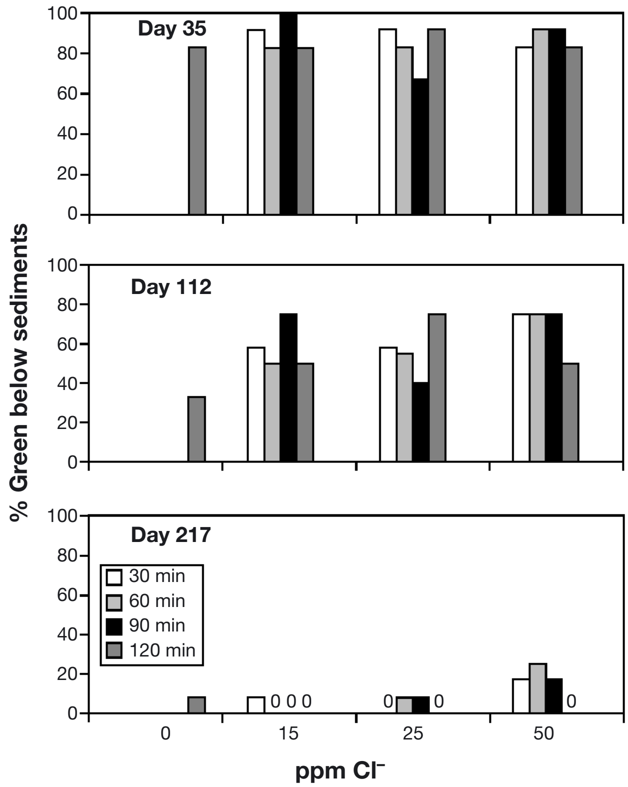

Fig. 3. *Caulerpa taxifolia*. Percentage of fragments that were green below the surface of the sediments after treatment with chlorine at 10 to 11°C (Days 35 and 112) and at 20 to 23°C (Day 217). n = 12. Values of zero noted on *x*-axis

Fig. 4. *Caulerpa taxifolia*. Two fragments after chlorine treatment at cold temperature. The green portion of the thallus was below the sediment surface

ered, but exhibited the greatest reduction when the temperature dropped below 19°C. Only 26% of the original fronds remained after 4 wk (Fig. 5); the rest had disintegrated. By Week 6 all tissue above the sand was white. At Week 9, all buried portions of the fronds examined were green. When the temperature was increased during Week 12, no new growth was observed and the white fronds began to deteriorate. The fronds were examined a month later when both the aboveground and buried portions were white and disintegrating; none had grown. In contrast, in the control treatment under temperatures favorable to growth (20 to 23°C), *Caulerpa taxifolia* grew new fronds, increasing the total number over the same time (6 wk).

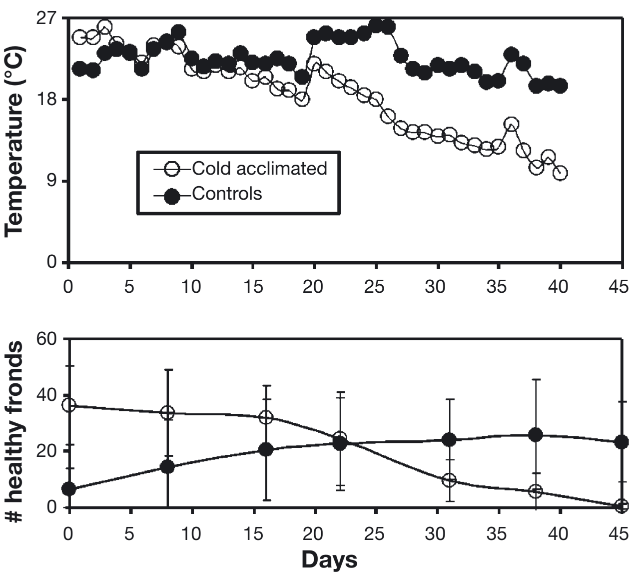

Fig. 5. *Caulerpa taxifolia*. Acclimation of the seaweed from water temperatures favorable to growth to temperatures along the open coast of northern California. Means ± SD are shown, n = 5 aquaria

Heat shock response

After exposure to hot seawater for 1 h, 9 fragments were pale green with white apical tips, and the remaining 1 was uniformly green. After 2 h, only the central axis of the fragments was green, indicating translocation of chloroplasts into the center of the siphon, except for 1 replicate which remained uniformly green. At the end of 2 wk, all heat-shocked fragments were dead except 1 (2 h treatment), which had a small area of green on the buried part of the thallus. Control fragments remained uniformly green.

DISCUSSION

Attempts to eradicate marine invasive species have been few (Culver & Kuris 2000, Kuris 2003), particularly for seaweeds (Curiel et al. 2001). Until prevention is effective at reducing introductions of marine invasive species, there is a need for controlled experiments on eradication techniques. In the laboratory, *Caulerpa taxifolia* was killed by exposure to chlorine over relatively short periods of 30 to 120 min, but only the highest concentration (125 ppm) killed all fragments. Death in some cases was not evident for several weeks. Poor survival of 1 cm fragments of fronds, including controls, was consistent with results from previous studies (Smith & Walters 1999).

Our results indicate that *in situ* eradication treatment should be maintained at a concentration of at least 125 ppm (0.25% bleach in seawater) for a minimum of 30 min. In the invasion sites in southern California, the oxidizable organic load is much higher than in our experiments because of the presence of native seagrass (*Zostera marina*, *Ruppia maritima*) and the associated community of other seaweeds and animals, in addition to the invading *Caulerpa taxifolia*. Sufficient chlorine must also penetrate 15 cm into the sediments to reach the rhizoids and buried portions of stolons (S. L. Williams pers. obs.). If concentrations are lower than 50 ppm, regeneration will be possible particularly under warmer temperatures. In the 50 ppm treatment, only 1 of 40 total fragments survived. This result emphasizes the importance of using a sufficiently large sample size to detect the rare survivor; invasive *C. taxifolia* readily establishes new populations from vegetative fragments (Ceccherelli & Cinelli 1999).

The volume of liquid bleach delivered in the field eradication at Agua Hedionda was estimated to achieve 0.5% bleach-in-seawater mixture, based on preliminary tests made in buckets containing only *Caulerpa taxifolia* (R. Woodfield pers. comm.). The concentration was not measured during or after delivery, so it is unknown whether the concentration remained sufficiently high long enough to kill the seaweed. Thalli were reported to appear white post-treatment, although the lower portions of the seaweed canopy were green in places following bleach treatments (K. Merkel & R. Woodfield pers. comm.). At Huntington Harbour and Agua Hedionda after the year 2000, solid disks of chlorine were used. Rather than estimating a dilution rate and chlorine concentration, disks were placed haphazardly roughly 5 cm apart on top of visible *C. taxifolia*.

With regard to the chlorination protocol for *Caulerpa taxifolia*, it is uncertain whether an effective concentration of chlorine is maintained long enough under the high organic loads of dense *C. taxifolia* and seagrass communities, and whether the chlorine penetrates 15 cm into the sediments to reach rhizoids. Although it is acknowledged that chemicals might be less effective when applied to dense aquatic vegetation (Willard et al. 1998, Major et al. 2003), plant biomass is rarely a controlled factor in eradication research (but see Gutierrez Lopez 1993). The average fresh biomass of the seaweed alone is 1020 g m^{-2} at its winter low in Huntington Harbour (Williams & Grosholz 2002, assuming 90% water content). Thus, the organic load is several orders of magnitude higher than the mass used in these experiments, in which ~0.1% Cl^{-} min^{-1} was oxidized. The use of chlorine is expensive and potentially hazardous, so no more should be applied than needed. Currently, it is unknown whether the reduction in the total area covered by *C. taxifolia* at each eradication site is attributable to maintaining chlorine at concentrations sufficient to kill *C. taxifolia*, or tarping and blocking light, or the combination. In general, the development of eradication strategies for invasive aquatic weeds is challenged by incomplete knowledge about the efficacy of multiple treatment types under the complete range of environmental conditions exhibited across different invasion sites (Ramaprabhu et al. 1982, Netherland & Shearer 1996, Getsinger 1998).

Caulerpa taxifolia varied considerably in survival and regeneration between fragments often taken from the same thallus and presumably from the single clone that invaded southern California (Jousson et al. 2000). This clone has not been determined as different from the Mediterranean clone (Jousson et al. 1998, Wiedenmann et al. 2001). Even under the controlled laboratory setting, surprising phenotypic plasticity was evident. The morphology of *C. taxifolia* in southern California can be remarkably different despite being grown in the same aquarium or even on the same thallus (authors' pers. obs.). Such clonal variation can have important ecological consequences (Poore & Fagerström 2000, Scrosati 2002). Intraplant variation in the secondary metabolite caulerpenyne has also been observed in 3 species of *Caulerpa*, although no mechanism for this pattern was been provided (Meyer & Paul 1992). Clones of invasive *C. taxifolia* thus might not respond similarly to eradication treatments.

The translocation of chloroplasts into buried portions of tissue when shocked by cold or heat is a new observation to add to the repertoire of cytoplasmic streaming and cellular organization reported for the genus *Caulerpa* (Sabnis & Jacobs 1967, Dawes & Barilotti 1969, Jacobs 1970). Typically, species of *Caulerpa* translocate chloroplasts away from portions of the thallus when buried or held in the dark (Williams et al. 1985, S. L. Williams pers. obs.). Although the temperature shock clearly elicited the translocation response, the mechanism involved is unknown. Chisholm et al. (2000) reported retraction of chlorophyll from aboveground fronds to the frond apical tips and stolons during acclimation from 22 to 9°C of a cold-tolerant strain of *C. taxifolia* from Moreton Bay, Australia. This strain is considered a potential source population for aquarium and invasive strains, and to be very closely related to the California strain (Wiedenmann et al. 2001, Meusnier et al. 2002, Schaffelke et al. 2002). It is interesting that chlorophyll remained in the aboveground biomass of the native cold-tolerant Australian strain at low temperatures, unlike the California invasive strain, which bleached completely over a similar acclimation regime. No observations of belowground chlorophyll content in the Australian strain were made. In any case, the sediments could conceivably serve as a seasonal refuge for *C. taxifolia*, i.e. it might be able to maintain itself when water temperatures become too cool for growth or even to support aboveground biomass. In the temperate waters off Croatia, the aboveground canopy of *C. taxifolia* dies back in winter over a 3 mo period when temperatures are sustained at 10°C, only to regrow again in spring when waters warm to 13°C (A. Zuljevic pers. comm.). No one, however, has examined the belowground biomass to determine whether it has green tissue when the aboveground biomass appears dead in the field.

Similar to our findings, Chisholm et al. (2000) reported a lethal temperature of 9°C at which regrowth of a presumably native strain of *Caulerpa taxifolia* was not possible after 4 to 6 wk exposure, even following reacclimation to a favorable temperature. Clearly, parallel comparisons of native and invasive strains in common garden-type experiments are needed to resolve how differently these strains respond to the environment.

The introduction of *Caulerpa taxifolia* fragments to the open coast in northern California is not likely to result in establishment of a new population, although it is very difficult to predict the response of a highly invasive species in a new environment (Ruiz et al. 1999). A caveat is that survival of detached *C. taxifolia* is size-dependent (Smith & Walters 1999), and a much larger thallus might be able to withstand cold better. Another caution is that this seaweed has already differentiated in aquarium culture (Jousson et al. 1998) and could do so again, which might lead to a strain more tolerant of cold waters. We are not suggesting that selection in aquaria led to cold tolerance in the invasive strain, because clearly there are native cold-tolerant strains (Chisholm et al. 2000), rather that further selection in aquaria could do so in the future. An introduced fragment would also have to anchor successfully under the rough hydrodynamic regime over much of the open coast of northern California, which seems very unlikely (see Smith & Walters 1999 for additional discussion).

Although information is accumulating on attempted eradiactions of *Caulerpa taxifolia* invasions worldwide (Meinesz 2002, Millar 2002, Zuljevic & Antolic 2002), approaches continue to be haphazard and have failed to benefit from results of well-designed experiments or field studies designed to evaluate eradication protocols. The broad tolerance of this species, and its wide availability in the aquarium trade (Gillespie et al. 1997, Withgott 2002), make new invasions possible along warmer coasts worldwide. *C. taxifolia* is one of at least 85 seaweeds that have become established in non-native habitats (Boudouresque & Verlaque 2002). It remains to be seen whether chlorine would be an effective eradication treatment for other invasive seaweeds. Chlorine application would be very difficult on open coasts, for example. Chlorination of invasive seaweeds that disperse by microscopic life history stages, such as the kelp *Undaria pinnatifida* (Forrest et al. 2000), would be an option only if used before reproduction or if applied repeatedly. The use of chlorine as a rapid method of eradication needs to be weighed against the value of the native community, because chlorine kills indiscriminately. These scenarios create a pressing need for more information about eradication techniques, combining controlled laboratory experiments with data from the field (Myers et al. 2000).

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