

Exploring the success of manual eradication of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta): the effect of habitat

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Abstract – *Caulerpa racemosa* var. *cylindracea* was first recorded in the Mediterranean Sea in 1990 and its aggressive spread is now considered one of the most serious invasive events ever documented in the Mediterranean basin. The aim of this paper is to evaluate whether manual eradication of *C. racemosa* var. *cylindracea* is a feasible approach and if its success depends on the habitat. We performed removals of the alga from patches of different sizes in 4 habitats – *C. racemosa* var. *cylindracea* on rock, *C. racemosa* var. *cylindracea* mixed with *Posidonia oceanica*, *C. racemosa* var. *cylindracea* on *P. oceanica* dead 'matte', *C. racemosa* var. *cylindracea* mixed with *Cymodocea nodosa* – and evaluated the success of repeated manual removal efforts with controls where removal was performed only once. The abundance of *Caulerpa racemosa* var. *cylindracea* greatly decreased with removal efforts, but the presence of the alga was still detectable after 3 months of treatments in all habitats. After four removal efforts, tiny fragments of the alga still occurred in experimental patches and, due to its fast growth rate, the fragments could quickly recreate a patch like the one removed. These data suggest that the manual method on its own cannot be effective for removal of *C. racemosa* var. *cylindracea*. A combination of destructive methods of control, such as a mechanical scraping and the use of a benthic vacuum, may enhance the probability of success and result in the best habitat conservation.

***Caulerpa racemosa* var. *cylindracea* / control / eradication / invasion / removal**

Résumé – Etude du succès d'arrachage manuel de *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) : le rôle de l'habitat. Cette étude a pour but d'évaluer si l'éradication manuelle de *Caulerpa racemosa* var. *cylindracea* est faisable et si est liée à l'habitat. Nous avons arraché des morceaux de taille différente de quatre habitats différents – *C. racemosa* var. *cylindracea* sur le rocher, *C. racemosa* var. *cylindracea* mêlée à *Posidonia oceanica*, *C. racemosa* var. *cylindracea* sur la matte morte de *P. oceanica*, *C. racemosa* var. *cylindracea* mêlée à *Cymodocea nodosa* – et comparé la réussite d'arrachage manuel répété à un seul arrachage. L'abondance de *Caulerpa racemosa* var. *cylindracea* diminuait fortement avec les arrachages, mais elle était encore décelable après 3 mois de traitement dans tous les habitats. En effet, après 4 arrachages, des fragments minuscules de l'algue étaient encore présents dans les pièces expérimentales et, en considération du taux élevé de

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recouvrement, ils auraient pu recréer rapidement un morceau égal à celui arraché. Ces données suggèrent que la simple méthode manuelle ne peut pas être efficace et que la seule technique qui augmente les chances de succès et préserve le plus d'habitat pourrait être en combinaison avec une méthode destructive de contrôle, par exemple le raclage mécanique et l'usage d'un vacuum benthique.

***Caulerpa racemosa* var. *cylindracea* / contrôle / déplacement / éradication / invasion**

INTRODUCTION

Several measures have been developed to control, contain or eradicate a wide range of invasive species. Where possible, eradication is the favoured approach, which necessitates the removal of every individual and propagule of an invasive species, such that only a reintroduction could allow its return. Therefore, eradication of invasive species is an increasingly important aspect of the conservation and management of natural ecosystems (Zavaleata *et al.*, 2001; Scott, 2001; Simberloff, 2001; Bax *et al.*, 2001).

Five factors are critical for successful eradication: 1) resources must be sufficient to fund the program; 2) lines of authority must be clear; 3) the biology of the target organism must make it susceptible to control procedures; 4) re-invasion must be prevented; 5) the pest must be detectable at relatively low densities and not widespread (Myers *et al.*, 2000).

Caulerpa racemosa (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman *et* Boudouresque (Verlaque *et al.*, 2003) was recorded for the first time in the Mediterranean in 1990 in Libya (Nizamuddin, 1991). It was subsequently reported for other regions of the Mediterranean basin (Panayotidis & Montesanto, 1994; Piazzi *et al.*, 1994) and showed traits of invasiveness since the first stages of spread (Piazzi *et al.*, 1997, 2005). At present, nearly the whole Mediterranean Sea has been colonized (Piazzi *et al.*, 1994; 1997, 2005; Modena *et al.*, 2000; Verlaque *et al.*, 2000; 2004) and the Canary Islands have just been reached (Verlaque *et al.*, 2004).

Such a rapid expansion must be linked to efficient ways of reproduction. Vegetative reproduction of *C. racemosa* var. *cylindracea* certainly occurs either through ramification and development of stolons. Fragments bearing all portions (frond, stolon and rhizoid) are able to re-establish in the field (Ceccherelli & Piazzi, 2001), but specialized propagules can be also involved in vegetative propagation (Renoncourt & Meinesz, 2002). Sexual reproduction has been observed in the field during summer in the South-Eastern Mediterranean (Panayotidis & Žuljević, 2001). The invasion of *C. racemosa* causes major modifications in benthic communities, mostly in the form of a considerable decrease in species richness (Argyrou *et al.*, 1999; Piazzi *et al.*, 2001; Ceccherelli & Campo, 2002).

At present, due to the widespread distribution in numerous countries, eradication of *C. racemosa* var. *cylindracea* from the Mediterranean has not been considered and would be hardly feasible on large scales. However, one measure developed in conserved sites, such as Marine Protected Areas, is to prevent the spread of introduced species. The settlement of new exotic species, known to be pests elsewhere, can be discovered by means of repeated visits of SCUBA divers that can directly survey the bottom of relatively small areas. Then, initiating the eradication program immediately after the discovery of the colony would reduce

the cost and maximise the benefits. Such effort has, for example, regularly been done since 1994 in the National Park of Port-Cros (Cottalorda *et al.*, 1996). Surveys and eradication efforts, such as by manual removal or using cloth soaked in copper salts, have been used to control the spread of the co-generic invasive species *Caulerpa taxifolia* (Vahl) C. Agardh (Robert, 1996; Robert & Gravez, 1998).

In the Mediterranean, the control of *Caulerpa taxifolia* has involved a variety of methods through the years, but efforts have merely slowed the spread of the alga. Manual removal has been performed at several sites, in both Italy and Spain, but the alga is still occurring at all of them (Riera *et al.*, 1994; Orestano, 1998). In Croatia, control measures have been implemented annually by covering isolated colonies with black plastic sheets or by using a benthic vacuum in, respectively, Stari Grad Bay and in Malinska (Žuljević & Antolić, 2001; Zavodnik *et al.*, 1998; 2001). However, despite these efforts, the eradication of the alga has not been achieved. A combination of mechanical and chemical methods has been recently utilised with satisfactory results for two infestations along the coast of southern California. This latter method consists in placing tarpaulins over patches of algae and injecting chlorine underneath to kill them (Dalton, 2001). Although eradication success has still to be evaluated, the damaging of native species due to the treatment means that this method may be useful only for very small patches of *C. taxifolia* and/or at relatively depauperate sites. To our knowledge, no scientific tests of methods of eradication of *C. racemosa* var. *cylindracea* have been performed or published.

The aim of this paper is to evaluate whether the manual eradication of *Caulerpa racemosa* var. *cylindracea* in the Mediterranean is a feasible approach and if the success depends on the habitat and the size of patches. The efficacy of the manual removal could differ among habitats depending on the accessibility to the alga, thus on co-occurring species and heterogeneity of the substratum. The success could be also enhanced in small patches, for which a more careful removal could be performed and less material would have to be removed.

MATERIALS AND METHODS

This study was carried out from September 2001 until November 2001 at Leghorn (Italy), on the coast of Tuscany, North-western Mediterranean Sea (43°28'24"N and 10°19'42"E). At this site, *Caulerpa racemosa* var. *cylindracea* has been invading since 1996 (Piazzi *et al.*, 1997) at a shallow depth (2 m) where a variety of species occur. At present, patches of *C. racemosa* var. *cylindracea* are found on rock covered by algal assemblages (Piazzi *et al.*, 2001), in sediments mixed with *Posidonia oceanica* (Linnaeus) Delile, on dead 'matte' of *P. oceanica* (interwined with rhizomes, roots and trapped compacted sediment), or mixed with *Cymodocea nodosa* (Ucria) Ascherson. Within this site, we randomly chose 2 areas (about 300 m² in size) for each habitat and, in each area, six large (about 1000 cm²) and six small (about 400 cm²) patches of *C. racemosa* var. *cylindracea*. The first removal was performed in all 96 patches on 6 September. After that, in each combination area × size, three patches were treated as controls and three as treatments. In controls, removal was not performed any further, while in treatments removal was performed repeatedly (approximately every three weeks), by

manually uprooting the alga from the substratum. The use of un-removed patches as controls would have not provided comparable data with treatments. A time of 15 min and 10 min was spent on the average to clear a large and a small patch, respectively. A buffer zone 10 cm wide was cleared around each experimental unit and maintained free of *C. racemosa* var. *cylindracea* during the study period, in order to avoid the re-colonization of the experimental units through stolons coming from surrounding areas. In the controls, removal was performed again only on 12 September 2001.

This experiment was started in late summer, because this is the time at which patches of *Caulerpa* spp. are most common. At that time, numerous isolated patches of different size suitable for the experiment, with a high coverage of *C. racemosa* var. *cylindracea* (about 90% of the substratum), were found. In treatments, subsequent removal was done on four occasions: 12 September (sampling was not done on this date), 25 September, 15 October and 5 November. Five removal efforts were therefore performed in treatments. On each sampling, the number of fragments and the total stolon length of fragments of *C. racemosa* var. *cylindracea* were counted in each entire patch. To obtain comparable estimates from the two experimental quadrat sizes, data collected in the small patches were multiplied by 2.5.

Four-way ANOVAs were performed for both variables on data collected on 25 September 2001 and 25 November 2001 (*i.e.* after two and five manual removals, in treatments). Time was not considered in the analyses to avoid problems of independence of data. 'Habitat', 'Removal' and 'Size' were treated as fixed factors, while 'Area' was a random factor nested in 'Habitat'. Cochran's test was used prior to ANOVA to test the assumption of variance homogeneity and SNK tests were used to compare means of significant sources of variation (Underwood, 1997).

RESULTS AND DISCUSSION

The abundance of *Caulerpa racemosa* var. *cylindracea* decreased significantly with removal efforts, but the alga was still present after 3 months of treatment. Nineteen days after the first removal effort, the number of fragments was, on average, 1 to 3.5 in large plots, depending on the habitat (Fig. 1). In small plots, the number of fragments was on average lower, but still around 2 per plot. The mean abundance of fragments was reduced by the subsequent removals; however, after five efforts *C. racemosa* var. *cylindracea* was found in all but a few plots, and generally one or few fragments per plot were still detected, even in the small plots.

On 25 September, treatments had been removed twice and controls once. On that sampling date, the number of fragments and the total stolon length of *C. racemosa* var. *cylindracea* was significantly dependent on the habitat (Fig. 1 and 2), being lowest in the dead 'matte' of *Posidonia oceanica* (Tables 1 and 2). Both response variables indicated the lower abundance of *C. racemosa* var. *cylindracea* in the removal treatments than in controls (SNK tests, Tables 1 and 2), suggesting that there was significant difference in the abundance of the alga already between two (treatments) and one (controls) removals few days after treatments.

On the following samplings dates the number of fragments of *C. racemosa* var. *cylindracea* in removed plots was slightly reduced, whereas in controls did not vary (Fig. 1), suggesting that establishment of new fragments did not occur

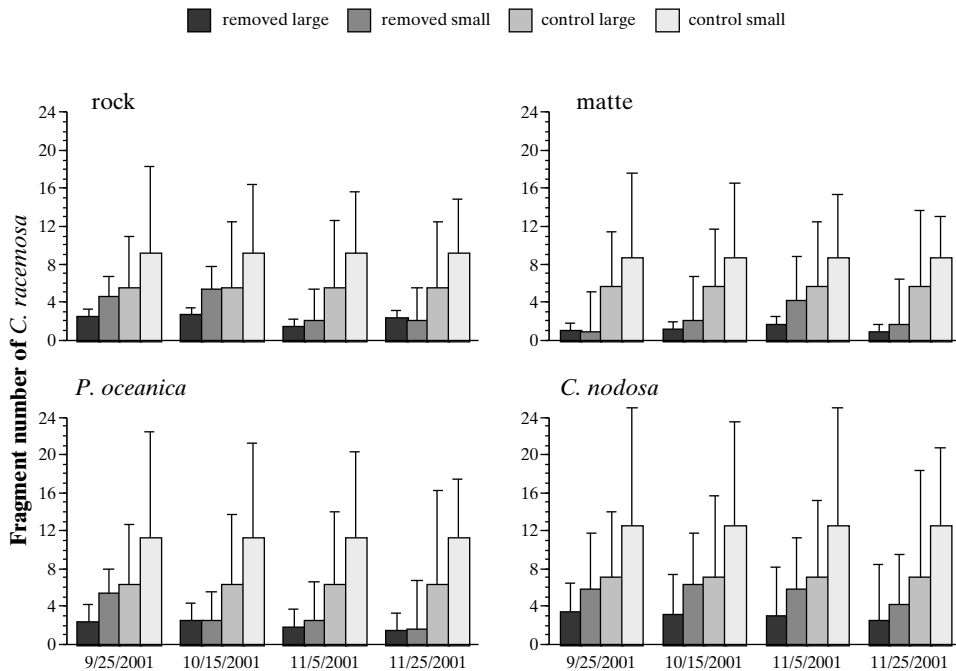


Fig. 1. Variation throughout the study period of number of *Caulerpa racemosa* var. *cylindracea* fragments (mean \pm SE) in large and small removal plots in the 4 habitats ($n=3$). Dates refer to sampling and removal.

during the study. Conversely, for the total length of the stolons of the alga, besides the little decrease in the removed plots, a consistent increase was observed in controls, suggesting that the abundance of the alga did not decrease naturally (Fig. 2). On 25 November, ten days after the fourth eradication effort, the total stolon length of the alga in the removal plots was, on average, 0.5 to 1.3 cm in large plots and 0.2 to 1.5 cm in small plots, depending on the habitat. Conversely, it varied between 100 and 220 cm in small and large controls, respectively.

The end of the experiment (25 November) was imposed by winter fragmentation of algal material. After that, these response variables were no longer taken, due to the difficulty in counting the number of fragments and measuring their length. The analysis performed on data collected on this sampling time did not detect any significant difference in the success of eradication across habitats, either for the number of fragments (Table 1) and for the total length of stolons of the alga (Table 2). The effect of removal treatment was significant throughout all samplings.

These data altogether suggest that manual removal of *Caulerpa racemosa* var. *cylindracea*, although repeated five times, does not lead to local eradication of the species, but can drastically reduce the abundance of the alga. Unexpectedly, even the smaller patch size did not experience a more successful eradication, as indicated by SNK test results on data collected at the start and at the end of the experiment for the number of fragments (Table 1). Furthermore, in autumn the

Table 1. Results of multifactorial ANOVAs on fragment number of *C. racemosa* on data collected the 25th September and 25th November. Habitat (rock, *Posidonia oceanica*, *P. oceanica* dead 'matte', *Cymodocea nodosa*); Area (2 areas). Significant results are given in **bold**.

Source of variation	df	25 th September			25 th November		
		MS	F	P	MS	F	P
Habitat =H	3	2.22	51.76	0.0012	0.95	2.92	0.1638
Removal =R	1	18.99	70.51	0.0011	32.46	80.06	0.0009
Size =S	1	3.17	20.67	0.0104	1.56	69.33	0.0011
Area(H) =A	4	0.04	0.22	0.9280	0.32	1.54	0.2023
H×R	3	1.15	4.28	0.0970	0.48	1.18	0.4226
H×S	3	0.16	1.09	0.4487	0.12	5.47	0.0671
R×S	1	0.11	0.56	0.4949	0.75	9.19	0.0387
R×A(H)	4	0.26	1.36	0.2568	0.40	1.91	0.1200
S×A(H)	4	0.15	0.78	0.5442	0.02	0.11	0.9800
H×R×S	3	0.14	0.72	0.5923	0.14	1.73	0.2987
S×R×A(H)	4	0.20	1.02	0.4062	0.08	0.38	0.8190
Residual	64	0.19			0.21		
Cochran's test			C=0.1336 ns Transf. ln(x+1)			C=0.1242 ns Transf. ln(x+1)	

25th September

SNK (SE=0.0423) on the 'Habitat' term: Rock=*Posidonia*=*Cymodocea*>Matte

SNK (SE=0.0649) on the 'Removal' term: Removed<Controls

SNK (SE=0.0566) on the 'Size' term: Large<Small

25th November

SNK (SE=0.0583) on the 'Size×Removal' term:

Removal	Size	Size	Removal
Removal<Control	Large	Large=Small	Removal
Removal<Control	Small	Large<Small	Control

abundance of *C. racemosa* var. *cylindracea* did not decrease naturally, as shown in the controls. Further evidence was provided by Piazzi *et al.* (2001) who found an increase in abundance of this alga on dead 'matte' and rocky substratum and cover increased from 80% up to 100% at this time of the year. On the whole, even in that study, no local extinction of patches was ever observed.

Unexpectedly, the success of the manual eradication of *C. racemosa* var. *cylindracea* does not change depending on the species of co-occurrence. On dead 'matte' of *P. oceanica*, *C. racemosa* var. *cylindracea* was initially eradicated with higher success, as indicated by the lower abundance obtained in this habitat after two manual removals. However, at the end of the experiment this difference could not be detected. These results are particularly discouraging, considering the high growth rate of *C. racemosa* var. *cylindracea*. Recent data have shown that recovery of this species, although strongly dependent on the period of the year, is so high that in three months of growth during summer the cover of *C. racemosa* var. *cylindracea* reaches about 80% on substrata covered by algal turfs (Piazzi *et al.*, 2003).

Table 2. Results of multifactorial ANOVAs on total stolon length of *C. racemosa* on data collected the 25th September and 25th November. Habitat (rock, *Posidonia oceanica*, *P. oceanica* dead 'matte', *Cymodocea nodosa*); Area (2 areas). Significant results are given in **bold**.

Source of variation	df	25 th September			25 th November		
		MS	F	P	MS	F	P
Habitat=H	3	8.59	15.30	0.0117	1.71	2.66	0.1839
Removal=R	1	50.09	163.04	0.0002	312.12	553.05	0.0000
Size=S	1	1.53	4.13	0.1118	1.17	7.41	0.0528
Area(H)=A	4	0.56	1.04	0.3931	0.64	1.79	0.1408
H×R	3	1.21	3.96	0.1085	0.64	1.14	0.4332
H×S	3	0.65	1.76	0.2929	0.21	1.36	0.3740
R×S	1	0.33	0.35	0.5853	0.64	3.96	0.1176
R×A(H)	4	0.31	0.57	0.6858	0.56	1.57	0.1928
S×A(H)	4	0.37	0.69	0.6013	0.16	0.44	0.7797
H×R×S	3	0.09	0.10	0.9569	0.12	0.78	0.5651
R×S×A(H)	4	0.94	1.74	0.1512	0.16	0.45	0.7709
Residual	64	0.53			0.36		
Cochran's test		C=0.1643 ns Transf. ln(x+1)			C=0.1301 ns Transf. ln(x+1)		

25th September

SNK (SE=0.1530) on the 'Habitat' term: Rock=*Posidonia*=*Cymodocea*>Matte

SNK on the 'Removal' term indicated Removed<Control (SE=0.0800).

25th November

SNK on the 'Removal' term indicated Removed<Control (SE=0.1084).

Furthermore, the capability to secure open space against propagation of stolons allows the alga to recover a 400 cm² surface quickly throughout a warm season. A tiny fragment can be therefore expected to grow quickly and recreate a patch of the same size as the one initially removed.

Tiny fragments could have occurred in the experimental removal plots either because they were missed during manual removal or/and because of vegetative and sexual reproduction in nearby meadows. Although re-establishment of fragments and release of gametes of *C. racemosa* var. *cylindracea* are unlikely in autumn (Ceccherelli & Piazzini, 2001; Panayotidis & Žuljević, 2001), reproduction of the algae established in the meadows next to the experimental site could potentially have occurred. However, this could occur at any sites where manual eradication is attempted, unless removal was carried out on very large scales. Moreover, in the field incomplete removal of the alga can occur, even when extreme care is used. Heterogeneity of the substratum is probably responsible for the re-occurrence of *C. racemosa* var. *cylindracea*, because portions of alga occurring in small crevices of rock and dead 'matte' of *P. oceanica* are difficult to remove entirely. The below-ground compartment in meadows of *Cymodocea nodosa* is also a habitat in which tiny fragments of alga are difficult to remove. These findings would suggest that manual removal on its own cannot be effective in eradicating *C. race-*

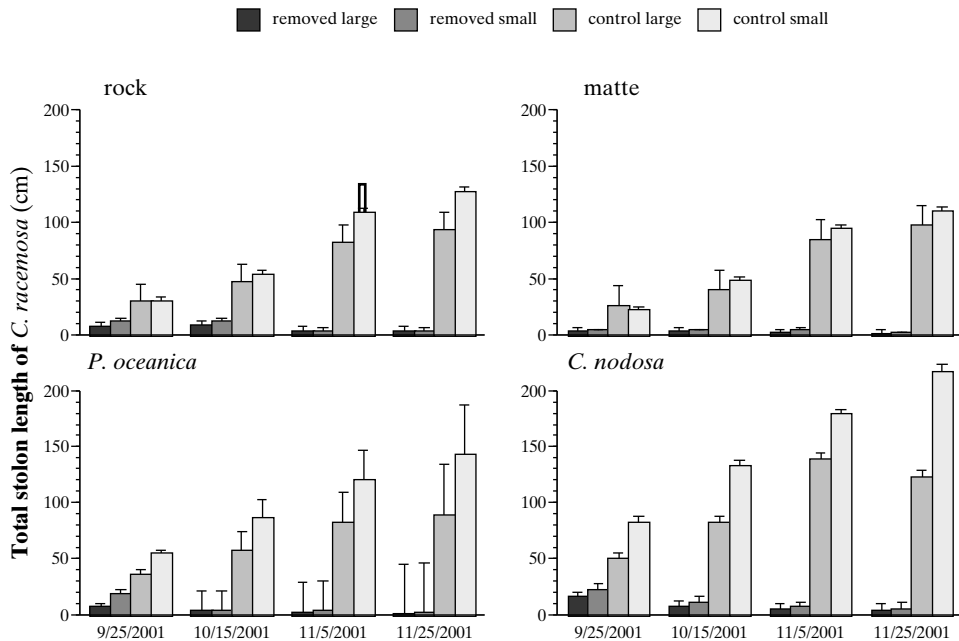


Fig. 2 Variation throughout the study period of *Caulerpa racemosa* var. *cylindracea* total stolon length (mean +SE) in large and small removal plots in the 4 habitats (n=3). Dates refer to sampling and removal.

mosa var. *cylindracea* in any of the habitats tested. Various destructive methods of control, such as a mechanical scraping and the use of a benthic vacuum, may enhance the probability of success whilst having the lowest impacts on native biota. However, when manual eradication of *C. racemosa* is performed in very localized sites next to established meadows, repeated eradication efforts are needed to cope with re-establishment of fragments and sexual reproduction.

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