

Spread of the invasive alga *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) along the Mediterranean coast of the Murcia region (SE Spain)

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Abstract

Spread of the invasive alga Caulerpa racemosa var. *cylindracea* (Caulerpales, Chlorophyta) along the Mediterranean Coast of the Murcia region (SE Spain).— The aim of this paper was to document the appearance and spread of the green alga *Caulerpa racemosa* along the coast of Murcia in south–eastern Spain. It was found for the first time in the area in 2005 and over the next two years the number of new sightings increased almost exponentially. In the period 2005–2007 the total surface area colonised by the alga in the region was estimated to be at least 265 ha. Benthic assemblages colonised by the alga were rocky bottoms with photophilic algae, dead *P. oceanica* rhizomes, infralittoral and circalittoral soft bottoms and maerl beds. No penetration of the alga was observed in *P. oceanica* meadows, except in one locality. Biometric analysis indicated high vegetative development in the established colonies in comparison to those described in other Mediterranean areas. Rapid spreading dynamics observed in the Murcia region is a potential threat for native benthic communities.

Key words: Biological invasions, *Caulerpa racemosa* var. *cylindracea*, Colonised surface area, Distribution, Mediterranean Sea, Spain.

Resumen

Introducción y expansión del alga invasora Caulerpa racemosa var. *cylindracea* en el litoral de la región de Murcia (SE España).— En el presente trabajo se documenta la aparición y dispersión del alga verde *Caulerpa racemosa* a lo largo de la costa de Murcia, región situada en el sureste español. El alga fue detectada por primera vez en el año 2005 y durante los dos años consecutivos se observó un crecimiento casi exponencial en el número de áreas colonizadas. La superficie total colonizada por el alga en Murcia durante el periodo 2005–2007 ha sido estimada en 265 ha., siendo las comunidades bentónicas afectadas algas fotófilas sobre sustrato rocoso, "mata muerta" de *P. oceanica*, fondos blandos infralitorales y circalitorales y fondos con comunidades de maerl. La presencia del alga dentro de praderas de *P. oceanica* solamente fue detectada en una localidad. Los estudios biométricos realizados muestran un elevado desarrollo vegetativo de las poblaciones de *C. racemosa* en Murcia en comparación con colonias de otras áreas del Mediterráneo, siendo esta rápida dinámica de expansión una amenaza potencial para las comunidades bentónicas nativas.

Palabras clave: Invasiones biológicas, *Caulerpa racemosa* var. *cylindracea*, Superficie colonizada, Mar Mediterráneo, España.

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Introduction

A total of 745 alien species have been reported in the Mediterranean Sea. Of these, 385 (52%) are already well established (Zenetos et al., 2005). Within this group, the green alga *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque (hereinafter *C. racemosa*) has been described as one of the most notorious invaders and has been included in the '100 worst invaders' list for the Mediterranean Sea (Streftaris & Zenetos, 2006). The biological characteristics of *C. racemosa* (high rates of vegetative dispersal, production of allelopathic substances, etc.) determine its high colonisation potential and its extraordinary ability to outcompete and alter native benthic assemblages, which make this species a particular potential threat for the Mediterranean coastal ecosystem (Piazzi et al., 2005).

C. racemosa was observed in the Eastern Mediterranean Sea for the first time along the coast of Libya in 1990 (Nizamuddin, 1991), the origin of this invasive variety as yet unknown (Verlaque et al., 2003; Durand et al., 2002; Panayotidis, 2006). Since then, the species has spread rapidly, gradually invading the Mediterranean Sea. This has been well documented in the western basin along the coasts of Italy, France and North Africa (Piazzi et al., 2005). Along the Mediterranean coast of Spain, the species was first sighted in the Balearic Islands in 1998 (Ballesteros et al., 1999). It reached the east coast of the Iberian peninsula (Castellón) in 1999 (Aranda et al., 1999) and began to spread quickly southward, being sighted in Alicante (SE Spain) in 2000 (Aranda et al., 2003). At that point, the algal spread seemed to stabilise (fig. 1A), but its presence was confirmed in the Murcia region in 2005, indicating that the colonising process was spreading southward.

Precise studies documenting the presence of the alga in newly colonised areas (*i.e.* colony size, depth range, substrate type, morphometric data and invaded native communities) are crucial to elucidate its colonising potential, spreading dynamics and mechanisms (vectors) at local and large spatial scales (Klein & Verlaque, 2008). Cartographic methods make it possible to measure the extent of the spread and can assist in helping to predict potential impacts and future scenarios (Meinesz, 2007). Detailed information on the spreading dynamics and extent of *C. racemosa* is available for a limited number of Mediterranean regions (Piazzi et al., 1997; Ruitton et al., 2005a). The goals of the present study were: (1) to document the spreading dynamics of *C. racemosa* along the coast of Murcia (SE Spain) from its appearance in 2005 to 2007, both at regional and local scales, and (2) to provide some quantitative estimate of the colonised surface area. Furthermore, the work includes several characteristics of the invaded sites (colonised assemblages, colonization depth) together with the vegetative development of several colonies in this geographical area.

Material and methods

Study area and field sampling programme

This study was carried out on the Mediterranean coast of Murcia, SE Spain (fig. 1A). After *C. racemosa* was first sighted in the region in 2005, an active detection programme was established to map the distribution of the alga and its spreading dynamics over time (Meinesz, 2007; Ruitton et al., 2005a). To this end, we initially selected 42 sampling stations unevenly distributed along 224 km of the Murcia coastline through a depth range from 2 to 30 m (fig. 1B, 1C). These stations were selected from different long-term sampling programmes that had already been initiated in the region for different purposes (scientific monitoring of *P. oceanica* meadows, environmental impact assessments and other scientific projects), but since they were visited at least once a year by specialised divers, this ensured reliable information about the date of appearance of the alga. Of course, this sampling strategy resulted in a non-systematic sampling design, but it provided insight into the colonisation process in a representative area of the Murcia coast. The period covered by this sampling programme was 2005–2007 *i.e.* the first three years of the colonisation process of *C. racemosa* in the Murcia region.

Once the alga was detected at a given station, divers surveyed a total surface area of 0.5 ha to characterise the colonised area (depth range, types of colonised substrate and benthic assemblages) and to estimate its surface area (*i.e.* colonisation levels *sensu* Ruitton et al., 2005a). Based on the field data obtained, invaded localities were assigned to one of the following five categories of colonisation level: (I) one or a few small colonies covering a surface area of less than 10 m²; (II) colonies of varying sizes covering a total surface area between 10 and 10⁴ m²; and (III) meadows covering surface areas between 10⁴ and 10⁵ m², (IV) 10⁵ and 10⁶ m² and (V) greater than 10⁶ m². For categories I and II, the surface area was estimated in a single survey within the sampling station using quadrats and transects. For cases belonging to categories III–V, where the colonised area extended beyond the area surveyed by divers at a single station, additional dives were necessary to determine the limits of the total colonised area. These additional dives were performed at neighbouring points separated from the sampling station by several hundreds of metres and at different depths and directions (a specific sampling design was established in each case). Once the limits of the invaded area were identified, these were determined by GPS and input into a Geographic Information System (ArcView microcomputer programme Version 9.0, Esri ©) to estimate the surface area of the polygon thus generated.

Biometric analysis

Biometric analysis of *C. racemosa* colonies was performed using data from summer 2007 (June 28th to August 9th), a season in which the vegetative development of the alga was close to its annual

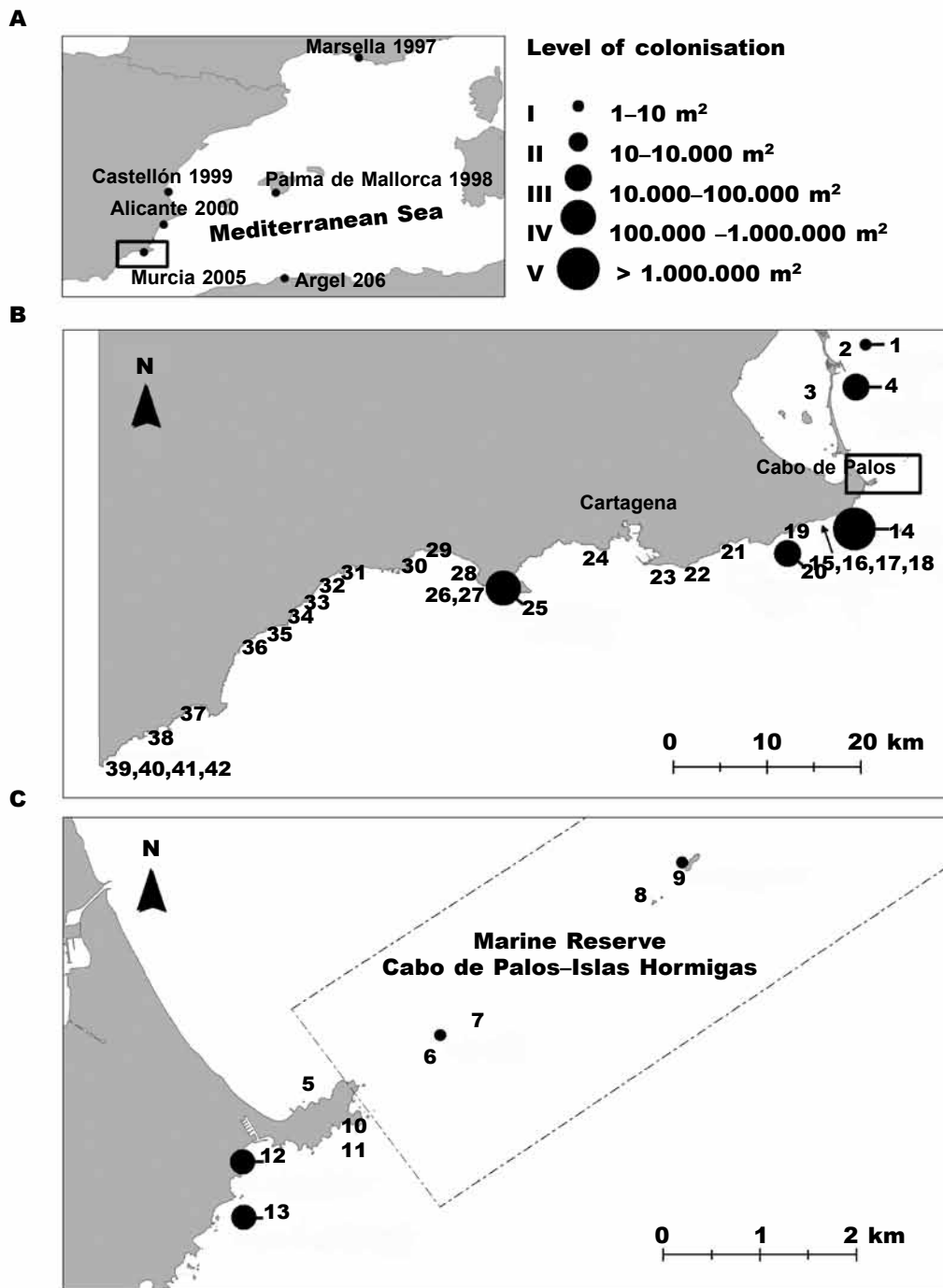


Fig. 1. A. Recent spread of *C. racemosa* in the Western Mediterranean basin; B. Distribution of sampling stations on the Murcia coast; C. Distribution of sampling stations in the Marine Reserve Cabo de Palos–Islas Hormigas. Invaded stations are indicated by black circles, the size of which corresponds to one of the five categories of colonisation level. Information related to the 42 sampling stations is included in appendix 1.

Fig. 1. A. Expansión reciente de *C. racemosa* en la cuenca mediterránea occidental; B. Distribución de las estaciones de muestreo en la costa de Murcia; C. Distribución de las estaciones de muestreo en la reserva marina del Cabo de Palos–Islas Hormigas. Las estaciones invadidas se indican mediante círculos negros, cuyo tamaño corresponde a una de las cinco categorías de nivel de colonización. En el apéndice 1 se incluye la información relacionada con las 42 estaciones de muestreo.

Table 1. Sampling stations colonised by *Caulerpa racemosa* along the coast of Murcia. For number of stations see figure 1 and appendix 1 and for level of colonisation see figure 1: S. Station; Y. Year of colonisation; L. Level of colonisation; N/A. Data not available.

Tabla 1. Estaciones de muestreo colonizadas por *Caulerpa racemosa* a lo largo de la costa murciana. Para el número de las estaciones ver la figura 1 y el apéndice 1 y para el nivel de colonización ver la figura 1: S. Estación; Y. Año de colonización; L. Nivel de colonización; N/A. Datos no disponibles.

S	Locality	Y	2005 (m ²)	2006 (m ²)	2007 (m ²)	L
1	La Manga	2007	0	0	10	I
4	Isla Grosa	2006	0	221	104	II
6	Piles I	2007	0	0	1	I
9	Isla Hormiga	2007	0	0	1	I
12	La Barra	2007	0	0	104	II
13	Los Punchosos	2007	0	0	4.5 x 10 ⁴	III
14	Calblanque	2005	300	N/A	2.5 x 10 ⁶	V
19	Cabo Negrete	2007	0	0	10 ⁴ –10 ⁵	III
25	C. Tiñoso	2006	0	13,724	9 x 10 ⁵	IV

maximum in this (unpublished data) and other regions of the Western Mediterranean (Klein & Verlaque, 2008; but see Cebrian & Ballesteros, 2009). Samples were collected at three of the most invaded stations: station 1 (–10 m), station 14 (–25 m), and station 25 (–22 m) (fig. 1B). Fronds, stolons and rhizoids were carefully collected by hand within six replicated 1,600 cm² quadrats that were randomly placed within fully colonised areas (*i.e.* 100% cover) along a 50 m transect. Samples were processed in the laboratory to determine the following biometric variables as described by Capiomont et al. (2005) and Ruitton et al. (2005b): the total length of stolons (m/m²), number of stolon apices (no. apices/m²), number of fronds

(no. fronds/m²) and frond height (cm). Total biomass (g dw/m²) was determined by drying the samples at 70°C until constant weight.

Results

Distribution and estimated colonised area

Field data obtained at the invaded localities are summarised in figure 1 (B and C) and table 1. *C. racemosa* was first detected in station 14 (locality of Calblanque) in 2005 as dispersed patches covering a total surface area of more than 10⁴ m². By 2007, the

Table 2. Biometric analysis of the *Caulerpa racemosa* populations studied (mean values ± SD): S. Station number; D. Depth (m); B. Total biomass (g dw/m²); A. Number of apices (No. apices/m²); F. Number of fronds (No. fronds/m²); Sl. Total stolon length (m/m²); Fh. Frond height (cm).

Tabla 2. Análisis biométrico de las poblaciones de *Caulerpa racemosa* estudiadas (valores medios ± DE): S. Número de estación; D. Profundidad (m); B. Biomasa total (g ps/m²); A. Número de ápices (nº de ápices/m²); F. Número de frondas (nº frondas/m²); Sl. Longitud total de los estolones (m/m²); Fh. Altura de las frondas (cm).

S	Locality	D	B	A	F	Sl	Fh
4	Isla Grosa	10	62.7 ± 42.7	1,133 ± 958	5,401 ± 2,631	3,487 ± 1,073	1.6 ± 0.4
14	Calblanque	25	16.9 ± 7.3	323 ± 187	1,260 ± 589	3,913 ± 1,133	3.1 ± 0.3
25	C. Tiñoso	22	49.7 ± 21.0	2,238 ± 832	6,256 ± 1,316	4,528 ± 798	1.9 ± 0.7

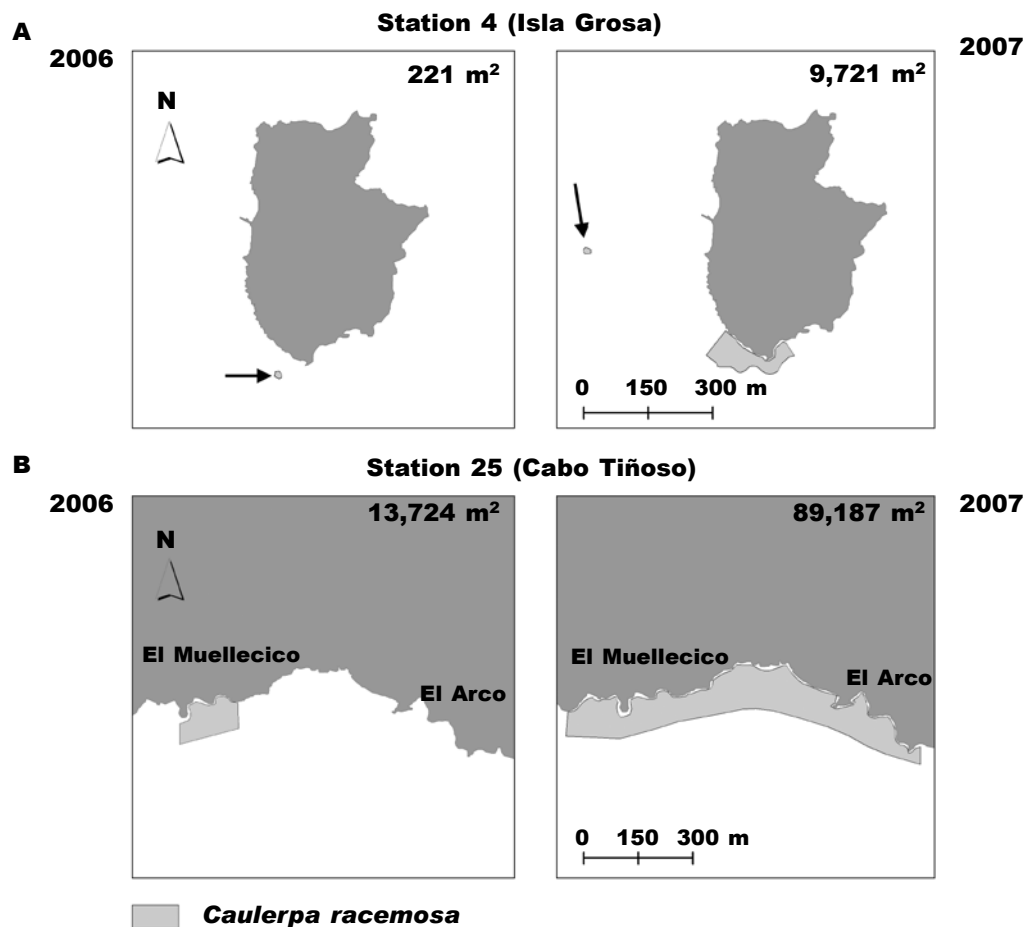


Fig. 2. Distribution and estimated surface area colonised by *Caulerpa racemosa* in: A. Station 4 (Isla Grosa); B. Station 25 (Cabo Tiñoso) in 2006 and 2007. Black arrows indicate the presence of new small patches of the alga.

Fig. 2. Distribución y superficie del área colonizada por *Caulerpa racemosa* en: A. Estación 4 (Isla Grosa); B. Estación 25 (Cabo Tiñoso) en 2006 y 2007. Las flechas negras indican la presencia de nuevas pequeñas zonas del alga.

colony had formed a more homogenous meadow of at least $2.5 \cdot 10^6$ m². After 2005, the number of newly invaded localities increased almost exponentially: two in 2006 and six in 2007 (table 1). In 2006, the population of station 4 (locality of Isla Grosa) was first found as a few small patches over a total surface area of 221 m² that increased to 10⁴ m² in 2007 (figs. 1B, 2B). In station 25 (locality of Cabo Tiñoso), the initial surface area in 2006 was estimated as 13,724 m² and this increased to 89,187 m² in 2007. In 2007, all new sightings were concentrated along the easternmost coast of the region (stations 1, 6, 9, 12, 13 and 19) with very different colonisation levels, ranging between categories I and III (fig. 1B, 1C; table 1). The cumulated field data gave a gross estimation of the total invaded area of 265 ha in 2007, which is probably an underestimation of the

real colonised area since information on areas deeper than 30 m was not available and some coastal zones were excluded from the survey.

Characteristics of the colonised areas

The depth of invaded areas ranged from 2–30 m, but the maximum colonised depth was greater than 30 m since deeper stands continued further to this isobath (annex I). Shallow colonies (< 10 m) were the least frequent while most of the studied colonies fell within 10–30 m. The alga colonised a wide range of substrates and native assemblages: rocky photophilic algae (boulders and vertical walls), infralittoral and circalittoral soft-bottoms, dead mats of *P. oceanica*, and maerl beds (annex 1). In most localities, *C. racemosa* formed compact multilayered

mats up to 12 cm thick over the substrate. Cabo Tiñoso was the only locality where the *P. oceanica* meadow was partially invaded by the alga, but no penetration of the seagrass canopy was observed at the other localities.

Biometric characterization of the colonies

Table 2 summarises the biometric characteristics of *C. racemosa* colonies at three selected stations: 4, 14 and 25. The total biomass varied between 9.4 and 13.9 g dw/m². The number of stolon apices ranged from 150 to 3,756/m², while the total number of fronds ranged from 937 to 9,018.7/m². In addition, the total length of stolons ranged from 1,684 to 5,777 m/m², while the height of fronds varied between 0.3 and 9.5 cm.

Discussion

C. racemosa was observed for the first time on the coast of Murcia as an isolated colony at the locality of Calblanque (station 14) in 2005. The origin and the introducing vector of the alga in the region is unknown, but two hypotheses can be given: (1) dispersion from the nearest colonies, located in the province of Alicante 90 km to the north; and (2) introduction through the nearby harbour at Cartagena, which is a crucial point for the very dense maritime traffic supported by this part of the Mediterranean Sea (fig. 1B). Further regional dispersion in subsequent years occurred in an almost exponential manner and new colonies appeared without a clear spatial pattern in localities separated by hundreds of metres to tens of kilometres. This rapid and discontinuous regional spread is similar to that described by Langar et al. (2002) on the Tunisian coast and by Ruitton et al. (2005a) along the French Mediterranean coast. This pattern of spread has been attributed to the efficient reproductive mechanisms reported for *C. racemosa*, both sexual (Panayotidis & Žuljević, 2001) and vegetative (Renoncourt & Meinesz, 2002), that determine its higher colonisation potential relative to other invasive Caulerpales (e.g. *C. taxifolia*, Meinesz, 2007).

In 2007 the most widespread population of *C. racemosa* was found in station 14 at the locality of Calblanque, the site where it was first sighted. However, there was no relationship between the actual colony size and the time elapsed since it was first observed, as indicated by the large variation in the estimated surface area between new colonies detected in 2006 and 2007 (1 to 10⁵ m²). This is because at some localities the alga appeared before the date of its first sighting, but was not detected in the preceding year. The alga was probably already present as one or a few small inconspicuous patches that would be difficult to find, even by trained divers, but this implies that *C. racemosa* is able to spread over a surface area of at least 1 ha in a 1-year period, which represents a very fast colonisation rate. Station 25 at the locality of Cabo Tiñoso is a good example: the invaded area increased 44-fold in 1 year (i.e. 7.5 ha/year). Similarly, the alga colonised a surface area of almost 1 ha in

2007 in station 4 from only few small patches found one year earlier (fig. 2).

Similar spreading dynamics have been reported from other Mediterranean localities (Piazzi et al., 1997; Piazzi & Cinelli, 1999; Piazzi et al., 2001; Ruitton et al., 2005b), showing that once the alga arrives at a locality, substrate colonisation can be a very rapid process. This could be due to the high stolon elongation rate (up to 2 cm/day) and reproductive capacity of the alga (Panayotidis & Žuljević, 2001; Ceccherelli & Piazzi, 2001; Renoncourt & Meinesz, 2002; Ruitton et al., 2005b), but habitat characteristics such as substrate type and the complexity of native communities are also a determinant. As described for other Mediterranean localities (Ruitton et al., 2005a; Žuljević et al., 2003; Piazzi et al., 2005; Klein & Verlaque, 2008; Piazzi & Balatta, 2009), the alga fully colonised a variety of substrates and biocenoses present throughout its depth distributional range (i.e. rocky photophilic algae, dead rhizomes of *P. oceanica*, detritic soft bottoms and maerl beds), with the exception of *P. oceanica* meadows and unstable soft-bottoms. Accordingly, other studies have shown that *P. oceanica* meadows are among the most resistant habitats to invasion (Occhipinti-Ambrogi & Savini, 2003; Piazzi & Cinelli, 1999; Katsanevakis et al., 2010; Infante et al., 2011). Penetration of the algae in *P. oceanica* meadows only occurs in low-density canopies (Ceccherelli et al., 2000; Montefalcone et al., 2007.)

Biometric analysis of *C. racemosa* meadows indicated a high degree of vegetative development in the studied colonies. The mean values of biometric descriptors were within the ranges observed in other invaded localities of the Western Mediterranean at a similar depth range (Buia et al., 2001; Ruitton et al., 2005b; Capiomont et al., 2005; Klein & Verlaque, 2008; Cebrian & Ballesteros, 2009). The mean values of total stolon length found in this study (3–4.5 km/m²) were higher than the maximum values reported in these studies for a similar season (summer–autumn: 0.3–1.2 km/m²; Capiomont et al., 2005; Ruitton et al., 2005b; Ivesa & Devescovi, 2006; Cebrian & Ballesteros, 2009). This extensive stolon development indicates overgrowth over the colonised substrate (Capiomont et al., 2005) and hence, a high potential impact on the native assemblages, particularly in those with lower vertical stratification (Balata et al., 2004; Piazzi & Balata, 2009) such as the maerl beds observed in deeper stations (e.g. station 14: Calblanque, 25 m). Nonetheless, the clear reduction of algal biomass with the depth of the sampling station (table 2) suggests that the potential future impact on these deep native assemblages could be lower than that on shallower ones. Of course, there is not a general, consistent vertical pattern of algal abundance across the different invaded localities of the Mediterranean coast, due to local differences in a great variety of abiotic and biotic factors (Cebrian & Ballesteros, 2009). However, the vertical pattern of biomass reported in this and other studies (e.g. De Biasi et al., 1999) suggests that changes of environmental factors associated to depth gradients such as light and temperature (which are also primary factors

of algal growth) could be responsible for the observed reduction of algal biomass with depth. The enlargement of *C. racemosa* fronds observed in the deepest station Calblanque is consistent with results obtained in other deep colonized areas (Cebrian & Ballesteros, 2009) and is a typical photoacclimatory response of these benthic macrophytes to light limitation (e.g. Ohba & Enomoto, 1987; Kirk, 1994), supporting the hypothesis that light reduction could contribute to explain (at least in part) the vertical pattern of algal biomass reported in this study.

After this study, new sightings of the alga has been reported since 2008 confirming its presence along the west coast of Murcia and in the neighbouring province of Almería, indicating that its geographical propagation is still continuing in a westerly direction. Accordingly, *C. racemosa* appeared in Algeria in 2006 (Ould-Ahmed & Meinesz, 2007) and in Ceuta in 2007 (Rivera-Ingraham et al., 2010). The estimated rate of spread of the alga in the Murcia region (at least 265 ha in three years) is higher than that reported in the Marseille region (France) over six years (350% increase; Ruitton et al., 2005a), but is similar to the highest values reported for southern latitudes (coast of Tuscany, Italy) for a similar time period (Piazzi et al., 1997). This evidence, together with the high degree of stolon development reported in this study, suggests that the highly invasive nature of *C. racemosa* on the SE coast of Spain may be favoured by the higher temperatures and irradiance characteristic of these southern Mediterranean latitudes. However, these regional variations should be interpreted with caution due to the low number of studied cases, and the use of different methodologies and experimental conditions (Klein & Verlaque, 2008). It is clear that the invasion by this and other introduced species represents a serious potential threat for native marine communities, but the real ecological consequences and their economic impact (local fisheries, tourism) are subjects that need to be addressed further.

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Appendix 1. Summary of the information related to the 42 sampling stations: localities, coordinates UTM, surveyed depth (colonized depth) and habitat/biocenosis present (RF. Rocky photophilous; RS. Rocky sciaphilous; IS. Infralittoral sands; D. Detritic; DM. Maerl; P. *P. oceanica* meadows; PU. *P. oceanica* meadows, upper limit; PD. *P. oceanica* meadows, deep limit; PM. *P. oceanica*-death 'matt'); S. Station number; L. Locality; D. Depth (m); C. Colonised; NC. Not colonised.

Apéndice 1. Resumen de la información relacionada con las 42 estaciones de muestreo: localidades, coordenadas UTM, profundidad estudiada (profundidad colonizada) y hábitat/biocenosis presente (RF. Rocosos fotófilo; RS. Rocosos esciófilo; IS. Arenas infralitorales; D. Detrítico; DM. Maerl; P. Praderas de *P. oceanica*; PU. Praderas de *P. oceanica*, límite superior; PD. Praderas de *P. oceanica*, límite de profundidad; PM. "Matas muertas" de *P. oceanica*); S. Número de la estación; L. Localidad; D. Profundidad (m); C. Colonizado; NC. No colonizado.

S	L	UTM			Habitat/biocenosis								
		X	Y	D	RF	RS	IS	D	DM	P	PU	PD	PM
1	La Manga	703019	4182444	26–27					C				NC
2	Tomás Maestre	700822	4179442	5–7								NC	
3	Isla Grosa	701766	4178400	4–8								NC	
4	Isla Grosa	701985	4177946	4–12	C							NC	C
5	Cala Túnez	703513	4168161	5–8			NC					NC	
6	Piles I	704710	4168591	18–22 (20)	C								
7	Piles II	704991	4168756	10–20	NC								
8	Bajo de en medio	706008	4168409	10–20	NC								
9	Isla Hormiga	707082	4170103	10–25	NC	NC			C				
10	C. Escalera–somera	703946	4167629	10–12	NC							NC	
11	C. Escalera–profunda	703966	4167543	20–24				NC				NC	
12	La Barra	702954	4167457	2–4	C							NC	
13	Los Punchosos	702866	4166766	5–13 (7)	C							NC	
14	Calblanque	700052	4161847	25–26					C				NC
15	Calblanque	693773	4160728	27					NC				NC
16	Calblanque	694689	4160208	25					NC				NC
17	Calblanque	695176	4159498	29					NC				NC
18	Calblanque	697283	4159339	27				NC					NC
19	Cabo Negrete	697404	4159360	27					C				NC
20	Cabo Negrete	697569	4159519	27					NC				NC
21	El Gorguel	687416	4160406	5–17	NC								
22	Cabo de Agua	683908	4158528	5–17	NC		NC				NC		
23	Punta del Aguilón	682494	4158927	5–17	NC			NC					NC
24	Isla de las Palomas	673129	4160784	19–22				NC					NC
25	C. Tiñoso	664394	4156520	5–30	C	C		C	C	C			
26	C. Tiñoso	663125	4156704	0–12	NC							NC	
27	C. Tiñoso	663172	4156648	21–25				NC					NC
28	La Azohía	661074	4157999	17–21			NC				NC		
29	Mazarrón	656472	4159510	17–21				NC					NC
30	Mazarrón	655306	4159294	17–21				NC					NC
31	Bolnuevo	646505	4157273	21				NC					NC
32	Bolnuevo	645186	4156116	24–25				NC					NC

