Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area

Stelios Katsanevakis, Yiannis Issaris, Dimitris Poursanidis, Maria Thessalou-Legaki

► To cite this version:

Stelios Katsanevakis, Yiannis Issaris, Dimitris Poursanidis, Maria Thessalou-Legaki. Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area. *Marine Environmental Research*, 2010, 70 (2), pp.210. 10.1016/j.marenvres.2010.05.003 . hal-00602594

HAL Id: hal-00602594

<https://hal.science/hal-00602594>

Submitted on 23 Jun 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area

Authors: Stelios Katsanevakis, Yiannis Issaris, Dimitris Poursanidis, Maria Thessalou-Legaki

PII: S0141-1136(10)00070-X

DOI: [10.1016/j.marenvres.2010.05.003](https://doi.org/10.1016/j.marenvres.2010.05.003)

Reference: MERE 3450

To appear in: *Marine Environmental Research*

Received Date: 12 March 2010

Revised Date: 6 May 2010

Accepted Date: 7 May 2010

Please cite this article as: Katsanevakis, S., Issaris, Y., Poursanidis, D., Thessalou-Legaki, M. Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area, *Marine Environmental Research* (2010), doi: 10.1016/j.marenvres.2010.05.003

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa*
var. *cylindracea* within a marine protected area**

Stelios Katsanevakis^{a*}, Yiannis Issaris^{a,b}, Dimitris Poursanidis^{c,d}, Maria Thessalou-
Legaki^b

^a *Institute of Marine Biological Resources, Hellenic Centre for Marine Research,
Anavyssos 19013, Greece*

^b *Department of Zoology-Marine Biology, School of Biology, University of Athens,
Panepistimioupolis, 15784 Athens, Greece*

^c *WWF Hellas, Glaukou 13, 71409 Knossos, Heraklion, Greece*

^d *Department of Marine Sciences, University of the Aegean, Mytilene, 81100, Greece*

* Corresponding author (tel: +30 2109856701, fax: +30 2109811713)

Emails:

SK: stelios@katsanevakis.com

YI: yissaris@gmail.com

DP: dpoursanidis@gmail.com

MT-L: mthessal@biol.uoa.gr

Abstract

The relative vulnerability of various habitat types to *Caulerpa racemosa* var. *cylindracea* invasion was investigated in the National Marine Park of Zakynthos (Ionian Sea, Greece). The density of *C. racemosa* fronds was modelled with generalized additive models for location, scale and shape (GAMLSS), based on an information theory approach. The species was present in as much as 33% of 748 randomly placed quadrats, which documents its aggressive establishment in the area. The probability of presence of the alga within randomly placed 20 x 20 cm quadrats was 83% on ‘matte morte’ (zones of fibrous remnants of a former *P. oceanica* bed), 69% on rocky bottoms, 86% along the margins of *Posidonia oceanica* meadows, 10% on sandy/muddy substrates, and 6% within *P. oceanica* meadows. The high frond density on ‘matte morte’ and rocky bottoms indicates their high vulnerability. The lowest frond density was observed within *P. oceanica* meadows. However, on the margins of *P. oceanica* meadows and within gaps in fragmented meadows relative high *C. racemosa* densities were observed. Such gaps within meadows represent spots of high vulnerability to *C. racemosa* invasion.

Key words: benthic ecology; biological pollutants; *Caulerpa racemosa*; invasive macrophytes; phytobenthos; seagrass; vulnerability; Mediterranean; MPA; Greece

1. Introduction

More than 900 introduced species have been reported so far from the Mediterranean Sea, of which ~500 are considered as established. Among them, there are more than 100 established alien macrophytes (Zenetos et al., 2008). Approximately one tenth of these species can be considered as invasive, i.e. they have overcome biotic and abiotic barriers, are able to disseminate away from their area of initial introduction, and have a noticeable ecological and economic impact. Decline of biodiversity, effects on phytobenthic, fish and invertebrate biota, threat to the ecological stability of invaded ecosystems, and pressures on economic activities are among the impacts of invasive macrophytes (Boudouresque and Verlaque, 2002; Zenetos et al., 2006; Schaffelke and Hewitt, 2007; Piazzì and Balata, 2009).

The green alga *Caulerpa racemosa* (Forsskål) J. Agardh *sensu lato* (Chlorophyta: Bryopsidales) is widely distributed in tropical and warm temperate seas (Verlaque et al. 2003). Three intraspecific taxa of *C. racemosa* have been identified in the Mediterranean Sea, among which *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman, and Boudouresque (Verlaque et al., 2003; Klein and Verlaque, 2008) (hereafter *C. racemosa*) is one of the most aggressive invaders in the basin (Streftaris and Zenetos, 2006; Verlaque et al., 2004; Klein and Verlaque, 2008). This variety was first found in the Mediterranean Sea along the Libyan coast in 1990 (Nizamuddin, 1991), being introduced from south-western Australia (Famà et al., 2000; Verlaque et al., 2003). It has rapidly spread throughout the entire basin, reaching as far as the Canary Islands in the Eastern Atlantic Ocean (Verlaque et al., 2004). The species has been reported from all kind of substrata with a variety of benthic assemblages,

between 0 and 70 m depth, in both polluted and unpolluted areas, and has demonstrated an excessive rate of proliferation and high adaptability to physical stressors (Piazzi et al., 2005; Streftaris and Zenetos, 2006; Klein and Verlaque, 2008; Tsiamis et al., 2008). Its stolons can quickly elongate and easily overgrow other macroalgal (Piazzi et al., 2001; Piazzi and Ceccherelli, 2006) or invertebrate species (Kružić et al., 2008; Baldacconi and Corriero, 2009). Quite often *C. racemosa* forms compact meadows in the Mediterranean Sea (Argyrou et al., 1999; Ruitton et al., 2005; Klein and Verlaque, 2008; Katsanevakis and Thessalou-Legaki, 2009), contrary to its native range in south-western Australia, where it does not form monospecific stands but grows intermixed with other algae (Verlaque et al., 2003). It has a high potential for dispersal by fragmentation and re-establishment of stolons (Ceccherelli and Piazzi, 2001), formation of propagules (Renoncourt and Meinesz, 2002), and sexual reproduction (Panayotidis and Žuljević, 2001).

C. racemosa was initially reported in Greece from Zakynthos Island and Pylos Bay (Ionian Sea) in 1993 (Panayotidis and Montesanto, 1994). Since then, the species has been spreading extremely rapidly along Greek coasts and it is now found on the majority of the Greek coastal areas (Tsiamis et al., 2010; Salomidi et al., 2009).

There are a few studies in the literature comparing invasion success of *C. racemosa* on different substrates such as varying shoot densities of *Posidonia oceanica* (L.) Delile meadows (Ceccherelli et al., 2000) or different types (encrusting, filamentous, and erect) of vegetation layers (Ceccherelli et al., 2002). However, the extent to which biotic and abiotic interactions influence the spread of the alga is not fully understood, although there are some relevant efforts (e.g., Bulleri and Benedetti-Cecchi, 2008). So

far, there is no comparative study investigating the relative vulnerability of the main Mediterranean habitat types to *C. racemosa* invasion. To provide such insight, in the present study we investigated the relative vulnerability of a variety of habitat types to *C. racemosa*, in one of the sites of its first appearance in the early 90's (Laganas Bay, Zakynthos Island, Ionian Sea). Special focus was given on various substrate types within fragmented *Posidonia oceanica* meadows. Hereafter, vulnerability is defined as the susceptibility to the introduction, establishment and spatial spread of the invader, i.e. how easily it may attain high densities.

Until lately Marine Protected Areas (MPAs) were established to protect biodiversity via the complete removal of human exploitation and other activities, and thus were intended to be "islands" of nature and tranquility in a sea of hostile environment (Simberloff 2000; Boudouresque et al. 2005). However, in many cases MPAs have not been able to stop or limit the expansion of invasive alien species. For example, the boundaries of many northwestern Mediterranean marine parks offered no protection to the invasion of the green alga *Caulerpa taxifolia*, once it was present along neighboring coasts (Meinesz 1999). International shipping is usually prohibited in MPAs but recreational or small fishing boats could equally well be the vectors of alien species introductions. Indeed an assessment of the importance of recreational boating carried out in 100 yachts in Scotland has confirmed that boating must be considered as a high risk vector for non-native species (Ashton et al. 2006). The spreading of both *Caulerpa racemosa* and *C. taxifolia* in the western Mediterranean was facilitated by fishing activity, in particular by bottom trawlers and trammel nets (Relini et al. 2000; Magri et al 2001). Restrictive measures on the activities of recreational and fishing boats within MPAs might have a positive effect by

decelerating the invasion rates of some alien macrophytes. Another aim of this study was to investigate whether protection measures within an MPA (the National Marine Park of Zakynthos) had any effect on *C. racemosa* spread.

Specifically, we tested the following hypotheses:

1. Various habitat types have different vulnerability to the invasive alga *C. racemosa*.
2. Within fragmented *Posidonia oceanica* meadows vulnerability differs among structural substrate types such as the meadow margin and gaps of various sizes without alive *P. oceanica*.
3. Protection measures in MPAs, especially restrictions on recreational boating and fishing activities, effectively restrain the expansion of *C. racemosa*.

For all the points above we aimed to quantify the potential differences among habitats/substrate types and management zones by appropriately modelling the frond density of *C. racemosa*.

2. Materials and Methods

2.1 Study area

The survey was conducted in the National Marine Park of Zakynthos (NMPZ) (Fig. 1). The bay of Laganas in Zakynthos Isl. hosts the most important known nesting aggregation of the endangered loggerhead sea turtle, *Caretta caretta* (Linnaeus,

1758), in the Mediterranean. NMPZ was founded in 1999, with the protection of the critical nesting habitat of the loggerhead sea turtle being its main objective.

Within the marine area of the NMPZ there are three main management zones (A, B, C) in Laganas Bay and a buffer zone (Ia) (Fig. 1). In Zone A no recreational boat activity is allowed, in Zone B boats are allowed to enter with a speed limit of 6 knots but anchoring is forbidden, and in Zone C boats are allowed to travel with a speed limit of 6 knots and also to anchor. Commercial fishing using mobile gear and recreational fishing is prohibited in all zones (including the buffer zone) and commercial fishing using static gears is prohibited in Zone A from the beginning of May until the end of October. The management zones do not conform to some specific features of the marine environment, but they were established almost exclusively on criteria regarding undisturbed approach of turtles to the nesting beaches. The three management zones and the buffer zone provide a quasi-experimental setting of varying level of protection (from very low in Ia to high in zone A), which was exploited in this study to test our third hypothesis.

2.2 Field work

Field work was conducted during the first fortnight of October 2009. Two independent surveys were conducted. The aim of the first survey was to assess vulnerability of all main habitat types of the survey area, while the second survey focused on *Posidonia oceanica* beds, specifically on the relative vulnerability of different substrate types within fragmented meadows. Fragmented meadows are defined as those where the continuity of the meadow is interrupted by gaps of various

sizes without alive *P. oceanica* (mentioned as ‘patches’ hereafter), which could be either sandy or ‘matte morte’ areas, i.e. zones of fibrous remnants (dead rhizomes) of a former *P. oceanica* bed.

In the first survey, three 50-m line transects were surveyed with SCUBA diving in each of 23 sites. The selection of the 23 sites was based on the following requirements: (1) good spatial coverage of the study area; (2) representative samples on all major habitat types of the study area; (3) narrow range of surveyed depths. The latter requirement was to avoid adding depth as an extra covariate, as there is an important bathymetry-related variation of *C. racemosa* density (Salomidi et al., 2009), and also for logistical reasons for diving. Thus, all measurements were conducted at depths between 5 and 13 m. The above restrictions caused a non-uniform spatial distribution of the sampling sites in the study area. Specifically, the central regions of zones B and Ia were not sampled as they were generally deep (reaching 70 m in zone Ia), while the shallow regions (north part of zone B) had exclusively sandy bottoms (all other habitat/substrate types were absent).

The line transects were defined with a nylon line, marked with water-resistant numbered signs every five meters, that was deployed using a diving reel. After deploying each line transect, the number of *C. racemosa* fronds within a 20x20 cm quadrat was counted at each sign, i.e. eleven measurements were conducted along each line. For each quadrat, the habitat type was also logged. Five different habitat types were identified: unvegetated sandy bottoms (S); *Posidonia oceanica* meadows (PO); ‘matte morte’ (MM); margins of *P. oceanica* meadows, usually with sandy

bottoms or ‘matte morte’ (PM); and rocky bottoms (R). Although, PM is strictly not a separate ‘habitat type’ than PO, we catachrestically use this terminology hereafter.

In the second survey, the number of *C. racemosa* fronds within 20x20 cm quadrats was measured in a total of 44 patches distributed in 11 sites (Fig. 1), where fragmented *P. oceanica* meadows was the dominant ecotope. In each site, a 200-m line was deployed defining a straight line transect. When the line crossed a patch, frond measurements were conducted in six locations along the line, representing three different substrate types: at the two intersections of the patch margin and the line (PM), within the *P. oceanica* meadow at a 2 m distance from the margin of the patch (PO), and in two randomly chosen spots within the patch (Pa) (Fig. 2). The size of the patch, defined as its maximum dimension (in m), was also recorded.

2.3 Modelling frond counts

Some distinctive features of data collected on the abundance of organisms (count data) are: (i) the tendency to contain many zero values (zero-inflated dataset); (ii) overdispersion, defined as the extra variation, which is not explained by the Poisson distribution alone; (iii) heterogeneity of variances (where the variance of the distribution of the response variable changes with explanatory variables). Failure to account for these issues will cause bias in parameter estimations and the associated measures of uncertainty, can reduce the capability to detect relationships, or even lead to incorrect inference (Martin et al., 2005; Stasinopoulos and Rigby, 2007).

Herein, generalized additive models for location, scale and shape (GAMLSS; Rigby and Stasinopoulos, 2005) were applied to effectively confront the abovementioned issues. GAMLSS are regression type models that overcome many of the limitations associated with the popular generalized linear models (GLM; McCullagh and Nelder, 1989) and generalized additive models (GAM; Hastie and Tibshirani, 1990). In GAMLSS the exponential family distribution assumption for the response variable is relaxed and replaced by a general distribution family, and the systematic part of the model is expanded to allow modelling not only of the mean (or location) but also other parameters of the distribution (Stasinopoulos and Rigby, 2007). For fitting the models, the *gamlss* package (Stasinopoulos and Rigby, 2007) was used in R v.2.8.0 (R Development Core Team 2008).

Inference was based on the information theory approach (Burnham and Anderson, 2002), i.e. an integrated process of *a priori* specification of a set of candidate models, model selection based on the principle of parsimony according to Akaike's information criterion (AIC; Akaike, 1973), and the estimation of parameters and their precision.

For data collected in the first survey, two categorical variables, habitat type (*habitat*) and the corresponding management zone of the NMPZ (*zone*) were used as potential predictor variables of the *C. racemosa* frond counts. Seven different GAMLSS, g_i , $i = 0$ to 6, were fitted (Table 1). Either only the mean response (models g_1 – g_3) or both the mean response and the standard deviation (models g_4 – g_6) were modelled, with one or both predictor variables, i.e. in models g_1 – g_3 the standard deviation was assumed

constant, while in g_4 – g_6 dependent on the predictor variables. Model g_0 was the null model, with no predictor variable, while g_6 was the full model.

To identify the best underlying distribution of the response variable, six potential distributions were compared: Poisson, negative binomial (Anscombe, 1950), zero-inflated Poisson (Lambert, 1992), Poisson-inverse Gaussian (Dean et al., 1989), Sichel (Rigby et al., 2008), and Delaporte (Rigby et al., 2008). Comparisons among different error distributions were based on the full model (g_6), and selection was based on AIC.

The AIC differences, $\Delta_i = AIC_i - AIC_{\min}$, were computed over all candidate models.

Models with $\Delta_i > 10$ had essentially no support and could be omitted from further consideration, while all models with $\Delta_i < 2$ had substantial support (Burnham and Anderson 2002). To quantify the plausibility of each model, given the data and set of six models, the ‘Akaike weight’ w_i of each model was calculated, where

$w_i = \exp(-0.5\Delta_i) / \sum_j \exp(-0.5\Delta_j)$. The ‘Akaike weight’ is considered as the weight of

evidence in favour of model i being the actual best model of the available set of models (Akaike, 1983; Buckland et al., 1997; Burnham and Anderson, 2002).

Estimates of the relative importance of predictor variables j was made by summing the Akaike weights across all the models in the set where each variable occurs [$w_+(j)$]; the larger the sum of Akaike weights the more important that variable is, relative to the other variables (Burnham and Anderson, 2002).

For data collected in patches within *P. oceanica* meadows (second survey), two potential predictor variables were included in the candidate models, patch size (*size*),

which was a continuous variable, and the location of the sampled quadrat in the region of the patch (*location*). *Location* was a categorical variable with three levels (Pa, PM, and PO) (Fig. 2). Seven different GAMLSS, h_i , $i = 0$ to 6, were fitted (Table 2). Either only the mean response (models h_1 – h_3) or both the mean response and the standard deviation (models h_4 – h_6) were modelled, with one or both predictor variables. Model h_0 was the null model, with no predictor variable, while h_6 was the full model. The same approach as with the data of the first survey was followed for the identification of the best underlying statistical distribution and for model selection.

3. Results

3.1 Vulnerability of habitat types (Survey 1)

Among the six tested distributions, negative binomial gave the lowest AIC and was selected as the underlying distribution for modeling the counts of *C. racemosa* fronds (Table 3). The Delaporte distribution also had substantial support by the data with only 1.9 units higher AIC score, while all other distributional assumptions were not supported ($\Delta_i \gg 10$).

The best models of the expected number of *C. racemosa* fronds were g_4 ($w_4 = 49.7\%$) and g_6 ($w_6 = 50.3\%$) with almost equal support, while all other models had essentially no support ($\Delta_i \gg 10$). The habitat type was more important as predictor variable of *C. racemosa* frond counts [$w_+(habitat) = 1.00$] than zone [$w_+(zone) = 0.50$]. Model g_4 was nested within g_6 (full model), and their only difference was in the inclusion of

zone in g_6 . In relation to habitat type (*habitat*) the output of the two models did not differ, and thus only g_6 is presented herein (Fig. 3). Models that considered constant variance (g_0 – g_3) had essentially no support ($\Delta_i \gg 10$) indicating that heterogeneity of variances could not be ignored in our case. As assessed by normal QQ-plots (plots of the quantiles of the normal distribution against the distribution of the residuals), the normalized residuals of model g_6 behaved well, while in model g_3 , which is the same as g_6 for the mean response but did not include standard deviation modelling, the residuals deviated from the normal distribution (Figure 4).

C. racemosa was found in 245 of the 748 quadrats that were sampled (33%). The probability of presence within a randomly placed 20 x 20 cm quadrat was 83% in MM, 69% in R, 86% in PM, 10% in S, and 6% in PO. The frond density was the highest on matte morte and rocky bottoms (mostly rocky reefs with photophilic macroalgal assemblages, dominated by *Cystoseira* spp.) and the lowest within *P. oceanica* meadows (Fig. 3; Table 4). *C. racemosa* was completely absent in unfragmented robust *P. oceanica* beds, and was only recorded in a few spots of low *P. oceanica* density in fragmented meadows. On the margins of *P. oceanica* meadows, intermediate *C. racemosa* densities were observed. On sandy bottoms *C. racemosa* density was generally low and highly variable (Fig. 3). Similar values of frond density were observed in the three management zones of the NMPZ (A, B, and C), while a slightly lower mean value was observed in the buffer zone Ia (Fig. 3; Table 4).

3.2 Vulnerability of fragmented *P. oceanica* meadows (Survey 2)

Among the six tested distributions, negative binomial gave the lowest AIC and was selected as the underlying distribution for modeling the counts of *C. racemosa* fronds (Table 3). The Delaporte distribution also had substantial support, the Sichel distribution had substantially less support, while all other distributional assumptions were not supported ($\Delta_i \gg 10$).

The best model of the expected number of *C. racemosa* fronds in the region of patches within *P. oceanica* meadows was h_4 ($w_4 = 59.6\%$) (Table 2). Model h_6 also had substantial support ($w_6 = 23.0\%$), and models h_1 and h_3 had essentially less support. The other models were not supported by the data ($\Delta_i \gg 10$). Location was more important as a predictor variable of *C. racemosa* frond counts [$w_+(location) = 1.00$] than patch size [$w_+(size) = 0.33$]. Only model h_4 is presented herein; in h_6 size was included as an additional variable but it was not significant either for the mean ($p = 0.22$) or for the standard deviation ($p = 0.72$), thus h_6 did not provide any additional information and was not investigated further. Models that assumed constant variance (g_0-g_3) had less support [$w_+(g_0-g_3) = 17.4\%$] than models assuming heterogeneous variances [$w_+(g_4-g_6) = 82.6\%$]. Model h_4 provided a good absolute fit, as assessed by the Q-Q plot and the distribution of normalized residuals.

C. racemosa was present in 50% of the quadrats in PM, 19.3% of those in Pa, and only 4.5% of those in PO. The frequency of presence of the alga in PM was significantly higher than the frequency of presence in Pa (chi-square test, $p < 0.001$). The frond density in Pa (2.0–8.5 fronds in 20 x 20 cm quadrats; 95% CI) was not significantly different ($p = 0.19$) than the frond density in PM (4.7–11.3 fronds) but was significantly higher ($p < 0.001$) than the frond density in PO (0.03–0.32 fronds)

(Fig. 5). Conditional on presence, the counts of *C. racemosa* fronds did not differ between Pa (7.8–12.9 fronds) and PM (9.4–13.3 fronds) but were significantly lower in PO (1.1–3.3 fronds) (95% Tukey test, based on log-transformed counts to homogenize variances).

4. Discussion

The habitat types investigated were found to have markedly different vulnerabilities to the *Caulerpa racemosa* invasion. The highest frond densities were observed in matte morte and rocky habitat types, indicating their high vulnerability. On sandy/muddy bottoms the frond density was substantially lower indicating lower vulnerability of this habitat type, especially in unstable soft substrata. Similar qualitative observations have been reported elsewhere, e.g. at Marseilles, dense *C. racemosa* meadows have colonized matte morte areas and coarse sediment but the sandy bottoms were only poorly colonized by few stolons (Ruitton et al., 2005). Under certain conditions, *C. racemosa* may form compact dense mats on soft bottoms but such mats seem to mostly occur in matte morte zones (Argyrou et al., 1999; Ruitton et al., 2005) or areas where native meadows pre-existed, e.g. in *Caulerpa prolifera* beds (S.K., pers. obs.), and not in previously unvegetated sandy/muddy bottoms.

Probability of *C. racemosa* presence in *P. oceanica* meadows was low and the alga was mostly restricted in the narrow margins of the seagrass meadows and in gaps within fragmented meadows. As demonstrated by this study, *C. racemosa* average density was high in the meadow margins and in patches, while it abruptly declined to

practically zero within the meadow at a short distance from the margin. Thus, in fragmented seagrass meadows, the sandy or matte morte patches represent the spots of high vulnerability to *C. racemosa* invasion. As indicated by the significantly higher probability of *C. racemosa* presence in PM than in Pa locations, the margins of patches represent the preferable location within a fragmented meadow for the successful establishment of the alga. Other studies have indicated that both the vertical and horizontal algal growth is negatively influenced by seagrass density, and dense and healthy *P. oceanica* meadows represent a barrier to the colonization of *C. racemosa* (Ceccherelli et al., 2000; Klein and Verlaque, 2008).

The above results seem to be partly due to the dispersal mechanism of the species by fragmentation, which is one of the main reasons for the fast spread of *C. racemosa* in the Mediterranean. Fragments of the species, produced by either natural or anthropogenic (fishing gear, anchoring) processes, have to stabilize for some time on the substrate in order to get firmly anchored with their rhizoids and become resistant to hydrodynamic forces. Once anchored, the stolons will elongate and the clone will quickly expand locally. Matte morte, rocky habitats, and the margins of *P. oceanica* meadows provide appropriate substrates with many overhangs and other spots of easy attachment of algal fragments, contrary to sandy/muddy substrates. For example, fragments drifting by waves or currents will easily be brought to a halt when they reach the margin of a sea grass meadow as they will get entangled to sea grass rhizomes or leaves. Similarly, on sandy/muddy bottoms it is not uncommon to observe *C. racemosa* fragments attached to small 'hard' objects such as marine litter or sponge colonies, while the alga is completely absent from everywhere else in the surrounding soft bottom (SK, pers. obs.). Such hard objects probably act as anchoring

points of drifting *C. racemosa* fragments. We assume that this mechanism of attachment of algal fragments is the cause of a substantial part of the variation of *C. racemosa* frond density among habitat/substrate types.

In seagrass beds that are not in a good state (high fragmentation, large areas of *matte morte*, low shoot density) vulnerability to *C. racemosa* invasion would be higher. Scars created by anchoring in seagrass beds will also favour the distributional expansion of the alga. It has been demonstrated that boat anchoring has a destructive impact on seagrass meadows destroying many shoots and creating scars that can take a long-time to be re-colonized, especially in meadows of slow growth seagrass species such as *P. oceanica* (Francour et al., 1999; Milazzo et al., 2004; Ceccherelli et al., 2007; Montefalcone et al., 2008). Thus, anchoring in *P. oceanica* beds creates favourable microhabitats for the settlement and expansion of *C. racemosa*. In this respect, a ban of anchoring in seagrass beds, apart from being a management measure for the protection of the meadow, is a measure against the further distributional expansion of *C. racemosa*.

Fishing, especially with mobile gears, such as otter trawls and boat seines, but also with trammel nets, substantially contributes to the spread of *Caulerpa* spp (Relini et al. 2000; Magri et al 2001). Fragments of the species are collected by fishing gear and then discarded by fishermen together with all by-catch and debris, sometimes far from the fishing site. In a similar way recreational boats may assist the dispersion of *C. racemosa* by fragmenting its stolons during anchoring and even transferring such fragments to the surface seawater layers from where longer dispersion is likely. Currents may further disperse the fragments created by fishing gear or anchoring and

the alga may settle and get established in a very distant location. Even in zones of absolute protection within MPAs, where both fishing and anchoring are prohibited, such fragments may arrive with currents from neighbouring sites with no such restrictions. In addition, the ability of *C. racemosa* to reproduce sexually renders any protection measures in MPAs inefficient to prevent the introduction of the species from other locations.

Commercial fishing with nets is allowed in all management zones of NMPZ and in all neighbouring areas with only a six-month ban in zone A; anchoring is permitted within zone C. Due to the existence of the marine park and the developed eco-touristic industry focusing on turtle-watching, there is an increased number of recreational boats sailing in zones B and C and anchoring in zone C. As NMPZ is a semi-closed bay, *C. racemosa* fragments that are produced within any of the protection zones are likely to remain within the boundaries of NMPZ. Thus, the role of fishing gear and recreational boats to the further spread of the species in NMPZ cannot be avoided by the existing protection measures. The operation of NMPZ for the last eleven years did not seem to decelerate the spread of *C. racemosa* in Laganas Bay. On the contrary, the species is found in the NMPZ with very high probabilities of presence within randomly placed 20 x 20 cm quadrats on matte morte areas, rocky bottoms, and the margins of *P. oceanica* meadows. For the reasons stated above (role of fishing gear and anchoring, ability of sexual reproduction, further expansion of existing stolons) no direct effect of the protection measures to the spread of the species is anticipated in the future, unless the protection measures will prove effective to improve the status of *P. oceanica* meadows within NMPZ (mainly because of the ban of anchoring and use of mobile fishing gear) and thus reduce favourable microhabitats, such as patches

within meadows. However, the process of sea grass recovery is very slow and no short-term effects are likely. If the management measures prove ineffective to protect the *P. oceanica* meadows in the park and fragmentation increases in the future, *C. racemosa* will further spread covering all new matte morte areas.

Although the vulnerability of seagrass meadows to *C. racemosa* has been sufficiently investigated, there is a lack of knowledge on the vulnerability of rocky bottoms. The type of macroalgal assemblages (turf, encrusting, and erect) and the number of algal species seem to affect the *C. racemosa* invasion, with substrates dominated by turfing algae being more vulnerable (Ceccherelli et al., 2002). Turfs probably physically assist the settlement of *C. racemosa* by entrapment of fragments of the alga and/or enhancing the anchoring of stolons (Bulleri and Benedetti-Cecchi, 2008). However, the extent of the invasion of *C. racemosa* on rocky bottoms, the invasion rates, impacts to and competition with the benthic fauna, and the factors that favour successful settlement and high densities are issues that need further investigation. As found in this study, but also based on our personal observations in other areas of the Ionian and the Aegean Sea, the extent of *C. racemosa* invasion on rocky habitats is dramatic and the consequences largely unknown.

In conclusion: (1) we verified that vulnerability to *C. racemosa* invasion changes with habitat and the most vulnerable habitat/substrate types are matte morte areas, the margins of *P. oceanica* meadows, and rocky bottoms; (2) within fragmented *Posidonia oceanica* meadows vulnerability differs among structural substrate types and is higher along the margins of patches with no apparent dependence on patch size;

and (3) the boundaries of the NMPZ do not seem to offer any protection against *C. racemosa* invasion, which is explained by the dispersal mechanism of the species.

Acknowledgements

This study was part of the project “Survey of the Marine Benthic Fauna in the National Marine Park of Zakynthos”, funded by EU and national sources (coordinator: M.T.-L.). We thank the park authorities and staff for their valuable assistance during the survey. We are grateful to three anonymous reviewers who made valuable comments and suggestions that improved the quality of this paper.

References

- Akaike, H., 1973. Information theory as an extension of the maximum likelihood principle, in: Petrov, B.N., Csaki, F. (Eds.), Proceedings of the Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 267-281.
- Akaike, H., 1983. Information measures and model selection. Bulletin of the International Statistical Institute 44, 277-290.
- Anscombe, F.J., 1950. Sampling theory of the negative binomial and logarithmic series approximations. Biometrika 37, 358-382.
- Argyrou, M., Demetropoulos, A., Hadjichristophorou, M., 1999. Expansion of the macroalga *Caulerpa racemosa* and changes in soft bottom macrofaunal assemblages in Moni Bay, Cyprus. Oceanologica Acta 22, 517-528.

- 489 Ashton, G., Boos, K., Shucksmith, R., Cook, E., 2006. Risk assessment of hull fouling
490 as a vector for marine non-natives in Scotland. *Aquatic Invasions* 1, 214-218.
- 491 Baldacconi, R., Corriero, G., 2009. Effects of the spread of the alga *Caulerpa*
492 *racemosa* var. *cylindracea* on the sponge assemblage from coralligenous
493 concretions of the Apulian coast (Ionian Sea, Italy). *Marine Ecology* 30, 337-345.
- 494 Boudouresque, C.F., Verlaque, M., 2002. Biological pollution in the Mediterranean
495 Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin* 44, 32-
496 38.
- 497 Boudouresque, C.F., Cadiou, G., Le Diréach, L., 2005. Marine protected areas: a tool
498 for coastal areas management, in: Levner, E., Linkov, I., Proth, J.M. (Eds.),
499 Strategic management of marine ecosystems. Springer, Dordrecht, The
500 Netherlands, pp. 29-52.
- 501 Buckland, S.T., Burnham, K.P., Augustin, N.H., 1997. Model selection: An integral
502 part of inference. *Biometrics* 53, 603-618.
- 503 Bulleri, F., Benedetti-Cecchi, L., 2008. Facilitation of the introduced green alga
504 *Caulerpa racemosa* by resident algal turfs: experimental evaluation of underlying
505 mechanisms. *Marine Ecology Progress Series* 364, 77-86.
- 506 Burnham, K.P., Anderson, D., 2002. Model selection and multi-model inference.
507 Springer, Berlin.
- 508 Ceccherelli, G., Piazzzi, L., 2001. Dispersal of *Caulerpa racemosa* fragments in the
509 Mediterranean: Lack of detachment time effect on establishment. *Botanica*
510 *Marina* 44, 209-213.
- 511 Ceccherelli, G., Piazzzi, L., Cinelli, F., 2000. Response of the non-indigenous
512 *Caulerpa racemosa* (Forsskål) J. Agardh to the native seagrass *Posidonia*

- 513 *oceanica* (L.) Delile: effect of density of shoots and orientation of edges of
 514 meadows. *Journal of Experimental Marine Biology and Ecology* 243, 227-240.
- 515 Ceccherelli, G., Piazzzi, L., Balata, D., 2002. Spread of introduced *Caulerpa* species in
 516 macroalgal habitats. *Journal of Experimental Marine Biology and Ecology* 280,
 517 1-11.
- 518 Ceccherelli, G., Campo, D., Milazzo, M., 2007. Short-term response of the slow
 519 growing seagrass *Posidonia oceanica* to simulated anchor impact. *Marine*
 520 *Environmental Research* 63, 341-349.
- 521 Dean, C., Lawless, J.F., Willmot, G.E., 1989. A mixed Poisson-inverse Gaussian
 522 regression model. *Canadian Journal of Statistics* 17, 171-181.
- 523 Famà, P., Olsen, J.L., Stam, W.T., Procaccini, G., 2000. High levels of intra- and
 524 inter-individual polymorphism in the rDNA ITS1 of *Caulerpa racemosa*
 525 (Chlorophyta). *European Journal of Phycology* 35, 349-356.
- 526 Francour, P., Ganteaume, A., Poulain, M., 1999. Effects of boat anchoring in
 527 *Posidonia oceanica* seagrass beds in the Port-Cros National Park (north-western
 528 Mediterranean Sea). *Aquatic Conservation: Marine and Freshwater Ecosystems*
 529 9, 391-400.
- 530 Hastie, T.J., Tibshirani, R.J., 1990. *Generalized Additive Models*. Chapman and Hall,
 531 London.
- 532 Katsanevakis, S., Thessalou-Legaki, M., 2009. Spatial distribution, abundance and
 533 habitat use of the protected fan mussel *Pinna nobilis* in Souda Bay, Crete.
 534 *Aquatic Biology* 8, 45-54.
- 535 Klein, J., Verlaque, M., 2008. The *Caulerpa racemosa* invasion: A critical review.
 536 *Marine Pollution Bulletin* 56, 205-225.

- 537 Kružić, P., Žuljević, A., Nikolić, V., 2008. The highly invasive alga *Caulerpa*
 538 *racemosa* var. *cylindracea* poses a new threat to the banks of the coral *Cladocora*
 539 *caespitosa* in the Adriatic Sea. *Coral Reefs* 27, 441-441.
- 540 Lambert, D., 1992. Zero-inflated Poisson regression with an application to defects in
 541 manufacturing. *Technometrics* 34, 1-14.
- 542 Magri, M., Piazzzi, L., Serena, F., 2001. La présence de *Caulerpa racemosa* le long
 543 des côtes septentrionales de la Toscane et les conséquences possibles sur
 544 l'activité de pêche, in: Gravez, V., Ruitton, S., Boudouresque, C.F., Le Direac'h,
 545 L., Meinesz, A., Scabbia, G., Verlaque, M. (Eds.), Fourth International Workshop
 546 on *Caulerpa taxifolia*. GIS Posidonie publ., Marseilles, France, pp. 338-344.
- 547 Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J.,
 548 Tyre, A.J., Possingham, H.P., 2005. Zero tolerance ecology: improving
 549 ecological inference by modelling the source of zero observations. *Ecology*
 550 *Letters* 8, 1235-1246.
- 551 McCullagh, P., Nelder, J.A., 1989. *Generalized Linear Models*, second ed. Chapman
 552 and Hall, London.
- 553 Meinesz, A., 1999. *Killer algae*. University of Chicago Press, Chicago.
- 554 Milazzo, M., Badalamenti, F., Ceccherelli, G., Chemello, R., 2004. Boat anchoring on
 555 *Posidonia oceanica* beds in a marine protected area (Italy, western
 556 Mediterranean): effect of anchor types in different anchoring stages. *Journal of*
 557 *Experimental Marine Biology and Ecology* 299, 51-62.
- 558 Montefalcone, M., Chiantore, M., Lanzone, A., Morri, C., Albertelli, G., Bianchi,
 559 C.N. 2008. BACI design reveals the decline of the seagrass *Posidonia oceanica*
 560 induced by anchoring. *Marine Pollution Bulletin* 56, 1637-1645.
- 561 Nizamuddin, M., 1991. *The green marine algae of Libya*. Elga Publisher, Bern.

- 562 Panayotidis, P., Montesanto, B., 1994. *Caulerpa racemosa* (Chlorophyta) on the
 563 Greek coasts. *Cryptogamie Algologie* 15, 159-161.
- 564 Panayotidis, P., Žuljević, A., 2001. Sexual reproduction of the invasive green alga
 565 *Caulerpa racemosa* var. *occidentalis* in the Mediterranean Sea. *Oceanologica*
 566 *Acta* 24, 199-203.
- 567 Piazzì, L., Balata, D., 2009. Invasion of alien macroalgae in different Mediterranean
 568 habitats. *Biological Invasions* 11, 193-204.
- 569 Piazzì, L., Ceccherelli, G., 2006. Persistence of biological invasion effects: Recovery
 570 of macroalgal assemblages after removal of *Caulerpa racemosa* var. *cylindracea*.
 571 *Estuarine, Coastal and Shelf Science* 68, 455-461.
- 572 Piazzì, L., Ceccherelli, G., Cinelli, F., 2001. Threat to macroalgal diversity: effects of
 573 the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Marine*
 574 *Ecology Progress series* 210, 149-159.
- 575 Piazzì, L., Balata, D., Ceccherelli, G., Cinelli, F., 2005. Interactive effect of
 576 sedimentation and *Caulerpa racemosa* var. *cylindracea* invasion on macroalgal
 577 assemblages in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science* 64,
 578 467-474.
- 579 R Development Core Team, 2008. R: A language and environment for statistical
 580 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-
 581 900051-07-0. <http://www.R-project.org> (Accessed 10 November 2008)
- 582 Relini, G., Relini, M., Torchia, G., 2000. The role of fishing gear in the spreading of
 583 allochthonous species: the case of *Caulerpa taxifolia* in the Ligurian Sea. *ICES*
 584 *Journal of Marine Science* 57, 1421-1427.

- Renoncourt, L., Meinesz, A., 2002. Formation of propagules on an invasive strain of *Caulerpa racemosa* (Chlorophyta) in the Mediterranean Sea. *Phycologia* 41, 533-535.
- Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale and shape. *Applied Statistics* 54, 507-554.
- Rigby, R.A., Stasinopoulos, D.M., Akantziliotou, C., 2008. A framework for modelling overdispersed count data, including the Poisson-shifted generalized inverse Gaussian distribution. *Computational Statistics and Data Analysis* 53, 381-393.
- Ruitton, S., Verlaque, M., Boudouresque, C.F., 2005. Seasonal changes of the introduced *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its Mediterranean range. *Aquatic Botany* 82, 55-70.
- Salomidi, M., Tsiamis, K., Katsanevakis, S., Issaris, Y., Panou, A., Zenetos, A., 2009. Invasion of the green alga *Caulerpa racemosa* in the Hellenic coasts. *BIOLIEF*, World Conference on Biological Invasions and Ecosystem Functioning, Porto, Portugal, October 27-30, pp. 53.
- Schaffelke, B., Hewitt, C.L., 2007. Impacts of introduced seaweeds. *Botanica Marina* 50, 397-417.
- Simberloff, D., 2000. No reserve is an island: marine reserves and nonindigenous species. *Bulletin of Marine Science* 66(3), 567-580.
- Stasinopoulos, D.M., Rigby, R.A., 2007. Generalized additive models for location, scale, and shape (GAMLSS) in R. *Journal of Statistical Software* 23, 1-46.
- Streftaris, N., Zenetos, A., 2006. Alien marine species in the Mediterranean - the 100 'worst invasives' and their impact. *Mediterranean Marine Science* 7(1), 87-118.

- 609 Tsiamis, K., Panayotidis, P., Zenetos, A., 2008. Alien marine macrophytes in Greece:
610 a review. *Botanica Marina* 51, 237-246.
- 611 Tsiamis, K., Montesanto, B., Panayotidis, P., Katsaros, C., Verlaque, M., 2010.
612 Updated records and range expansion of alien marine macrophytes in Greece
613 (2009). *Medit Mar Sci* 11(1), in press
- 614 Verlaque, M., Durand, C., Huisman, J.M., Boudouresque, C.F., Le Parco, Y., 2003.
615 On the identity and origin of the Mediterranean invasive *Caulerpa racemosa*
616 (Caulerpales, Chlorophyta). *European Journal of Phycology* 38, 325-339.
- 617 Verlaque, M., Afonso-Carrillo, J., Candelaria Gil-Rodríguez, M., Durand, C.,
618 Boudouresque, C.F., Le Parco, Y., 2004. Blitzkrieg in a marine invasion:
619 *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the
620 Canary Islands (North-East Atlantic). *Biological Invasions* 6, 269-281.
- 621 Zenetos, A., Çinar, M.E., Pancucci-Papadopoulou, M.A., Harmelin, J.G., Furnari, G.,
622 Andaloro, F., Bellou, N., Streftaris, N., Zibrowius, H., 2006. Annotated list of
623 marine alien species in the Mediterranean with records of the worst invasive
624 species. *Mediterranean Marine Science* 6(2), 63-118 [2005].
- 625 Zenetos, A., Meriç, M., Verlaque, P., Galli, P., Boudouresque, C., Giangrande, A.,
626 Çinar, M.E., Bilecenoglu, M., 2008. Additions to the annotated list of marine
627 alien biota in the Mediterranean with special emphasis on Foraminifera and
628 Parasites. *Mediterranean Marine Science* 9(1), 119-165.

Figure captions

Fig. 1: Map of the study area. The sampling sites and the protection zones of the National Marine Park of Zakynthos are indicated.

Fig. 2: Location (representing three different substrate types) of *C. racemosa* frond measurements within patches in fragmented *P. oceanica* meadows. Quadrats were placed at the two intersections of the patch margin and the line (PM), within the *P. oceanica* meadow at a 2 m distance from the margin of the patch (PO), and in two randomly chosen spots within the patch (Pa).

Fig 3: Estimated values of the two categorical variables (*habitat type* and *zone*) for model g_6 of *C. racemosa* frond counts within 20 x 20 cm quadrats. Graphs of the mean response are given in the top panel and graphs of the standard deviation in the bottom panel. Values are given in the response (log) scale and the corresponding 95% confidence intervals are indicated with dotted lines. MM: ‘matte morte’; PM: margins of *P. oceanica* meadows; PO: *Posidonia oceanica* meadows; R: rocky bottoms; S: unvegetated sandy bottoms.

Fig. 4: Normal QQ plots for models g_6 (full model) and g_3 .

Fig. 5: Estimated mean response and standard deviation of *C. racemosa* frond counts in 20 x 20 cm quadrats in relation to the levels of the categorical predictor variable *location*, based on model h_4 . Values are given in the response (log) scale and the

655 corresponding 95% confidence intervals are indicated with dotted lines. The three
656 levels of the predictor variable are defined in Fig. 2.

657

ACCEPTED MANUSCRIPT

Table 1: Survey 1: Summary of the parameterisation of the seven candidate models g_i of the counts of *C. racemosa* fronds in 20 x 20 cm plots.

Model	Modeled distribution parameters	Predictor variables	df	AIC	Δ_i	w_i (%)
g_0	-	Null	2	2748.19	462.82	0.0
g_1	μ	<i>habitat</i>	6	2440.25	154.88	0.0
g_2	μ	<i>zone</i>	5	2744.40	459.03	0.0
g_3	μ	<i>habitat + zone</i>	9	2441.58	156.21	0.0
g_4	μ, σ	<i>habitat</i>	10	2285.39	0.03	49.7
g_5	μ, σ	<i>zone</i>	8	2712.07	426.70	0.0
g_6	μ, σ	<i>habitat + zone</i>	16	2285.37	0.00	50.3

For each model, the Akaike Information Criterion AIC, Akaike differences Δ_i , Akaike weights w_i , and degrees of freedom (df) are given. Models with substantial support ($\Delta_i < 2$) are given in bold. μ, σ are the mean and the standard deviation of the distribution of the response variable.

Table 2: Survey 2: Summary of the parameterisation of the seven candidate models h_i of the counts of *C. racemosa* fronds in 20 x 20 cm plots in the second survey (patches in *P. oceanica* meadows).

Model	Modeled distribution parameters	Predictor variables	df	AIC	Δ_i	w_i (%)
h_0	-	Null	2	825.01	56.21	0.0
h_1	μ	<i>location</i>	4	772.92	4.12	7.6
h_2	μ	<i>size</i>	3	826.80	58.00	0.0
h_3	μ	<i>location + size</i>	5	772.41	3.61	9.8
h_4	μ, σ	<i>location</i>	6	768.80	0.00	59.6
h_5	μ, σ	<i>size</i>	4	828.21	59.41	0.0
h_6	μ, σ	<i>location + size</i>	8	770.71	1.91	23.0

For each model, the Akaike Information Criterion AIC, Akaike differences Δ_i , Akaike weights w_i , and degrees of freedom (df) are given. Models with substantial support ($\Delta_i < 2$) are given in bold. μ, σ are the mean and the standard deviation of the distribution of the response variable.

Table 3: Comparisons among different error distributions of the response variable (count of *C. racemosa* fronds in 20 x 20 cm quadrats), based on the full models (g_6 for survey 1 and h_6 for survey 2).

Model comparisons based on g_6		
Distribution	df	AIC
Negative Binomial	16	2285.4
Delaporte	17	2287.3
Sichel	17	2336.4
Poisson-inverse Gaussian	16	2415.8
Zero Inflated Poisson	16	3083.9
Poisson	8	5067.3

Model comparisons based on h_6		
Distribution	df	AIC
Negative Binomial	8	770.7
Delaporte	9	772.8
Sichel	9	778.0
Poisson-inverse Gaussian	8	839.8
Zero Inflated Poisson	8	916.8
Poisson	4	2249.2

Table 4: Survey 1: Expected counts of *C. racemosa* fronds in 20 x 20 cm quadrats, as predicted by model *g6*.

habitat type	zone A	zone B	zone C	zone Ia
MM	13.5 (9.1-20.1)	14.1 (10.7-18.6)	12.4 (7.7-19.7)	7.9 (5.0-12.3)
PM	5.1 (3.1-8.2)	5.3 (3.2-8.7)	4.6 (2.6-8.3)	2.9 (1.7-5.2)
PO	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.1 (0.1-0.2)
R	10.1 (6.7-15.1)	10.5 (8.2-13.5)	9.2 (5.6-15.0)	5.9 (3.9-8.7)
S	1.0 (0.3-3.9)	1.1 (0.3-3.9)	0.9 (0.2-3.7)	0.6 (0.2-2.3)

95% confidence intervals are given in parenthesis.

standard deviation

standard deviation