Modelling distribution patterns and habitat preference of the invasive green alga *Caulerpa racemosa* in the Saronikos Gulf (Eastern Mediterranean)

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ABSTRACT: Although the invasive alga *Caulerpa racemosa* has exhibited an excessive rate of proliferation and has invaded the entire Mediterranean Sea basin within the last 18 yr, its spatial distribution patterns remain largely unknown. A 2-component modelling approach based on generalized additive models was applied to model the density of *C. racemosa* fronds in relation to spatial and environmental variables. A bimodal distribution of presence probability in relation to depth was observed, with higher probabilities of occurrence at very shallow bottoms (<4 m) and at depths between ~15 and 30 m. Presence probability steadily decreased at greater depths. Frond density was markedly higher in rocky habitats than in *Posidonia oceanica* beds or on sandy/muddy bottoms. The highest frond densities were observed in the southeastern part of the Saronikos Gulf, which is considered to be the area of initial establishment of the species in the Gulf; this indicates that time favours the steady expansion and further dominance of this species.

KEY WORDS: *Caulerpa racemosa* · Generalized additive models · Greece · Invasive macrophytes · Spatial distribution patterns

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INTRODUCTION

Biological invasions in marine habitats represent a recognized worldwide threat that has a strong impact on biodiversity and local economies (Pimentel et al. 2005, Streftaris & Zenetos 2006). The Mediterranean Sea is one of the regions most severely affected by alien marine invasions. According to a recent update, 947 alien species had been reported in the Mediterranean Sea by December 2009, raising the rate of introductions to 1 species every 1.5 wk (Zenetos 2010). Of these introductions, >100 exotic macrophytes have reportedly been established in the Mediterranean Sea (Zenetos et al. 2008).

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). In the Mediterranean, the species is encountered in 3 distinct varieties: (1) *Caulerpa racemosa* var. *turbinata* (J. Agardh) Eubank / *uvifera* (C. Agardh) J. Agardh, which has been observed in the Levantine Sea and Tunisian coasts, (2) *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse, which is mainly observed in the Levantine Sea, and (3) the recently introduced *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman, and Boudouresque, which is widely distributed in the entire Mediterranean (Verlaque et al. 2000, 2003, in press). The last variant was first observed in Libya in 1990 (Nizamuddin 1991), but its identity and origin remained puzzling for at least a decade, until ad hoc morphological and genetic studies classified it as *C. racemosa var. cylin-*

dracea (Sonder) Verlaque, Huisman, and Boudouresque, of southwestern Australian origin (Verlaque et al. 2003, Klein & Verlaque 2008).

This new variety (hereafter, *Caulerpa racemosa*) has rapidly spread throughout the entire Mediterranean basin, reaching as far as the Canary Islands in the eastern Atlantic Ocean (Verlaque et al. 2004). The rate of expansion of *C. racemosa* appears to be among the most significant rates ever recorded for an invasive species (Streftaris & Zenetos 2006, Klein & Verlaque 2008). The species has been reported to occur in at least 14 Mediterranean countries (Albania, Algeria, Croatia, Cyprus, France, Greece, Italy, Libya, Malta, Monaco, Montenegro, Spain, Tunisia, and Turkey) and all major Mediterranean islands. It grows on all kinds of substrata, including both polluted and unpolluted areas between 0 and 70 m depth, and shows an excessive rate of proliferation (Streftaris & Zenetos 2006, Macic & Kascelan 2007, Klein & Verlaque 2008, Tsiamis et al. 2008, P. Francour pers. comm.).

Caulerpa racemosa has stolons that can quickly elongate, and easily overgrow other macroalgal (Piazzi et al. 1997) or invertebrate species (Kružić et al. 2008, Baldacconi & Corriero 2009). It has a high potential for dispersal by fragmentation and re-establishment of stolons (Ceccherelli & Piazzi 2001), formation of propagules (Renoncourt & Meinesz 2002), and sexual reproduction (Panayotidis & Žuljević 2001). This latter characteristic has been suggested to be the most probable explanation for the species' continuous spread in both Mediterranean basins as opposed to its congeneric alien invasive *C. taxifolia,* which only reproduces vegetatively (Meinesz & Hesse 1991, Panayotidis 2006).

In Greece, small patches of *Caulerpa racemosa* were initially reported from 2 oligotrophic eastern Ionian coasts (Zakynthos Island and Pylos Bay) (Panayotidis & Montesanto 1994). These first occurrences were soon followed by new records from other distant Aegean locations (Panayotidis & Montesanto 1998). In the Saronikos Gulf, *C. racemosa* was first observed in 1996, established in an unpolluted bay near the southeastern entrance of the Gulf (Panayotidis & Montesanto 2001), and has rapidly spread from this location (Panayotidis et al. 2006).

Records of *Caulerpa racemosa* in the literature are often imprecise. Simple maps indicating the presence of the species have been compiled, and the length of affected coastlines has been roughly estimated in some countries (Piazzi et al. 2005). However, these analyses were performed without a standardized method (Klein & Verlaque 2008). The patterns of spatial distribution of the species in the Mediterranean Sea and the dependence of algal density on spatial and environmental factors have not been properly assessed so far.

In the present study, generalized additive models (GAMs; Hastie & Tibshirani 1990) were utilized to model the density of *Caulerpa racemosa* fronds to increase our knowledge on the distribution patterns of the species. GAM is a nonparametric technique that offers the required flexibility and does not limit the analysis to the concept of a strict parametric shape. GAMs are increasingly used in ecological studies of the spatial distribution and abundance of marine fauna (e.g. Daskalov et al. 2003, Katsanevakis 2007, Maravelias et al. 2007, Herr et al. 2009, Murase et al. 2009, Laidre et al. 2010) because of the ecological interpretability of the nonparametric response curves and the flexibility of GAMs to fit the data closely. To our knowledge, GAMs have not been used to investigate the spatial distribution and density of any marine flora until the present study.

MATERIALS AND METHODS

Study area. The Saronikos Gulf (see Fig. 1) is an embayment of the south Aegean Sea (E. Mediterranean, Greece) that is surrounded by the Attica peninsula coast in the north and the Peloponnesus peninsula coasts in the west and southwest. The total surface area of the Saronikos Gulf is ~2850 km^2 and stretches along a coastline length of ~744 km. The bottom of the eastern part of the Gulf is relatively flat with a mean depth of 90 m. The western part is deeper and has an elongated north–south trough with maximum depths of ~200 m in the north and ~450 m in the south (Kontoyiannis et al. 2005). Tidal effects are negligible, while seasonal flows are basically induced by thermohaline effects and strongly modified by winds (H. Kontoyiannis pers. comm.). A 48 km wide opening connects the Saronikos Gulf with the Aegean Sea, thus allowing a fast renewal of its water masses (5–10 d for the eastern part of the Gulf; Zeri et al. 2009).

The Saronikos Gulf receives treated sewage effluents from the Athens metropolitan area through a deep underwater outlet situated near Psitallia islet on the northeast of the Gulf. These effluents, as well as pollution from the industrialized areas in the northern and northwestern coasts and the port of Piraeus, are known to account for a distinct ecological quality gradient, which ranges from poor near the outfall, moderate in the western region, and high in the eastern and southern subregions (Orfanidis et al. 2001, Simboura et al. 2005). The Saronikos Gulf is considered as a hotspot of alien macrophytes, partly because of intense shipping (Piraeus is one of the most important ports globally) and the role of the South Aegean Sea as a migration route for Red Sea species entering through the Suez Canal (Lessepsian migrants) and further expanding westwards (Tsiamis et al. 2010).

Fieldwork. Twenty line transects extending from the shore perpendicular to the depth contours were defined in the study area. Unbiased design-based inference requires that the line transects in the study area be randomly placed, or be on a grid of systematically spaced lines that are randomly superimposed on the study area. However, with a model-based approach such as the one used in this study, the line transects are not required to be located according to a formal and restrictive survey sampling scheme, although a good spatial coverage of the study area is desirable. Thus, a sampling scheme that provided good spatial coverage as well as sufficient samples in all primary habitat types was applied. Sampling effort was increased (i.e. more closely placed transects) in areas of high *Caulerpa racemosa* abundance to increase the sample size of nonzero counts. The transect lengths were 200 m, unless a maximum depth of ~40 m before obtaining this length was reached.

A more or less pronounced winter regression has been observed in the cover, biomass, and frond density of *Caulerpa racemosa* in various Mediterranean regions (Klein & Verlaque 2008 and references therein). Due to this seasonal variation in *C. racemosa* frond density, the period of peak density (summer–autumn) was chosen as the most appropriate time for conducting this survey. Thus, all field measurements were performed between 1 August and 15 November 2008.

Each line transect was defined with a nylon line that was deployed using a diving reel while SCUBA diving. The line was marked with water-resistant numbered signs every 5 m. After deploying the line, the number of algal fronds (erect axes) within a 20×20 cm stainless steel quadrat was counted at each sign. For each quadrat, the habitat type and depth (measured by a dive computer) were logged on an underwater plastic slate. Although sampling was principally non-destructive, careful removal of various filamentous epiphytes (mainly Ectocarpales and Cladophorales) was at times necessary to maintain visual access to the understory.

Modelling of frond counts. Inference was based on the information theory approach (Burnham & Anderson 2002). According to this approach, data analysis is taken to mean the integrated process of *a priori* specification of a set of candidate models (based on the science of the problem), 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. Model selection based on information theory is a relatively new paradigm in biological sciences and has been recommended as an improved and more robust alternative than traditional approaches of hypotheses testing (Akaike 1981, Burnham & Anderson 2002).

A distinctive feature of data collected on the abundance of organisms is the tendency to contain many 0 values. When the number of 0s is large such that the data are not readily fitted to standard distributions (e.g. the Poisson distribution), the data set is referred to as 'zero-inflated' (Welsh et al. 1996, Barry & Welsh 2002, Martin et al. 2005). Failure to account for zero-inflation will cause bias in parameter estimations and the associated measures of uncertainty, can reduce the capability to detect relationships, or lead to incorrect inference in the worst scenario (Martin et al. 2005). Several methods have been proposed to deal with zero-inflated datasets (Welsh et al. 1996, Martin et al. 2005). One such approach is to use 2-component models (also known as conditional or hurdle models). In the 2-component modelling approach, the response is modelled as having 2 states: a state in which no 'objects' (fronds in this case) occur and a state in which objects occur with varying levels of abundance. Using this approach, the probability that a species is present is first estimated, and, given that it is present, the relative mean number of individuals observed is then modelled.

GAMs (Hastie & Tibshirani 1990) have the general formulation

$$f(y_i) = LP_i = c + \sum_m s_m(z_{mi}) + \sum_r F_{ri} \quad (1)$$

where y is the response variable, f is the link function, LP is the linear predictor, c is the intercept, $s_m(\cdot)$ is the 1-dimensional smooth function for predictor variable m, z_{mi} is the value of covariate m for plot i, and F_r are categorical predictors.

To model the probability, p, of *Caulerpa racemosa* presence, a binomial error distribution was assumed and a logit link (canonical link for the binomial family) was used, where $f(\mathrm{p}) = \log(\mathrm{p}/1-\mathrm{p})$, i.e. the natural logarithm of the odds of presence (Welsh et al. 1996, Barry & Welsh 2002). Thus, the general GAM becomes

$$\mathrm{logit}(\hat{\mathrm{p}}) = \log(\hat{\mathrm{p}}/1-\hat{\mathrm{p}}) = c + \sum_m s_m(z_{mi}) + \sum_r F_{ri} \quad (2)$$

Four predictor variables were used: (1) *depth*, (2) habitat type (*htype*), (3) longitude (*lon*), and (4) latitude (*lat*). Habitat type was a categorical predictor variable. Four different habitat types were identified: *Posidonia oceanica* meadows (PO), *Cymodocea nodosa* beds (CN), unvegetated sandy/muddy bottoms (S), and rocky bottoms (R). The other 3 covariates were continuous, with depths ranging between 0.3 and 42 m, east longitudes between 23.034° and 24.010°, and north latitudes between 37.460° and 37.975°.

Eight different GAMs (h_i, $i = 0$ to 7) were fitted (Table 1). Model h_0 was the null model, with no predictor variable. The smooth functions $s_m(\cdot)$ were represented using regression splines that were estimated by penalized iterative least squares (Wood 2006). The optimum degree of smoothing was defined by mini-

Table 1. Summary of the parameterisation of the 8 candidate models h_i of the probability of *Caulerpa racemosa* presence. For each model, the Akaike information criteria AICs, Akaike differences Δ_i, Akaike weights w_i, unbiased risk estimator (UBRE) scores, deviance explained, and estimated degrees of freedom (edf) are given. Models with substantial support ($\Delta_i < 2$) are shown in **bold**. *lon*: longitude, *lat*: latitude, *htype*: habitat type

	Model	AIC	Δ_i	w_i (%)	UBRE score	Deviance explained (%)	edf
h_0	Null	351.9	130.7	0.0	0.003	0.0	1.0
h_1	s(*lon*) + s(*lat*)	236.1	14.8	0.0	−0.681	36.2	6.4
h_2	s(*depth*)	353.1	131.8	0.0	−0.522	0.2	2.0
h_3	*htype*	308.1	86.9	0.0	−0.039	14.2	4.0
h_4	**s(*lon*) + s(*lat*) + s(*depth*)**	**221.3**	**0.0**	**82.8**	**−0.701**	**42.8**	**10.5**
h_5	s(*lon*) + s(*lat*) + *htype*	235.0	13.8	0.1	−0.682	37.9	8.9
h_6	s(*depth*) + *htype*	307.2	85.9	0.0	−0.584	15.1	5.0
h_7	s(*lon*) + s(*lat*) + s(*depth*) + *htype*	224.4	3.2	17.1	−0.696	43.5	13.4

mizing the unbiased risk estimator (UBRE; Craven & Wahba 1979). AIC and AIC differences, $\Delta_i = \mathrm{AIC}_i - \mathrm{AIC}_{\min}$, were computed over all candidate models h_i. Models with $\Delta_i > 10$ have essentially no support and were omitted from further consideration, while all models with $\Delta_i < 2$ have substantial support (Burnham & Anderson 2002). To quantify the plausibility of each model, given the data and a set of 6 models, the 'Akaike weight' w_i of each model was calculated as

$$w_i = \frac{\exp(-0.5\Delta_i)}{\sum_j \exp(-0.5\Delta_j)} \qquad (3)$$

The Akaike weight is considered as the weight of evidence in favour of model *i* being the actual best model in the available set of models (Akaike 1983, Buckland et al. 1997, Burnham & Anderson 2002).

The expected number of *Caulerpa racemosa* fronds in 20 × 20 cm experimental plots, conditional on the species being present, was related to predictor variables using GAMs of the general formulation

$$f(\mathrm{E}[\hat{n}_i]) = LP_i = c + \sum_m s_m(z_{mi}) + \sum_r F_{ri} \qquad (4)$$

A left truncated negative binomial error distribution was assumed with a log link (canonical link for the negative binomial) (Welsh et al. 1996). The smooth function $s_m(\cdot)$ was represented using cubic regression splines. The optimum degree of smoothing was defined based on the AIC, following an optimization procedure described in Stasinopoulos & Rigby (2007). Three predictor variables were used: (1) *time*, (2) *depth*, and (3) *htype*. *C. racemosa* was never observed in *Cymodocea nodosa* beds; thus, *htype* included only 3 habitat types (PO, R, and S). *Time* was included as a covariate to account for possible temporal

variation in frond density. This was measured in days, with the value 1 corresponding to 1 August 2008, and ranged from 1 to 105. Eight different GAMs (g_i, $i = 0$ to 7) were fitted, where model g_0 was the null model, with no predictor variable (Table 2). Model selection in the set of candidate models was based on AICs.

For fitting the GAMs, the packages mgcv (Wood 2000, 2006), gamlss, and gamlss.tr (Stasinopoulos & Rigby 2007) were used in R v. 2.5.1 (R Development Core Team 2007).

RESULTS

Rocky bottoms were more common at depths <10 m, *Posidonia oceanica* meadows were more frequent at depths between 15 and 25 m, and *Cymodocea nodosa* was the least abundant habitat type, which was mostly confined to shallow waters (Table 3). *Caulerpa racemosa* was observed in 6.6% of the sampled quadrats with a maximum observed density of 67 fronds 400 cm^{-2}

Table 2. Summary of the parameterisation of the 8 candidate models g_i of the abundance of *Caulerpa racemosa* fronds in 20 × 20 cm plots, conditional on presence. For each model, the Akaike information criteria AICs, Akaike differences Δ_i, Akaike weights w_i, deviance explained, and estimated degrees of freedom (edf) are given. Models with substantial support ($\Delta_i < 2$) are given in **bold**. *htype*: habitat type

	Model	AIC	Δ_i	w_i (%)	Deviance explained (%)	edf
g_0	Null	322.9	19.5	0.0	0.0	2.0
g_1	s(*time*)	307.2	3.8	8.5	6.8	5.0
g_2	s(*depth*)	323.9	20.5	0.0	0.3	3.0
g_3	*htype*	314.3	10.9	0.2	4.0	4.0
g_4	s(*time*) + s(*depth*)	310.6	7.2	1.6	7.9	8.5
g_5	**s(*time*) + *htype***	**303.4**	**0.0**	**57.5**	**9.2**	**7.0**
g_6	s(*depth*) + *htype*	313.8	10.4	0.3	6.9	8.4
g_7	**s(*time*) + s(*depth*) + *htype***	**304.6**	**1.2**	**31.8**	**9.9**	**8.7**

Table 3. Relative distribution of habitat types in different depth strata in the study area, based on the recorded data for the sampled quadrats. PO: *Posidonia oceanica* meadows; CN: *Cymodocea nodosa* beds; R: rocky bottoms; S: unvegetated sandy/muddy bottoms

Depth strata (m)	% of sample in each habitat type				Sample size
	PO	CN	R	S	
0–5	16.2	9.7	37.4	36.7	278
5–10	27.7	2.4	28.3	41.6	166
10–15	27.5	3.8	8.8	60.0	80
15–20	35.6	2.3	5.7	56.3	87
20–25	37.7	1.6	6.6	54.1	61
25–30	5.7	0.0	8.6	85.7	35
>30	0.0	0.0	3.1	96.9	32

(Table 4). The species was absent from the western and northern Saronikos Gulf; the highest frond densities were observed in the southeastern part of the gulf (Fig. 1).

The best model of the probability, p, of *Caulerpa racemosa* presence was h_4 (Table 1), which included univariate smooth functions of *lon*, *lat*, and *depth*. All other models had essentially no support ($\Delta_i > 10$), except for model h_7, which had some support ($\Delta_7 = 3.2$, $w_7 = 17.1\%$). Model h_4 was nested within h_7, with h_7 having *htype* as an extra predictor variable. However, model h_7 was not further considered, and h_4 was selected as the single model for inference, since $\Delta_7 > 2$ and *htype* was not significant (χ^2, p = 0.46). The expression of h_4 was

$$\text{logit}(\hat{p}) = \qquad\qquad (5)$$
$$-5.11 + s_1(lon, 4.87) + s_2(lat, 1.00) + s_3(depth, 3.60)$$

where the smooth functions of predictors $s_m(z_m, \text{edf})$ (edf being the estimated df) are given in Fig. 2. To get actual probabilities of presence for specific values of *lon*, *lat* and *depth*, the corresponding values of the smooth terms (from Fig. 2) were entered in the above expression of logit($\hat{p}$). There was a bimodal distribution of presence probability in relation to depth, with higher probabilities at very shallow bottoms (<4 m) and at depths between ~15 and 30 m. The highest probabilities of presence were observed at the highest longitudes, representing the eastern part of the study area.

To further demonstrate that the observed bimodal bathymetric distribution of the species was not due to the bathymetric distribution pattern of seagrasses, the same analysis was repeated excluding PO and CN habitat types. The same bimodal distribution was still observed (not shown here), indicating that this result is not related to the bathymetric distribution of seagrass beds.

The best model of the expected number of *Caulerpa racemosa* fronds (conditional on presence) was g_5 ($w_5 = 57.5\%$), with g_7 also having substantial support ($\Delta_7 = 1.2$, $w_7 = 31.8\%$) (Table 2). Thus, both models were used for inference. The other models had substantially less or essentially no support from the data. The expression of g_5 was log($\hat{n}$) = s(*time*, 3.00) + *htype*. The univariate smooth function of *time* and the estimated

Table 4. Aggregated results for each of the 20 line transects. The total number of sampled quadrats, the number of quadrats with *Caulerpa racemosa*, and the average and maximum measured densities at each transect are given

| Site	Latitude (°)	Longitude (°)	Sampling date (2008)	Quadrats sampled	Number of non-zero counts	Density (fronds 400 cm^{-2})		
						Overall mean	Mean of non-zero counts	Maximum
Thymari	37.6922	23.9380	1/8	40	14	2.3	6.6	26
Kalogerolimano	37.8228	23.1462	23/8	21	0	0.0	–	0
Sideronas	37.8440	23.0391	23/8	37	0	0.0	–	0
Koudounes	37.7554	23.9020	26/8	41	9	4.1	18.7	67
Legraina	37.6694	23.9434	28/8	21	9	14.4	33.6	63
South Perdika	37.6877	23.4496	13/9	41	0	0.0	–	0
West Marathonas	37.7240	23.4500	13/9	41	0	0.0	–	0
Peninta Braxia (east)	37.7275	23.5407	14/9	23	0	0.0	–	0
Bagies-Agioi (north)	37.7726	23.5174	14/9	41	2	0.2	4.0	7
Proto Limanaki	37.8011	23.7855	17/9	39	1	0.3	13.0	13
Lemonodasos (south)	37.4601	23.5108	20/9	41	1	0.1	4.0	4
Kalavria - Poros	37.5157	23.5186	20/9	33	0	0.0	–	0
Methana	37.6399	23.3800	21/9	41	1	0.0	1.0	1
Epidauros	37.6229	23.1558	21/9	33	0	0.0	–	0
Peristeria	37.8773	23.4538	24/9	41	0	0.0	–	0
Myloi Kokkini	37.9184	23.0342	30/9	41	0	0.0	–	0
Kineta	37.9747	23.2389	17/10	41	0	0.0	–	0
Ag. Kosmas	37.8918	23.7165	1/11	41	0	0.0	–	0
Galini	37.9556	23.4386	2/11	41	0	0.0	–	0
Thiafi	37.6552	24.0100	15/11	41	10	0.8	3.3	14
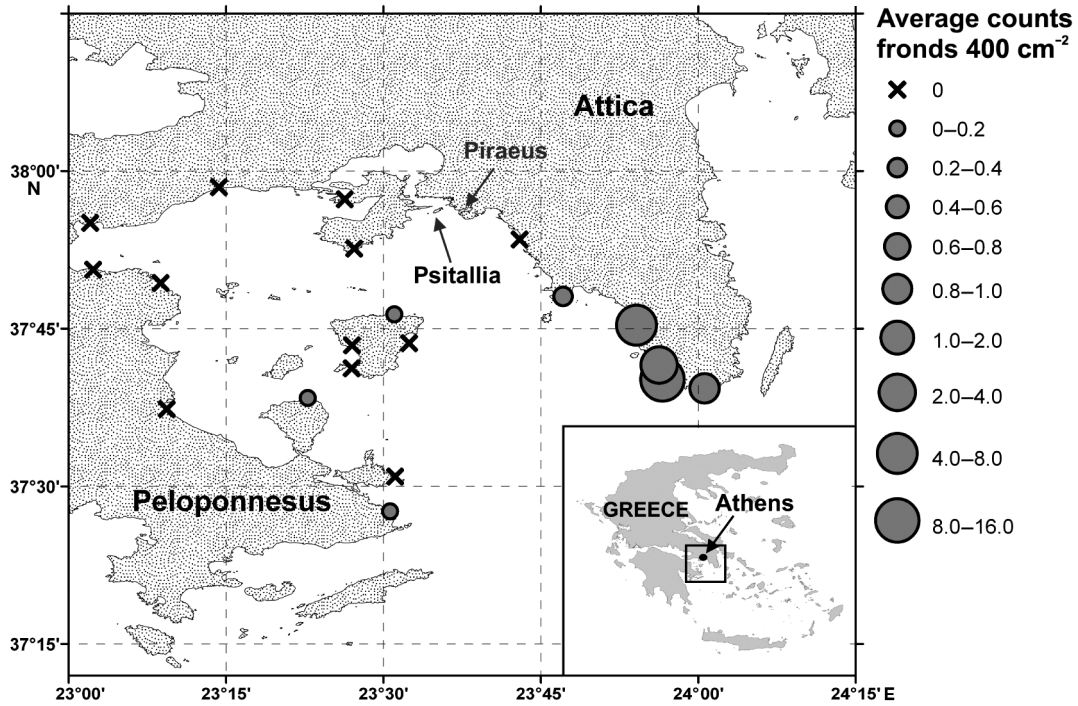
Fig. 1. Map of Saronikos Gulf showing the average counts of *Caulerpa racemosa* fronds in the 20 × 20 cm plots of each transect
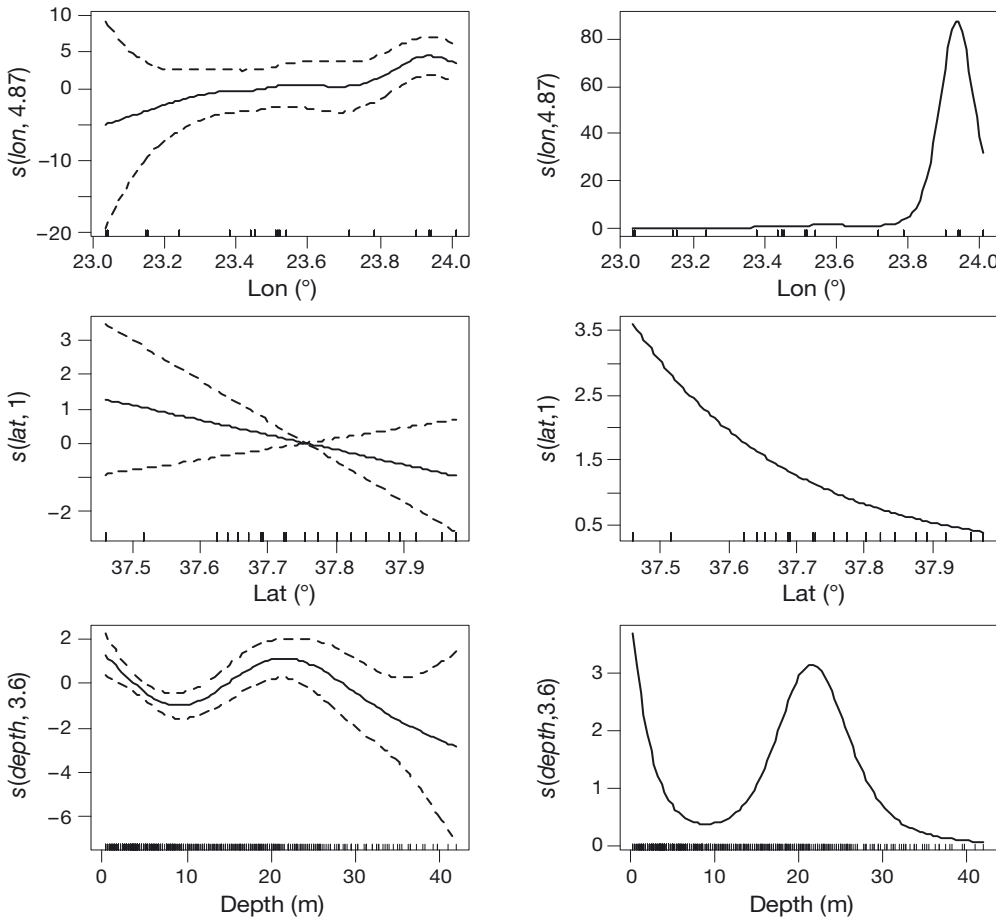

Fig. 2. Estimated smooth terms for the h_4 model of the probability of *Caulerpa racemosa* presence. In the left panels, the smooth terms are given in the linear predictor scale, i.e. in $\log(p/1-p)$, and the corresponding 95% CIs are indicated with dashed lines. In the right panels, the smooth terms are given in the 'odds of presence' scale, i.e. the smooth terms were exp-transformed. The numbers after the commas in the y-axis titles are the estimated df of the smooth term. A 1-dimensional scatterplot (vertical bars) is given at the bottom of each graph to illustrate the distribution of available data
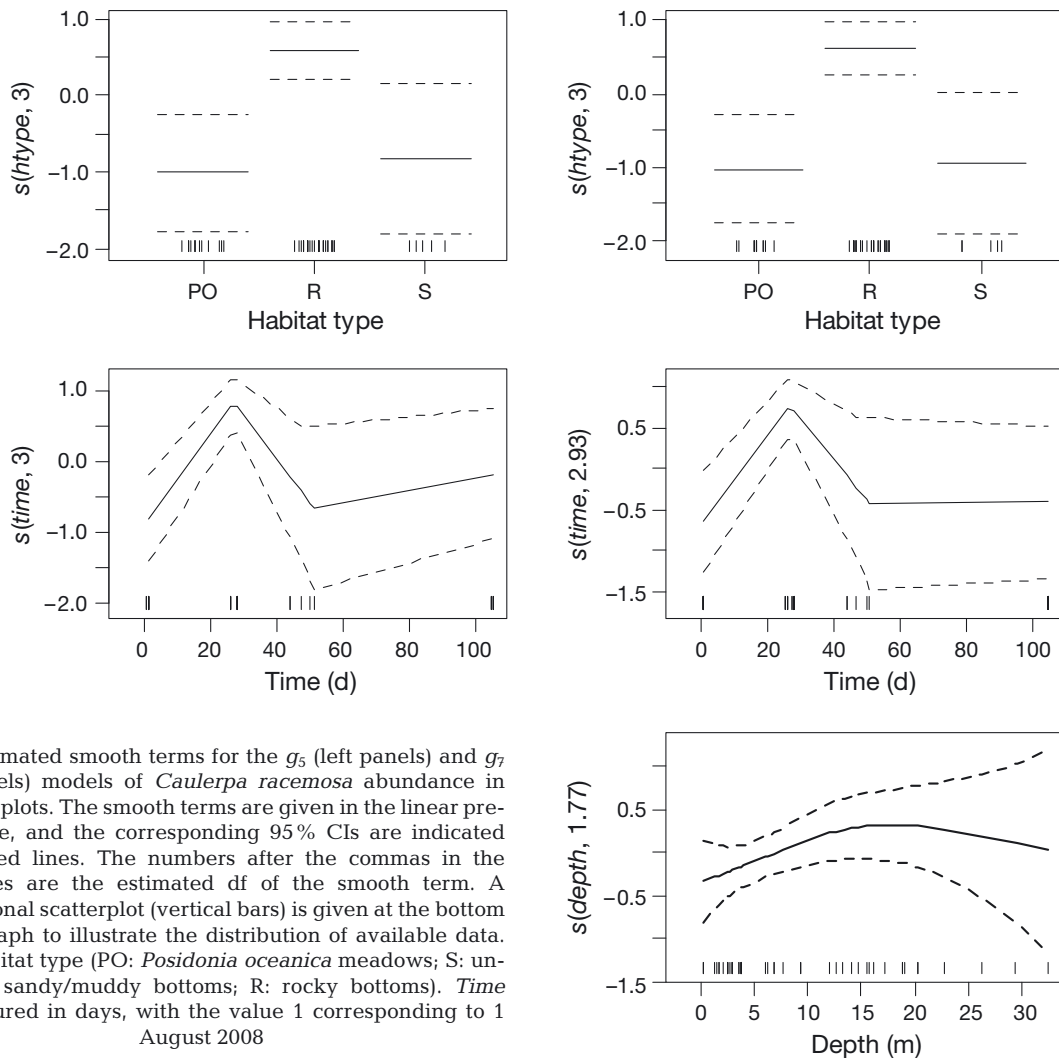
Fig. 3. Estimated smooth terms for the g_5 (left panels) and g_7 (right panels) models of *Caulerpa racemosa* abundance in 20×20 cm plots. The smooth terms are given in the linear predictor scale, and the corresponding 95% CIs are indicated with dashed lines. The numbers after the commas in the *y*-axis titles are the estimated df of the smooth term. A 1-dimensional scatterplot (vertical bars) is given at the bottom of each graph to illustrate the distribution of available data. *htype*: habitat type (PO: *Posidonia oceanica* meadows; S: unvegetated sandy/muddy bottoms; R: rocky bottoms). *Time* was measured in days, with the value 1 corresponding to 1 August 2008

levels of *htype* are given in Fig. 3. The expression of g_7 was $\log(\hat{n}) = s_1(time, 2.93) + s_2(depth, 1.77) + htype$. The univariate smooth functions and the levels of *htype* are also given in Fig. 3.

Frond density was markedly higher in rocky habitats than in *Posidonia oceanica* beds or on sandy/muddy bottoms. The *Caulerpa racemosa* fronds within *P. oceanica* beds were mostly observed in the meadow limits or in sites of low seagrass density. In very shallow bottoms, although the probability of *C. racemosa* presence was high (model h_4; Fig. 2), the corresponding frond density was low compared to that at greater depths (Fig. 3). According to both g_5 and g_7, there was a peak in frond density at the end of August. However, this result should be viewed with caution due to the uneven temporal distribution of transects with *C. racemosa* and the low number of distinct values of *time* containing records of *C. racemosa* (8 values).

DISCUSSION

In the native range of *Caulerpa racemosa* in southwestern Australia, the bathymetric range of the species is restricted between the intertidal zone and a depth of 6 m, and it is primarily found on reef flats and in intertidal pools (Klein & Verlaque 2008). In the Mediterranean, *C. racemosa* features a much wider bathymetric range and has been observed to grow from the intertidal zone, sometimes partially emerged, down to a depth of 70 m (Raniello et al. 2006, Klein & Verlaque 2008). At the lower limit of its bathymetric distribution, *C. racemosa* presence is sparse and occasional, while high abundances have been reported mostly in the range of 0 to 30 m (see Appendix A of Klein & Verlaque 2008). A comparative investigation of the spatial distribution of *C. racemosa* over a wide bathymetric range has not been conducted prior to the present study.

In the Saronikos Gulf, the probability of *Caulerpa racemosa* presence exhibited a bimodal bathymetric distribution with a first peak at very shallow depths (<4 m) and a second peak at depths between 15 and 30 m. At greater depths, the probability of *C. racemosa* presence steadily decreased and was practically 0 at 40 m. The lower limit of the bathymetric distribution is mainly affected by the efficiency of light exploitation through photochemical reactions, as light is a major factor that affects the photosynthetic performance and ultimately the growth performance of plants (Lobban et al. 1985). Raniello et al. (2006) reported on the photoacclimation capacity of *C. racemosa*, and indicated that the pigment siphonaxanthin is involved in acclimation to deep light regimes as well as photoprotection of shallow stands through regulated conversion into lutein.

The bimodal pattern of *Caulerpa racemosa* presence with depth (instead of a smooth decline from shallow to deeper areas) is difficult to interpret and is most likely related to the complexity of underlying effects. In conjunction with the nonlinear variation in light intensity with depth and the complex photochemical cycles, inherently nonlinear effects of abiotic parameters (temperature, hydrodynamics, salinity, dissolved oxygen, and nutrients) and ecological interactions with other species (e.g. predation or competition for space, light, and nutrients) may result in nonlinear patterns of spatial distribution. Moreover, moderate depths are much more stable environments in terms of both light availability and wave action, than are their deeper and shallower counterparts, respectively. These latter environments could thus be more prone to invasion by opportunists, such as *C. racemosa*, which is well known for its ability to exploit low light conditions (Raniello et al. 2006) and is commonly found in wave-exposed shores (Klein & Verlaque 2008).

Bimodal bathymetric distribution has been observed for laminarian algae in subtidal regions of northern New Zealand, with peaks of abundance at the 3 to 6 m and 10 to 17 m bathymetric zones (Choat & Schiel 1982). This bimodal feature was consistent over a wide geographic range of the northern coastal sites and for semi-exposed offshore islands in New Zealand. This pattern was attributed to the bathymetric distribution of invertebrate herbivores (echinoids and gastropods) that were primarily abundant at intermediate depths (6 to 10 m). Prediction models of the expansion of the congeneric species *Caulerpa taxifolia* suggested a single depth stratum (between 5 to 10 m) of high risk of invasion (Hill et al. 1998), indicating different patterns of bathymetric distribution between the 2 species.

The abundance (conditional on presence) of *Caulerpa racemosa* in *Posidonia oceanica* beds was lower than that on rocky bottoms, and appeared to be influenced by seagrass density. In a multifactorial experiment, Ceccherelli et al. (2000) tested the effect of *P. oceanica* canopy structure on frond size, stolon growth, and percent cover of *C. racemosa*. Their results indicated that both the vertical and horizontal algal growth was negatively influenced by seagrass density, and that dense and healthy *P. oceanica* meadows represent a barrier to the colonization of *C. racemosa*. Other studies (see Klein & Verlaque 2008 for a review) have also reported that *C. racemosa* is seemingly unable to penetrate dense *P. oceanica* meadows but is often found on the rhizomes at the margins of the meadow or in sparse beds. Although a significant increase in the abundance of *C. racemosa* on and around dead 'mats' of *P. oceanica* has been frequently reported in the literature (Klein & Verlaque 2008 and references therein), the absence of this type of substrate did not permit the substantiation of such a relationship in the surveyed region. Large patches of dead mats seem to be more or less absent from the Saronikos Gulf (authors' pers. obs., P. Panayotidis pers. comm.). Indeed, the only documentation of such a conspicuous ecomorphosis dates back to 1988 and is geographically restricted to the greater area of Piraeus port (Panayotidis 1988).

Dense beds of *Cymodocea nodosa* might also represent an obstruction to *Caulerpa racemosa* invasion. In this study, zero *C. racemosa* abundance was observed in all quadrats within *C. nodosa* beds (n = 37). However, no conclusions can be reached on the vulnerability of *C. nodosa* meadows to *C. racemosa* invasion since all *C. nodosa* beds (found in 4 line transects) were observed in the western Saronikos Gulf where *C. racemosa* was generally absent.

Caulerpa racemosa abundance on unvegetated sandy/muddy bottoms was lower than that on rocky bottoms and similar to that in *Posidonia oceanica* beds. Under certain conditions, *C. racemosa* may form compact dense mats on soft bottoms (Argyrou et al. 1999, Klein & Verlaque 2008). Although such mats have been observed in the Aegean Sea (e.g. in the outer Souda Bay in Crete Island; Katsanevakis & Thessalou-Legaki 2009), no dense mats were observed in soft-substrate areas in this study in the Saronikos Gulf.

Since 1996 when *Caulerpa racemosa* was first observed in the southeastern Saronikos Gulf (Panayotidis & Montesanto 2001), the species has progressively colonized numerous other sites, exhibiting an anticlockwise propagation along the coastline (Fig. 4; Panayotidis et al. 2006, K. Tsiamis pers. comm.). The species is still absent from the westernmost parts of the gulf and is still very infrequent from the sites of recent detection (which is the reason why we did not record it during this study in some sites of documented presence). In this study, the highest densities were found in
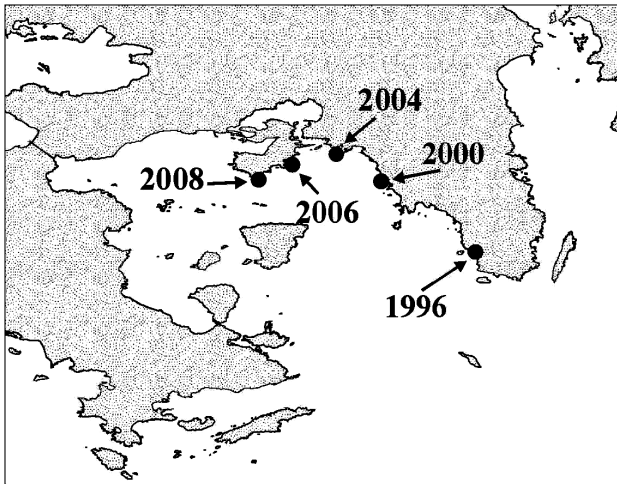
Fig. 4. Historical records of *Caulerpa racemosa* in the Saronikos Gulf from the date of first occurrence (1996) to 2008 (Panayotidis et al. 2006, K. Tsiamis pers. comm.). These sites have been monitored in the framework of the ecological monitoring of the Saronikos Gulf conducted by the Hellenic Centre for Marine Research

the reported area of first colonization (southeastern Saronikos). When a site is invaded by *C. racemosa*, the alga appears to continue its range extension and increase its cover and frond density. In most of the invaded sites in the Mediterranean, no decline in colonized surfaces has been reported (Klein & Verlaque 2008). The length of the time period from the initial colonization appears to be an important factor that determines the frond density of the species. Therefore, high frond densities, such as those currently observed in the southeastern Saronikos Gulf, are expected to occur in most of the eastern coast during the following decade.

In practice, the usual approach followed when investigating the relations between ecological variables is to assume a specific parametric form (often linear) and apply multiple regression techniques. A more appropriate approach is to fit a flexible nonparametric model that could attain a great variety of patterns and does not assume functional relationships between the predictor and response variables, rather than to adopt a specific parametric form. Ecological phenomena, such as the spatial distribution of a species, are usually complex and are being driven apart from the main dominant effects by many other smaller effects that are difficult to detect. The variable environment, composite interspecific relationships (e.g. competition and predation), individual heterogeneity, and rare events may have important effects and add further complexity. In the present study, GAMs were used to offer the required flexibility and overcome the limiting concept of a strict parametric shape in ecological relationships. The use of such flexible models is recommended in studies relating abundance of marine fauna or flora to environmental and spatial predictors. In addition, the information theory approach for inference strengthens the validity of the results and provides increased robustness compared to classical approaches for modelling and model selection (Burnham & Anderson 2002, Katsanevakis & Maravelias 2008).

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