

# Spatio-temporal variations and recruitment of *Sargassum flavifolium* Kützing in sublittoral cobble bottoms: relationships with environmental variables

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**Abstract** Dynamics of *Sargassum flavifolium* Kützing were studied in sublittoral cobble bottoms. The analyses showed that some morphological attributes (thallus length and number of branches, blades, stipes and receptacles) were related to selected environmental variables (depth, cobble size, temperature, wave exposure and day length), and spatio-temporal differences in selected variables (thallus length, reproductive capability, recruitment and number of thalli per cobble) were found. *S. flavifolium* showed an annual life cycle with marked variations in habit along the year and between locations. The longest thalli were found in May–July coinciding with annual increases in seawater temperature and day length, and the decrease in wave exposure. However, depth and cobble size were also variables that interacted with this seasonal pattern, and as a result, differences in *Sargassum* development could be observed within a site or at the same depth. The longest thalli were found at the deepest bottoms and on larger cobbles. The highest number of receptacles occurred on the largest individuals, and the greatest percentage of fertile thalli was observed in the deeper samples. Reproduction and recruitment were coupled, and recruits were conspicuous and more numerous in shallow cobbles. Significant differences in number of thalli per cobble were observed between months, although changes related to recruitment were only observed in small cobbles.

**Keywords** Macroalgae · Thallus length · Phenology · Reproduction · Canary Islands · NE Atlantic Ocean

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## Introduction

*Sargassum* is one of the most diverse genera of macroalgae, with about 400 species currently accepted and a high intra-specific morphological variability (Mattio and Payri 2011). *Sargassum* is distributed worldwide, especially in tropical and subtropical regions, where they are dominant elements in eulittoral and shallow sublittoral benthic communities (Ateweberhan et al. 2005; Ang 2006), forming dense forests equivalent to those by temperate species of *Fucus*, *Cystoseria* or kelps (Steneck et al. 2002; Thibaut et al. 2005). *Sargassum* is among the most productive macrophytes due to the large size and rapid growth (Murase et al. 2000; Rivera and Scrosati 2006), and in addition, they are key engineering species that provide essential support to invertebrate and fish populations (Wells and Rooker 2004).

Most *Sargassum* spp. are ‘pseudoperennial’ species, consisting of a perennial holdfast and short stipes, which produce varying numbers of annual lateral primary branches, secondary branches, primary and secondary blades, vesicles and receptacles (Díaz-Villa et al. 2005). They show strong seasonal variations in thallus length and reproduction (Ateweberhan et al. 2005, 2009). Usually, their life cycle is completed yearly, with successive periods of slow vegetative growth, rapid growth, reproductive recruitment, and senescence and dieback phases (Ang 2006; Plouguerné et al. 2006; Ateweberhan et al. 2008). This pattern may vary, and some species reveal a bimodal growth pattern, with two periods of high growth rates and two periods of high degenerative rates (May-Lin and Ching-Lee 2013).

Studies on the phenology of several *Sargassum* species have found a strong relation between thallus morphology and changes in seawater temperature (Yoshida et al. 1998; Gillespie and Critchley 1999; Hwang et al. 2004; Yu et al. 2012). As other macroalgae, *Sargassum* species present optimum temperature of growth and limits of tolerance (Hwang

et al. 2004; Plouguerné et al. 2006). Maximum development of temperate species occurs in summer when seawater temperature is higher, while differences exist in tropical species, most of them with maximum peaks in thallus length and reproduction in the coolest season, but others in the warmest season (Yoshida et al. 1998; Gillespie and Critchley 1999; Plouguerné et al. 2006). Day length, separately or in combination with temperature, has also been documented in both the field and experimentally as major regulator of *Sargassum* growth, with maximum rates occurring with larger daylight (Uchida 1993; Plouguerné et al. 2006). Some authors point out that seasonal variations in *Sargassum* are related to nutrient availability, and low concentrations thereof limit the development of thalli (Hwang et al. 2004; May-Lin and Ching-Lee 2013); others show the role played by herbivores (McCook 1997) and the effects of wave exposure (Andrew and Viejo 1998; Engelen et al. 2005).

Species of *Sargassum* colonize multiple rocky habitats in the shallow sublittoral (Schiel and Foster 2006), including cobble beds as one of the most extensive (Scheibling et al. 2009) and where *Sargassum* populations may be abundant (Harries et al. 2007; Kawamata et al. 2011). Shallow cobble beds are dynamic habitats strongly subject to the effect of wave exposure and sedimentation process (Lieberman et al. 1984), both factors affecting population dynamics of algae (Davis and Wilce 1987b; Airoidi 2000, 2003). Most species that grow on cobble beds are ephemeral and turf-forming species, but *Sargassum* species are one of the few exceptions (Davis and Wilce 1987a; Airoidi 2003). Moreover, within cobble beds, the high variability in algae composition and community structure is linked to cobble size, since environmental disturbance is lower for species on the greatest cobbles (Scheibling et al. 2009). In any case, the degree of development of *Sargassum* thalli in cobble beds depends on multiple factors, whose effects altogether may be additive, opposite or interactive. Following this hypothesis, some environmental variables and different cobble sizes are analysed here together to determine their effects on dynamics of *Sargassum* populations. *Sargassum* species are common in sublittoral communities of La Palma Island (Canarian archipelago, eastern subtropical Atlantic) (Sangil et al. 2011, 2014), with *Sargassum flavifolium* Kützinger as a characteristic element in cobble beds (Sangil 2012).

*S. flavifolium* is a species with a distribution restricted to the northeastern Atlantic from France to the Canary Islands and the Mediterranean (Guiry and Guiry 2014). Although it was reported from the Canary Islands by Børgesen (1926) as *Sargassum vulgare* var. *flavifolium* (Kützinger) Grunow, this report has been overlooked in recent checklists. Thalli growing on stable rocky substrates are erect and reach up to 50 cm

high, with perennial holdfast and stipes, from which annually arise primary branches with primary blades and secondary branches with secondary blades, vesicles and receptacles (Fig. 1a). *S. flavifolium* is characterized by brown terete branches, smooth or spinous; blades, yellow-brown, linear-lanceolate, serrate margins with long teeth and irregularly scattered cryptostomata; few numerous subspherical vesicles; and stocky, male or female receptacles and fertile up to the base (Børgesen 1926; Cormaci et al. 2012). However, in previous unpublished observations, we have observed that thalli growing on unstable cobble bottoms (Fig. 1b) at the Canary Islands are usually smaller, the primary branches seem to be decumbent and arranged in a rosette, lacking secondary branches and vesicles, and with receptacles arising directly from primary branches.

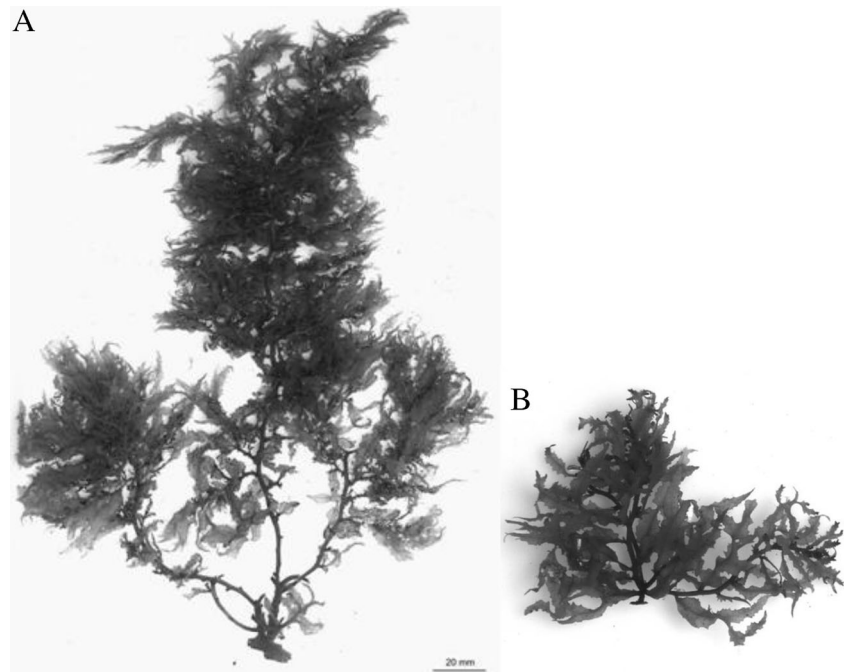
We analyse the morphological attributes, reproductive capability, recruitment and number of thalli per cobble of *S. flavifolium* over an annual cycle, and especially we examine these parameters by means of multivariate analyses and by a fully hierarchical design to test the following: (1) the relationships between thallus morphology and selected environmental variables (wave exposure, temperature, day length, depth and cobble size) and (2) the differences in spatio-temporal patterns of several morphological and population attributes (thallus length, number of receptacles, number of recruits and number of thalli per cobble).

## Material and methods

### Sampling

Fieldwork was conducted in three sites of La Palma Island (28.6 N, 17.8 O) (Fig. 1S). Samplings were carried out in the sublittoral at two different depths: shallow (6–9 m) and deep (15–18 m). Sampled bottoms consisted of pebbles, cobbles and sand, and macroalgal assemblages are characterized by diverse turf-forming species in which *S. flavifolium* grows together with *Padina pavonica* (Linnaeus) Thivy in Taylor, *Liagora ceranoides* Lamouroux, *Jania adhaerens* Lamouroux, *Lobophora variegata* (Lamouroux) Oliveira and *Halopteris scoparia* (Linnaeus) Sauvageau. At each site and depth, isodiametric cobbles of three different sizes (about 50, 120 and 210 mm in diameter) were haphazardly collected every 2 months from May 2005 to July 2006. Six cobbles of each size were collected at each site and depth. Care was taken that cobbles collected have roughly spherical shapes and protruding from the sand in over 50 % of their size. Each cobble was scraped and samples were preserved in 4 % formalin in seawater. In the laboratory, the number of thalli per cobble was noted, specimens were isolated, and selected morphological vegetative and reproductive parameters from each individual (total length and number of stipes, branches,

**Fig. 1** *Sargassum flavifolium*: specimen from stable rocky substrates (a) and specimen from unstable cobbles bottoms (b)



blades and receptacles) were studied. Thallus length was measured with a ruler, while all stipes, branches, blades and receptacles were counted. A total of 925 thalli were examined. In addition, to estimate the number of *Sargassum* recruits, four concrete experimental blocks (15×15×7.5 cm) were deployed at each site and depth (see Sangil et al. 2012). Experimental blocks were recovered and replaced by new ones every 2 months, from May 2005 to September 2006 ( $n=216$ ). Each experimental block was also scraped and samples were preserved in 4 % formalin in seawater for further identification.

#### Environmental variables

Variables that potentially drive the morphology of *Sargassum* (wave exposure, temperature and day length) were measured bimonthly (see Fig. 2S). Data on wave exposure were obtained from an oceanic virtual data point of wave height from Spanish coasts (<http://www.puertos.es>). This website provides historical records from Spanish coasts through a set of points. For our study, data were obtained from the point situated in the east of the island. According to Enola project (<http://www.enola.ihcantabria.com>), the three localities have a similar exposition to the waves. Pattern of seawater surface temperature (SST, °C) from La Palma was obtained from a worldwide database (<http://www.esrl.noaa.gov/psd/data/gridded/data.ncep.reanalysis.html>), which creates time series for the selected coordinates. Data on day length were taken from online source for latitude and longitude of the island (<http://www.eurometeo.com>).

#### Data analyses

*Relationships between thallus morphology and environmental variables* A distance-based linear model routine (DistLM) (Legendre and Anderson 1999) was applied to analyse the relationships between all vegetative and reproductive parameters of *S. flavifolium* and environmental variables (depth, cobble size, temperature, wave exposure and day length). The analysis, based on similarity matrices, used the stepwise selection method, an adjusted  $R^2$  criterion and 9,999 permutations. Prior to the DistLM analysis, the draftsman plot method with the corresponding Pearson correlation coefficients was used to explore the relationships between the environmental variables (Clarke and Gorley 2006). This test was performed to detect any possible relationship between variables to discard strongly correlated variables. Because the correlations between variables were low in our case ( $R<0.5$ ), no variable was excluded. Following recommendations by Clarke and Gorley (2006), morphological parameters were square root transformed, while environmental variables were normalized. A distance-based redundancy analysis (dbRDA) was used to perform an ordination of fitted values from the given model. The plot allows to visualize the structure of the data cloud and the relationships between morphological parameters and environmental variables according to the multivariate regression model generated by the DistLM (McArdle and Anderson 2001).

*Spatio-temporal patterns in thallus length and number of receptacles* Differences in thallus length and number of receptacles per thallus were examined using distance-based permutational ANOVAs. Resemblance matrices were generated using

Euclidean distances of data (Anderson et al. 2008). Four factors were tested in these analyses: depth, cobble size, month and site. Due to the lack of samples to test the interaction ‘cobble size × depth × month’, it was decided to perform two three-way different models. In the first model, the interaction between the factors ‘depth’ (two levels: shallow, deep), ‘month’ (eight levels: bi-monthly samplings, from May 2005 to July 2006) and ‘site’ (three levels) was tested. Depth and month were treated as fixed factors, while site as random factor. In the second model, the interaction ‘depth × cobble size × site’ was tested. Depth and site were treated as in the first model. ‘Cobble size’ consisted in a fixed factor with three levels: small (cobbles of about 50 mm diameter), medium (≈120 mm) and large (≈210 mm). For these models, sums of squares type III (Anderson et al. 2008) were employed to prevent the effects of the unbalanced number of samples in the different levels of each factor of analysis. Some significant terms in the full model were examined using appropriate a posteriori pairwise comparisons (Anderson 2004). When the number of possible permutations was not large, Monte Carlo *p* values ( $n=4,999$ ) were instead obtained by random sampling from the asymptotic permutation distribution (Anderson and Robinson 2003).

**Spatio-temporal patterns in number of recruits** Patterns in recruitment on experimental blocks were analysed with a permutational ANOVA. Number of recruits was tested using a three-way model, with depth (two levels) and month (nine levels: bi-monthly samplings, from May 2005 to September 2006) treated as fixed factors, and site (three levels) as random factor.

**Spatio-temporal patterns in number of thalli per cobble** Spatio-temporal differences in number of thalli per cobble were tested with permutational ANOVAs. Analyses were performed separately per cobble size (small, medium and large). The analyses consisted in three-way models, using depth (two levels) and month (nine levels: bi-monthly samplings, from May 2005 to September 2006) as fixed factors and site (three levels) as random factor. Some significant terms were examined with a posteriori pairwise comparisons.

All statistical analyses were performed using PRIMER-E v.6 + PERMANOVA+ ([www.primers-e.com](http://www.primers-e.com); Clarke and Gorley 2006).

## Results

Relationships between thallus morphology and environmental variables

The DistLM analysis showed significant relationships between morphological vegetative and reproductive parameters

of *Sargassum* and all environmental variables studied (Table 1). When variables were individually considered in the marginal test (Table 1), the highest percentages of variation of *Sargassum* parameters were explained by temperature (11.16 %) and depth (9.19 %), while the rest of the variables contributed less [wave exposure (4.92 %), day length (3.63 %) and cobble size (1.89 %)]. When the variables were analysed altogether in the sequential test (Table 1), the percentages of variation varied slightly: depth (9.19 %), temperature (6.15 %), wave exposure (4.92 %), day length (2.74 %) and cobble size (1.48 %). The total variation explained for this model was of 24.56 %. The dbRDA analysis showed that wave exposure was positively correlated with the first axis. Depth and day length were negatively correlated with the first axis, while cobble size was correlated positively with the second axis (Fig. 2a). The overlaid trajectories of variation of *Sargassum* parameters showed that thallus length, number of branches and stipes increased their values following the negative values of the first axis and in the direction of increasing depth and day length (Fig. 2b), while number of blades and receptacles were correlated with negative values of the first axis and positive values of the second axis and following the increasing of cobble size. The distribution of the cloud of samples on the ordination plot showed that many samples from May and July were located along the negative values of the first axis. These samples also showed large distances between them along the second axis. Conversely, samples from September to March occupied positive values of the first axis, or negative values close to 0. These samples showed

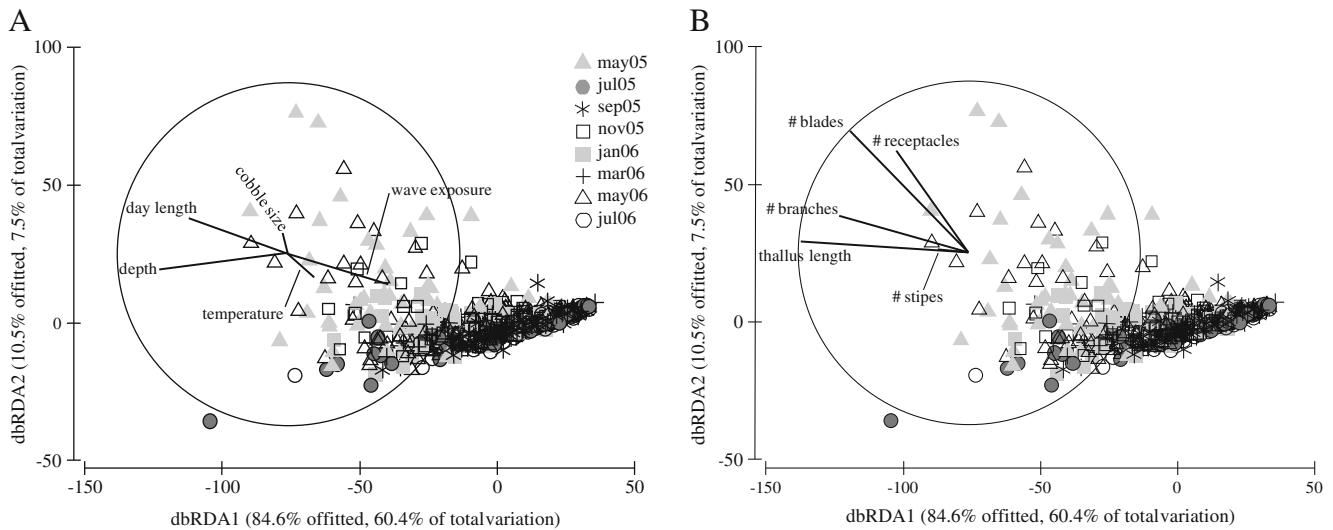
**Table 1** Results of distance-based linear model routine (DistLM)

Variable	Pseudo- <i>F</i>	<i>p</i>	Prop	Cumul
Marginal test <sup>a</sup>				
Depth	93.518	<0.001	9.19	
Cobble size	8.291	<0.05	1.89	
Temperature	11.369	<0.001	11.16	
Wave exposure	47.793	<0.001	4.92	
Day length	34.847	<0.001	3.63	
Sequential test <sup>b</sup>				
Depth	93.518	<0.001	9.19	9.19
Temperature	71.157	<0.001	6.15	15.34
Wave exposure	52.847	<0.001	4.92	20.28
Day length	17.026	<0.001	2.74	23.07
Cobble size	9.062	<0.05	1.48	24.56

<sup>a</sup> Test for relationships between individual environmental variables and vegetative and reproductive parameters of *Sargassum flavifolium*

<sup>b</sup> Test for relationships between environmental variables and *Sargassum* parameters considering environmental variables altogether in a multiple regression model





**Fig. 2** Distance-based redundancy analysis (dbRDA). Relationships between samples based on vegetative and reproductive parameters of *Saragassum flavifolium* and environmental variables (depth, temperature, wave exposure, day length, cobble size) (a). Direction of increases in morphological parameters (thallus length, number of stipes, branches, blades and receptacles) (b) ( $n=925$ )

short distances between them. Along the second axis, samples were arranged in a narrow range of values.

**Spatio-temporal patterns in thallus length and number of receptacles**

Analyses on thallus length showed a significant interaction between factors ‘depth × site’ (pseudo- $F=8.83$ ;  $p<0.001$ ) and differences for month (pseudo- $F=20.974$ ;  $p<0.001$ ) and cobble size (pseudo- $F=5.798$ ;  $p<0.05$ ) (Table 2). Thallus was longer in the deeper samples (Fig. 3a). A posteriori comparisons for the interaction depth × site and for pairs of factor depth showed that thallus length was different in the three sites, while for factor site, significant differences were found in five of the six pairs (Table 1S). The longest thalli occurred in May (2005 and 2006), while the smallest in November (Fig. 3b). Differences were detected between most pairs of months (Table 1S). The longest thalli were recorded in large cobbles, while the smallest in small ones (Fig. 3c). Pairwise comparisons found differences between large and small cobbles. The distribution of frequencies of thallus size classes changed with month, depth and cobble size (Fig. 4). In May and July, and in deeper and large cobbles, more classes with long thalli were detected. However, especially in November and in shallow and small and medium cobbles, thalli mainly belonged to smaller classes.

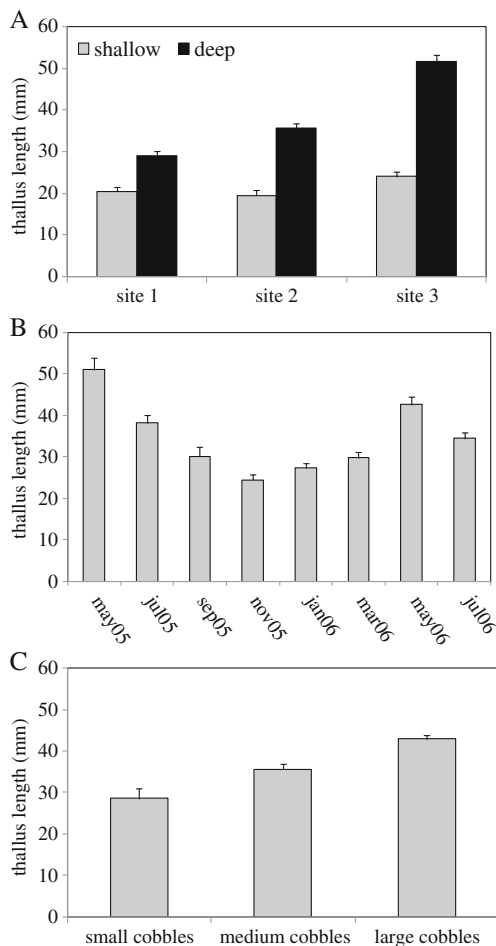
Analyses on the number of receptacles per thallus showed only a significant interaction between ‘depth × month’ (pseudo- $F=5.047$ ;  $p<0.01$ ) (Table 3). The number of receptacles was higher in deeper samples. Receptacles were present in thalli from shallow cobbles only in May and July, while in the deeper from May to November. At both depths, the highest number of receptacles was found in May (Fig. 5a). A

posteriori comparisons found some significant differences when comparing both the two depths in the same month and

**Table 2** Results of distance-based permutational ANOVAs comparing thallus length

Source of variation	df	MS	Pseudo-F	p (perm)
Model testing the interaction between the factors depth, month and site				
Depth (D)	1	249.500	16.857	0.050
Month (M)	7	20.982	20.974	<0.001
Site (S)	2	42.988	24.834	<0.001
D × M	7	2.624	1.363	0.287
D × S	2	15.288	8.831	<0.001
M × S	14	0.946	0.546	0.899
D × M × S	14	1.939	1.120	0.327
Res	877	1.731		
Total	924			
Model testing the interaction between depth, cobble size and site				
Depth (D)	1	100.040	13.945	0.101
Cobble size (C)	2	6.159	5.798	<0.05
Site (S)	2	9.400	4.470	<0.05
D × C	2	0.565	0.333	0.699
D × S	2	7.855	3.735	<0.05
C × S	4	0.986	0.469	0.753
D × C × S	4	1.664	0.791	0.520
Res	907	2.102		
Total	924			

Depth (two levels: shallow 6–9 m and deep 15–18 m), month (eight levels: bimonthly samplings, from May 2005 to July 2006) and cobble size (three levels: small, about 50 mm diameter; medium ≈120 mm; large ≈210 mm) were treated as fixed factors and site (three levels) as random factor



**Fig. 3** Mean thallus length (+standard error) of *Sargassum flavifolium* per depth and site (a), per month (b) and per cobble size (c) ( $n=925$ )

pairs of months at the same depth (Table 2S). The highest percentage of fertile thalli was reached in the deeper samples in May and July (Fig. 5b).

#### Spatio-temporal patterns in number of recruits

The number of recruits per sample showed interaction in ‘depth  $\times$  month  $\times$  site’ (pseudo- $F=2.313$ ;  $p<0.01$ ) (Table 4). Significant differences in the number of recruits were detected not only between months but also between depths and sites. The recruitment period took place from May to September, with the highest number of recruits in July (Fig. 6a, b). Recruits were more numerous in shallow samples. The temporal pattern differed slightly per site and at each depth.

#### Spatio-temporal patterns in number of thalli per cobble

The number of thalli showed significant differences in month in small cobbles (pseudo- $F=4.680$ ;  $p<0.05$ ) and an interaction between depth  $\times$  month  $\times$  site in medium (pseudo- $F=2.310$ ;  $p<0.01$ ) and large cobbles (pseudo- $F=2.238$ ;  $p<0.01$ )

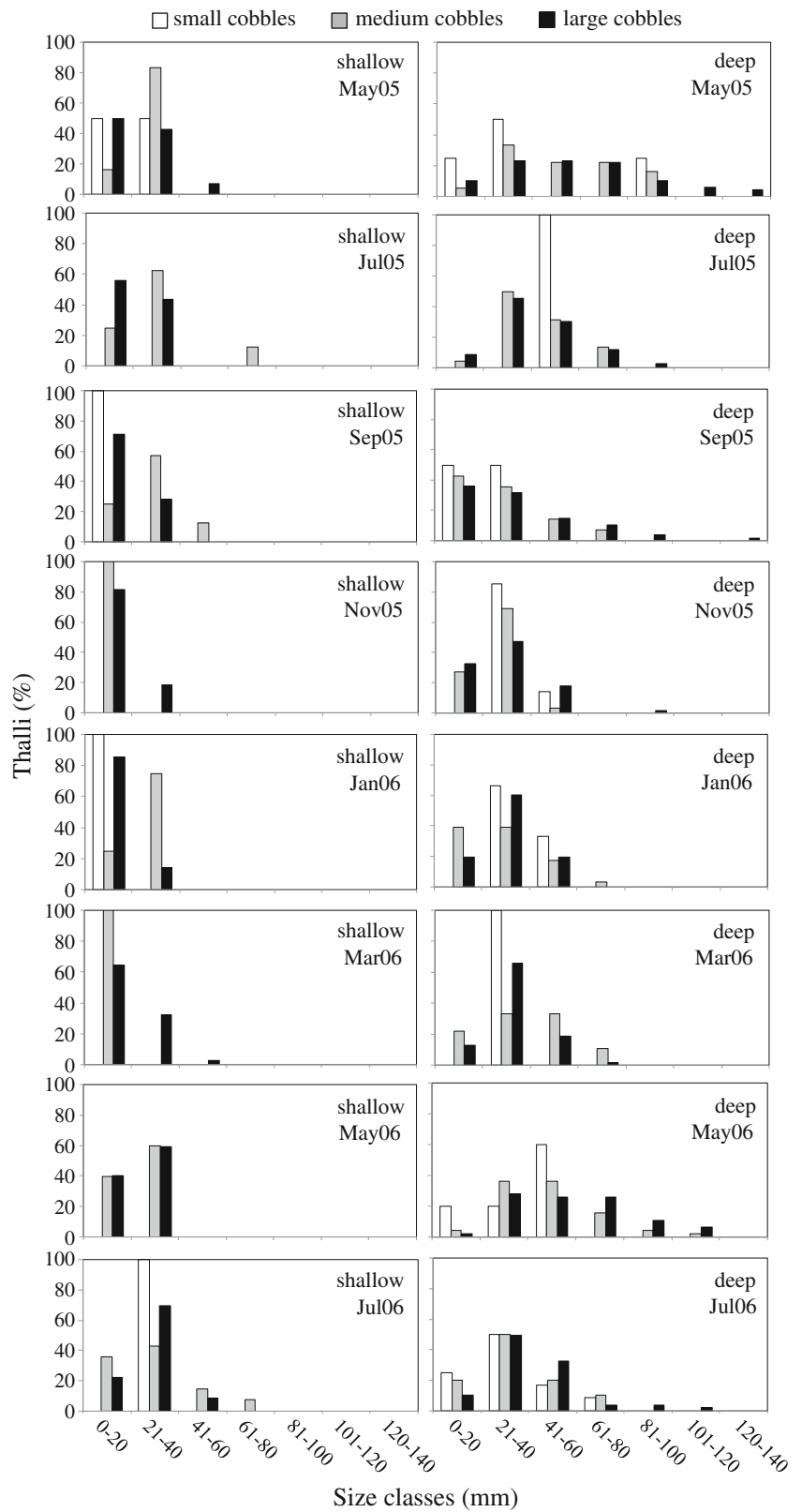
(Table 5). In small cobbles, a slight seasonal trend was observed. The highest number of thalli per cobble was found in summer (September 2005 and July 2006), while the minimum in spring (May 2005 and March 2006) (Fig. 7a). Pairwise comparisons found significant differences when comparing September 2005 and July 2006 with the rest of the months (Table 3S). Number of thalli per cobble exhibited a similar temporal pattern in shallow medium and small cobbles. No temporal patterns were observed in deep medium cobbles or in large cobbles (Fig. 7b, c). In medium and large cobbles and in most sites and months, number of thalli per cobble tended to be superior in deeper samples.

## Discussion

In cobble bottoms, the morphology of *S. flavifolium* exhibits marked temporal and spatial variations in relation to the environmental variables studied. All variables, i.e. depth, cobble size, wave exposure, temperature and day length, considered both individually and in a multiple regression model involve significant influence on *Sargassum* morphology. Nevertheless, the variables that explained most of the morphological vegetative and reproductive variability are depth and temperature. As showed in the ordination analysis (dbRDA), values of morphological attributes in *S. flavifolium* increase as depth, day length and cobble size increase, whereas they decrease as temperature and wave exposure increase. Annual renewable parameters (thallus length and number of branches, blades and receptacles) are those that experienced major changes. Multivariate and univariate analyses in thallus length and number of receptacles show that *S. flavifolium* has the typical seasonal cycle described for most *Sargassum* species (Umar et al. 1998; Ang 2006; Plouguerné et al. 2006; Ateweberhan et al. 2008). The temporal variation observed, with higher values in May–July and lower in November–January, matches with shifts in environmental variables, such as temperature, wave exposure and day length. However, depth and cobble size are variables that interact with this seasonal pattern, and as a result, differences in morphological attributes of *S. flavifolium* could be observed between and within depths and cobble sizes.

In general, the greatest development of thalli of *Sargassum* coincides with a rise in seawater temperature in late spring. However, during warmer temperatures in summer, *Sargassum* populations are usually in senescence and dieback phase. *S. flavifolium* shows a distinct pattern with the largest thalli during periods of intermediate temperatures, a similar pattern to that documented in *Sargassum orotavicum* from the Canary Islands by Díaz-Villa et al. (2005). Patterns in development of thalli of *Sargassum* seem to be regulated by optimum growth temperatures and follow the latitudinal gradient in seawater temperature (Yoshida et al. 1998). This optimum period

**Fig. 4** Distribution of frequencies of length classes of *Sargassum flavifolium* per cobble size and depth ( $n=925$ )



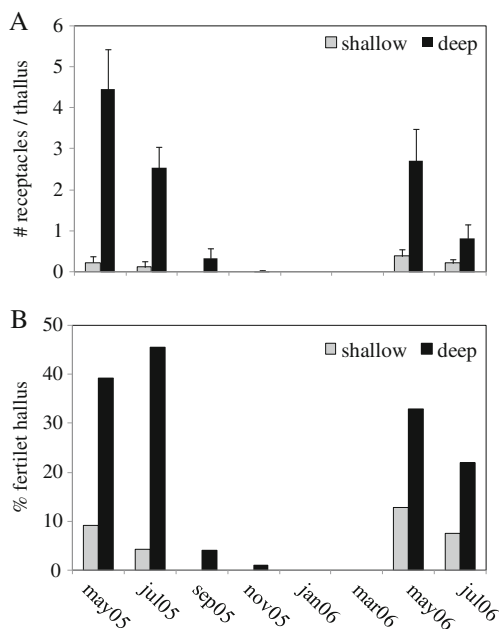
occurs in summer for cold temperate species, but in winter for most tropical species (Yoshida et al. 1998; Gillespie and Critchley 1999; Plouguerné et al. 2006) and spring subtropical

species, such as in the Canarian *Sargassum* (Díaz-Villa et al. 2005). In combination with temperature, the photoperiod must also be taken into account; most of algal developmental

**Table 3** Results of distance-based permutational ANOVAs comparing number of receptacles per thallus of *Sargassum flavifolium*

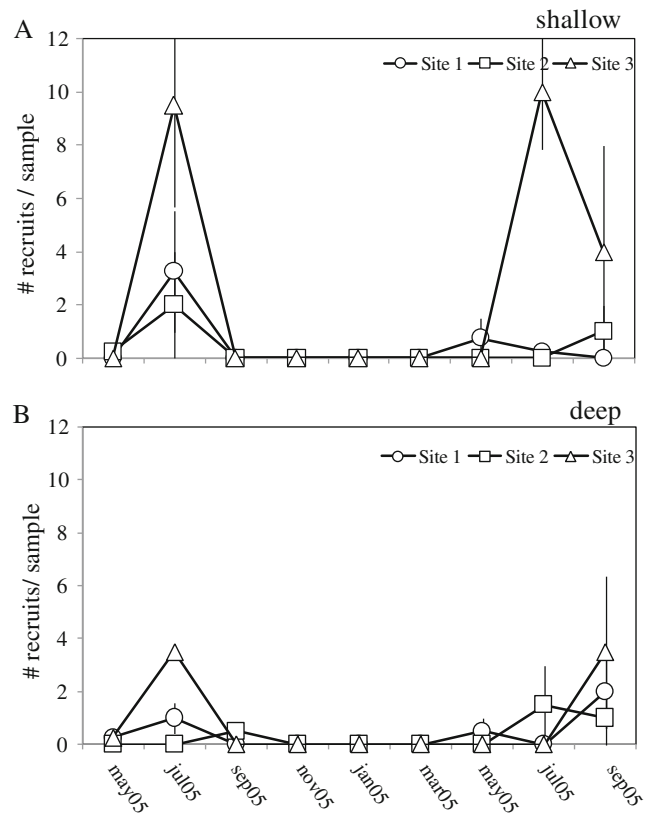
Source of variation	df	MS	Pseudo-F	p (perm)
Model testing the interaction between the factors depth, month and site				
Depth (D)	1	11.897	5.642	0.187
Month (M)	7	3.731	7.588	<0.01
Site (S)	2	0.891	1.160	0.289
D × M	7	2.677	5.047	<0.01
D × S	2	2.158	2.811	0.063
M × S	14	0.480	0.625	0.788
D × M × S	14	0.512	0.667	0.751
Res	877	0.767		
Total	924			
Model testing the interaction between depth, cobble size and site				
Depth (D)	1	3.209	16.930	0.082
Cobble size (C)	2	0.215	0.904	0.507
Site (S)	2	0.152	0.169	0.374
D × C	2	0.186	0.263	0.758
D × S	2	0.094	0.103	0.829
C × S	4	0.189	0.209	0.869
D × C × S	4	0.692	0.766	0.434
Res	907	0.037		
Total	924			

Depth (two levels: shallow 6–9 m and deep 15–18 m), month (eight levels: bimonthly samplings, from May 2005 to July 2006) and cobble size (three levels: small, about 50 mm diameter; medium ≈120 mm; large ≈210 mm) were treated as fixed factors and site (three levels) as random factor

**Fig. 5** Mean number of receptacles (+standard error) per thallus of *Sargassum flavifolium* per depth and month (a) and percentage of fertile thalli per depth and month (b) ( $n=925$ )**Table 4** Results of distance-based permutational three-way ANOVAs comparing the number of recruits per sample using depth (two levels: shallow 6–9 m and deep 15–18 m) and month (nine levels: bimonthly samplings, from May 2005 to September 2006) as fixed factors and site (three levels) as random factor

Source of variation	df	MS	Pseudo-F	p (perm)
Model testing the interaction between the factors depth, month and site				
Depth (D)	1	1.043	0.767	0.605
Month (M)	8	4.623	3.275	<0.01
Site (S)	2	3.791	9.632	<0.001
D × M	8	1.149	1.262	0.322
D × S	2	1.360	3.456	<0.05
M × S	16	1.411	3.585	<0.001
D × M × S	16	0.910	2.313	<0.01
Res	162	0.393		
Total	215			

processes (Lüning and Dieck 1989), and specifically suggested for *Sargassum* species (Uchida 1993; Arenas et al. 1995; Plouguerné et al. 2006; Thomsen et al. 2006), are under photoperiodic or combined temperature/day length control. In our study, both temperature and day length may be operating together; thus, the highest values of morphological attributes of *S. flavifolium* coincide with longer days and temperature

**Fig. 6** Mean number of recruits (+standard error) of *Sargassum flavifolium* per sample, month and site, in shallow 6–9 m (a) and deeper 15–18 m (b) bottoms ( $n=216$ )



**Table 5** Results of distance-based permutational three-way ANOVAs comparing the number of thalli per cobble size, using depth (two levels: shallow 6–9 m and deep 15–18 m) and month (nine levels: bimonthly samplings, from May 2005 to September 2006) as fixed factors and site (three levels) as random factor

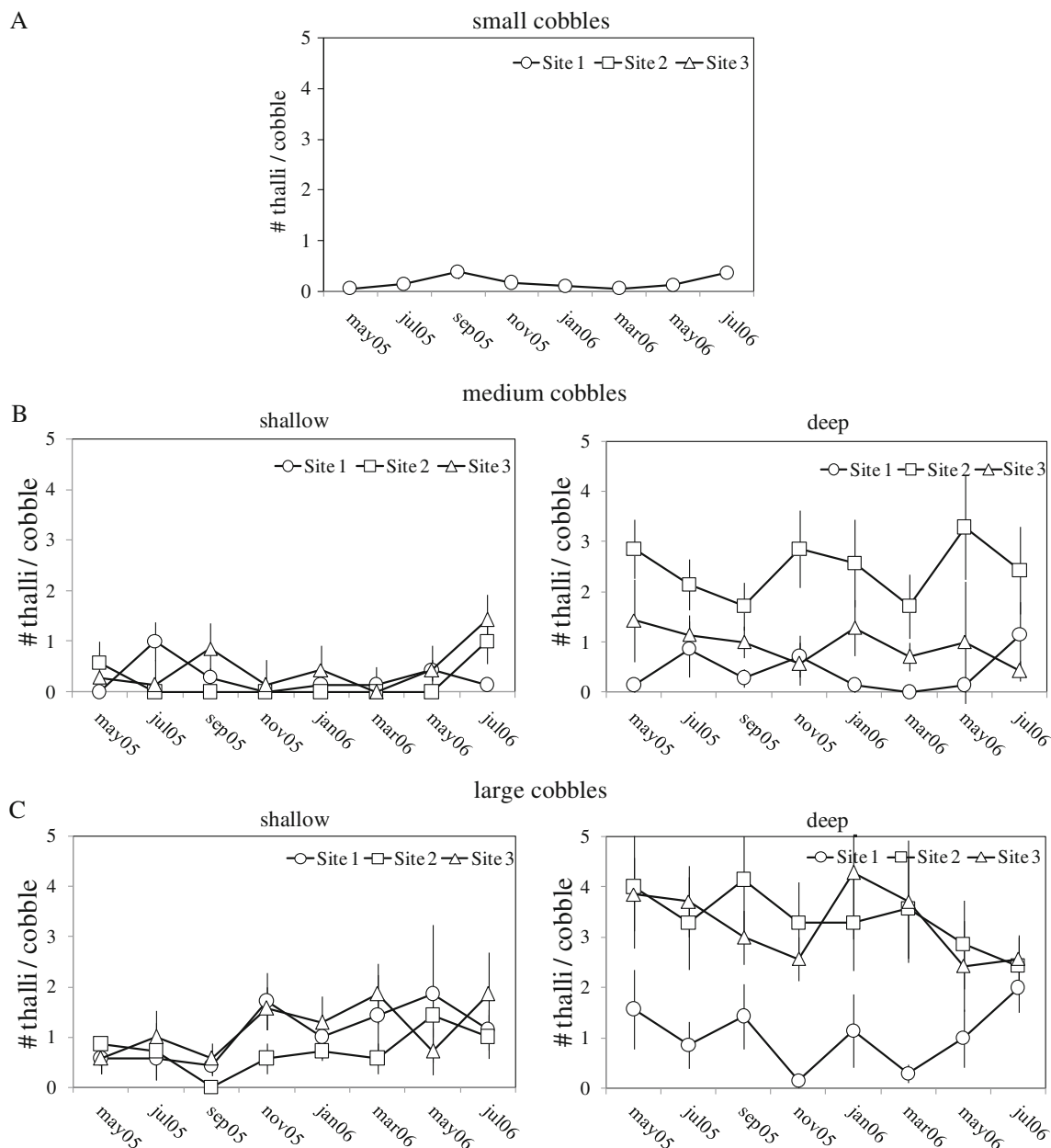
Source of variation	df	MS	Pseudo-F	p (perm)
<b>Small cobbles</b>				
Depth (D)	1	4.074	10.293	0.117
Month (M)	7	0.710	4.680	<0.05
Site (S)	2	0.008	0.039	0.963
D × M	7	0.217	1.024	0.452
D × S	2	0.395	1.757	0.174
M × S	14	0.151	0.674	0.807
D × M × S	14	0.212	0.942	0.511
Res	288	0.225		
Total	335			
<b>Medium cobbles</b>				
Depth (D)	1	19.482	4.044	0.238
Month (M)	7	1.110	1.585	0.227
Site (S)	2	3.663	10.268	<0.001
D × M	7	0.538	0.653	0.702
D × S	2	4.817	13.502	<0.001
M × S	14	0.700	1.963	<0.05
D × M × S	14	0.824	2.310	<0.01
Res	288	0.356		
Total	335			
<b>Large cobbles</b>				
Depth (D)	1	14.174	1.287	0.400
Month (M)	7	1.813	1.806	0.153
Site (S)	2	4.728	9.021	<0.001
D × M	7	1.523	1.297	0.311
D × S	2	11.007	21.001	<0.001
M × S	14	1.003	1.915	<0.05
D × M × S	14	1.173	2.238	<0.01
Res	288	0.524		
Total	335			

increase. Although the relationship between thallus morphology and nutrient concentrations could not be evaluated in this study, the highest nutrient concentrations in the Canary Islands also occur in spring, when after seasonal thermocline rupture in winter, there is a rise of nutrients from deep water (García-Braun and Molina 1984).

Wave exposure is a main factor that affects *Sargassum* morphology (Andrew and Viejo 1998), but its effects on population dynamics are variable and depend on depth (Engelen et al. 2005); and as we showed in this study, in sublittoral cobble bottoms, effects also depend on cobble size. In many algae, there is a general trend of decreasing thallus size with increasing wave exposure (Hurd 2000), but this does not occur with some species of *Sargassum*. In the eulittoral and shallow sublittoral and in

stable rocky substrates (e.g. boulders, platforms, terraces), *Sargassum* shows the largest thalli in areas under high wave exposure regimes (Engelen et al. 2005; Baer and Stengel 2010), although if wave exposure is extreme, thallus length can be affected (De Paula and Oliveira Filho 1982). On the contrary, in cobble bottoms, the larger thalli of *S. flavifolium* occur on the deeper bottoms examined (15–18 m), although with some differences between sites. This also coincides in months with less intense wave exposure. Like the rest of the variables studied, wave exposure shows in the Canary Islands noticeable seasonality changes (Pérez et al. 2003). Shallow cobbles form unstable bottoms, especially in autumn and winter, when waves are more intense and cobbles become unfavorable for development of many macroalgae (Davis and Wilce 1987b; Airoidi 2000, 2003). Ruyter et al. (1987) and Thomsen et al. (2006) suggested that sublittoral *Sargassum* could be light-limited. However, we show in this paper that rocky stability seems to play a more important role than light in sublittoral cobble bottoms. At both studied depths, cobble size is decisive in thallus length. The largest thalli are found in the more stable largest cobbles, where greater weight prevents the detachment of thalli. Thomsen et al. (2006) found similar results in *Sargassum muticum* in which thallus size is strongly dependent on substrate type, with a positive correlation to more stable boulders but negative to stones. In addition, waves remove sediments, which are abrasive for thalli and can bury them. *Sargassum* can tolerate certain levels of sedimentation, but thallus length decreases as sedimentation increases (Espinoza and Rodríguez 1987; McCook 1997; Umar et al. 1998). Although we did not evaluate the role of sedimentation on the morphology of *S. flavifolium*, it is expected that sedimentation plays more negative effects in shallow cobbles where waves shake the sand and cause abrasion on thalli. The pseudoperennial and flexible life cycle of *Sargassum* is an ecological advantage to grow in stressful environments (Engelen et al. 2005; Kawamata et al. 2011). Seasonal portions of the thallus reach their optimum development during favorable periods, while the resistant and perennial holdfast and stipes ensuring the maintenance of the population during unfavorable period (Ateweberhan et al. 2005).

Reproduction in *S. flavifolium* is strictly seasonal, as in most of the species of this genus (e.g. Ateweberhan et al. 2008; Zhanga et al. 2009; Yu et al. 2012), but shows differences in relation to depth. The optimum reproduction period is May–July, coinciding with the longest thalli, as has been documented for other *Sargassum* (Gillespie and Critchley 1999; Rivera and Scrosati 2006; Yu et al. 2012). During this period, both temperature and day length increase, playing a key role in promoting the formation of reproductive tissues (Yoshida et al. 1998; Yu et al. 2012). In our study, deeper populations of *S. flavifolium* show a greater reproductive capability than shallow populations, and the number of receptacles per thallus in the deeper was ten times superior to the shallower. In deep samples,



**Fig. 7** Mean number of thalli per cobble (+standard error) of *Sargassum flavifolium* per cobble size: small 50 mm (a), medium 120 mm (b) and large 210 mm (c) cobbles ( $n=336$ )

fertile thalli contribute with 22–45 % to the total population and reproductive period also include September and November, while shallow samples only reach 4–13 % and they are restricted to May and July. Depth-related reproductive differences have previously been documented, suggesting that depth should be considered as an important factor regarding maturation in *Sargassum* (Chan and Ang 2000; Engelen et al. 2005; Yu et al. 2012). In cobble bottoms, however, environmental disturbances (cobble movement, sedimentation) cause a reduction in thallus size and reproductive capability, as in other type of habitats with high sedimentation (Espinoza and Rodríguez 1987).

*Sargassum* reproduction and recruitment are coupled, and recruits are conspicuous shortly after the reproductive period (Ang 1985). Recruitment in *S. flavifolium* shows a similar seasonal pattern to that of reproduction, although the peak of recruits occurs 2 months later than optimum reproduction. The zygotes of *Sargassum* have limited dispersion and usually germinate near the adults, and only vegetative drifting fragments with viable reproductive structures can increase their dispersion (Kendrick and Walker 1995; Stiger and Payri 1999). This means that recruitment patterns and recruit density are related to adult phenology and density (Kendrick 1994). Similar to that found in other fucal (Vadas et al. 1990; Ang

1991), recruitment in *S. flavifolium* shows high spatial variability. Settlement is a critical and unpredictable phase, where apart from disturbance and abiotic characteristics of the environment, factors such as competition and predation can acquire an important role (Schiel and Foster 2006; Monteiro et al. 2012). Differences in the number of recruits between depths are evident, and they are higher in shallow bottoms. It is possible that during the first phase of settlement, as studied here, and in the first weeks of development, recruits may have better conditions for settlement, e.g. light irradiation. Furthermore, an incorporation of propagules from the near shallow rocky platforms might have been possible. At a depth between 0 and 5 m, populations of *S. flavifolium* are common at the study sites (Sangil et al. 2011, 2014), but not under 5 m, because from about this threshold rocky platforms are occupied by urchin barrens and *Sargassum* populations are absent. Although sand abrasion and burial can be stressful to small recruits (Thomsen et al. 2006), settlement of *S. flavifolium* coincides with the period of less wave energy.

Finally, number of thalli per cobble of *S. flavifolium* is different on medium and large cobbles and between depths. The number of thalli on both cobble sizes is superior in deeper than in shallower bottoms, following the same trend observed in thallus length and reproduction. In small cobbles, there are no differences with depth. Number of thalli per cobble is the parameter with less seasonal variability, as some previous studies on other *Sargassum* have showed (Kendrick and Walker 1995; Ateweberhan et al. 2009). Nevertheless, seasonal patterns were observed in small cobbles and shallow medium cobbles, where the highest number of thalli coincides with the settlement period and the lowest number of thalli can be related to the mortality of recruits after summer (Kendrick and Walker 1995; Rivera and Scrosati 2000).

In conclusion, population dynamics of *S. flavifolium* in subtidal cobbles seems directly linked to the substrate stability. Depth and cobble size interact with seasonal pattern, which is here showed as changes in day length, temperature and wave exposure. Consequently, the most developed thalli of this species and the highest reproductive capability as well as the largest number of thalli are found in the most stable deep and large cobbles.

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