

# Biomass variation and reproductive phenology of *Gracilaria gracilis* in a Patagonian natural bed (Chubut, Argentina)

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**Abstract** The biomass variation and the reproduction of the natural *Gracilaria gracilis* bed in Bahía Bustamante (Patagonia, Argentina) were analyzed for 2 years, with the aim of determining the present situation of the population for an updated status overview; establishing the relevant features of the temporal variation in both biomass and reproductive states in relation to environmental factors, epiphytes and associated algae; and assessing carpospore availability for future spore-culture development. Field measurements and sampling were performed monthly between March 2006 and February 2008. In both years, *G. gracilis* biomass presented marked seasonal variations, with a minimum in winter and a maximum in late spring and in summer. During both years, coexistence of the three life-cycle phases was found, with dominance of tetrasporophytes. Two data sets from individuals originated from sexual reproduction (tetraspores and carpospores) and from asexual reproduction by thallus fragmentation were analyzed separately. In the fragmentation fraction, tetrasporophyte frequencies remained higher than those for gametophytes. However, in the spore-originated fraction, a

generation ratio close to 0.5 was observed. Female gametophytes bearing cystocarps were always present, with a maximum in summer and autumn. Biological data were related to environmental factors by means of canonical correspondence analysis (CCA). The first year was characterized by higher biomass values of *G. gracilis* and *Undaria pinnatifida*, lower epiphytism, larger *Gracilaria* thalli and greater proportion of mature tetrasporophytes and gametophytes. The second year was characterized by a high proportion of *Gracilaria* vegetative thalli and high epiphyte density. The best time to obtain spores from cystocarpic thalli would be in summer and early autumn.

**Keywords** *Gracilaria gracilis* · Carposporophyte · Biomass · Phenology · Natural bed

## Introduction

The genus *Gracilaria* Greville (Gracilariaceae, Rhodophyta) includes species of worldwide economic importance, being the main source of agar (Critchley 1993; Zemke-White and Ohno 1999; Smit 2004). The increasing interest in agar as a commercial product has encouraged study of *Gracilaria* from biological, genetic, reproductive, ecological and cultural viewpoints (e.g., Santelices and Doty 1989; Kain and Destombe 1995; Oliveira et al. 2000; Buschmann et al. 2001; Guillemain et al. 2008a). As to the market economy, *G. gracilis* (Stackhouse) Steentoft, Irvine & Farnham and *G. chilensis* Bird, Mc Lachlan & Oliveira are the most important species due to their agar yield and quality (Oliveira et al. 2000). *Gracilaria gracilis* is found in diverse places in Europe and it was recently identified by the barcoding approach in Northern Africa (Engel et al. 2001; Skriptsova and Yakovleva 2002; Polifrone et al.

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2006; Rebello et al. 1996; Wakibia et al. 2001, Guillemín et al. 2008b). In Argentina, this species identified by using the barcoding approach (Destombe et al. 2010), grows exclusively in Chubut province. The commercial beds are restricted to relatively small bays in the north of Golfo San Jorge. In order of importance according to their biomass and extension, these beds are Bahía Bustamante, Bahía Melo, and Bahía Arredondo. During the 1980s and 1990s, various studies of *G. gracilis* biology, population dynamics, and ecology were carried out (Mayer 1981; Boraso de Zaiuso 1983, 1984, 1987, 1989, 1995a; Boraso de Zaiuso and Paternoster 1985; Boraso de Zaiuso et al. 1997; Romanello et al. 1993). Like other *Gracilaria* species (Guillemín et al. 2008a), *G. gracilis* reproduces sexually through haploid and diploid spores, which produce both haploid and diploid individuals consisting of erect thalli that grow from a perennial holdfast attached to the substratum. In addition, haploid and diploid individuals are able to reproduce vegetatively by thallus fragmentation. These fragments are unable to form new holdfasts and to re-attach themselves to hard substrata. Contrary to hard substrata populations, spore recruitment in soft-bottom populations is unlikely, and the reproduction is mainly vegetative (Simonetti et al. 1970).

The population in Bahía Bustamante is particularly interesting because it has been one of the most productive places with harvests since the end of the sixties. In spite of the fact that the harvests were restricted to algae on the beach as wave-cast drift material, the annual *Gracilaria* production in the seventies (3,000–4,000 dry tons) decreased to average values of 2,000 dry tons during the 1980s (Borasó de Zaiuso 1995b). The decline was even more noticeable from the 1990s, reaching only 66.1 dry tons in 2003 (Borasó et al. 2006).

There has been a worldwide depletion of the *Gracilaria* natural beds due to overexploitation, which has led to the development of various culture techniques to increase the biomass (Santelices and Ugarte 1987; Critchley 1993). However, the applied vegetative-propagation techniques produce culture aging in the long run, forcing the constant inocula re-planting from the natural population (Buschmann et al. 1995, 2001).

The use of spores as seeds allows obtaining high biomass from a small thallus number (Santelices and Doty 1989). In Chile, the main agar-producing country in the world, the aging of *G. chilensis* populations has caused a major decline in farm productivity. Therefore, some stocks were renewed by means of spores from field-collected fertile thalli (Infante and Candia 1988; Alveal et al. 1997). Carpospores are the most commonly employed spores because, unlike the tetrasporophytic thalli, cystocarps (carposporophyte) formed after fertilization on female gametophyte, can be detected by the naked eye (Santelices

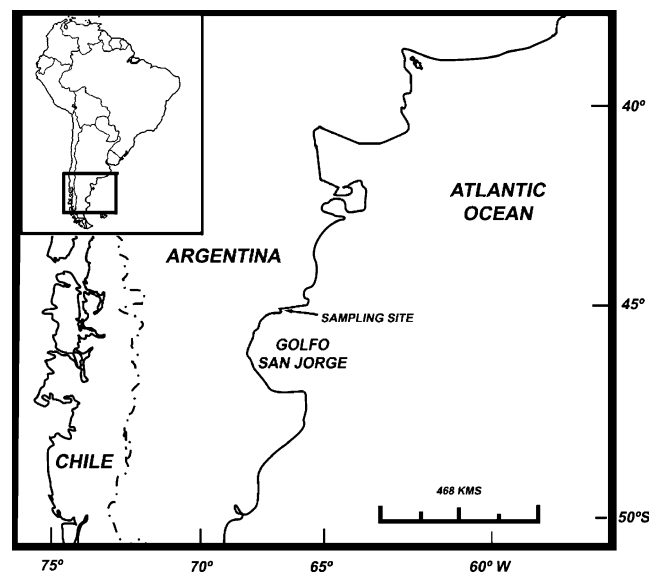
and Doty 1989). The spore cultures put an interesting perspective on the application of genetic-manipulation techniques and the development of strain selection processes. For spore culture development, it is necessary both to have reproductive material and to know about its seasonal availability as well as its spatial distribution (Dawes 1995).

In Argentina, there is no experience in *G. gracilis* culture, except for a few attempts at vegetative-propagation experiments in Golfo Nuevo (Borasó de Zaiuso 1984; Casas and Piriz 1998).

In this study, we analyzed biomass variation and reproduction in a natural *G. gracilis* bed in Bahía Bustamante during 2 years, aiming at: (1) knowing the present situation of the population in order to obtain an updated status overview, (2) establishing the relevant features of temporal variation in both biomass and reproductive states in relation to environmental factors, epiphytes and associated algae, and (3) assessing carpospore availability for future spore-culture development.

## Materials and methods

Bahía Bustamante (45° 08 S, 66° 32 W) is located in the department of Escalante, province of Chubut, in the north of Golfo San Jorge (Fig. 1). The *G. gracilis* bed is found in the south of the bay, between 0 and 8 m below mean low water (MLW) level. Field measurements and sampling were performed monthly for 2 years, between March 2006 and February 2008. In March 2006 samples of sediment at each sampling site were analyzed using the traditional granulometric approach (Wentworth 1922). Three fractions: gravel, sand and silt/clay were separated.



**Fig. 1** The research area in Argentina. Location of the sampling site at Bahía Bustamante in Golfo San Jorge

For each sampling site in situ data of temperature, salinity (*Pinpoint*<sup>®</sup> Salinity Monitor, American Marine INC, USA) and pH (*Pinpoint*<sup>®</sup> pH Monitor, American Marine INC, USA) were taken from the subsurface water. The day length was determined at the sampling date. Water samples were also collected for nutrient analysis, keeping them refrigerated until arrival to the laboratory. Nutrient concentrations were analyzed by colorimetric techniques on a Technicon II autoanalyzer, at the Marine Chemistry Laboratory of the Instituto Argentino de Oceanografía (IADO). Nitrates, nitrites, and phosphates were determined as described in Treguer and Le Corre (1975); Grasshoff et al. (1983) and Eberlein and Kattner (1987), respectively.

*Gracilaria gracilis* samples were collected by scuba diving. Each monthly sample consisted of 20 sampling units of 0.25 m<sup>2</sup> each. On four transects parallel to the shore, five sampling units were located 100 m apart from each other. Each sampling unit was relocated during the following month using a GPS. Due to the low ratio between each sample and the total sampled area, it is assumed that each sampling did not affect the subsequent ones. For each sampling unit, drained wet biomass of *G. gracilis*, larger-size epiphytes, associated algae and *Undaria pinnatifida* were determined separately. In particular, the latter was considered because of its importance in the area as a recently present invasive alga (Casas et al. 2004). At each date, 100 whole *Gracilaria* thalli were taken randomly. When the total number of thalli was less than 100, all individuals were studied. The reproductive status for each thallus was determined by observation with binocular and optical microscopes. The female gametophyte is identified only after fertilization, when the cystocarp is formed. The thallus' length and weight (AND FK-400,  $\pm 0.001$  g) were also assessed. The presence of basal disk in the thalli was recorded as an indicator of its origin from spore germination. The epiphytic density was evaluated by observation with a binocular microscope, and expressed as number of epiphytes per cm<sup>2</sup> of *G. gracilis* thallus. Biological data were related to environmental factors by means of a canonical correspondence analysis (CCA; ter Braak 1986; ter Braak and Smilauer 1998). Untransformed quantitative data were used and the significant variables ( $P=0.05$ ) were selected. A previous detrended correspondence analysis (DCA) was made to calculate the gradient length and to verify the CCA was the appropriate procedure (ter Braak 1995). The biological variables considered in the multivariate analysis were the following: B: *G. gracilis* biomass (kg m<sup>-2</sup>); ED: epiphyte density (epiphytes cm<sup>-2</sup> on *G. gracilis* thallus); UB: *U. pinnatifida* biomass (kg m<sup>-2</sup>); AB: associated-alga biomass (with the exception of *U. pinnatifida*) (kg m<sup>-2</sup>); W: mean weight of *G. gracilis* thalli (g); L: mean length of *G. gracilis* thalli (cm); C: percentage of *G. gracilis*

cystocarpic thalli; M: percentage of *G. gracilis* mature male thalli; T: percentage of *G. gracilis* mature tetrasporophytic thalli; V: percentage of *G. gracilis* vegetative thalli; BD: percentage of *G. gracilis* thalli with basal disk. The abiotic factors considered in the CCA were the following: N: nitrates ( $\mu\text{moles L}^{-1}$ ); Ni: nitrites ( $\mu\text{moles L}^{-1}$ ); P: phosphates ( $\mu\text{moles L}^{-1}$ ); D: length of daily light period; Temp: temperature ( $^{\circ}\text{C}$ ); Y: annual period (2006–2007:1; 2007–2008:2).

## Results

### Environmental characterization

More than 80% of the sampling places showed a substratum with medium to fine sand, with only three sites with gravel preponderance. The surface water temperature varied between 7°C in August 2007 and 18°C in February 2008 (Fig. 2). The pH ranged from pH 7.3 in August 2007 and pH 7.8 in January 2008 (Fig. 2). The salinity ranged between 33.8 and 34.4 PSU.

Both nitrates and nitrites had their maximum values in autumn for both years: 5.65  $\mu\text{moles L}^{-1}$  for nitrates in May 2006 and 0.34  $\mu\text{moles L}^{-1}$  for nitrites in May 2007 (Fig. 3). Phosphate values ranged between 0.63 and 2.73  $\mu\text{moles L}^{-1}$ , with their maximum values in autumn and spring for each year (Fig. 3).

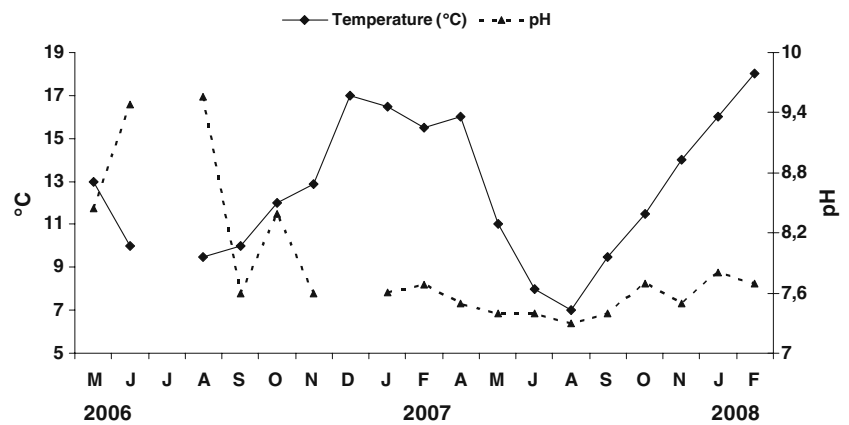
### Biomass *G. gracilis* variation

During the first period the annual mean *G. gracilis* biomass was 1.79 kg m<sup>-2</sup> and it amounted to 0.79 kg m<sup>-2</sup> in the second period (Fig. 4). Significant differences were found between both years (ANOVA,  $P<0.05$ ). The highest mean biomass was  $4.27\pm 5.23$  kg m<sup>-2</sup> in December 2006. After a heavy storm, the minimum mean biomass was  $0.02\pm 0.04$  kg m<sup>-2</sup> in September 2007. The mean biomass was high (3.7 kg m<sup>-2</sup>) in November 2006, but it showed a large variation (standard error=6.14), due to very high values in some sampling units and zero values in others. In both years, the greatest biomass values were observed during late spring and summertime, and the lowest mean values appeared in winter amounting to 1.02 kg m<sup>-2</sup> in 2006 and 0.27 kg m<sup>-2</sup> in 2007. These values agree with the CCA image of inter-annual and seasonal *Gracilaria* biomass variation.

### Reproductive phases

The proportion of gametophytes and sporophytes was compared with Chi-square test, between the population fraction originated from spore germination (with basal disk)

**Fig. 2** Variation of pH and temperature in the area from March 2006 to February 2008



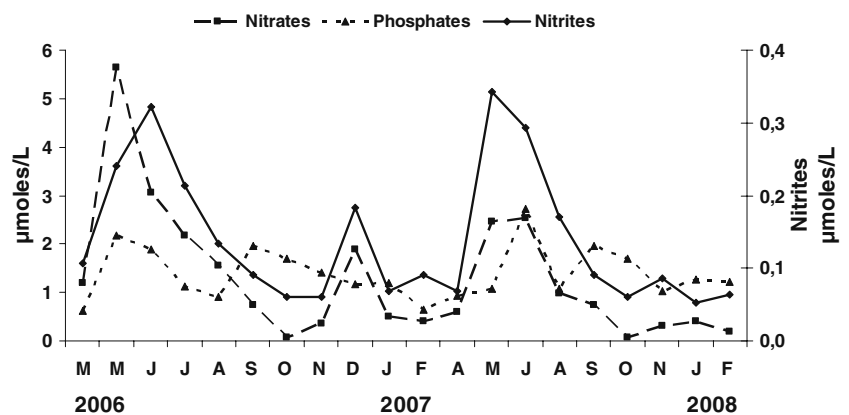
and the fraction produced by thallus fragmentation. Significant differences were found for the first year (Chi-square=4.82, 1 *df*,  $P < 0.05$ ) and very significant differences for the second year (Chi-square=20.8, 1 *df*,  $P < 0.01$ ). The fraction originated from spores had generation ratios (diploid thalli/diploid thalli+haploid thalli) ranging from 0.14 in May 2007 to 1 in January 2007, with an average of 0.5. The generation ratios in the thalli produced by fragmentation were usually more than 0.5, amounting to 0.86 in August 2007 and February 2008 (Fig. 5). Taking into account their different composition, the population fractions were separated in the next analysis. In the fraction produced by fragmentation (Fig. 6a), the proportion of tetrasporophytes was greater than the one of gametophytes, reaching 75% in February 2008. Gametophytes surpassed tetrasporophytes only in Jun 2006, but with a generation ratio close to 0.5. Carposporophytes were absent in July 2006 and August 2007; the maximum percentage was observed in May 2006, with 30% of carposporophytes. Male gametophytes were also present during both years. Their proportion ranged between 3.4% in February 2008 and 27.1% in March 2006, without an evident seasonal variation. The proportion of vegetative thalli had a maximum in spring 2007, representing 69.4% of the population in November. The sex ratio (male

gametophyte/male gametophyte+female gametophyte) ranged between 0.2 and 1 in the fragmentation fraction. When the total thalli of both sampled years were taken into account, the sex ratio was 0.54.

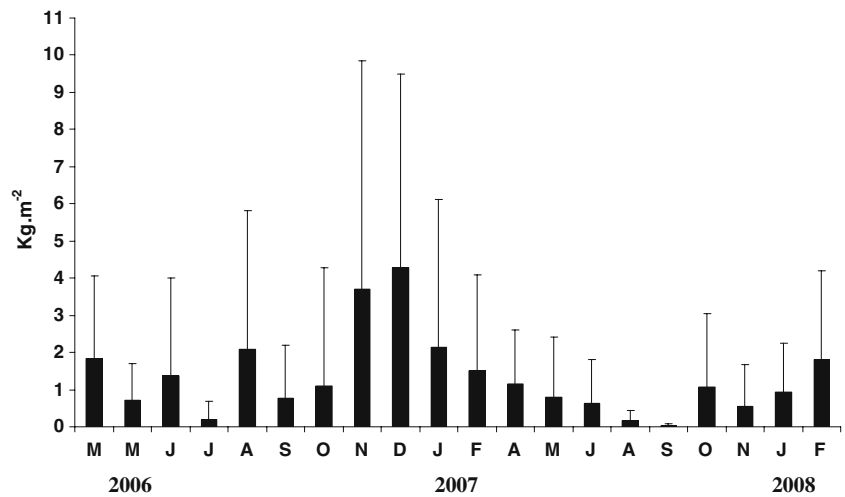
In the fraction originated from spores (Fig. 6b), the proportion of tetrasporophytes was more variable than in fragmentation fraction, reaching 100% in January 2007, but with a total number of four thalli. Tetrasporophytes percentage ranged between 9.1% and 81.2% in October and November 2006, respectively. Female gametophytes were absent in October 2006, June and August 2007. In May 2007, a maximum of 40% was observed. Male gametophytes had a maximum of 43% in October 2007. Vegetative thalli had seasonal variation, with greater proportion at the end of winter and in spring of both years, reaching a maximum of 61% in November 2007. The sex ratio ranged from 0 to 1, with an average of 0.54. When the total thalli of both sampled years were taken into account, the sex ratio was 0.56.

Mixed-phase thalli with cystocarpic branches grown on the tetrasporophytes were observed in ten sampling dates in both fractions. The maximum proportion was registered in February 2007, with six mixed-phase thalli on a total of 65, all of them without basal disk (Fig. 6a, b).

**Fig. 3** Variation in the concentration of phosphates, nitrates and nitrites in the area from March 2006 to February 2008



**Fig. 4** Monthly mean ( $\pm$ SE) wet biomass of *G. gracilis* during March 2006 to February 2008



*G. gracilis* thallus size

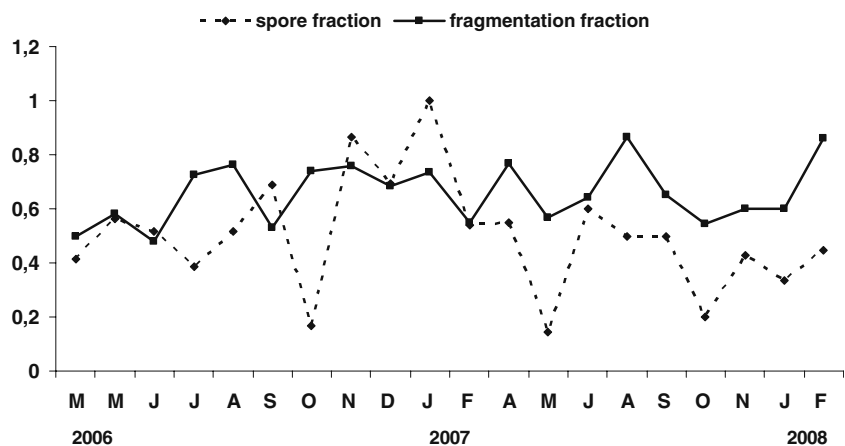
In the fraction produced by fragmentation, the highest mean weight was 15.1 g in March 2006, while the minimum weight was 1.2 g in November 2007 (Fig. 7a). The highest mean length was 67.6 cm in March 2006, while a minimum length of 14.8 cm was observed in September 2007 (Fig. 7b). In the fraction originated from spores, the highest mean weight was 16.4 g in January 2007, while the minimum weight was 2.4 g in May 2007 (Fig. 7a). The highest mean length was 52.4 cm in May 2006, while a minimum length of 11.5 cm was observed in September 2007 (Fig. 7b).

No significant differences were found between both fractions, neither for the average weight (ANOVA, 38 df,  $P=0.054$ .) nor for the average length (ANOVA, 38 df,  $P>0.2$ ).

Associated algae and epiphytes

For both years, the highest associated algal biomass values were observed at the end of winter and in spring.

**Fig. 5** Generation ratio in fractions produced by fragmentation and coming from spores



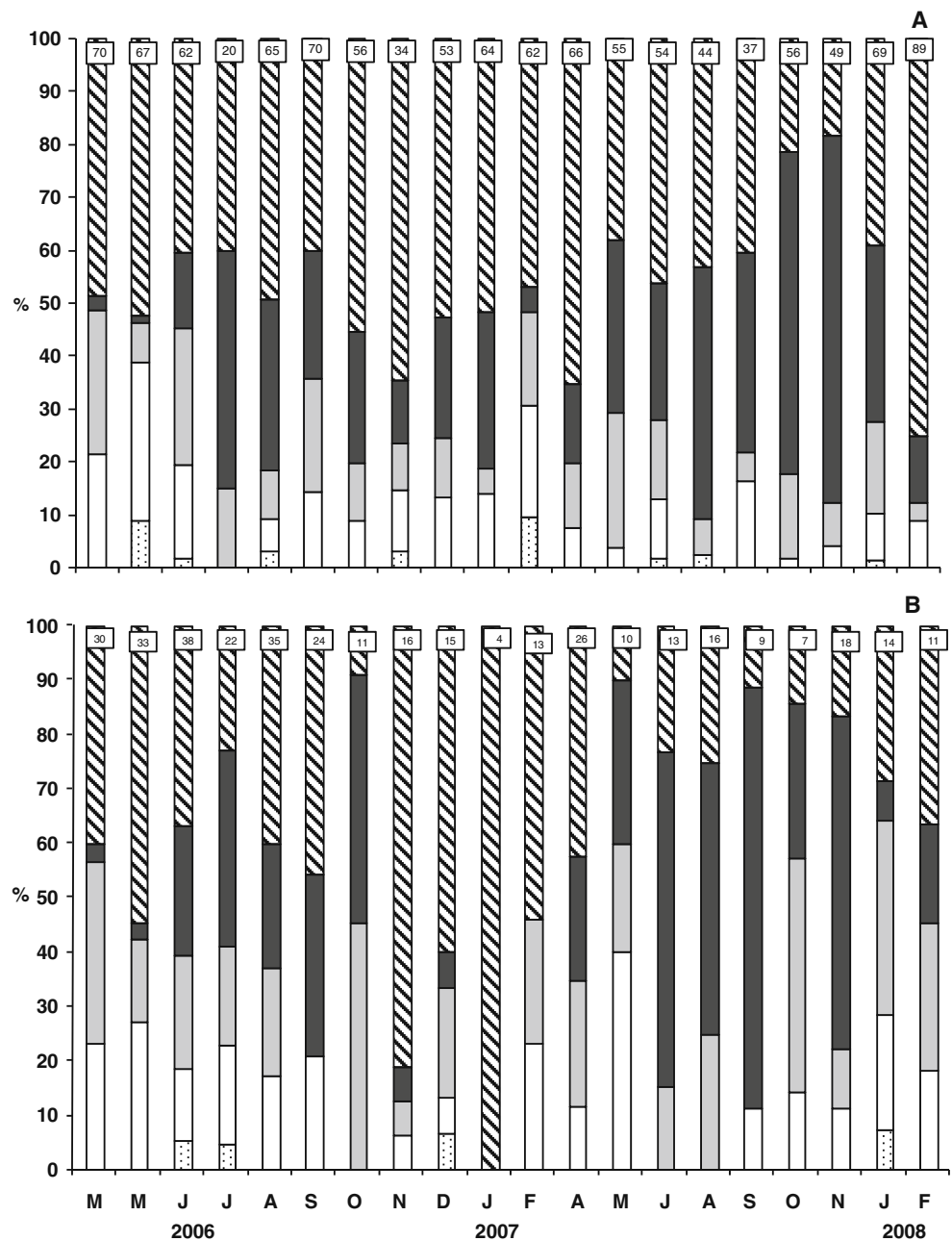
In spring–summer 2006, *U. pinnatifida* represented 60–90% of the associated algae biomass, reaching a maximum of 1.34 kgm<sup>-2</sup> in November 2006 (Fig. 8). During the second period, *U. pinnatifida* biomass decreased deeply, whereas the rest of the associated algae, mainly Ceramiales, reached the highest biomass.

The mean biomass of *U. pinnatifida* and *G. gracilis* exhibited similar variations with higher values in spring–summer 2006 and lower ones in autumn–winter 2007 (Fig. 8). A biomass recovery was observed in spring–summer 2007 without reaching the values achieved during the first year. In February 2008, the values of *G. gracilis* biomass and its relationship with the associated algal biomass were both recomposed and similar to those shown at the beginning of the study (Fig. 8).

Epiphyte density on *G. gracilis* ranged between 0.037±0.015 epiphytes cm<sup>-2</sup> in November 2006 and 35.27±14.67 epiphytes cm<sup>-2</sup> in April 2007. This value remained high during the whole second sampling year (Fig. 9). In both years, a similar seasonality was observed with maximum values in autumn and summer.



**Fig. 6** Percentage of different reproductive stages of the *G. gracilis* fraction produced by fragmentation (a) and the fraction coming from spores (b). Female gametophytes (white bars); tetrasporophytes (striped bars); male gametophytes (gray bars) and vegetative thalli (black bars); mixed phases (dotted bars). The total numbers of thalli are indicated on the top of each bar



Relationship between the observed biological variables and the environmental factors

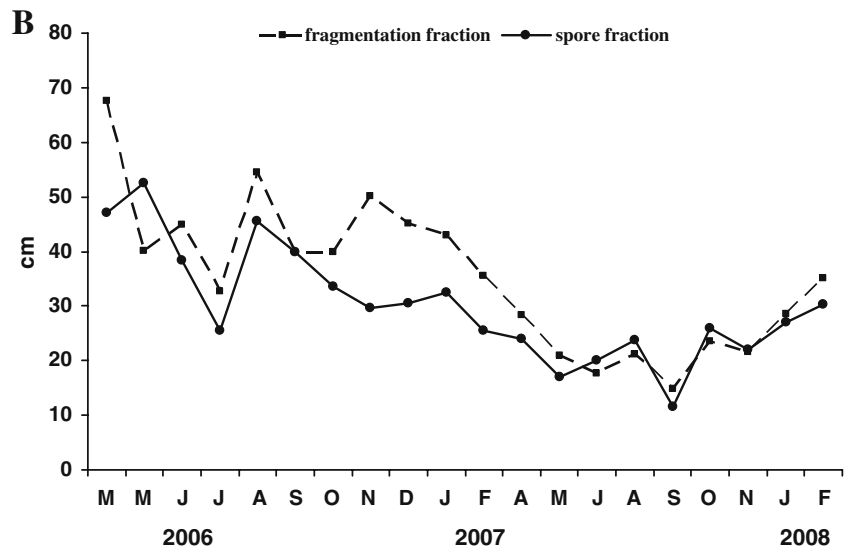
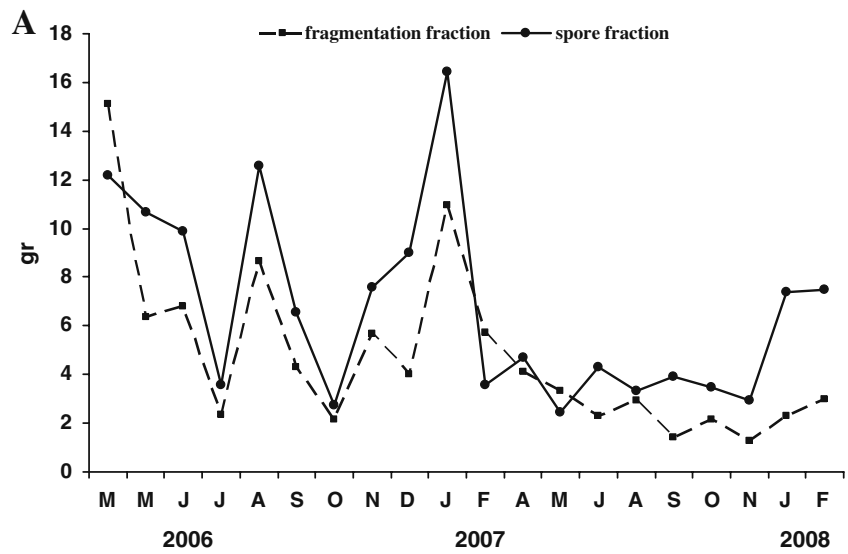
The day length, temperature, and nitrogen concentration as nitrates were significant among the abiotic factors included in the CCA. The concentration of phosphates was removed by the program in the explanatory model because it was not significant. The information provided by the nitrite concentration resulted redundant because it had the same variation as the nitrate one.

In the CCA, the first three axes explain 97.4% of the joint variation of environmental factors and biological variables (Table 1). Axis I is mainly related to the inter-

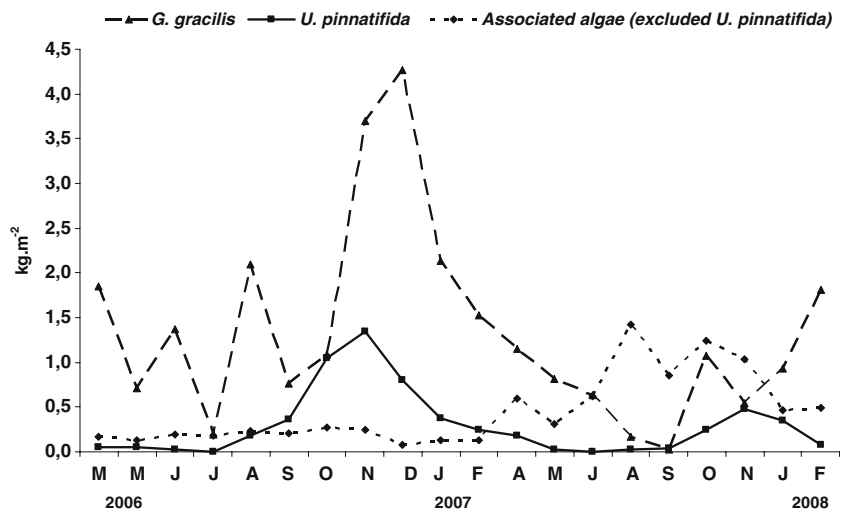
annual differences between the first (March 2006 to February 2007) and the second (April 2007 to February 2008) period (Fig. 10).

The first year was characterized by higher biomass values of *G. gracilis* and *U. pinnatifida*, lower epiphytism, larger *Gracilaria* thalli and greater proportion of reproductive individuals. These characteristics were located in the negative semi-plane of axis I. The second year of sampling is located on the positive semi-plane of axis I. This period was characterized by low *G. gracilis* and *U. pinnatifida* biomass, high biomass of the other associated algae, high *G. gracilis* vegetative thallus proportion and high density of epiphytes.

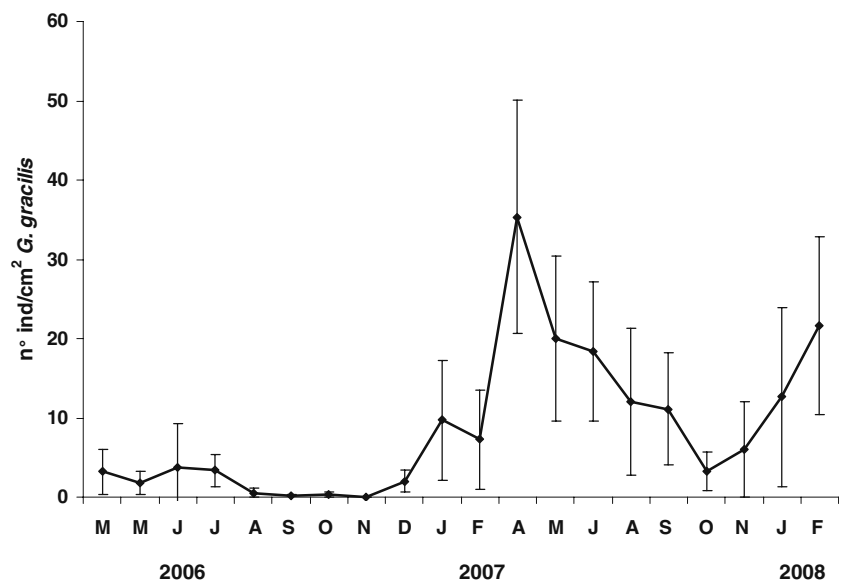
**Fig. 7** *G. gracilis* mean weight (a) and mean length (b) in fractions produced by fragmentation and fractions coming from spores



**Fig. 8** Monthly mean wet bio-masses of *G. gracilis*, *U. pinnatifida* and other associated algae



**Fig. 9** Epiphyte density ( $\pm$ SE) on *G. gracilis* thalli



The combination of axes I–III reflects the seasonal order of sampling dates. Axis II separates the summer–autumn samples from the winter–spring ones. The third axis reinforces the seasonal nature of biological variations by separating autumn and winter on the positive semi-plane from spring and summer on the negative one. The relatively high nitrate level in the autumn also helps the definition of this axis.

A second CCA was performed from biomass and proportion of reproductive phase data separated in fractions produced both by fragmentation and from spores. The results were similar to the first CCA, without adding relevant information (no data illustrated).

## Discussion

During both years, we found coexistence of *G. gracilis* male gametophytes, female gametophytes, and tetrasporophytes, as in Bahía Arredondo (Boraso de Zaixso 1995a). There was not exclusive alternation between gametophytic and sporo-

phytic phases as had been observed in Golfo Nuevo by Boraso de Zaixso and Paternoster (1985). The reproductive periods, showing the lowest proportion of vegetative thalli, were characterized by more tetrasporophytes than gametophytes. Dominance of tetrasporophytic phase has also been indicated in natural populations of several species of the genus, including *Gracilaria edulis*, *G. foliifera*, *G. chilensis*, *G. bursapastoris*, *G. coronopifolia*, *Gracilaria* (“verrucosa” type); *G. dominguensis*, *G. cervicornis*, *G. cornea* and *G. damaecornis* (Umamaheswara Rao 1973; Hoyle 1978; Romo and Alveal 1979; Whyte et al. 1981; Hay and Norris 1984; Pinheiro-Joventino 1986; Orduña-Rojas and Robledo 2002; Brito and Silva 2004). With respect to *G. gracilis*, tetrasporophyte dominance was indicated for populations in France and Italy (Marinho-Soriano et al. 1998; Polifrone et al. 2006). On the other hand, populations without phase dominance have also been observed in France and Argentina (Destombe et al. 1989; Boraso de Zaixso 1989), while populations with predominance of gametophytes were found in India and Philippines (Trono and Azanza-Corrales 1981; Oza et al. 1989).

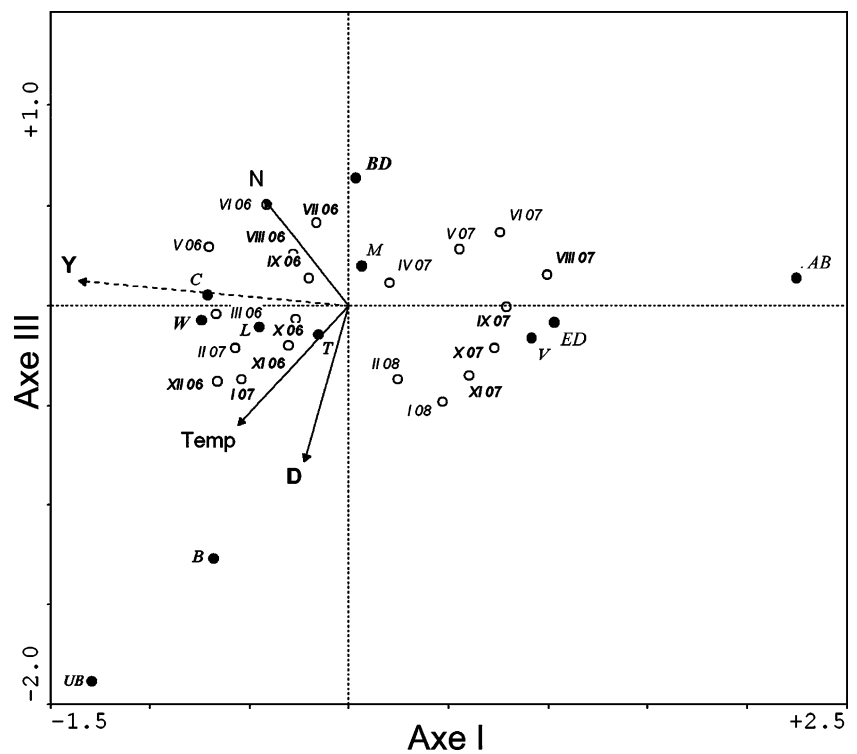
Hoyle (1978) suggested that in *Gracilaria* species, the gametophytic and the tetrasporophytic phases should ideally occur in 1:1 ratio, but Destombe et al. (1989) and Thornber and Gaines (2004) developed models in Rhodophyta where, at equal fertility, the proportion of diploid thalli was lower than the one of haploid thalli. Engel et al. (2001) considered that generation ratios different from 1:1 could be due to asexual reproduction, at least in some cases. In Bahía Bustamante, the proportion of gametophytes in the population fraction coming from spore germination was greater than in the fraction produced by fragmentation. The first fraction had an average generation ratio of 0.5, while the one produced by thallus fragmentation was usually

**Table 1** Summary of the CCA results for biologic and environmental variables

Axis		I	II	III	IV
Eigenvalues		0.092	0.038	0.008	0.0004
Biologic-environment correlations		0.906	0.806	0.536	0.460
Cumulative percentage variance	Of environment variables	34.1	48.4	51.3	052.7
	Of biologic-environment relation	64.7	91.8	97.4	100.0



**Fig. 10** CCA biplot: association between biologic and environmental variables. *B* *G. gracilis* biomass; *ED* epiphyte density, *UB* *U. pinnatifida* biomass, *AB* associated-alga biomass, *W* mean weight of *G. gracilis* thalli; *L* mean length of *G. gracilis* thalli; *C* percentage of cystocarpic thalli; *M* percentage of male thalli; *T* percentage of tetrasporophytic thalli, *V* percentage of vegetative thalli, *BD* percentage of thalli with basal disk; *N* nitrates, *D* length of daily light period, *Temp* temperature, *Y* annual period



greater than 0.5 (0.86 in August 2007 and February 2008). This fact suggests some advantages for the tetrasporophytes when environmental conditions favor fragmentation over reproduction. A factor that might favor tetrasporophytes is the different survival rates after reproduction. For farmed populations of *G. chilensis* Guillemin et al. (2008a) hypothesized that tetrasporophyte predominance could be due to its greater capacity of fragmentation. However, Santelices and Varela (1995) found a greater regeneration capacity of vegetative thalli for *G. chilensis* in comparison with the tetrasporophytes and female gametophytes. This is understandable because a reallocation of resources may be expected. The perception of the relative abundance of thalli in one phase depends on the time length while the phase appears as vegetative. Both in this work and in the Golfo Nuevo population (Boraso de Zaixso 1983), tetrasporophyte maxima succeeded the relative maxima in abundance of vegetative thalli after approximately 3 months. Thus, it is possible that most of the vegetative thalli were undifferentiated tetrasporophytes.

Despite a predominance of soft substrate in Bahía Bustamante, the gravel fraction was enough to allow recruitment from spores, as indicated by significant percentages of *G. gracilis* thalli with basal disk. The proportion of thalli produced in Bahía Bustamante from spore germination was usually lower than the one in Golfo Nuevo and Bahía Melo, as was reported by Boraso de Zaixso (1983) and Boraso de Zaixso et al. (1997). It is interesting to note that shells and small pebbles were well represented in the substratum of the last two sites (Boraso de Zaixso 1989).

Even so, over 50% of thalli were small and with basal disk in July 2006, reinforcing the idea of recruitment at this date. According to Kain and Destombe (1995), the substratum influences the phase ratio since spore recruitment is difficult in a soft substratum; then, one of the phases can predominate through vegetative propagation.

The presence of mixed phases has repeatedly been observed in this one and in other species of the genus (Oliveira and Plastino 1984; Destombe et al. 1989; Prieto et al. 1991; Brito and Silva 2004). Even though several hypotheses have been proposed in order to explain the causes of the presence of these mixed phases (Kain and Destombe 1995), the kind of abnormality found in our case, i.e., tetrasporophytes with epiphyte female gametophytes, could be attributed mainly to the in situ germination of tetraspores (Oliveira and Plastino 1984; Candia 1988; Destombe et al. 1989; Polifrone et al. 2006). This idea is supported by the laboratory observations by Destombe et al. (1989), who obtained the same kind of abnormal individuals when they grew *G. gracilis* diploid thalli.

Seasonality in growth and reproduction has been found for natural populations of different *Gracilaria* species, especially at high latitudes, exhibiting the highest growth rates in spring and/or summer and low growth rate in winter (Kain & Destombe 1995). Laboratory assays support these observations on the effect of temperature. Levy et al. (1990) obtained the lowest values of growth at 10°C and higher ones between 16 and 28°C, when they worked with specimens of *G. gracilis* from Argentina. Macchiavello et

al. (1998) also obtained higher growth rates at different light intensities between 25°C and 28°C in an Argentinean *G. gracilis* strain from Puerto Madryn. On the other hand, in an outdoor *G. gracilis* cultivation at Namibia Rebello et al. (1996) observed, a maximum daily growth in spring, between 21.5°C and 25.5°C and a minimum growth in autumn.

In both years, the biomass of *G. gracilis* in Bahía Bustamante showed marked seasonal variation, with a minimum in winter and the highest biomass in late spring and in summer. This biomass increment was associated with higher temperature and longer days. These observations are consistent with those reported for other natural populations of the same species in Golfo Nuevo and Bahía Melo (Boraso de Zaixso 1983; Boraso de Zaixso et al. 1997). Likewise, Casas and Piriz (1998) obtained the maximum biomasses in spring and summer through experiences of culture in Golfo Nuevo.

Regarding the nutrients, the phosphate levels seem not to limit the development of *G. gracilis* biomass or of any other biological descriptor in this study. On the contrary, nitrate and nitrite levels showed some inverse relationship with *G. gracilis* and *U. pinnatifida* biomasses on the same or forthcoming dates. The decreasing trend of nutrients from its maximum in autumn (nitrates and phosphates) and spring (phosphates) until late summer may be due to the consumption by *G. gracilis*, its epiphytes and its associated algae. These results are consistent with the existing knowledge about the strategy of *G. gracilis* and other species with respect to nutrients. Lapointe (1985) and Smit et al. (1997) indicate that *G. gracilis* can store nitrogen, enduring long periods with low levels of nutrients in the medium so that this nutrient can be assimilated by the alga when environmental conditions, mainly temperature and light radiation, are more appropriate for growth, as in spring and early summer.

Biomass is added through recruitment and growth and it is reduced mainly by losses of whole individuals and thallus fragments. Some of these mechanisms respond to seasonal factors, while others depend on the intensity, frequency and timing of storms; thus, in September 2007 the lowest biomass was recorded a few days after heavy storms. In November 2006, a large spatial variation with many empty sampling units was found despite the high average biomass. This fact may also be attributed to a post-storm sampling. According to Santelices and Fonck (1979) and Santelices et al. (1984), the rapid spatial and temporal changes in *G. chilensis* biomass are the result of underground thalli, which are resistant to losses by storms and regenerate erect thalli when the adequate conditions emerge. This mechanism was also observed in the population of *G. gracilis* from Argentina, especially in Bahía Melo (Boraso de Zaixso et al. 1997).

In the second year of sampling, lower quantities of *G. gracilis* were found, together with a tenfold increase in epiphyte density. Epiphytism usually implies lower algal growth rates and higher biomass loss (Kuschel and Buschmann 1991; Buschmann and Gómez 1993, Buschmann et al. 1995, 2001). The mean weight and length of the individuals also showed differences between the two years sampled. Both had a decreasing trend with steep variations from the beginning of 2006 to the end of 2007. Both values changed to a positive trend during the first months of 2008. The decrease in average thallus size may have been caused by either the incorporation to the population of new thalli through recruitment, or the fragmentation of big thalli already existing in the population.

In this study, the average values of *G. gracilis* biomass obtained for the period between 2006 and 2008 were higher than those observed in the same locality 20 years ago (Boraso de Zaixso 1989). Biomass values during the first year duplicated those registered during 1986–1987; however, no notable differences were observed in the physico-chemical variables (Boraso et al. 2006). This increase in biomass may be partly due to the natural recovery of the bed, but it may also reflect the lower exploitation by the concessionaires, as indicated by statistical data on *Gracilaria* harvesting from the beach cast material in Chubut province in the recent decades (Boraso et al. 2006). This would allow the drift material on the beach to be dragged out to sea again, favoring vegetative propagation.

With respect to the associated algae, the presence and abundance of *U. pinnatifida* was especially evaluated because this brown alga is an invasive species (Casas and Piriz 1996; Piriz and Casas 2001). It was present during most of the time, and important values of biomass were reached in each summer. The simultaneous presence of *U. pinnatifida* and *G. gracilis* is allowed by the kind of substrate because the invasive alga preferably developed on hard substrata (Martin and Bastida 2008), while *G. gracilis* in Bahía Bustamante mostly grows on sandy substrata. Nevertheless, through *U. pinnatifida* manipulative experiments in Golfo Nuevo, Casas et al. (2004) proved that *G. gracilis* moves away from the invasive algae. Therefore, although it would apparently not affect most of *G. gracilis* population in Bahía Bustamante, *U. pinnatifida* may be preventing a greater expansion of this bed and manipulative experiments would be necessary to test this hypothesis.

In short, vegetative propagation and sexual spore reproduction are both involved in bed-keeping. There is coexistence of reproductive thalli throughout the year, rather than an exclusive alternation between gametophytic and tetrasporophytic phases. The best time to obtain spores from cystocarpic thalli would be in summer and early autumn, when mature female gametophytes are found together with high biomass values. The biological varia-

bles, including biomass, show clear inter-annual differences. These results indicate the importance of extending the studies through several years.

The results obtained allow a better understanding of *G. gracilis* life cycle and the population biology. These aspects are relevant to build and/or improve management programs within the framework of a sustainable exploitation of the natural population. Regarding the predominance of vegetative propagation, implementation of repopulation methods through spore cultures is suggested. This method would allow an increase both in standing-stock and in population genetic variability.

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