



Reproductive biology of *Gigartina skottsbergii* (Gigartinaceae, Rhodophyta) from Chile

M. Avila¹, A. Candia¹, M. Núñez¹ & H. Romo²

¹División de Fomento de la Acuicultura, Instituto de Fomento Pesquero, Casilla 665, Puerto Montt, Chile

²Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Chile

E-mail: mavila@ifop.cl

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Abstract

Reproductive phenology and spore viability were studied in a natural bed of *Gigartina skottsbergii* at the locality of Ancud, Chile. Monthly sampling of biomass and density disclosed a decrease of both parameters in time, from 40 g m⁻² in June 1996 to 1 g m⁻² December 1997 and from almost 4 thalli m⁻² to 1 thallus m⁻² in the same period, respectively. Mature reproductive structures, cystocarps and tetrasporangial sori, were observed over the whole study period. Greatest cystocarp densities occurred in October through February (16 to 29 cystocarps cm⁻²) and of tetrasporangial sori between July and October (66 to 88 sori cm⁻²). In both types of reproductive structures, sporulation is more frequent in winter and early spring. High mortality of carpospores and tetraspores was observed in laboratory experiments performed between June and September.

Introduction

Gigartina skottsbergii Setchell & Gardner is a subtidal carrageenophyte of commercial importance in Chile (Avila & Seguel, 1993; Norambuena, 1996; Bixler, 1996). Its distribution is from Niebla (39° 53' S) to Cape Horn (54° 56' S) and it is endemic to the southernmost part of South America (Ramírez & Santelices, 1991). It is also found on the southern coasts of Argentina and the sub-antarctic islands (Piriz, 1988; 1996). In Chile this species has been exploited since the late 1980s reaching up to 8000 dry tons in 1996 (Avila et al., 1997), and the annual quantity of carrageenan extracted has gradually increased, up to 1700 t in 1996. The landing zone of this resource extends from Faro Corona (41° 47' S; 73° 53' W) to Puerto Aguirre (45° 10' S; 73° 32' W) (Schnettler, pers. com.).

The information available on this species refers mostly to its geographical distribution (Ramírez & Santelices, 1991; Santelices, 1989), vegetative frond morphology (Santelices, 1989; Alveal et al., 1990), reproductive structures (Kim, 1976) and chemical composition (Schnettler, pers. com.). Other recent studies

deal with the fluctuation of biomass and density in natural populations (Piriz, 1988; 1996; Zamorano, pers. com.) as well as with the species phenology (Zamorano & Westermeier, 1996; Avila et al., 1997; Candia, pers. com.).

The reproductive biology of this species is little documented, although sexual reproduction is known to occur seasonally (Zamorano & Westermeier, 1996; Westermeier & Sigel, 1997); an alternation in the dominance of the reproductive phases, as described for other species of the Gigartinales (e.g. *Iridaea splendens*, Ang et al., 1990) also occurs seasonally. Kim (1976) indicated that the species has a triphasic life cycle with alternation of isomorphic generations.

In contrast to other Gigartinales where the population is maintained by perennial holdfasts which produce or regenerate new blades (May, 1986; Santelices & Norambuena, 1987; Gómez & Westermeier, 1991), *G. skottsbergii* adheres to the substratum by means of secondary haptera which develop in juvenile stages (Piriz, 1996). Studies conducted at the Island of Chiloé showed that natural recruitment occurs in winter time between June and August, being

strongly associated to the period of greatest abundance of mature reproductive phases (Avila et al., 1997).

The relative importance between the abundance of cystocarpic and tetrasporic phases, spore production (carpospores and tetraspores) and recruitment has not been established for *G. skottsbergii*. The aim of this work was to determine plant fecundity and spore viability in order to establish the importance of the reproduction through spores in the maintenance of *G. skottsbergii* natural beds.

Materials and methods

To determine the abundance of the tetrasporophyte, gametophyte and carposporophyte phases in *G. skottsbergii*, a subtidal population was sampled at the locality of San Antonio, Ancud ($41^{\circ} 52' S$; $73^{\circ} 51' W$), Chiloe Island, Chile. The depth in the sampling area was between 6 and 10 m below mean tide level, the subtidal algal community was dominated by *G. skottsbergii* and covered approximately an area of 12 ha. Monthly samplings were done from June 1996 to February 1998 by hooka diving. On each occasion two non-permanent transects of 100 m each were established, perpendicular to the coastline. A 1×1 m quadrat was sampled every 10 m along the transect, making a total of 20 samples in each month. Fronds were collected with the substrate and placed in polyethylene bags and labelled, subsequently analyzed in the laboratory, reproductive phases and sizes were separated, and wet biomass (g m^{-2}) and frond density (fronds m^{-2}) were determined. Plants below 1 cm were directly counted on the substratum under a stereomicroscope in the laboratory.

Since mature fronds were scarce in the San Antonio locality, reproductive thalli of the tetrasporophyte and carposporophyte phases to study plant fecundity were sampled in a nearby natural bed. Two wet kg of each mature tetrasporophyte and cystocarpic were sampled for 21 months from June 1996 until February 1998, at Bahía de Ancud, Chiloé ($41^{\circ} 55' S$; $73^{\circ} 51' W$). Of these, 10 fronds bearing cystocarps and 10 thalli with tetrasporangial sori were separated in the laboratory. Wet biomass was determined for each frond. Subsequently, the density of reproductive structures (cystocarps or tetrasporangial sori) was determined in the 10 thalli and from each phase, by counting under the microscope the structures present in ten 1 cm^2 random locations on each frond. In tetrasporophytic thalli, the number of mature, immature

and empty tetrasporangial sori present in each sampled area was determined. Maturity in sori was determined by colour scale (Santelices & Martínez, 1997), as follows: mature sori, brown to black; immature sori, orange to light brown, and empty sori, white (Figure 1).

Gigartina skottsbergii cystocarps originate in papillae which jut out from the surface of the female gametophytic thallus. Due to this arrangement, we first counted the number of papillae cm^{-2} , then the number of cystocarps per papilla and finally the total number of cystocarps cm^{-2} . With these data, we estimated the density and abundance of reproductive structures in an area of 1 cm^2 of the tetrasporophytic and carposporophytic phases.

Carpospore and tetraspore cultures were started to determine their viability during June, July and September, when mature tetrasporophyte and carposporophyte are abundant. Sections of $2 \times 2 \text{ cm}$ were cut from gametophytic thalli with cystocarps and from tetrasporophytic thalli with tetrasporangial sori. Three pieces of each phase were placed into Petri dishes ($20 \times 100 \text{ mm}$) with filtered seawater enriched with Provasoli solution (Provasoli, 1968). After the liberation of carpospores and tetraspores, 1 ml aliquots of each spore type were drawn separately. They were put in dishes with culture medium to obtain germination and development of carpospores and tetraspores.

These cultures were kept at 10°C and 16:8 h light: dark cycle, with a $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and observed after 5 days. Two Petri dishes with carpospores and tetraspores were sampled under an inverted microscope, with 10 ocular fields ($10 \times 10 \times$) corresponding to an approximate area of 2.443 mm^2 . Live and dead spores were counted in each field. The percentage of mortality and of survival was estimated for each spore type.

Data of density of reproductive structures and biomass were tested using a correlation analysis. Mortality rates were tested using a two-way ANOVAs with *a posteriori* Tukey HSD for multiple comparisons (Sokal & Rohlf, 1981).

Results

During the study period (June 1996 to February 1998), a decrease was observed in the total and reproductive biomass (reproductive phases) in the bed studied at San Antonio. This biomass fell from about 40 g m^{-2} in winter (June, 1996) to under 1 g m^{-2} the subsequent

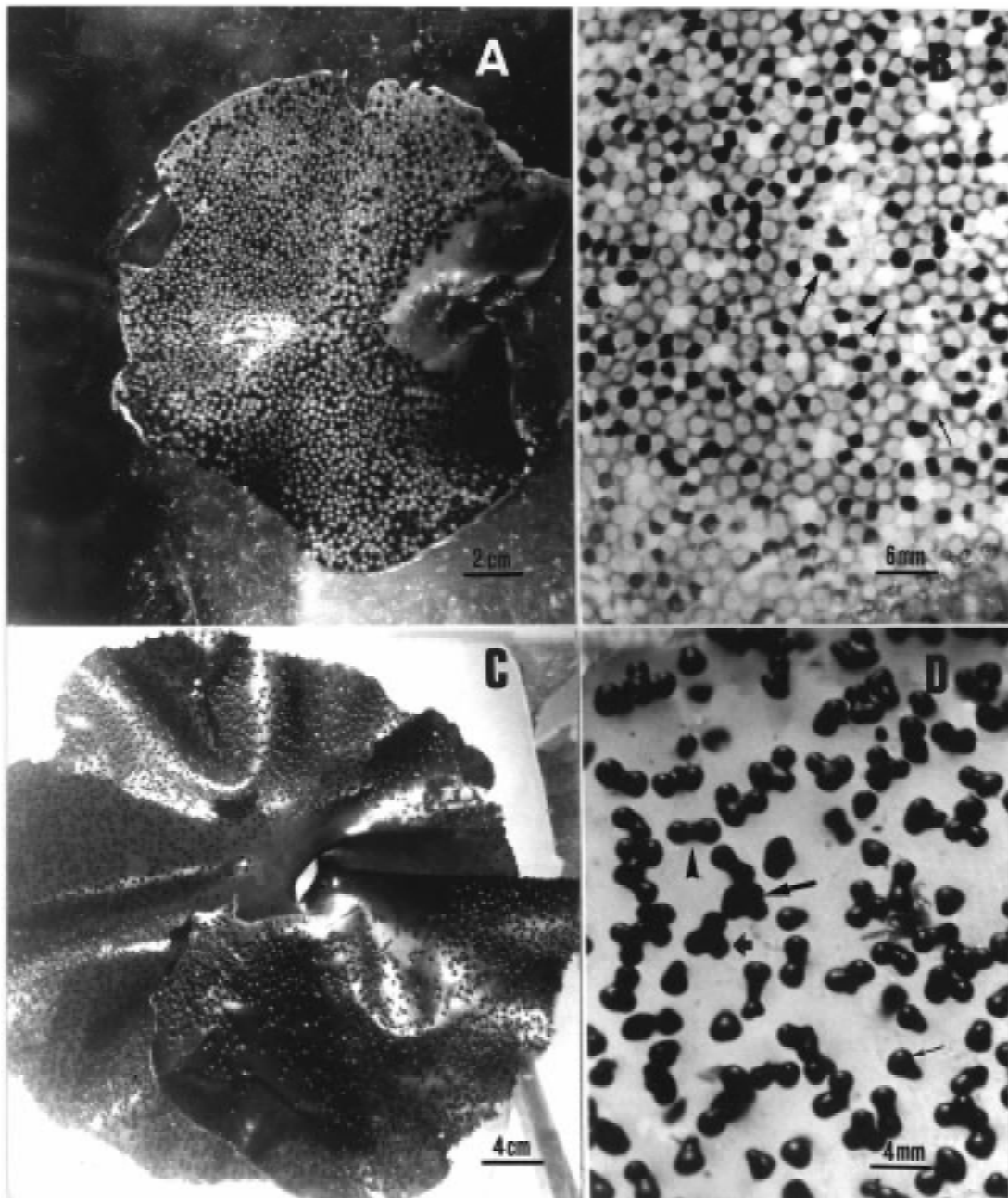


Figure 1. Morphology of the female gametophytic and tetrasporophytic thallus of *G. skottsbergii* and cystocarps and tetrasporangial sori disposition in a gametophytic and tetrasporophytic thallus. A: Tetrasporophytic thallus with tetrasporangial sori distribution. B: Mature sori (●), immature (→) and sporulated sori (▶). C: Female gametophytic thallus with papillae and cystocarps. D: Papillae and cystocarps distributions, one papilla with one cystocarp (→), with two (▶), three (◆) or four (↔).

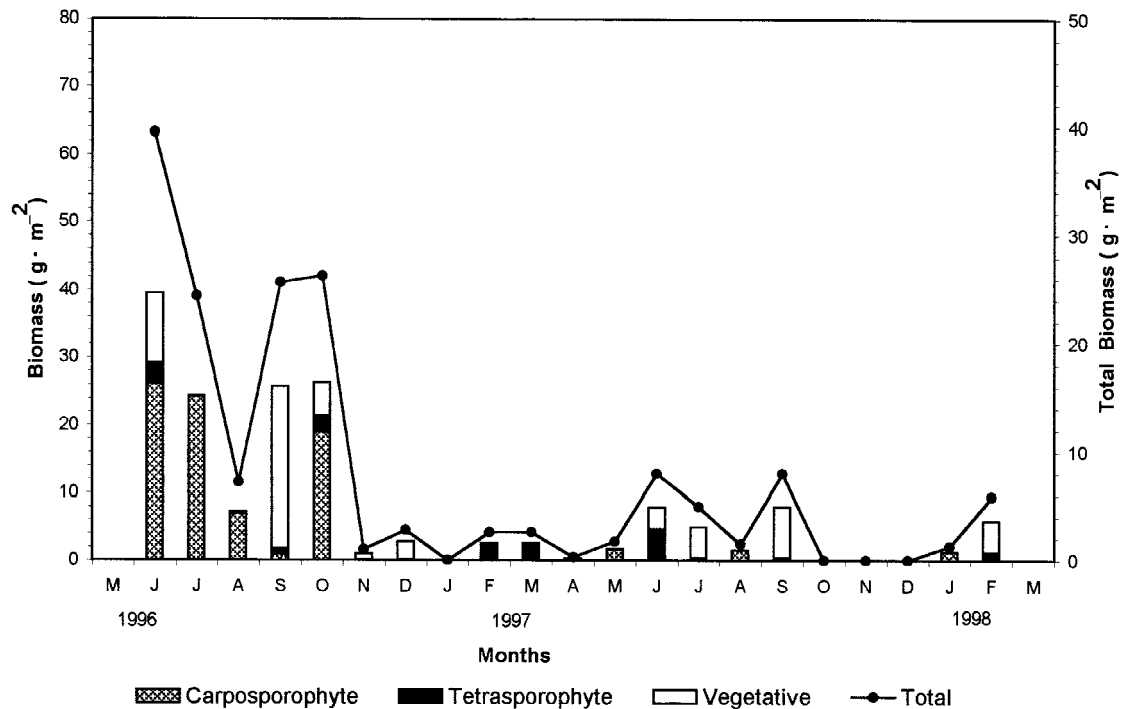


Figure 2. Monthly fluctuations in biomass (g m^{-2}) of *G. skottsbergii*.

autumn (Figure 2). A similar trend was observed in the density of reproductive thalli which strongly fluctuated from less than 4 thalli m^{-2} early winter (June, 1996) to less than 1 thallus m^{-2} in summer (February to March, 1997) and from late winter 1997 to summer 1998 (Figure 3). A clear dominance of carposporophytic biomass was recorded during winter/spring 1996 (except September), from about 26 to 8 g m^{-2} , followed by 16 months in which the reproductive biomass was less than 6 g m^{-2} until the end of the sampling period (Figure 2). Contrasting with the dominance of reproductive biomass, a very high density of vegetative fronds dominated by young / small thalli was observed during 1996–1997 (Figure 3). Notwithstanding, a simultaneous and continuous drop of plant density was recorded throughout the study period (Figures 2 and 3).

Over the whole study period, gametophytic thalli with cystocarps and tetrasporophytic thalli with tetrasporangial sori were observed. The abundance of these reproductive structures fluctuated during the study. The total density of tetrasporangial sori in the tetrasporophytic fronds remained relatively constant

over the annual cycle, with a mean of 60 sori cm^{-2} (Figure 4). A higher density of sori was observed in winter and early spring. The highest density of mature sori was seen in autumn and winter, with 10 to 28 sori cm^{-2} , and a lower abundance in spring-summer with 1 to 10 sori cm^{-2} . As for immature sori, density was high in spring and summer, ranging between 15 to 58 sori cm^{-2} . The density of dehiscenced sori showed great fluctuations, with a peak at the end of winter (August and September).

In the female gametophytic thallus, the density of papillae and cystocarps in winter months was less than 6 papillae cm^{-2} with less than 10 cystocarps cm^{-2} (Figure 5). In both cases mean values fell to 0 in early spring. Between October and November a rapid increase was observed in the density of papillae and cystocarps, with mean values of 8 to 13 papillae cm^{-2} and of 16 to 29 cystocarps cm^{-2} over the spring-summer months (Figure 5). The minimal density observed in winter-spring may have derived from the massive carpospore liberation and subsequent necrosis of the papillae, which caused their detachment and perforations, which subsequently healed.

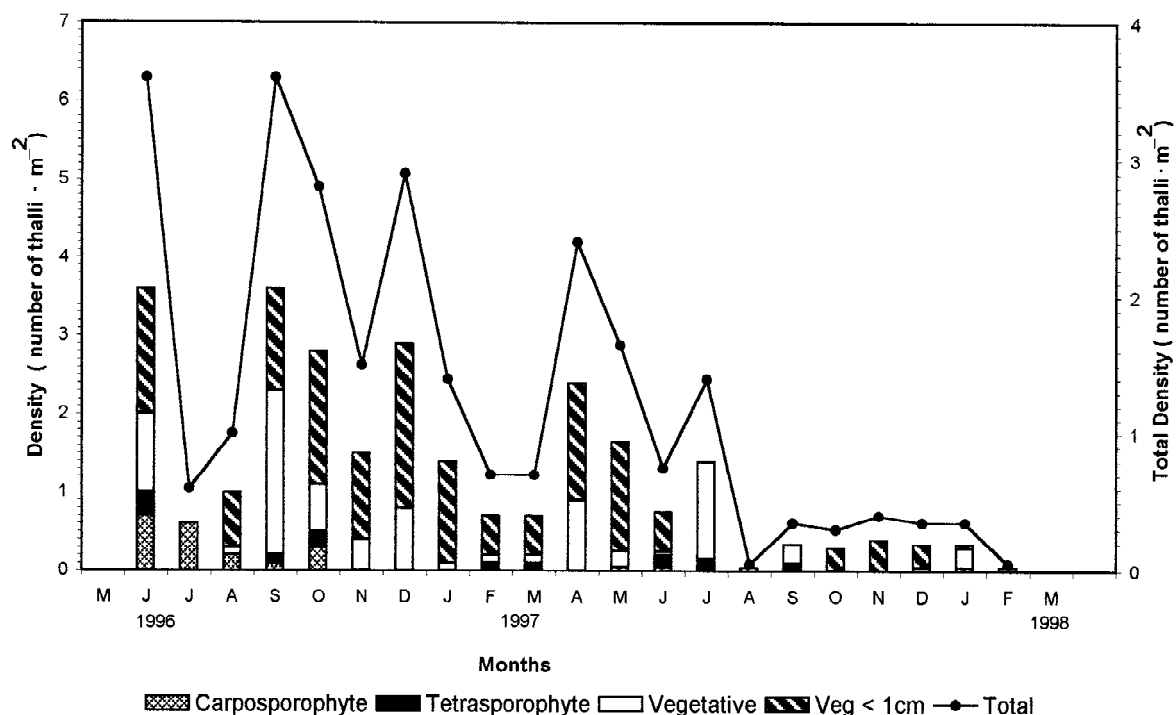


Figure 3. Monthly variations in thallus density (thalli m^{-2}) of *G. skottsbergii*.

When comparing the wet biomass of blades with tetrasporangial sori with the density of reproductive structures, we did not find any correlation between the biomass and density of reproductive structures, $r = 0.17$, while there was a better correlation between density of cystocarps and wet biomass of gametophytic fronds, $r = 0.504$. Figures 6 and 7 show monthly variations of number of tetrasporangial sori or cystocarps cm^{-2} and the variation of the biomass of the corresponding reproductive blades.

Based on our culture studies of the viability of carpospores and tetraspores performed during June, July and September, a high natural mortality for both types of spores was observed. No significant differences were found between months in mortality of tetraspores and carpospores ($p < 0.05$; two-way ANOVA). Comparisons among types of spores, indicated significant ($p < 0.05$; two-way ANOVA) differences in mortality between types of spores. For inoculated tetraspores mortality ranged from 79 to 88% and for inoculated carpospores ranged from 87% to 92%. The spores that survived germinated and developed holdfasts.

Discussion

Fluctuations of biomass and density over the study period indicated that the natural bed of *G. skottsbergii* is decreasing its standing stock. This is shown by the progressive decrease in biomass even during the growing season (spring-summer). In Chiloé Island commercial exploitation has increased since 1993 due to the great importance of Chilean raw materials for the production of carrageenans (Zamorano & Westermeier, 1996). Results show that there is no evidence of recuperation on the biomass specially during spring and summer, probably as a consequence of commercial exploitation of the resource. In relation to commercial harvesting there are 2 important aspects to consider: (1) harvesting pressure on natural beds has been increasing; and (2) harvest of attached plants by diving is permitted since there are no regulations for this species by law.

The biomass of mature tetrasporic fronds was under $5 g m^{-2}$ over the whole study period, so the availability of this type of spore for recruitment is quite scarce. The biomass of cystocarpic fronds fluctuates

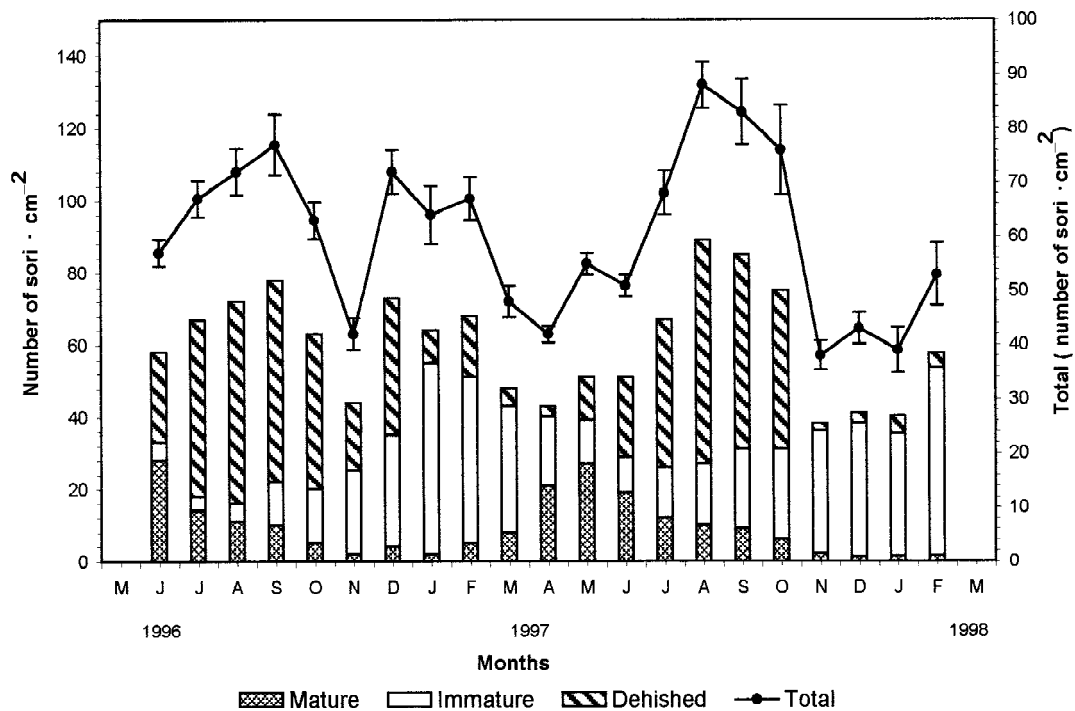


Figure 4. Monthly fluctuations in density of tetrasporangial sori in *G. skottsbergii* thalli; sori were identified as mature, immature and dehiscent sori over the study period (\pm standard error).

tuated during the study period between 26 g m^{-2} in June 1996, to less than 1 g m^{-2} from November 1996 to February 1998.

Reproductive phenology is similar to that obtained by other authors for *Gigartina skottsbergii* (Piriz, 1996; Zamorano & Westermeier, 1996). Vegetative fronds are found throughout the year where the peak of biomass is recorded in September (spring). Nevertheless carposporophytes were found only at the beginning of this study recording the highest biomass between June and October of 1996 (winter and early spring), on Argentina coasts this phase is found to be abundant in autumn (Piriz, 1996). Zamorano & Westermeier (1996) has described a bimodal cycle for cystocarpic plants, with a maximum in late winter and late summer. This second peak was not observed in this study. All authors agree that tetrasporophytes may be found throughout the year but in small proportions (Piriz, 1996; Zamorano & Westermeier, 1996; Avila et al., 1997).

The density of vegetative, tetrasporophyte and carposporophyte phases is low, under 2 thalli m^{-2} over the study period. However there was a continuous

presence of *Gigartina* small fronds, under 1 cm. This can be only explained as a result of germination of tetraspores or carpospores in spite of the low density of mature reproductive plants and the fact that spore mortality is high. The density of small thalli fluctuated along the study period and was also under 2 thalli m^{-2} . Regrowing from holdfasts was not observed during the study period, in any of the samples, probably due to harvesting methods. In other *Gigartina* species, like *Iridaea cordata*, *Mazzaella laminarioides* and *Sarcotrichia crispata* it has been found that fronds are able to propagate through regeneration of the perennial holdfast (May, 1986; Santelices & Norambuena, 1987; Poblete et al., 1984).

Although all stages of tetrasporangial sori were present throughout the year, the proportions at different seasons showed that new sori developed mainly in the summer, matured mainly in autumn/winter and dehiscent mainly in spring. For female gametophytes a cycle was also observed in the development of cystocarps. Papillae were more abundant from December to April (summer and early autumn), the same as cystocarps. The number of cystocarps slowly decreases

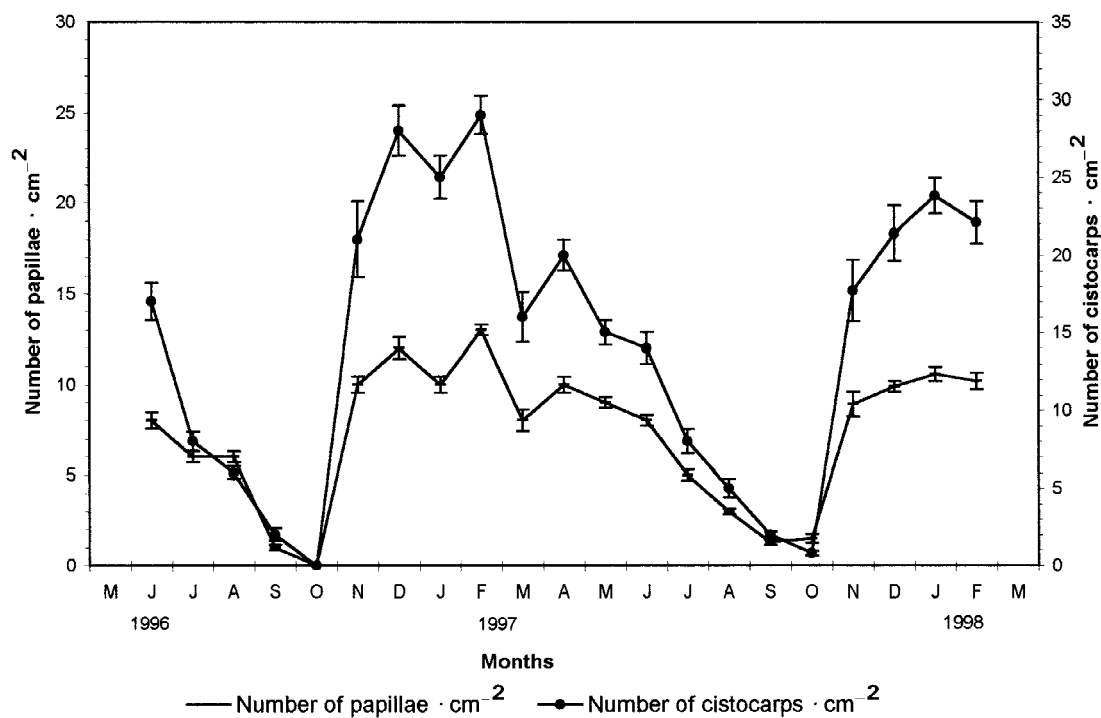


Figure 5. Monthly fluctuations in number of papillae and cystocarps in *G. skottsbergii* thalli, over the study period 1996–1998 (\pm standard error).

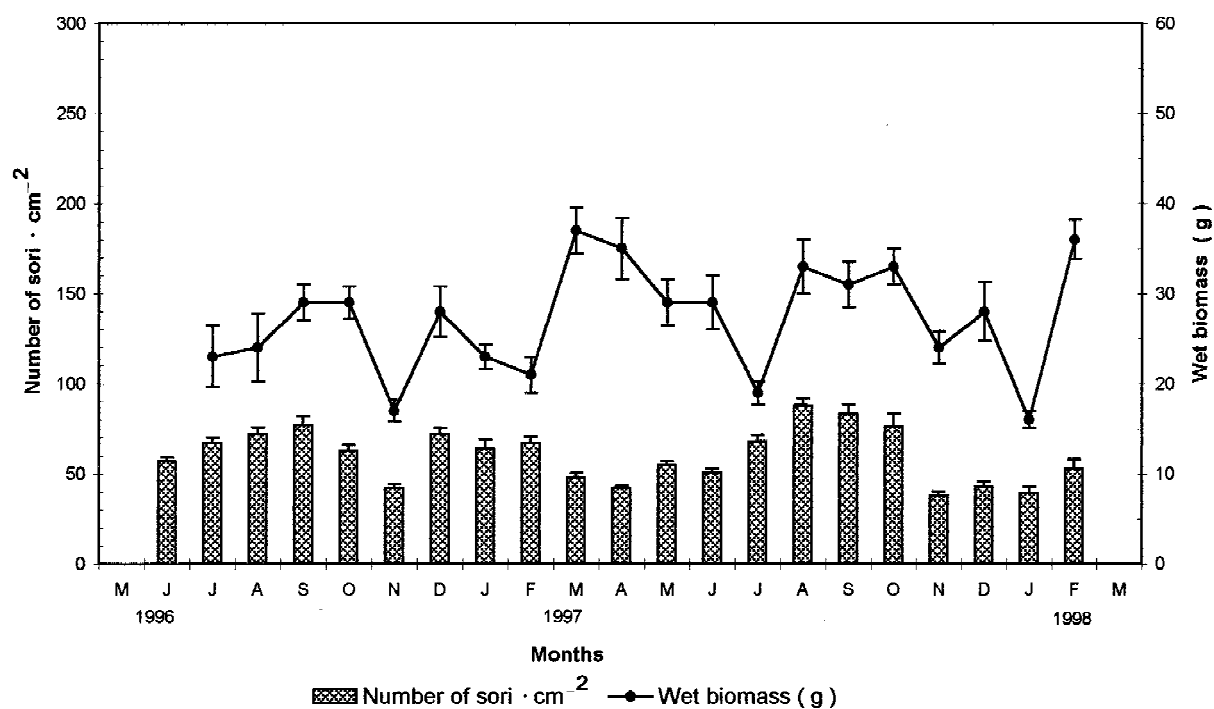


Figure 6. Monthly fluctuations in number of sori and biomass of the fronds in *G. skottsbergii* (\pm standard error).

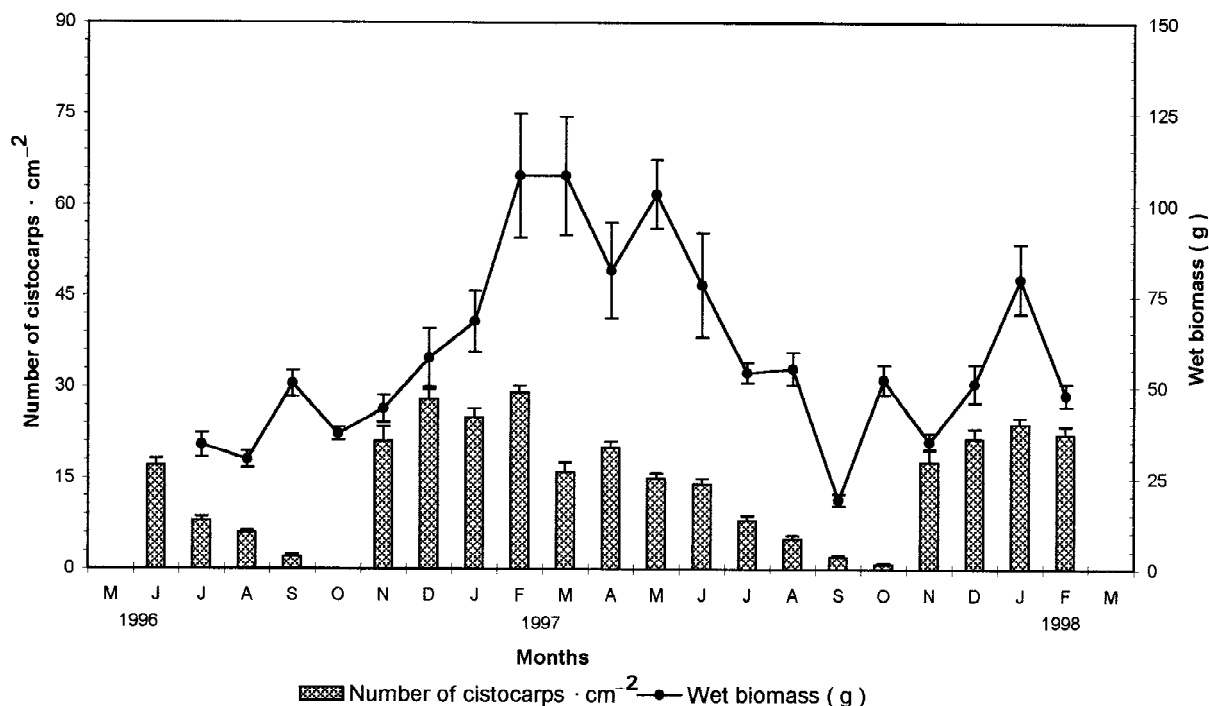


Figure 7. Monthly fluctuations in number of cystocarps and biomass in *G. skottsbergii* female gametophytic thalli (\pm standard error).

until June (winter), after July carpospores are released and the number of cystocarps decreases to low values during September-October (spring).

Field observations suggest that regrowth of the holdfast does not occur in this species. Fronds of < 1 cm that occurred during the study period could correspond to new fronds that have been developed from spores. We suggest that propagation and dispersal in a *Gigartina skottsbergii* natural bed is mainly due to spores. Results obtained under controlled conditions in the laboratory indicated that mortality in tetraspores and carpospores is high during the period of abundance of mature reproductive structures. Although viability of carpospores and tetraspores was low, spores that survive do germinate and develop holdfasts.

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