Hydrobiologia **326/327**: 223–228, 1996.
S. C. Lindstrom & D. J. Chapman (eds), Fifteenth International Seaweed Symposium.

Fecundity, spore recruitment and size in *Gelidium sesquipedale* (Gelidiales, Rhodophyta)

Rui Santos[1] & Pedro Duarte[2]
[1] *Universidade do Algarve, Unidade de Ciências e Tecnologias dos Recursos Aquáticos, Gambelas, 8000 Faro, Portugal; e-mail: rosantos@mozart.si.ualg.pt*
[2] *Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologia, Departamento de Ciências e Engenharia do Ambiente, Quinta da Torre, Monte da Caparica, 2825 Almada, Portugal*

Key words: fecundity, *Gelidium sesquipedale*, Portugal, reproduction, seaweed, size, spore recruitment

Abstract

Gelidium sesquipedale fecundity was quantified by counting tetrasporangial sori and cystocarps per meter squared and by estimating the number of spores contained inside them. These were obtained by regression on a size metric of reproductive structures. Tetrasporangial sori length and cystocarp thickness were the best estimators of spore number. To assess spore recruitment, 12 pottery tiles were fixed to the bottom, and the appearance of small fronds was monitored.

No clear seasonal pattern of reproduction was found. Tetraspore production peaked in March 1990 with 10.4 $\times$ 10^6 spores m^{-2}, whereas the carpospore peak was lower, 4.9 $\times$ 10^5 spores m^{-2} in July 1989. Recruitment followed tetraspore peaks. The probability of a *G. sesquipedale* tetraspore making the transition to a recruit was 4.7 $\times$ 10^{-5}. Frond length was significantly related to tetrasporangial sori number, while cystocarp number was only related to frond branching order. Minimum size for reproduction was 6.9 cm for gametophytes and 5.4 cm for tetrasporophytes; very rarely were cystocarpic fronds smaller than 9 cm, while tetrasporic fronds were often longer than 15 cm. Cystocarpic fronds were significantly shorter and had more branches than tetrasporic fronds.

Introduction

Gelidium sesquipedale (Clem.) Thur. is an important commercial species in the northeastern Atlantic (Juanes *et al.*, 1991; Santos & Duarte, 1991). Although the utilization of agar from *G. sesquipedale* in the food industry is decreasing due to the cheaper prices for *Gracilaria* agar and for carrageenan, the species is still the main source of raw material for specialized, highly priced products such as bacteriological grade agar and agarose (Santos *et al.*, 1993).

Gelidium sesquipedale has a modular type of construction in which erect shoots (fronds) develop from a basal system of prostrate axes that are attached to the substratum by rhizoids (Dixon, 1958). Recruitment, i.e. the addition of new fronds to the population, can be done sexually through the production, attachment, and development of spores, or asexually, through vegetative growth of new fronds from prostrate axes (Santelices, 1988), a process comparable to clonal growth in higher plants. Although the latter is generally considered to be the most important process of population recovery after physical disturbances such as commercial harvest or storms (Santos, 1994), the importance of spore production (tetraspores and carpospores) to recruitment is not clear. Gorostiaga (1990), based on visual observations of permanent quadrats, reported that new *G. sesquipedale* fronds developed independently of established clones. He interpreted this as spore recruitment.

In other *Gelidium* species the production and development of spores has been studied fairly well (see review in Santelices, 1990; Anderson *et al.*, 1991; Melo & Neushul, 1993), but how many spores actually make the transition to a visible plant has not been assessed. The probability associated with the transition

from spore to recruit is of major importance if demographic models of populations of seaweeds are to be developed (Ang & De Wreede, 1990; Santos, 1993b).

One of the objectives of this study was to assess for the first time the fecundity and spore recruitment of *G. sesquipedale*. Fecundity time variability was measured by monitoring both the abundance of tetrasporangial sori and cystocarps in a natural population and by determining the number of spores (tetraspores and carpospores) produced. The number of spores that successfully developed into new recruits was assessed by monitoring newly developed fronds on artificial substrata. The size structure of fertile fronds (cystocarpic and tetrasporic), was also monitored through time to assess what part of the population contributed to spore production. The existence of a minimum pre-reproductive size, the relationships between frond size and fecundity, and the morphological differences between life cycle phases were also investigated.

Materials and methods

The study was conducted in one of the most important *Gelidium sesquipedale* commercial beds, off Cape Espichel, Portugal, described in Santos (1993a). Five quadrat samples of 40 × 40 cm each were collected monthly from July 1989 to July 1990, except during the months of November and December 1989 and February 1990, when storms prevented diving. A second sampling period was carried out from November 1990 to October 1991, except during the months of February, March and September 1991. The number and size of quadrats were determined so as to minimize sampling errors and sampling time (Santos, unpublished data).

Samples were preserved by freezing. *Gelidium sesquipedale* fronds in each quadrat were observed under the dissecting microscope to select the fertile ones, i. e. those bearing tetrasporangial sori or cystocarps. These fronds were weighed and their length measured to the nearest mg and mm, respectively, and the maximum number of orders of frond branches was recorded. Prior to measuring, surplus water was removed from thawed fronds by blotting with paper towels.

Population fecundity was assessed by counting all cystocarps and tetrasporangial sori present in fertile fronds and by estimating the number of spores contained inside reproductive structures. Simple regression analyses were done between three size metrics of reproductive structures, length, width and thickness and the number of spores they carried to find the best estimator of spore number. Spore numbers for regression analysis were counted under the microscope after squashing reproductive structures. Sixty cystocarps collected in July 1987 and July 1989 and 45 tetrasporangial sori collected in October 1989 and January 1990 were used to calculate regressions.

To test if the relationships between reproductive structure size and spore number varied with time, we validated the regressions obtained with the above data for each month when fertile fronds were found. The number of spores counted inside five reproductive structures was compared with the confidence limits (p=0.05) of the number of spores estimated using regressions. The probability of finding more than one value outside the confidence limits is p=0.023 (1-[p^5+p^4 (1-p)], and p=0.95). In no case did more than one value fall outside the confidence limits; therefore, it was assumed that relationships did not vary with time.

Spore production was estimated only for the first sampling period, from July 1989 to July 1990. The average length of tetrasporangial sori and the average thickness of cystocarps in each month, estimated in a sub-sample of n>120, were used to calculate spore production per reproductive structure. This value was multiplied by the number of reproductive structures found in each month to calculate the population spore production per unit area.

Regression analyses were carried out to assess the relationships among the number of reproductive structures on *G. sesquipedale* fronds and three measures of frond size: maximum frond length, frond weight and maximum number of orders of frond branches. Morphological differences between gametophytes and tetrasporophytes were investigated by comparing their size metrics.

To assess the number of spores that successfully developed into new *G. sesquipedale* recruits, 12 pottery tiles were fixed to the bottom at depths of 9 to 12 m in July 1989. Pottery was used as it had proved to be a good substratum for spore attachment (Hanic & Pringle, 1978). Four tiles, fastened with plastic cable ties to each of three grids made of plastic-covered wire, were screwed to the rocky bottom. Ceramic tiles were carefully monitored, and the number of small fronds that could be identified as *Gelidium* (about 1 cm length) were counted monthly *in situ*.

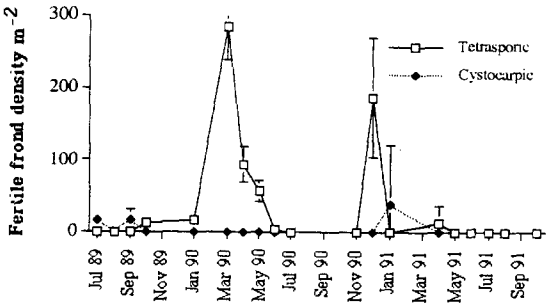

Figure 1. Temporal variation in fertile frond density (cystocarpic and tetrasporophytic). Vertical bars are standard deviations.

Results

No clear seasonal pattern was detected in the reproduction of *Gelidium sesquipedale* (Figure 1). Cystocarpic fronds, much less frequent than tetrasporic fronds, were present only three times, twice during summer (July 1989 and September 1989) and once in winter (January 1991). Tetrasporic fronds were found in all seasons except summer, from October 1989 to June 1990, in December 1990, and in April 1991. They peaked in March 1990 and in December 1990.

Regression analysis among the size metrics of reproductive structures and the number of spores inside them showed that tetrasporangial sori length and cystocarp thickness were the best estimators of spore number. Tetraspore number was best estimated by the linear function, No. of tetraspores = -88 + 860 sorus length (mm), with r^2=0.54, p<0.0001, n=45. The best linear function to estimate carpospore number was No. of carpospores = -387 + 1686 cystocarp thickness (mm), with r^2=0.31, p<0.0001, n=60.

A significant variation in average tetrasporangial sorus length was found from October 1989 to May 1990 (Oct. 0.893 mm, n=66; Jan. 1.236 mm, n=27; Mar. 0.967 mm, n=334; Apr. 1.209 mm, n=171; May 1.602 mm, n=123; ANOVA, F=53.432, p=0.0001). Tukey HSD multiple comparison tests showed that all pairwise comparisons between months were significantly different (p<0.02), except between October and March (p=0.72), and between January and April (p=1.00). Average cystocarp thickness decreased significantly from July 1989 to September 1989 (0.517 mm, n=37 versus 0.445 mm, n=96; ANOVA, F=22.157, p=0.0001). The variances of mean values for both ANOVAs were homogeneous (p<0.001, Bartlett test).

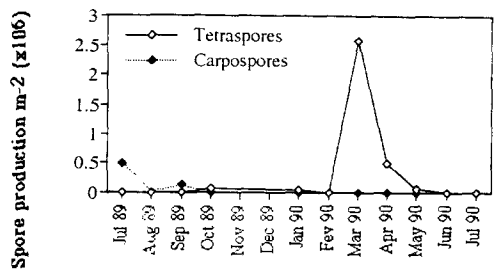

Figure 2. Temporal variation in carpospore and tetraspore production m^{-2}.

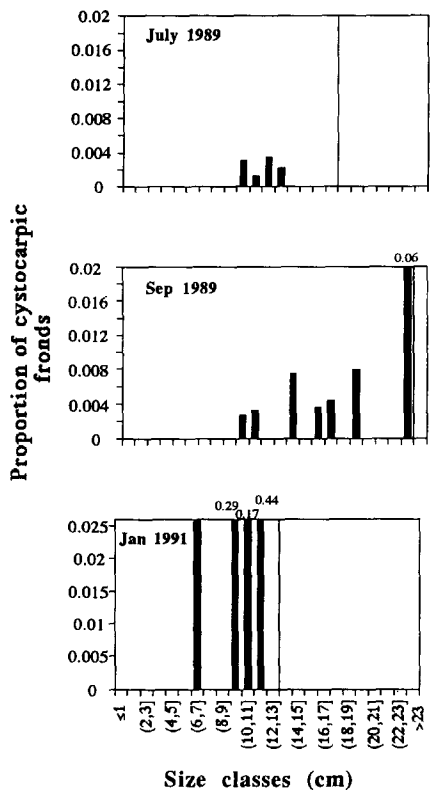

Figure 3. Proportion of cystocarpic fronds in each size class of population. Vertical lines show the maximum frond length in each month.

The number of carpospores produced per m^2 decreased from July 1989 to September 1989 (Figure 2), following both a decrease in cystocarps m^{-2} (from 1014 to 313) and a decrease in cystocarp thickness. Tetraspore production peaked in March 1990, with 10.4 × 10^6 spores m^{-2}, in contrast to the relatively low values in other months. The time variation of tetrasporangial sori m^{-2} followed the same pattern as tetrasporic fronds (Figure 1), peaking in March 1990 with 13 778 sori m^{-2}.

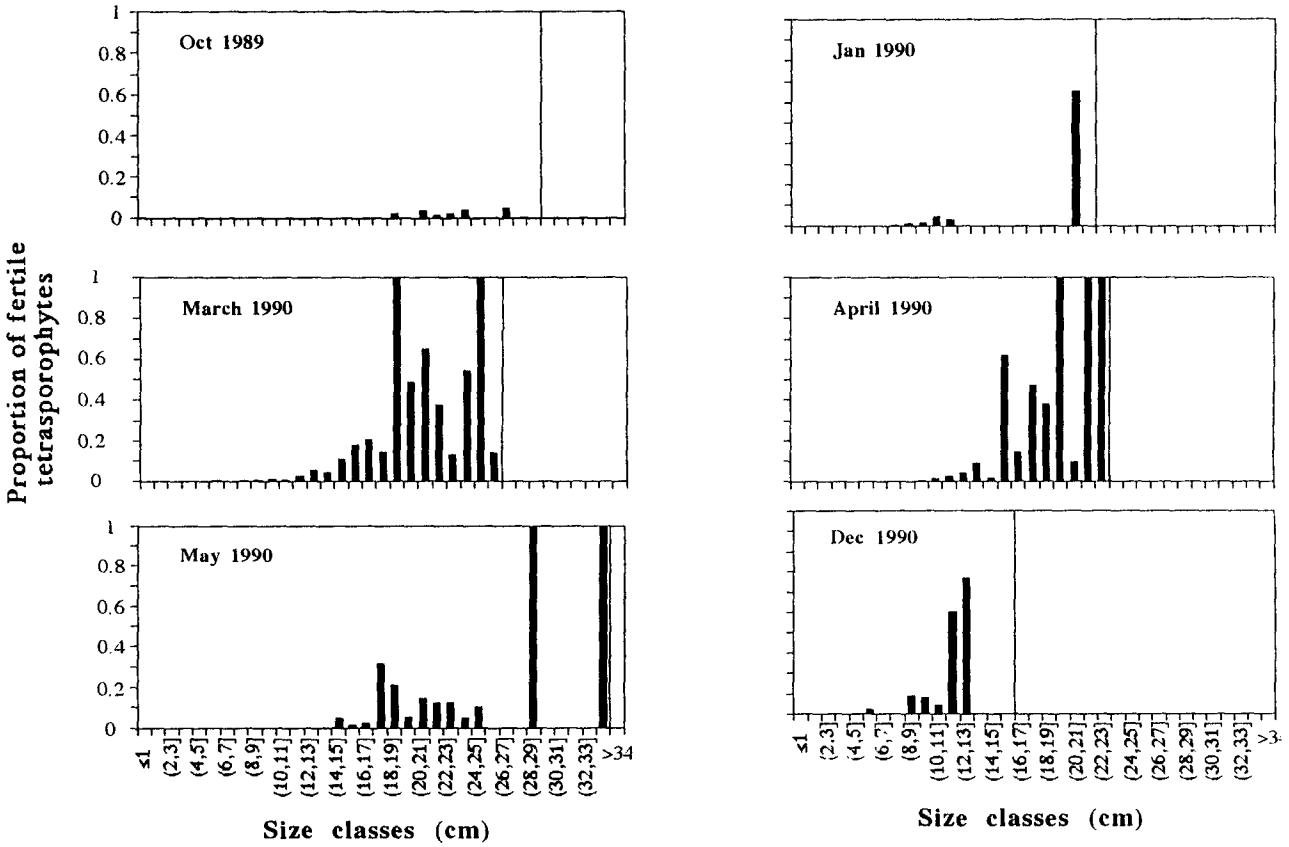

Figure 4. Proportion of tetrasporic fronds in each size class of population. Vertical lines show the maximum frond length in each month.

The frequency of cystocarpic fronds in the population is much lower than tetrasporic fronds (Figures 3 & 4). The largest fronds in the population in each month have a greater chance of being fertile independent of the maximum frond size in the population (Figures 3 & 4). The minimum frond size for reproduction was 6.9 cm for female gametophytes and 5.4 cm for tetrasporophytes, but gametangial fronds larger than 9 cm were fertile more often (Figure 3). Fifteen cm and larger appears to be the optimum size for reproduction in tetrasporic fronds (Figure 4).

Regression analysis showed that *G. sesquipedale* weight was not a good estimator of how many reproductive structures will develop on fronds. Both regressions of number of tetrasporangia and number of cystocarps on frond weight were not significant at $p>0.05$. Frond length was highly significantly related to the number of tetrasporangia ($p=0.0001$), but the predictive power of the regression model was low ($r^2=0.16$). The number of cystocarps per frond was not related to frond length ($p=0.42$), but it was significantly related to frond branching order ($p=0.028$, $r^2=0.23$). This result suggested that morphological differences exist between the two life history stages. The average length of fertile tetrasporophytes was significantly greater (t-test, $p<0.0001$, mean=15.912 cm, sd=4.128, $n=349$) than fertile female gametophytes (mean=11.880 cm, sd=3.134, $n=30$). In contrast, female gametophytes were much heavier ($p<0.0001$, mean=0.466 g, sd=0.293, $n=22$) than tetrasporophytes (mean=0.256 g, sd=0.249, $n=332$).

Maximum number of orders of branches of tetrasporophytes and gametophytes was not significantly different (mean=2.615, sd=0.763, $n=52$ *versus* mean=2.667, sd=0.789, $n=30$, respectively), suggesting that gametophytic fronds are heavier than tetrasporophytic fronds because they have more branches as opposed to higher branching order. No data on number of branches per frond was available to test this.

Algal colonization of pottery tiles went through a regular ecological succession: after one to two

weeks tiles were colonized by diatoms, followed after 1.5 months by opportunistic macroalgae such as Ulvales, *Colpomenia peregrina* Sauv., *Dictyota dichotoma* (Huds.) Lamour., and *Asparagopsis armata* Harv.. After 2.5 months the same algal cover was present, plus some Ceramiales. In January 1990, after 6 months, tiles were covered mostly by encrusting Corallinaceae. At this time, 2 tiles were lost due to storms. In April 1990, algal cover was similar to surrounding bottom flora: *A. armata* (also *Falkenbergia rufolanosa* (Harv.) F. Schmitz form), *Bonnemaisonia asparagoides* (Woodw.) C. Agardh., Ceramiaceae, encrusting Corallinaceae, *Cryptopleura ramosa* (Huds.) Kylin ex L. Newton, *Plocamium cartilagineum* (L.) Dixon, *Sphaerococcus coronopifolius* Stackh., *C. peregrina*, *Cutleria multifida* (Sm.) Grev. (*Aglaozonia parvula* (Grev.) Zanard. form), *D. dichotoma*, Ectocarpaceae, *Chaetomorpha* sp., *Cladophora* sp. and *Monostroma* sp.. The first *Gelidium sesquipedale* recruits were observed at this time (22 fronds m^{-2}). They showed a fairly homogeneous distribution among the tiles. The second time *G. sesquipedale* recruits were observed was in July 1990 (4 fronds m^{-2}). In January 1991, 37 recruits m^{-2} were counted.

Discussion

Our observations do not show defined seasonal patterns of reproduction perhaps due to the fact that the dates sampled were not the same between the two sampling periods (Figure 1). Reproductive structures in all seasons have been reported for *Gelidium sesquipedale* (Seoane-Camba, 1965, 1969; Gorostiaga, 1990) and other Gelidiales (Santelices, 1988), suggesting that their presence is not determined by environmental factors. The significant seasonal variation in both tetrasporangial sori length and cystocarp thickness indicates that environmental factors may influence their development and thus the quantity of spores produced.

Tetraspore production peaked in March 1990 with 10.4×10^6 spores m^{-2}, whereas carpospore production peaked at 4.9×10^5 spores m^{-2} in July 1989. The total wet weight of fertile fronds that produced these spores was 52.455 g m^{-2} and 7.371 g m^{-2}, respectively, which gave a peak spore production per unit weight of about 2.0×10^5 tetraspores g^{-1}, and 6.6×10^4 carpospores g^{-1}. These values are of the same order of magnitude as other *Gelidium* spp. reported in the literature: 10^4-10^6 spores/g for *Gelidium amansii* (Lamour.) Lamouroux in Japan (Suto, 1950 in Santelices, 1990), 2×10^5-10^6 spores per thallus of 12-15 cm for *Gelidium robustum* (Gardn.) Hollenberg & Abbott in California (Guzmán del Proó *et al.*, 1972).

The timing of *G. sesquipedale* recruitment on pottery tiles related well to the peaks in tetraspore production. Recruits were first observed in April 1990, when the diversity of macroalgae colonizing tiles suggested a late stage of succession. The other important recruitment period was January 1991. Both of these periods followed peaks of tetrasporic fronds by one month (Figure 1), suggesting that recruits originated from the settlement and germination of tetraspores rather than carpospores.

In only a very few phycological studies have the probabilities of a spore or zygote making the transition to a visible plant been estimated. Chapman (1984) estimated probabilities of 10.01×10^{-9} and 8.9×10^{-9} for *Laminaria digitata* Pyl. and *L. longicruris* Pyl., respectively, in eastern Canada, and Ang (1991) showed that the probability for *Fucus distichus* L. in western Canada ranged between 1×10^{-5} and 3.6×10^{-2}. The probability of a *G. sesquipedale* spore making the transition to a recruit was calculated as 4.7×10^{-5}, assuming that the recruits observed in April 1990 originated from the release, attachment, germination, and development of spores estimated in March 1990 (Figure 2). This is not an exact figure because we have no information both on release rates and on how much time a spore of this species takes to develop into a recruit. In many red algae tetraspores are known to be released continuously through a period of up to a week, contrary to carpospores which are all released at the same time within a period of only a few hours (Santelices, 1990). Studies on spore attachment indicate that *Gelidium* species have the strongest capacity for attachment immediately after shedding (Santelices, 1990).

The positive relationships between frond length and both the number of fertile fronds and their fecundity suggest that the reproductive potential of this population would be substantially higher if bigger fronds were more frequent. In fact, reproductive potential may have been unusually low because this was a period of population decline, due to unusually strong disturbances (combined effects of harvest and storms, see Santos, 1993b, 1994, 1995). Fronds longer than 15 cm, with more chances of being fertile, had unusually low frequencies throughout this study.

As in other species of Gelidiales, *G. sesquipedale* gametophytic and tetrasporophytic phases are considered isomorphic (Santelices, 1988). Morphomet-

ric data from this study suggest that this is not exactly right. Cystocarpic fronds were significantly shorter and had more branches than tetrasporic fronds, supporting our visual sensation that these fronds were 'bushier'.

As has been reported for *G. sesquipedale* (Seoane-Camba, 1965, 1969; Gorostiaga, 1990) and other Gelidiales (Santelices, 1988), cystocarpic fronds were much less abundant than tetrasporic fronds (0.2% and 2% of total number of fronds, respectively). The relative abundance of gametophytes and tetrasporophytes and the evolutionary persistence of diplohaplontic alternation of generations is an unexplained issue of rhodophyte life histories (see discussions in Hawkes, 1990; Santelices, 1990). This work does not assess why Gelidiales populations are dominated by tetrasporophytes, but results suggest that spore recruitment is mostly from tetraspores rather than from carpospores. If more tetraspores develop into gametophytes than carpospores into tetrasporophytes, then the relative abundance of both phases should be balanced after a number of generations. Are then the observed differences in relative abundance an artifact resulting from the fact that only when a frond is fertile is it possible to assign it to a life hystory phase, i. e. the proportion of fertile tetrasporophytes to fertile gametophytes does not reflect the proportion of total tetrasporophytes to total gametophytes, or are diploid spores and fronds better fitted (more vegetative recruitment, less mortality, more growth) than haploid? These questions are challenges yet to be assessed in life history research on the Gelidiales.

Acknowledgments

We thank A. Morais, A. Nabais and A. Afonso for their invaluable assistance in field, laboratory work and data analysis. The first author thanks H. Mooney for making his laboratory computer facilities at Stanford University available to complete this manuscript. S. Lindstrom and P. Åberg comments greatly improved the manuscript. This study was developed at INETI, Instituto Nacional de Engenharia e Tecnologia Industrial, and supported by a grant to Dr. Constança Peneda, to whom we are very grateful.

References

Anderson, R. J., R. H. Simons, N. G. Jarman & G. J. Levitt, 1991. *Gelidium pristoides* in South Africa. Hydrobiologia 221: 55–66.

Ang, P. O., 1991. Age- and size-dependent growth and mortality in a population of *Fucus distichus*. Mar. Ecol. Prog. Ser. 78: 173–187.

Ang, P. O. & R. E. De Wreede, 1990. Matrix models for algal life history stages. Mar. Ecol. Prog. Ser. 59: 171–181.

Chapman, A. R. O., 1984. Reproduction, recruitment and mortality in two species of *Laminaria* in southeast Nova Scotia. J. exp. mar. Biol. Ecol. 78: 99–110.

Dixon, P. S., 1958. The structure and development of the thallus in the British species of *Gelidium* and *Pterocladia*. Ann. Bot. 22: 353–368.

Gorostiaga, J. M., 1990. Aspectos demográficos del alga roja *Gelidium sesquipedale* (Clemente) Thuret. Discusión sobre su adequada gestión como recurso explotable. Ph. D. Dissertation, Universidad del Pais Vasco, Bilbao, Spain, 313 pp.

Guzmán del Proó, S. A., S. de la Campa de Guzmán & J. Pineda-Barrera, 1972. Shedding rhythm and germionation of spores in *Gelidium robustum*. Proc. int. Seaweed Symp. 7: 221–228.

Hanic, L. A. & J. Pringle, 1978. Pottery, a substrate for algal culture. Br. phycol. J. 13: 25–33.

Hawkes, M. W., 1990. Reproductive strategies. In K. M. Cole and R. G. Sheath (eds), Biology of the red algae, Cambridge University Press, Cambridge: 455–476.

Juanes J. A., B. Santelices & J. L. McLachlan (eds), 1991. International workshop on Gelidium, Kluwer Academic Publishers, London, 203 pp.

Melo, R. A. & M. Neushul, 1993. Life history and reproductive potential of the agarophyte *Gelidium robustum* in California. Proc. int. Seaweed Symp. 14: 223–229.

Santelices, B., 1988. Synopsis of biological data on the seaweed genera *Gelidium* and *Pterocladia* (Rhodophyta). FAO Fisheries Synopsis 145, 55 pp.

Santelices, B., 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanogr. mar. Biol. Annu. Rev. 28: 177–276.

Santos, R., 1993a. A multivariate study of biotic and abiotic relationships in a subtidal algal stand. Mar. Ecol. Prog. Ser. 94: 181–190.

Santos, R., 1993b. Plucking or cutting *Gelidium sesquipedale*? A demographic simulation of harvest impact using a population projection matrix model. Proc. int. Seaweed Symp. 14: 269–276.

Santos, R., 1994. Frond dynamics of the commercial seaweed *Gelidium sesquipedale*: effects of size and of frond history. Mar. Ecol. Prog. Ser. 107: 295–305.

Santos, R., 1995. Size structure and inequality in a commercial stand of the seaweed *Gelidium sesquipedale*. Mar. Ecol. Prog. Ser., 119: 253–263.

Santos, R. & P. Duarte, 1991. Marine plant harvest in Portugal. J. appl. Phycol. 3: 11–18.

Santos, R., J. M. Gorostiaga, R. Armisén, J. M. Salinas & J. C. Oliveira, 1993. *Gelidium sesquipedale* resource management in the Northeast Atlantic. In R. Santos, Population ecology of the commercial seaweed *Gelidium sesquipedale*: biological input for resource management. Ph. D. Dissertation, Dalhousie University, Halifax, Canada, 149 pp.

Seoane-Camba, J., 1965. Estudios sobre las algas bentónicas en la costa sur de la Peninsula Ibérica (litoral de Cadiz). Inv. Pesq. 29: 3–216.

Seoane-Camba, J., 1969. Crecimiento, producción y desprendimiento de biomassa en *Gelidium sesquipedale* (Clem.) Thuret. Proc. int. Seaweed Symp. 6: 365–374.

Suto, S., 1950. Studies on a counting method of spores of seaweeds in the sea. Bull. Jap. Soc. Sci. Fish., 15: 674–677.