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IS THERE AN ECOPHYSIOLOGICAL EXPLANATION FOR THE GAMETOPHYTE–TETRASPOROPHYTE RATIO IN *GELIDIUM SESQUIPEDALE* (RHODOPHYTA)?[1]

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In the fall, when 61% of the fronds of the *Gelidium sesquipedale* (Clem.) Born. et Thur. population located in Albufeira (southern Portugal) were reproductive, about 90% of these fronds were tetrasporophytes, whereas an equal percentage of female and male gametophytes was found (5%). The comparison of physiological performances of the reproductive phases (males, females and tetrasporophytes) did not reveal a physiological advantage of tetrasporic fronds. There were no significant differences either in the photosynthesis, nitrogen uptake, nitrate reductase activity, or biochemical composition of adult fronds. On the other hand, vegetative recruitment and spore production in the laboratory were significantly different. The re-attachment to calcareous substrate and the subsequent rhizoidal growth were faster in tetrasporophytes. Particular levels of temperature, rather than irradiance, had an important effect on the phase differences in the spore release, attachment, and germination rates. Significant results were the higher release of carpospores at all irradiances at 17° C, and the higher attachment percentage of carpospores at 13° C versus tetraspores. Under higher temperatures (21° C), tetraspores showed higher attachment rates while carpospores germinated more. *G. sesquipedale* cystocarps released carpospores for 2 months, while tetrasporangia stopped shedding tetraspores after 1 month, resulting in a 3-fold higher production of carpospores than tetraspores. Results showed that vegetative and spore recruitment may explain the low gametophyte–tetrasporophyte ratio of the studied population of *G. sesquipedale* as opposed to the physiological performance of phases.

Key index words: **ecophysiological differentiation; frond re-attachment; gametophyte: tetrasporophyte ratio; *Gelidium sesquipedale*; recruitment; spore vital rates**

Abbreviations: **G:T, gameophyte:tetrasporophyte ratio; K_s, light half-saturation point; LCP, light compensation point; NP_{max}, maximum photosynthetic rate; RPC, phycocyanin; RPE, phocoerythrin; SGR, specific growth rate.**

The relative abundance of isomorphic life-history phases is well documented in the Rhodophyta. Some studies reported the dominance of gametophytes (Dyck et al. 1985, Hannach and Santelices 1985, May 1986, Littler et al. 1987), others found a dominance of tetrasporophytes (Hansen and Doyle 1976, Norall et al. 1981, Akatsuka 1986, DeWreede and Green 1990, Santos and Duarte 1996), while still others observed similar numbers of gametophytes and tetrasporophytes (Hay and Norris 1984, Destombe et al. 1989). The deviation from a 1:1 gametophyte–tetrasporophyte ratio (G:T) in biphasic life cycles has been used in past studies to infer an evolutionary advantage of diploids. This assumes that tetrasporophytes are diploid, in unstable or stressed environments (Allender 1977, Akatsuka 1986), because they can mask deleterious mutations more effectively than haploids (Perrot et al. 1991), as opposed to the relative advantage of haploids in terms of energy efficiency, rates of evolutionary change, expression of mutations, and DNA repair (Bernstein et al. 1981, Kondrashov and Crow 1991, Perrot et al. 1991). More recently, demographic models have revealed that the use of distribution and abundance patterns as a basis for inferring adaptive differences that underlie phase dominance is problematic (Fierst et al. 2005). Scrosati and DeWreede (1999) showed that it is possible to expect stable G:T ratios different from the one for ecologically similar phases. Simulations suggested that phase dominance may result from differences in spore production between generations (Scrosati and DeWreede 1999, Thornber and Gaines 2004) or fertilization rate (Fierst et al. 2005) and not from phase-specific adaptations that might confer different mortality rates.

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Gelidium sesquipedale (Clem.) Born. et Thur. is a rhodophycean alga (Gelidiales) that has a *Polysiphonia*-type life cycle with two free-living isomorphic generations, the tetrasporophytes and the gametophytes, and a small carposporophyte phase that develops directly on the female thallus (Dixon 1959). The vegetative growth of erect fronds, both from the prostrate axis and from the re-attached frond fragments, has been suggested as the major process of population recovery from disturbances such as storms and commercial harvest (Santos 1994, 1995). Sexual reproduction and recruitment from spores has also been observed in this species (Gorostiaga 1990, Santos and Duarte 1996). The populations of *G. sesquipedale* show a predominance of tetrasporophytes (Dixon 1959, Gorostiaga 1990, Salinas 1991, Santos and Duarte 1996), which is a common feature of this genus (Montalva and Santelices 1981, Carter 1985, Akatsuka 1986, Macler and West 1987, Santelices 1990, Melo and Neushul 1993, Sosa et al. 1993), independent of the latitude and the environmental conditions. The deviation of the G:T ratio toward the tetrasporophytes appears to be characteristic of the evolutionary branch of Gelidiales, as opposed to the Gigartinales, whose populations are often dominated by gametophytes (Dyck et al. 1985, Hannach and Santelices 1985, May 1986, Littler et al. 1987, DeWreede and Green 1990).

There is no general explanation for tetrasporophyte predominance in Gelidiales. The few reports that assess the differences between life-history phases investigate only a few particular aspects of the ecophysiology of adult fronds that might explain the observed G:T ratio overlooking recruitment processes, both sexual and asexual. No clear physiological differences between the two life-history phases have been found in *G. pristoides* (Carter 1985), *G. coulteri* (Macler and West 1987), and in *G. canariensis* (Grunow) Seoane-Camba (Sosa et al. 1993). Matshuhiro and Urzúa (1988, 1990) reported similar agar composition for gametophytes and tetrasporophytes in *G. lingulatum* Kützing and *G. rex* Santelices et Abbott, but agar yield was higher in tetrasporophytes of the former species and in gametophytes of the latter. Concerning asexual recruitment processes, Maggs (1988) and Santelices (1990) suggested a possible explanatory mechanism for tetrasporophyte dominance, related to a more effective cycling of diploids through apomeiosis, but it was not investigated. On the other hand, tetrasporophyte fronds of *G. sesquipedale* were observed to re-attach more efficiently than gametophytes, as they produced more rhizoidal clusters (Salinas 1991, Juanes and Puente 1993), and a higher vegetative growth of erect axis from re-attached tetrasporic fronds was observed in *G. amansii* (Lamour.) Lamouroux (Akatsuka 1986).

This work includes both the comparison of ecophysiological performances of isomorphic life-cycle stages of *G. sesquipedale* and demographic processes related to phase-specific vegetative and spore recruitment. Novel data on the physiology of male individuals of *G. sesquipedale* are reported here for the first time. A preliminary objective of this study was to determine whether the studied population complies with the tetrasporophyte dominance reported for the *Gelidium* genus. The specific objectives were to compare the life-cycle phases for differences in: (a) re-attachment capacity and subsequent vegetative growth; (b) physiological capabilities revealed by photosynthetic performance, nitrogen uptake, nitrate reductase activity, and the biochemical composition of adult fronds; (c) spore release, attachment, and germination, and (d) germling growth rate.

MATERIAL AND METHODS

Sampling site and plant material. Sampling was performed by SCUBA diving at the rocky shore of Sâo Rafael, in Albufeira (South Portugal: 37°00″N, 7°58″W). A systematic sampling survey was used to assess the life-cycle phase population structure of *G. sesquipedale* in November 2001 and February, July, and October 2003. Three transect lines, separated by 10 m, were laid perpendicular to the shoreline until the depth limit of the species. Within each transect, 10×10 cm quadrats were scrapped every meter. Samples were transferred inside a cooler to the laboratory where they were separated into tetrasporophytes, female, and male gametophytes under a dissecting microscope. Because the species has an isomorphic life cycle it was not possible to classify non-reproductive fronds. Also there are no chemical methods available to assess the life-cycle phase of vegetative *G. sesquipedale* fronds because it is the case of Gigartinales (Garbary and DeWreede 1988).

For the ecophysiological experiments, fronds of the three reproductive types were collected haphazardly during the spring of 2002 at a depth of about 3 m. They were transferred to the laboratory as described above, cleaned of epiphytes, and maintained in a culture chamber in natural seawater at 17° C and 75 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of white light (Osram Lumilux Plus L18W/21-840 Osram, Munich, Germany) for 1 or 2 days before the experiments. These temperature and irradiance levels mimic the natural conditions available to the adult thalli.

Re-attachment and rhizoidal growth. The effects of photoperiod (6:18 and 18:6 L:D) on the re-attachment and subsequent growth of tetrasporophyte, female, and male fronds were investigated using a spray chamber system (Salinas 1991). Six-centimeter fronds of the three groups were laid on pottery tiles previously coated with a commercial plaster, "Estolite" (Asfaltex S. A., SantCugat delvallés, Spain), which contains calcium and magnesium carbonate. The temperature was set at 17° C, and the irradiance was set at 15 μmol photons $\cdot m^{-2} \cdot s^{-1}$. A low irradiance was selected to mimic the natural irradiance of the fronds under the canopy. In addition, Salinas (1991) observed that this irradiance level prevented positive phototropism and favored the attachment of the apical parts of *G. sesquipedale*. Two pottery tiles, each of them containing eight fronds of the same reproductive phase, were incubated at each photoperiod. The number of fronds that re-attached after 7 and 14 days was counted. The re-attachment was expressed as the percentage of the initial number of fronds on each tile. The apical growth rate of the rhizoids that developed from the re-attached fronds was calculated as the slope of the linear regression between the length of re-attached apices and time, monitored for 4 weeks. Nutrients were added to the seawater tank weekly, as 5 mg/L^{-1} $NaNO_3$ and 0.5 mg/L^{-1} $NaHPO_4$, as well as 0.5 mg/L^{-1} GeO_2 to inhibit diatom growth.

Photosynthetic measurements. Photosynthesis versus irradiance curves ($n = 5$) were measured over sequentially increas-

ing irradiance levels from darkness to 940 μmol photons $\cdot m^{-2} \cdot s^{-1}$ with a Clark-type oxygen electrode connected to a DW3 measuring chamber (Hansatech Instruments, Norfolk, UK). Light was provided by a slide projector (Pradovit 150, Leica, Heidelberg, Germany) equipped with a halogen lamp (Osram Xenophot 150W, Osram), using neutral density filters to obtain the different irradiance levels. The temperature was maintained at 17° C by a re-circulation water bath (RayPa, Tarassa, Spain). Curves were plotted according to Edwards and Walker (1983) and fitted by iteration (Kaleida Graph, Synergy software, Reading, PA, USA) to obtain the values for photosynthetic parameters.

Nitrogen uptake and nitrate reductase activity. Short-term measurements of ammonium and nitrate uptake rate were performed on apical parts of fronds maintained at 17° C and saturating light (300 μmol photons $\cdot m^{-2} \cdot s^{-1}$). Samples (approximately 0.1 g FW) were placed in 50 mL transparent plastic screw-top tubes containing 45 mL of artificial seawater spiked with either nitrate or ammonium. Nitrogen levels assayed were 5, 10, and 20 μM while inorganic phosphorus was added to a constant N:P molar ratio of 10:1. At incubation times of 10 and 20 min, medium samples were collected and nitrate and ammonium concentrations were determined with an autoanalyzer (SKALAR Sans Plus, Breda, The Netherlands). Four replicates were used per N concentration. Nitrate reductase activity of apical parts of the three types of fronds was determined ($n = 5$), following the *in situ* method described by Corzo and Niell (1991).

Biochemical composition. The biochemical composition of apical fragments (1.5–2 cm in length) of tetrasporophyte, female, and male gametophyte thalli was analyzed. Algal material was frozen in liquid N_2 and stored at −20° C until analysis. Chlorophyll *a* and total carotenoids were extracted in *N*,*N*-dimethyl-formamide at 4° C overnight and quantified spectrophotometrically using the equation of Inskeep and Bloom (1985) and Wellburn (1994), respectively. Triplicate samples (20–40 mg) were ground in phosphate buffer 0.1 M, pH 6.5 at 4° C, extracted overnight, and centrifuged at 6000 *g* for 20 min (Heraeus Megafuge 1.0 R, Kendro Lab Products, Langensbold, Germany). The contents of phycobiliproteins (phycoerythrin, RPE; phycocyanin, RPC) and soluble proteins were determined spectrophotometrically (Beckman DU 650, Beckman Coulter, Fullerton, CA, USA) from the supernatant. Phycobiliproteins were determined using the equations of Beer and Eshel (1985), and soluble proteins according to Bradford (1976). Insoluble carbohydrates were determined from the pellet fraction after treatment with 5% (w:v) trichloroacetic acid for 3 h at 80° C–90° C (Bird et al. 1982), following the phenol–sulfuric acid method (Kochert 1978). Soluble carbohydrate content was also quantified from the supernatant fraction using the same method. Insoluble proteins were extracted by treating the pellet resulting from the insoluble carbohydrate digestion with 1 N NaOH overnight (Bird et al. 1982), followed by centrifugation at 6000 *g* for 20 min. The fresh weight:dry weight relationship was determined to normalize the results on a dry weight basis (slope of regression line = 0.35; $r^2 = 0.98$, $n = 30$). Total intracellular carbon and nitrogen contents were estimated from triplicate samples oven-dried at 60° C for 48 h, using a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy).

Spore release, attachment, and germination. Fertile fronds-bearing sori (tetrasporophytes) and cystocarps (female gametophytes) were collected in February 2002 and transported to the laboratory as described above. After blotting on paper towels, reproductive structures were cut leaving some tissue from the parental branch, because this has been shown to improve spore release and survival (West and McBride 1999, Kamiya and Kawai 2002). Three structures of each type were placed in a Petri dish containing 8 mL of autoclaved seawater enriched with Provasoli (1:1000), adding germanium oxide (0.5 mg/L^{-1}) and G-Penicillin (0.5 mg/L^{-1}) to prevent the growth of diatoms and cyanobacteria, respectively. Three by three factorial designs with four replicates were used to test for the effects of phase (gametophyte, tetrasporophyte) irradiance (30, 75, and 150 μmol photons $\cdot m^{-2} \cdot s^{-1}$) and temperature (13, 17, and 21° C) on spore release, attachment, and germination, and on germling growth, under a photoperiod of 12:12 L:D. The released spores were counted after 1 day of incubation. The number of spores attached was quantified after handshaking the Petri dishes, disposing the suspension, and replacing the medium. After 9 days, the germinated spores were counted. The growth rate of the resulting germlings was assessed by monitoring their length every week for 30 days, using an ocular meter. The specific growth rate (SGR) was expressed as percentage of length increase per day: SGR (% day^{-1}) $= \ln (L_2/L_1)/t \times 100$, where t is the time in days, L_1 is the initial length, and L_2 is the length on day t. The medium in the Petri dishes was changed every week. The maximum spore output per reproductive structure was also assessed by daily counting the spores released from both tetrasporangial sori and cystocarps, at 17° C and 30 μmol photons $\cdot m^{-2} \cdot s^{-1}$, until no more spores were shed. A dissecting microscope was used to perform all counts and measurements.

Statistical analysis. Data were analyzed by one-, two-, or three-way analysis of variance (ANOVA). Spore release data were square-root transformed, while attachment and germination data were transformed to the arcsin of the squared-root to accomplish the data distribution assumptions of ANOVA. The post hoc SNK test was applied to identify differences among treatments. All these tests were performed using the Sigma Stat 2.0 (SPSS Inc.) software package. Apical growth rates of the re-attached fronds were compared using the test for comparison of slopes described in Sokal and Rohlf (1981). In all statistical tests, significant differences were considered at probability values of $P < 0.05$.

RESULTS

The main reproductive period of the *G. sesquipedale* population was during fall. In November 2001 and October 2003, the proportion of fertile fronds peaked at 61% and 58%, respectively, decreasing to 41% in February 2003 and to 10% in July 2003. The proportions of male and female gametophytes in the fall months were 2.7 ± 1.0% and 3.5 ± 0.6%, respectively, whereas the tetrasporophytes were 53.4 ± 0.6%.

Re-attachment and rhizoidal growth. The spray system promoted the appearance of rhizoidal clusters and encouraged the re-attachment of *G. sesquipedale* on the tiles. On day 4–5, the first rhizoidal clusters appeared on the lower apical face of the thalli, penetrating into the tile. The process was much faster in the tetrasporophytes than in the female and male gametophytes (Fig. 1), because almost 100% of the former fronds were attached to the substratum within the first week of culture, while it took one more week for gametophytes to reach their maximum percentage of re-attachment. There was no significant effect of photoperiod on the re-attachment, regardless of the life-history phase.

All of the re-attached fronds developed prostrated rhizoids during the experimental period. The growth of tetrasporophyte rhizoids was significantly higher

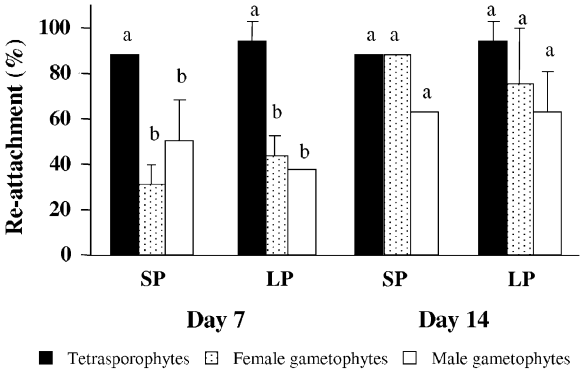

FIG. 1. Re-attachment of tetrasporophytes (black), female (dotted), and male (white) gametophytes of *Gelidium sesquipedale*, as percentage of initial number of fronds at short- and long-day photoperiod (SP and LP, respectively). Bars represent SD ($n = 2$). Values with the same letter in the superscript are not significantly different ($P > 0.05$).

TABLE 2. Mean values of photosynthesis parameters of life-history phases of *Gelidium sesquipedale*.

	Tetrasporophytes	Female gametophytes	Male gametophytes	*P*
NP_{max}	228 (33.7)	213 (37.7)	173 (41.5)	0.13
LCP	5.01 (1.49)	4.79 (3.20)	4.32 (2.88)	0.91
K_s	70.9 (13.20)	54.1 (12.0)	55.9 (3.06)	0.08
α	1.65 (0.33)	1.85 (0.26)	1.49 (0.30)	0.28
R_d	13.8 (5.96)	15.6 (6.12)	7.38 (1.98)	0.11

Maximum net photosynthesis (NP_{max}) is expressed as µmols $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$. The light compensation point (LCP) and the half-saturation light irradiance (K_s) are expressed as µmol photons $\cdot m^{-2} \cdot s^{-1}$. Initial photosynthetic efficiency (α) is expressed as µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$ (µmol photons $\cdot m^{-2} \cdot s^{-1})^{-1}$. Dark respiration rate (R_d) is in µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$. Standard deviations are shown in parentheses and *P* values are from the one-way ANOVA.

than gametophytes and there were no significant differences in the growth rate of female and male rhizoids (Table 1). The photoperiod did not affect rhizoidal growth.

Photosynthetic measurements. No differences were found among any of the three stages in respiration, maximum photosynthetic rate (NP_{max}), light compensation point (LCP), light half-saturation point (K_s), and photosynthetic efficiency (α) (Table 2). The mean values of maximum photosynthesis rate reached 200 µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$, whereas α showed mean values of 1.5 µmols $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$ (µmol photons $\cdot m^{-2} \cdot s^{-1})^{-1}$. No photoinhibition was observed within the irradiance range assayed.

Nitrogen uptake. No significant differences of *in situ* nitrate reductase activity were observed among tetrasporophytes, females, and males of *G. sesquipedale* (Fig. 2). The average nitrate reductase activity was 10 µmol $NO_2^- \cdot g^{-1}$ DW $\cdot h^{-1}$. The N uptake differences among tetrasporophytes and gametophytes depended on the N concentration, because there was a significant interaction between life phase and concentration ($P < 0.01$). Uptake rates were higher for ammonium than for nitrate ($P < 0.001$), except in tetrasporophytes at 5 µM, that showed similar values. At 5 µM, there were no significant differences among the three stages both in nitrate or ammonium uptake. At 10 µM, the tetrasporophytes showed a higher nitrate uptake rate than gametophytes, whereas the ammonium uptake rate of female gametophytes was lower than both tetrasporophytes and males. At the highest N concentration assayed, 20 µM, the female fronds presented a higher nitrate uptake but lower ammonium uptake than tetrasporophytes and male gametophytes (Fig. 3).

Biochemical composition. There were no consistent differences between reproductive phases in biochemical composition (Table 3). Chlorophyll *a*, total carotenoids, insoluble protein, and tissue N content were similar in all individuals. Male gametophytes presented the highest content of phycoerythrin and total soluble proteins, but the lowest insoluble carbohydrate concentration. On the other hand, female gametophytes showed the highest soluble carbohydrate concentration and more insoluble carbohydrate content than male individuals. An overall

TABLE 1. Growth rate of rhizoidal apices of re-attached fronds of different life-history phases of *Gelidum sesquipedale* under short- (SP) and long-day (LP) photoperiod.

	GR (mm/day)	R^2	*n*
SP			
Tetrasporophytes	0.23[a]	0.96	79
Female gametophytes	0.20[b]	0.92	76
Male gametophytes	0.19[b]	0.88	78
LP			
Tetrasporophytes	0.24[a]	0.96	77
Female gametophytes	0.20[b]	0.89	64
Male gametophytes	0.19[b]	0.78	76

Also shown are the coefficient of determination (R^2) and number of observations (*n*) of each regression equation used to calculate the growth rate (regression slope). Values with the same letter in superscript are not significantly different ($P > 0.05$).

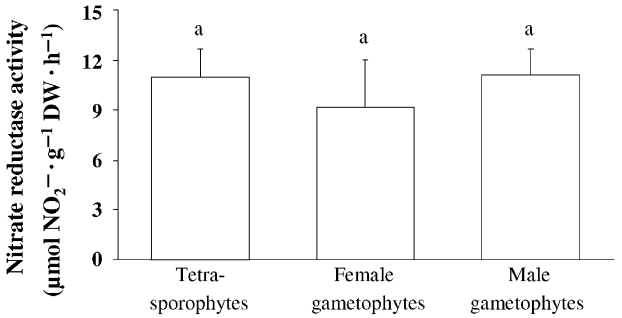

FIG. 2. *In situ* nitrate-reductase activity of *Gelidium sesquipedale* life-history stages. Bars represent SD ($n = 5$). Values with the same letter in the superscript are not significantly different ($P > 0.05$).

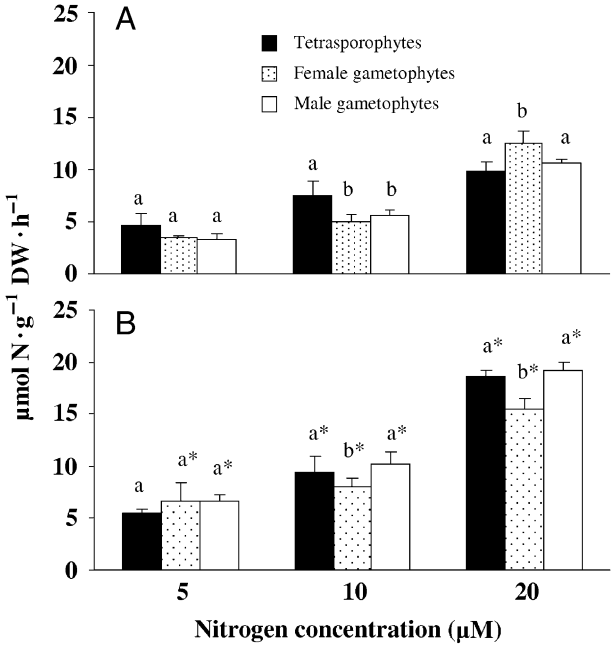

FIG. 3. Nitrate (A) and ammonium (B) uptake of tetrasporophytes (black), female (dotted), and male (white) gametophytes of *Gelidium sesquipedale* at different N concentrations. Bars represent SD ($n = 4$). Values with the same letter in the superscript are not significantly different ($P > 0.05$). *Significant differences between ammonium and nitrate uptake rates at each concentration.

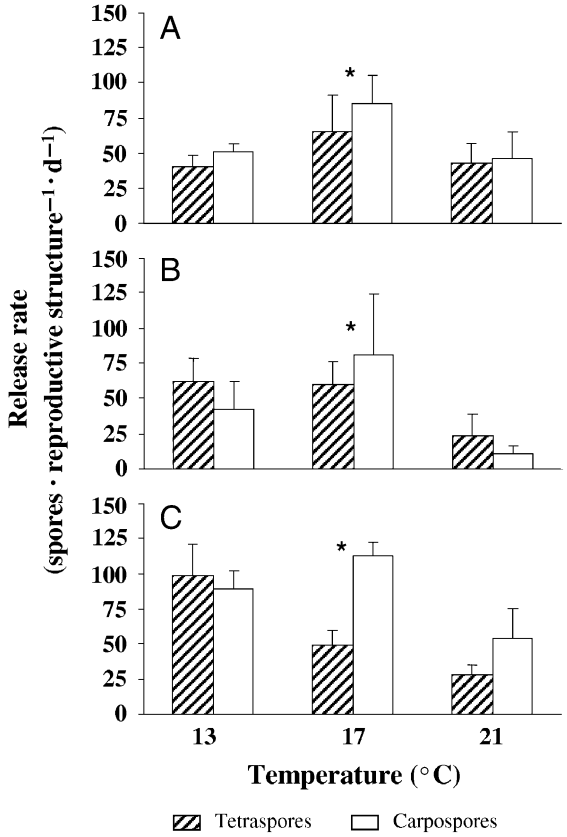

FIG. 4. Effects of temperature and irradiance (A) 30 µmol photons · m^{-2} · s^{-1} (B) 75 µmol photons · m^{-2} · s^{-1} (C) 150 µmol photons · m^{-2} · s^{-1}) on the release of tetraspores (lined) and carpospores (white) of *Gelidium sesquipedale*. Bars represent SD ($n = 4$). *Significant differences between tetraspores and carpospores.

higher tissue C content was found in gametophytic fronds than in tetrasporophytes.

Rates of spore release, attachment, germination, and germling growth. Regardless of the ploidy of the spores, the release and attachment rates were not affected by the irradiance level. The only significant difference between spores was found at 17° C, when carpospore release to the medium was consistently higher than tetraspore at all irradiance levels (Fig. 4). Temperature affected carpospore release, which was maximum at 17° C and minimum at 21° C.

The release of both carpospores and tetraspores, monitored at 17° C and 30 µmol photons · m^{-2} · s^{-1}, was not constant through time, showing a release cycle for carpospores of about 10 days (Fig. 5). A striking difference was found in the length of the release period: carpospore release extended up to 67 days, while tetraspores were only discharged for 30 days. Carpospores showed a higher capacity to attach to the substrate than tetraspores at 13° C independent of the irradiance level assayed, whereas the opposite response was observed at 21° C and both 30 and 75 µmol photons · m^{-2} · s^{-1} (Fig. 6). Tetraspore and carpospore germination rates were similar and independent of temperature ($P = 0.295$), except at 21° C and 75 µmol photons · m^{-2} · s^{-1}, when more carpospores germinated (Fig. 7). The lowest temperature associated with the highest irradiance level resulted in a reduced germination rate ($P < 0.001$). All the new

TABLE 3. Biochemical composition of life-history phases of *Gelidium sesquipedale*.

	Tetrasporophytes	Female gametophytes	Male gametophytes
Chl *a* (mg · g^{-1} DW)	2.45 (0.17)^a	2.32 (0.13)^a	2.59 (0.10)^a
Carotenoids (mg · g^{-1} DW)	0.50 (0.03)^a	0.48 (0.02)^a	0.52 (0.02)^a
RPE (mg · g^{-1} DW)	14.3 (1.07)^a	12 (0.63)^b	16.1 (0.82)^c
RPC (mg · g^{-1} DW)	1.26 (0.06)^a	1.06 (0.04)^b	1.35 (0.7)^a
Soluble proteins (mg · g^{-1} DW)	69.5 (4.53)^a	67.0 (5.64)^a	84.1 (1.41)^b
Insoluble proteins (mg · g^{-1} DW)	107 (17.73)^a	95.02 (7.24)^a	97.3 (5.16)^a
Soluble carbohydrates (mg · g^{-1} DW)	8.83 (1.91)^a	14.67 (1.13)^b	11.7 (0.64)^c
Insoluble carbohydrates (mg · g^{-1} DW)	126 (6.62)^a	139 (11.14)^a	107 (5.92)^b
N (% DW)	3.15 (0.05)^a	3.18 (0.14)^a	3.65 (0.05)^a
C (% DW)	36.7 (1.29)^a	38.78 (0.81)^b	39.0 (1.31)^b

Standard deviations are shown in parenthesis ($n = 3$); Values with the same letter in the superscript are not significantly different ($P > 0.05$). RPE—phycoerythrin, RPC—phycocyanin.

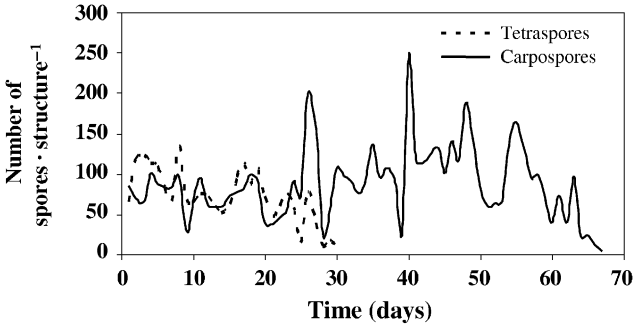

FIG. 5. Daily pattern of *Gelidium sesquipedale* tetraspore (dashed line) and carpospore (solid line) release at 17° C and 30 μmol photons · $m^{-2} \cdot s^{-1}$.

fronds that developed from the germinated spores presented positive growth under all experimental conditions. The temperature effects depended on light level (Fig. 8). The growth rate of both gametophytic and tetrasporic germlings was the highest at 30 μmol photons · $m^{-2} \cdot s^{-1}$and 21° C, while the lowest growth rate was observed at 13° C and 150 μmol photons · $m^{-2} \cdot s^{-1}$. The only significant difference in growth was found at 150 μmol photons · $m^{-2} \cdot s^{-1}$ and 13° C, when tetrasporophyte plantlets grew faster (Fig. 8).

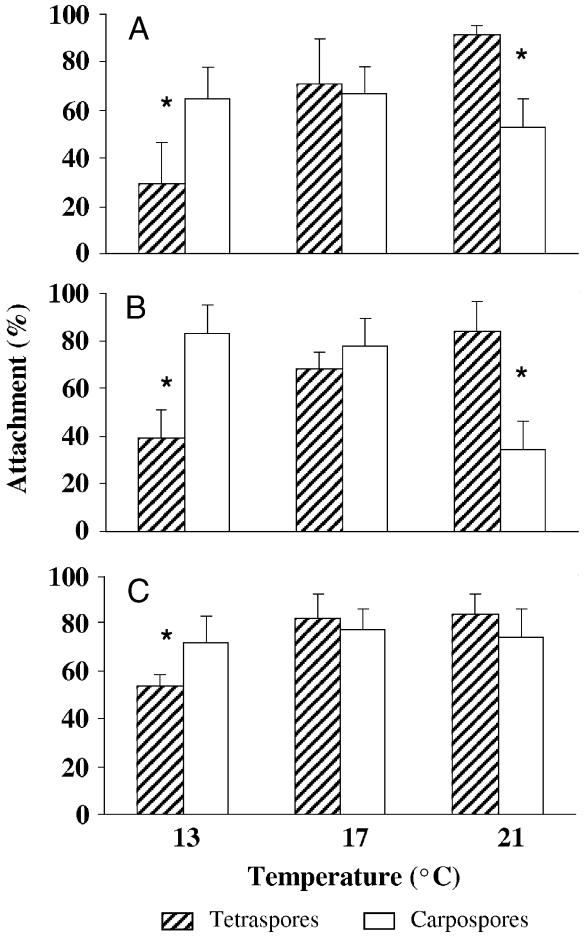

FIG. 6. Effects of temperature and irradiance (A) 30 μmol photons · $m^{-2} \cdot s^{-1}$ (B) 75 μmol photons · $m^{-2} \cdot s^{-1}$, and (C) 150 μmol photons · $m^{-2} \cdot s^{-1}$ on the attachment rate (as percentage of released spores) of tetraspores (lined) and carpospores (white) of *Gelidium sesquipedale*. Bars represent SD ($n = 4$). *Significant differences between tetraspores and carpospores.

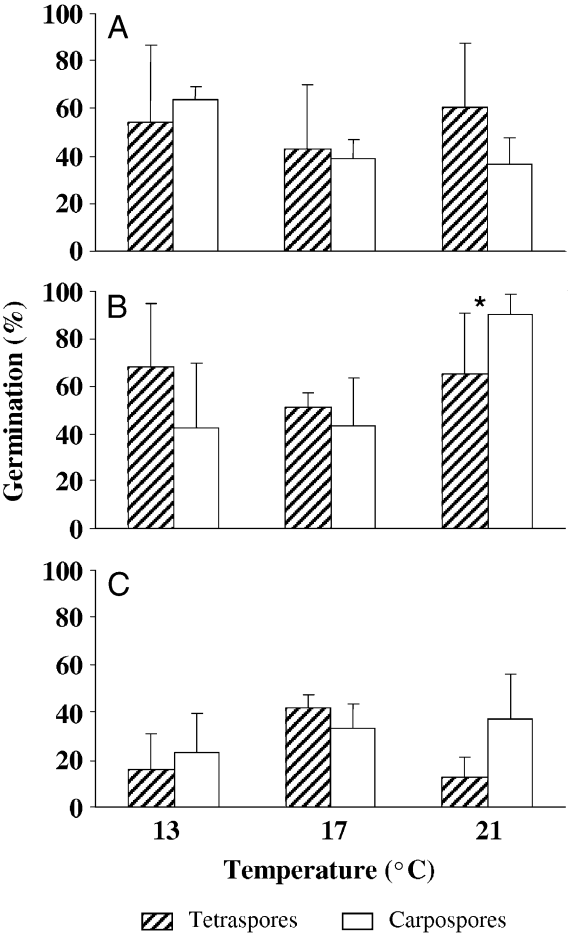

FIG. 7. Effect of temperature and irradiance (A) 30 μmol photons · $m^{-2} \cdot s^{-1}$ (B) 75 μmol photons · $m^{-2} \cdot s^{-1}$, and (C) 150 μmol photons · $m^{-2} \cdot s^{-1}$ on the germination rate (as percentage of attached spores) of tetraspores (lined) and carpospores (white) of *Gelidium sesquipedale*. Bars represent SD ($n = 4$). *Significant differences between tetraspores and carpospores.

DISCUSSION

The reproductive subpopulation of *G. sesquipedale* of São Rafael, southern Portugal, was dominated by tetrasporic fronds, as described elsewhere for other populations of the same species (Gorostiaga 1990, Salinas 1991, Santos and Duarte 1996) or of the same genus (Montalva and Santelices 1981, Carter 1985, Akatsuka 1986, Macler and West 1987, Santelices 1990, Melo and Neushul 1993, Sosa et al. 1993). The G:T ratio of São Rafael reproductive subpopulation varied between 1:7 and 1:10 in the fall, the most fertile season, when up to 61% of the fronds were reproductive. In the order Gelidiales, one can only report on the

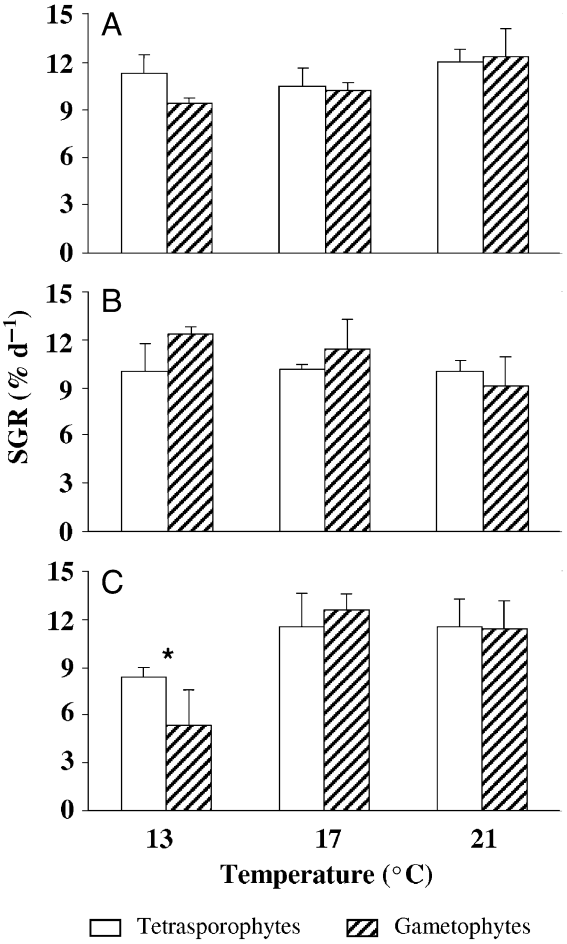

FIG. 8. Effect of temperature and irradiance (A) 30 μmol photons · m^{-2} · s^{-1} (B) 75 μmol photons · m^{-2} · s^{-1}, and (C) 150 μmol photons · m^{-2} · s^{-1} on the growth of tetrasporophyte (white) and gametophyte (lined) germlings of *Gelidium sesquipedale*. Bars represent SD ($n = 4$). *Significant differences between tetrasporophytes and gametophytes.

species phase-structure of the reproductive subpopulation, because of the difficulty in discriminating the isomorphic life-cycle phases of the non-reproductive individuals. There is no rapid method for determining the ploidy of individuals as is the case, for example, of the resorcinol chemical test for the Gigartinales (Garbary and DeWreede 1988). However, there is no indication that the phase proportions of the non-reproductive subpopulation would be different for the reproductive population. In the Gigartinales species *Iridaea splendens* (Setchell et Gardner) Papenfuss, DeWreede and Green (1990) observed similar G:T patterns within the reproductive and non-reproductive subpopulations.

To our knowledge, this is the only study including the males in the assessment of the G:T ratio of *G. sesquipedale*. Other studies have been reported on the species carposporophyte:tetrasporophyte ratio because inconspicuous spermatangial sori of male fronds were not reported. In a commercially harvested population of the central western coast of Portugal, Santos and Duarte (1996) observed a carposporophyte:tetrasporophyte ratio of 1:10, higher than in São Rafael, where it was up to 1:20 (because half of the gametophytes were carposporophytes). Other commercially harvested populations of *G. sesquipedale* in northern Spain had carposporophyte:tetrasporophyte ratios of 1:46 (Salinas 1991) and 1:9 (Gorostiaga 1990), indicating no clear effects of harvest on the phase structure of this species. Despite the inter-population differences, the tetrasporophyte dominance of *G. sesquipedale* is independent of geographical location or season of the year, as for other species of the genus (Santelices 1988). It is noteworthy that the female:male ratio observed here was close to 1:1, as reported for several populations of *G. robustum* (Gardn.) Hollenberg & Abbott (Guzmán del Próo et al. 1972, Melo and Neushul 1993). This sex ratio can be considered as evidence of recruitment from tetraspores, because this is the expected outcome of germination of meiotic tetraspores. This finding does not support the suggested absence of meiosis in tetrasporangia as an explanation for tetrasporophyte dominance in Gelidiales (Dixon 1961, Maggs 1988). Furthermore, meiosis was proven to occur in *G. latifolium* from Northern Ireland (Maggs and Rico 1991).

Physiology of adult fronds. Our observations on *G. sesquipedale* showed little or no physiological differentiation between adult fronds of different phases. Some reports on other species also documented no differentiation (Littler et al. 1987, Britting and Chapman 1993), while others have found photosynthesis and growth differences that might result in an advantage for the most abundant phase (Hannach and Santelices 1985, Matshuhiro and Urzúa 1990, Destombe et al. 1992, 1993, Dyck and DeWreede 1995, Zucarello et al. 2001). In the particular case of *Gelidium* species, such as *G. pristoides* (Turn.) Kützing (Carter 1985), *G. coulteri* Harvey (Macler and West 1987), and *G. canariensis* (Sosa et al. 1993), no significant differentiation between phases was ever observed. The Gelidiales is thus characterized by consistent deviations of the G:T ratio toward the tetrasporophytes and no obvious physiological differentiation between adult fronds. On the other hand, differences between phases that may explain the dominance of one phase seem to be characteristic of the Gigartinales. This is the case of *Gracilaria gracilis* (Stackhouse) M. Steentoft, L. M. Irvine, & W. F. Farnham (as *Gracilaria verrucosa* (Hudson) Papenfuss), whose haploid individuals dominate under low-nutrient conditions because of their higher growth rates, while the opposite response was observed when nutrients were abundant (Destombe et al. 1993). In *G. sesquipedale*, there were no patterns of life-cycle phase advantage in relation to nitrogen uptake rates (Fig. 3). The only advantage for tetrasporophytes versus gametophytes was observed at 10 μM NO_3^-, while at 20 μM NO_3^- and 10 and 20 μM NH_4^+, females presented lower uptake values than tetrasporophytes and males.

An important aspect of life-phase studies in *Gelidium* and other Rhodophyta is that most of them were performed on tetrasporophytes and female gametophytes, while male gametophytes were usually ignored, because of insufficient descriptions of spermatangial morphology (Carter 1985, Santelices and Flores 1995). The present work presents novel data on the physiology of male fronds that was not generally different from female fronds. Also, the biochemical composition was only slightly different among the three stages. The most relevant difference in the internal composition of the thalli was the higher tissue C in gametophytes as compared with tetrasporophytes. This fact may be of economical interest, as agar content is directly related to C content in agarophytes (Carmona et al. 1998).

Vegetative and spore recruitment. In contrast to no physiological differences found between adults, our results show significant differences between life-cycle phases of *G. sesquipedale* both in vegetative recruitment and in the spore production and survival, which may support the tetrasporic dominance of natural populations. Vegetative recruitment by re-attachment of broken body parts is a common process, observed in other red macroalgae, such as *Chondracanthus chamissoi* (C. Agardh) Kützing (Macchiavello et al. 2003), *Solieria filiformis* (Kützing) Gabrielson (Perrone and Cecere 1997), and in coral species of the genus *Acropora* (Smith and Hughes 1999). This process seems to play an important role in *Gelidium* populations, usually exposed to intensive harvesting and storms that cause frond fragmentation (Santos 1994). In fact, it has even been suggested as the main mechanism of recruitment in *G. sesquipedale* (Gorostiaga 1990). Akatsuka (1986) reported higher vegetative growth rates of *Gelidium* tetrasporophytes than gametophytes by re-attachment. Salinas (1991) and Juanes and Puente (1993) also observed a better capability of tetrasporophytes for re-attachment, because of their higher capacity to produce rhizoidal clusters. Salinas (1991) reported that female gametophytes with cystocarps did not readily re-attach and produce germlings, which was not confirmed by our results that showed that gametophyte fronds, both female and male, were also able to re-attach to the substrata, even though in a less efficient way than tetrasporophytes. The faster re-attachment and growth of tetrasporophyte frond fragments of *G. sesquipedale* observed here emphasizes the possible importance of this mechanism in maintaining the low G:T ratio of this species.

The re-attachment and subsequent growth of the prostrate *G. sesquipedale* thalli were not affected by photoperiod. Studies on this subject are scarce and in some cases contradictory. Salinas and Valdés (1993) observed a higher number of rhizoidal clusters and growth rate under long day photoperiod in *G. sesquipedale*, similar to the results found in *G. robustum* (D'Antonio and Gibor 1985), *G. latifolium* (Greville) Born. et Thur. (Rico 1991), and two Chilean species (Correa et al. 1985). In contrast, in *G. chilense* (Montagne) Santelices & Montalva, short day photoperiod conditions promoted higher growth rates than long photoperiods, although day length did not affect the production of bundles of attachment cells (Santelices and Varela 1994). In spite of this, it is reasonable to accept that this growth process is not strongly dependent on photoperiod, because it occurs at very low irradiances in the field, under the canopy of the frond tufts. In fact, low irradiances favor this process, because higher light levels would bring about positive phototropism, preventing the thalli from attaching to the substrata (Salinas 1991).

In natural populations of the genus *Gelidium*, there are about 20 times more fertile tetrasporophyte fronds than carposporophytes, which appears to be inconsistent with low G:T ratios. To explain this, it has been suggested that there might be a failure in the viability of tetraspores to develop into gametophyte fronds and/or a better capability of carpospores to germinate and produce tetrasporic fronds (Abbott 1980). Contrary to what was observed in some species of *Gelidium*, our results do not show any failures in the viability of *G. sesquipedale* tetraspores or gametophyte germlings. In *G. latifolium* from Ireland, Maggs and Rico (1991) found that the viability of tetrasporelings stages was very poor and only one of several hundred grew to maturity, suggesting that the lack of gametophytes in the local population may be a result of the poor viability of haploid tetraspores. Similarly, Rueness and Fredriksen (1989) observed that cultured tetraspores from the same species in Norway did not reach reproduction. Carter (1985) reported that *G. pristoides* carpospores germinate more successfully than bispores and Melo and Neushul (1993) observed that, although tetraspore release in *G. robustum* occurs all year round in California, not all spores have the potential to germinate.

The experiments developed here showed that particular levels of temperature, rather than irradiance, have an important effect on the ploidy differences in the spore release, attachment, and germination rates. Significant results were the higher release of carpospores at all irradiances at 17° C, which is the most common seawater temperature in the study site throughout the year (Spring, Summer, and Autumn, Fig. 9) and the higher attachment rate of carpospores than tetraspores at 13° C (Winter). Under higher temperatures (21° C), different sign effects were observed as tetraspores showed higher attachment rates while carpospores showed higher germination rates. The lack of significant effects of irradiance on the studied spore-related rates of *G. sesquipedale* does not support the conclusions of Santelices (1990), that spore release and attachment in *Gelidium* species are higher under low light conditions, or the observations of Garza-Sánchez et al. (2000), who found higher spore release at high light levels (140 μmol photons $\cdot m^{-2} \cdot s^{-1}$) in *G. pacifica*. In other *Gelidium* species, contradictory results on the effects of environmental variables on these spore recruitment rates have been observed, e.g. a

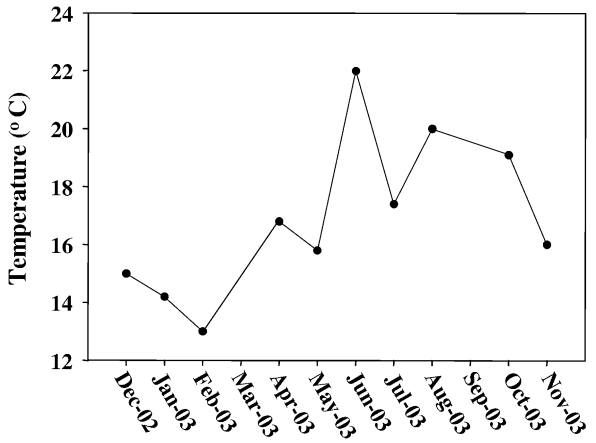

FIG. 9. Temporal variation of water temperature in Albufeira, Southeast of Portugal. Point temperature values were taken at monthly visits to the study site, at around noon time.

positive effect of temperature on spore germination in *G. amansii* (Santelices 1974, Akatsuka 1986) and the lack of any effect of temperature and light on both types of spores of *G. lingulatum* and *G. chilense* (Correa et al. 1985).

The previous statement that tetrasporophyte dominance of *Gelidium* populations appears inconsistent with the fact that there are many more fertile tetrasporophytic fronds than carposporic fronds needs to be clarified. Even though in lower numbers, the spore output of carposporophytes may be close to the output of tetrasporophytes. Our spore release experiment showed that *G. sesquipedale* cystocarps released carpospores for 2 months in the laboratory, while tetrasporangia stopped shedding tetraspores after 1 month, resulting in a total average number of 5774 carpospores produced per cystocarp compared with 2026 tetraspores produced per tetrasporangial sorus. Guzmán del Próo et al. (1972) reported similar observations for *G. robustum*. In the case of *G. sesquipedale*, not only is the carpospore output per reproductive structure higher but also the number of reproductive structures per frond is greater in gametophytes than in tetrasporophytes (unpublished data). The recent demographic modeling approaches of Scrosati and DeWreede (1999), Thornber and Gaines (2004), and Fierst et al. (2005) have shown that fecundity (no. of spores produced) and fertility (no. of juveniles produced by spore) can drive the pattern of phase dominance in the life cycle of isomorphic seaweeds. Thus, it is likely that the observed tetrasporophyte dominance of *G. sesquipedale* is determined by the recruitment processes, both by spore production and by vegetative growth of reattached fronds (not considered in the above models). To further understand the determinants of the G:T ratio of algal species it is crucial to quantify the season-specific fecundity of each phase and to integrate it with the season-specific vital rates of the spores. In fact, the models developed by Santos and Nyman (1998) fail to predict the population phase structure of *G. sesquipedale* probably because the carposporophyte fecundity was not corrected for the longer period of spore production of cystocarps compared with production of tetrasporangial sori.

In conclusion, the results of this work show that vegetative and spore recruitment may be related to the low G:T ratio of the studied population of *G. sesquipedale*, as opposed to the physiological characteristics of phases. Even though there were no physiological differences between adult fronds, there was a temperature effect on the spore recruitment-related rates that favor tetrasporophytes and a faster vegetative recruitment from re-attached tetrasporic fronds. These are processes common to all Gelidiales, but the way in which they determine the phase dominance of this evolutionary group is not general, but species-specific. A complete understanding of their relative importance may progress further by integrating sound demographic data with model simulations.

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Abbott, I. A. 1980. Seasonal population biology of some carragenophytes and agarophytes. *In* Abbott, I. A., Foster, M. S. & Eklund, I. F. [Eds.] *Symposium on Useful Algae*, pp. Seagrant Program, La Jolla, CA, 45–53.

Akatsuka, I. 1986. Japanese Gelidiales (Rhodophyta), especially *Gelidium*. *Oceanogr. Mar. Biol. Annu. Rev.* 24:171–263.

Allender, B. M. 1977. Ecological experimentation with the generations of *Padina japonica* Yamada (Dictyotales: Phaeophyta). *J. Exp. Mar. Biol. Ecol.* 26:225–34.

Beer, S. & Eshel, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. *Aust. J. Mar. Fresh. Res.* 36:785–92.

Bernstein, H., Byers, G. S. & Michod, R. E. 1981. Evolution of sexual reproduction, importance of DNA repair, complementation and variation. *Am. Nat.* 117:537–49.

Bird, K. T., Habig, C. & DeBusk, T. 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J. Phycol.* 18:344–8.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–54.

Britting, S. A. & Chapman, D. J. 1993. Physiological comparison of the isomorphic life history phases of the high intertidal alga *Endocladia muricata* (Rhodophyta). *J. Phycol.* 29:739–45.

Carmona, R., Vergara, J. J., Lahaye, M. & Niell, F. X. 1998. Light quality affects morphology and polysaccharide yield and composition of *Gelidium sesquipedale* (Rhodophyceae). *J. Appl. Phycol.* 10:323–31.

Carter, A. R. 1985. Reproductive morphology and phenology, and culture studies of *Gelidium pristoides* (Rhodophyta) from Port Alfred in South Afriapproximately. *Bot Mar.* 28:303–11.

Correa, J., Avila, M. & Santelices, B. 1985. Effects of some environmental factors on growth of sporelings in two species of *Gelidium* (Rhodophyta). *Aquaculture* 44:221–7.

Corzo, A. & Niell, F. X. 1991. Determination of nitrate reductase activity in *Ulva rigida* C. Agardh by the *in situ* method. *J. Exp. Mar. Biol. Ecol.* 146:181–9.

D'Antonio, C. M. & Gibor, A. 1985. A note on the influence of photon flux density on the morphology of germlings of

Gelidium robustum (Gelidiales, Rhodophyta) in culture. *Bot. Mar.* 28:313–6.

Destombe, C., Godin, M., Lefebvre, C., Dehorter, O. & Vernet, P. 1992. Differences in dispersal abilities of haploid and diploid spores of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Bot. Mar.* 35:93–8.

Destombe, C., Godin, J., Nocher, M., Richerd, S. & Valero, M. 1993. Differences in response between haploid and diploid isomorphic phases of *Gracilaria verrucosa* (Rhodophyta: Gigartinales) exposed to artificial environmental conditions. *Hydrobiologia* 260/261:131–7.

Destombe, C., Valero, M., Vernet, P. & Couvet, D. 1989. What controls haploid-diploid ratio in the red alga, *Gracilaria verrucosa*? *J. Evol. Biol.* 2:317–38.

DeWreede, R. E. & Green, L. G. 1990. Patterns of gametophyte dominance of *Iridaea splendens* (Rhodophyta) in Vancouver Harbor, Vancouver, British Columbia, Canada. *J. Appl. Phycol.* 2:27–34.

Dixon, P. S. 1959. The structure and development of the reproductive organs and carposporophyte in two British species of *Gelidium* and *Pterocladia*. *Ann. Bot.* 22:397–407.

Dixon, P. S. 1961. On the classification of the Florideae with particular reference to the position of the Gelidiaceae. *Bot. Mar.* 8:1–16.

Dyck, L. J. & DeWreede, R. E. 1995. Patterns of seasonal demographic changes in the alternate isomorphic stages of *Mazzaella splendens* (Gigartinales, Rhodophyta). *Phycologia* 34:390–5.

Dyck, L. J., DeWreede, R. E. & Garbary, D. 1985. Life history phases in *Iridaea cordata* (Gigartinaceae). Relative abundance and distribution from British Columbia to California. *Jpn. J. Phycol.* 33:225–32.

Edwards, G. & Walker, D. A. 1983. *C3, C4: Mechanisms and Cellular and Environmental Regulation of Photosynthesis*, 733 pp. Blackwell Sci. Pub, Oxford.

Fierst, J., terHorst, C., Kübler, J.E & Dudgeon, S. 2005. Fertilization success can drive patterns of phase dominance in complex life histories. *J. Phycol.* 41:238–49.

Garbary, D. & DeWreede, R. E. 1988. Life history phases in natural populations of Gigartinaceae (Rhodophyta): quantification using resorcinol. *In* Lobban, C. S., Chapman, D. J. & Kremer, B. P. [Eds.] *Experimental Phycology: A Laboratory Manual*, pp. Cambridge University Press, Cambridge, 174–8.

Garza-Sánchez, F., Zertuche-González, J. A. & Chapman, D. J. 2000. Effect of temperature and irradiance on the release, attachment and survival of spores of *Gracilaria pacifica* Abbott (Rhodophyta). *Bot. Mar.* 43:205–12.

Gorostiaga, J. M. 1990. *Aspectos demográficos del alga Gelidium sesquipedale (Clem.) Born. et Thur. Discusión sobre su adecuada gestión como recurso explotable*. Ph.D. thesis, University of Basque Country, 313 pp.

Guzmán del Proó, S. A., de la Campa de Guzmán, S. & Pineda-Barrera, J. 1972. Shedding rhythm and germination of spores in *Gelidium robustum*. *Proc. Int. Seaweed Symp.* 7:221–8.

Hannach, G. & Santelices, B. 1985. Ecological differences between the isomorphic reproductive phases of two species of *Iridaea* (Rhodophyta: Gigartinales). *Mar. Ecol. Prog. Ser.* 22:291–303.

Hansen, J. E. & Doyle, W. T. 1976. Ecology and natural history of *Iridaea cordata* (Rhodophyta; Gigartinnaceae): population structure. *J. Phycol.* 12:273–8.

Hay, M. & Norris, J. N. 1984. Cultivation biology of *Gracilaria*: seasonal reproduction and abundance of six sympatric species of *Gracilaria* Grev. (Gracilariaceae, Rhodophyta) on a Caribbean subtidal sand plain. *Hydrobiologia* 116/117(Dev. Hydrobiol. 22):63–94.

Inskeep, W. & Bloom, P. R. 1985. Extinction coefficients of chlorophyll *a* and *b* in *N*,*N*-Dimethylformamide and 80% acetone. *Plant Physiol.* 77:483–5.

Juanes, J. A. & Puente, A. 1993. Differential re-attachment capacity of isomorphic life history phases of *Gelidium sesquipedale*. *Hydrobiologia* 260/261:139–44.

Kamiya, M. & Kawai, H. 2002. Dependence of the carposporophyte on the maternal gametophyte in the three ceramiaceans (Rhodophyta), with respect to the carposporophyte development, spore production and germination success. *Phycologia* 41:107–15.

Kochert, G. 1978. Carbohydrate determination by the phenol-sulfuric acid method. *In* Hellebust, J. A. & Craigie, J. S. [Eds.] *Handbook of Phycological Methods: Physiological and Biochemical Methods*, pp. Cambridge University Press, England, 95–7.

Kondrashov, A. S. & Crow, J. F. 1991. Haploidy or diploidy: which is better? *Nature* 351:314–5.

Littler, M. M., Littler, D. S. & Taylor, P. R. 1987. Functional similarity among isomorphic life-history phases of *Polycavernosa debilis* (Rhodophyta, Gracilariaceae). *J. Phycol.* 23: 501–5.

Macchiavello, A. J. E., Bulboa, C. C. R. & Edding, M. V. 2003. Vegetative propagation and spore-based recruitment in the carrageenophyte *Chondracanthus chamissoi* (Gigartinales, Rhodophyta) in Northern Chile. *Phycol. Res.* 51:45–50.

Macler, B. A. & West, J. A. 1987. Life history and physiology of the red alga, *Gelidium coulteri* in unialgal culture. *Aquaculture* 61:281–93.

Maggs, C. 1988. Intraspecific life history variability in the Florideophycidae (Rhodophycea). *Bot. Mar.* 31:465–90.

Maggs, C. A. & Rico, J. M. 1991. A karyological demonstration of meiosis in *Gelidium latifolium* (Gelidiaceae, Rhodophyta) from Ireland. *Phycologia* 30:487–94.

Matshuhiro, B. & Urzúa, C. C. 1988. Agarans from tetrasporic and cystocarpic *Gelidium lingulatum*. *Bol. Soc. Chil. Quím.* 33:135–40.

Matshuhiro, B. & Urzúa, C. C. 1990. Agars from *Gelidium rex* (Gelidiales, Rhodophyta). *Hydrobiologia* 204/205:545–9.

May, G. 1986. Life history variations in a predominantly gametophytic population of *Iridaea cordata* (Gigartinaceae, Rhodophyta). *J. Phycol.* 22:448–55.

Melo, R. A. & Neushul, M. 1993. Life history and reproductive potential of the agarophyte *Gelidium robustum* in California. *Hydrobiologia* 1:1–7.

Montalva, S. & Santelices, B. 1981. Interspecific interference among species of *Gelidium* from Central Chile. *J. Exp. Mar. Biol. Ecol.* 53:77–88.

Norall, T. L., Mathieson, A. C. & Kilar, J. A. 1981. Reproductive ecology of four subtidal red algae. *J. Exp. Mar. Biol. Ecol.* 54:119–36.

Perrone, C. & Cecere, E. 1997. Regeneration and mechanisms of secondary attachment in *Solieria filiformis* (Gigartinales, Rhodophyta). *Phycologia* 36:120–7.

Perrot, V., Richerd, S. & Valero, M. 1991. Transition from haploidy to diploidy. *Nature* 351:315–7.

Rico, J. M. 1991. Field studies and growth experiments on *Gelidium latifolium* from Asturias (northern Spain). *Hydrobiologia* 221:67–75.

Rueness, J. & Fredriksen, S. 1989. Field and culture studies of *Gelidium latifolium* (Grev.) Born. et Thur. (Rhodophyta) from Norway. *Sarsia* 74:177–85.

Salinas, J. M. 1991. Spray system for re-attachment of *Gelidium sesquipedale* (Clem.) Born. et Thur. (Gelidiales: Rhodophyta). *Hydrobiologia* 221:107–1.

Salinas, J. M. & Valdés, L. 1993. Influence of temperature and photoperiod on the re-attachment process of *Gelidium sesquipedale* (Clem.) Born. et Thur. (Gelidiales: Rhodophyta). *J. Appl. Phycol.* 5:317–26.

Santelices, B. 1974. *Gelidioid algae, a brief resumé of the pertinent literature*, Tech. Rep. Mar. Agron. Sea Grant Program, Hawaii No. 1. 111 pp.

Santelices, B. 1988. Synopsis of biological data on the seaweed genera *Gelidium* and *Pterocladia* (Rhodophyta). *FAO Fish. Symp.* 145:1–55.

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

Santelices, B. & Flores, V. 1995. Spermatangial sori on cystocarpic branchlets of species of *Gelidium* and *Pterocladia* (Gelidiales, Rhodophyta). *Phycologia* 34:337–41.

Santelices, B. & Varela, D. 1994. Abiotic control of re-attachment in *Gelidium chilense* (Montagne) Santelices & Montalva (Gelidiales; Rhodophyta). *J. Exp. Biol. Ecol.* 177:145–5.

Santos, R. 1994. Frond dynamics of the commercial seaweed *Gelidium sesquipedale*: effects of size and 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: 253–6.

Santos, R. & Duarte, P. 1996. Fecundity, spore recruitment and size in *Gelidium sesquipedale* (Gelidiales: Rhodophyta). *Hydrobiologia* 326/327:223–8.

Santos, R. & Nyman, M. 1998. Population modelling of *Gelidium sesquipedale* (Rhodophyta, Gelidiales). *J. Appl. Phycol.* 10:261–72.

Scrosati, R. & DeWreede, E. 1999. Demographic models to simulate the stable ratio between ecologically similar gametophytes and tetrasporophytes in populations of the Gigartinaceae (Rhodophyta). *Phycol. Res.* 47:153–7.

Smith, L. D. & Hughes, T. P. 1999. An experimental assessment of survival, re-attachment and fecundity of coral fragments. *J. Exp. Mar. Biol. Ecol.* 235:147–64.

Sokal, P. R. & Rohlf, F. J. 1981. *Biometry.* 2nd ed, 859 pp. W. H. Freeman and Co, San Francisco.

Sosa, P. A., Jiménez del Río, M. & García-Reina, G. 1993. Physiological comparison between gametophytes and tetrasporophytes of *Gelidium canariensis* (Gelidiaceae: Rhodophyta). *Hydrobiologia* 260/261:445–9.

Thornber, C. S. & Gaines, S. D. 2004. Population demographic in species with biphasic life cycles. *Ecology* 85:1661–74.

Wellburn, A. R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various spectrophotometers of different resolution. *J. Plant Physiol.* 144:307–13.

West, J. A. & McBride, D. L. 1999. Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia* 398/399: 101–13.

Zucarello, G. C., Yeates, P. H., Wright, J. T. & Bartlett, J. 2001. Population structure and physiological differentiation of haplotypes of *Caloglossa leprieurii* (Rhodophyta) in a mangrove intertidal zone. *J. Phycol.* 37:235–44.