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Effects of photon flux density and agricultural fertilizers on the development of *Sarcothalia crispata* tetraspores (Rhodophyta, Gigartinales) from the Strait of Magellan, Chile

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Abstract Tetraspores of *Sarcothalia crispata* from San Juan Bay, Strait of Magellan, Chile, were cultivated under different combinations of photon flux densities and agricultural fertilizers in the laboratory. In the experiment, the *S. crispata* specimens were cultured in combinations of different photon flux densities (50, 100, 150 μmol photons m^{-2} s^{-1}) and enriched seawater solutions (sodium nitrate + monocalcium phosphate, urea + monocalcium phosphate, ammonium nitrate + monocalcium phosphate), always adjusting the N and P concentrations to 10 and 3 mg L^{-1}, and in sea water as control. After 45 days, the tetrasporeling plants were found to be larger at photon flux densities of 50 and 100 μmol photons m^{-2} s^{-1} in the nutrient enrichment experiments; growth was greatest in the sea water enriched with ammonium nitrate and urea. An analysis of the combined effect of the photon flux density and nutrients revealed that the best combination for sporeling growth was the ammonium nitrate and urea solution at 50–100 μmol photons m^{-2} s^{-1}.

Keywords Early development · Sporeling culture · Mass cultivation · Nutrients · Growth

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Introduction

Phycocolloid extraction and macroalgae farming are currently important productive activities in Chilean fisheries, demonstrating sustained growth and development in recent years. Phycocolloid production using carrageenan-producing algae from the genera *Iridaea*, *Gigartina*, *Mazzaella*, and *Sarcothalia* is second only to that using *Gracilaria chilensis* Bird, McLachlan & Oliveira, the most economically important macroalgae group in Chile.

Sarcothalia crispata (Bory) Leister, commonly known as "luga negra" and previously called *Iridaea ciliata*, is endemic to Chile. This red alga is distributed along the cold temperate coasts of central to southern Chile, from its northern limit in Valparaíso (36°S) to Tierra del Fuego (54°S; Ramírez and Santelices 1991; Hommersand et al. 1993; Hoffmann and Santelices 1997). Commercial extraction of this alga began in Chile in the 1960s (Romo et al. 2001).

Uninterrupted fishery statistics from 1986 are available for *S. crispata*. The first landing record reports 23,449 wet tons of this alga, and landings in 2005 reached 24,942 wet tons. *S. crispata* harvests are largest in central-southern Chile, between approximately 36° and 44°S (Sernapesca 2003). However, in spite of continuous landing records for over 20 years, landings that included *S. crispata* through the year 2000 were listed under the common name "luga-luga", which included at least two other carrageenophytes (*Gigartina skottsbergii* or "luga roja" and *Mazzaella sp.* or "luga cuchara"). Therefore, prior to 2000, the exact volume of *S. crispata* landings is not known, making it difficult to evaluate the resource's current state of sustainability based on landing records.

The opinions of experts and subsistence fishers, as well as information found in the fishery statistics, allow an

estimate of steady production levels that have remained at around 20,000–30,000 wet tons per year during the 30 years of "luga negra" extraction. However, with its increased national importance, populations of this species have begun to present symptoms of overexploitation. Two pieces of evidence indicate a decline in this resource: (1) although the extraction effort has increased during recent years, the volume of landings has remained approximately the same for several years, and (2) currently, populations available for extraction are found in harder to reach areas (Avila et al. 1994, 1996). Consequently, if the current extraction pressure continues, it is highly likely that the natural *S. crispata* populations will collapse.

Considering the increasing vulnerability of natural populations of this and other species of high commercial interest, together with the growing demand for the product on national and international markets, mass farming of *S. crispata* is a highly interesting alternative for maintaining—or even increasing—the productivity of this raw material (Mansilla et al. 2002). Experiments in the laboratory and in the field have shown that culturing algae from spores can be an effective method of mass production (Alveal et al. 1991, 1994, 1995, 1997; Candia et al. 1993) as long as aquaculturists are able to produce large enough quantities of initial stages under adequate conditions to assure good initial growth and the subsequent development of adult algae.

For this, certain aspects of the culturing methodology are particularly relevant for the successful implementation of new farming techniques; these aspects require research and innovation. As stated by many authors, the sporeling supply creates a bottleneck in successful commercial cultivation methodologies (Buschmann et al. 1999; Liu et al. 2004). Therefore, much research has been done in this area. Zhao et al. (2006) reported on early sporeling development in *Gracilaria asiatica*, and Mansilla et al. (2006), Avila et al. (2003), and Romo et al. (2001) on the effects of environmental factors on germination and growth in initial stages of commercial species from southern Chile. Other topics of study in the area include the effect of light period on *Undaria pinnatifida* gametophyte egg-discharge (Liu et al. 2004), the use of *Undaria* gametophyte clones in sporeling cultures (Wu et al. 2004), new methods of rearing sporelings using gametophyte clones and strain selection in *Laminaria japonica* (Li et al. 1999; Zhang et al. 2007), vegetative propagation through tissue fragmentation in *Gigartina skottsbergii* (Buschmann et al. 2001; Hernandez-Gonzalez et al. 2007), and spore viability and survivorship (Buschmann et al. 1999; Romo et al. 2001).

When cultivating algae from spores, low-cost agricultural fertilizers can be used to stimulate sporeling and germling growth during the industrial seed production stage (Werlinger 1998), replacing the expensive nutrients that are used in the experimental phase and design of the culture methodology. The most appropriate environmental conditions can also be estimated for this stage. According to Avila et al. (1999b), innovations in this phase would unquestionably have an important impact on the amount of money required to produce these seaweeds, as well as affecting the technological aspects of their mass production under farmed conditions.

The objective of this study is to improve the method for culturing *S. crispata* from spores, focusing on sporeling production using spores of individuals collected from natural populations in the Strait of Magellan that have not yet been subjected to exploitation. We used studies on the improvement of methodologies for the commercial cultivation of *G. chilensis* as references (Alveal et al. 1994, 1997).

Materials and methods

Mature tetrasporic fronds of *S. crispata* were collected at the end of September 2004 (austral spring) from natural intertidal algae beds in San Juan Bay. These beds are located in the western section of the Strait of Magellan (53° 37′S, 70°59′W), Chile, approximately 70 km southwest of the city of Punta Arenas. Following collection, the specimens were transported in glass containers with seawater to the marine biology laboratory of the Departamento de Ciencias Naturales y Recursos Marinos, Facultad de Ciencias, Universidad de Magallanes, Chile.

We performed laboratory experiments using a combination of four different culture medium under three different photon flux densities. Three of the media used were different enriched seawater solutions and one was non-enriched seawater (control); the combinations and concentrations of agricultural fertilizers used are indicated in Table 1. Each of the media was made using filtered and sterilized natural seawater (provided by the "Centro Cultivos Marinos"). The nitrogen and phosphorous concentrations were adjusted to 10 and 3 mg L^{-1}, respectively, based on the optimal values detected in similar experiments with *Gracilaria chilensis*; sodium nitrate values were 0.06 L^{-1} and sodium monocalcium phosphate values were 0.01 L^{-1} (Alveal et al. 1991).

In addition, we used three photon flux densities (50, 100, 150 μmol photons m^{-2} s^{-1}) on the experiments. The different levels were obtained by using different combinations of PAR Phillips TLT 20W/54 daylight fluorescent tubes. The culture chamber was maintained at 8°C±1°C, with a 12 h:12 h light:dark photoperiod.

Experimental treatments

Fertile frond fragments collected from natural populations were carefully washed under tap and distilled water to

Table 1 Types of agricultural fertilizers used in laboratory experiments, with corresponding concentrations of nitrogen and phosphorous

Fertilizer	Control	Solution 1	Solution 2	Solution 3
Sodium nitrate (Chilean nitrate)	-	0.061 $g.L^{-1}$ (10 $mg.L^{-1}$ nitrogen)	-	-
Urea	-	-	0.043 $g.L^{-1}$ (10 $mg.L^{-1}$ nitrogen)	-
Ammonium nitrate	-	-	-	0.057 $g.L^{-1}$ (10 $mg.L^{-1}$ nitrogen)
Monocalcium phosphate	-	0.007 $g.L^{-1}$ (3 $mg.L^{-1}$ phosphorous)	0.007 $g.L^{-1}$ (3 $mg.L^{-1}$ phosphorous)	0.007 $g.L^{-1}$ (3 $mg.L^{-1}$ phosphorous)

remove epiphytes and organic matter remains. To stimulate the opening of the sporangia and induce the spore release according to the methodology of Romo et al. (2001) and Avila et al. (2003), we desiccated mature fronds fragments (1–2 cm^2), placing them in trays with 200 mL of 0.45 μm filtered and sterilized seawater with continuous shaking for 24 h. The resulting spore suspension (average density: 33,000 spores mL^{-1}) was then used to inoculate 36 glass slides.

After the spores had settled onto the glass slides (average density: 133 spores cm^{-2}), they were separated into 12 containers, each having three slides and one of the four culture media. In the incubation chamber, the containers were separated into three compartments corresponding to the three photon flux densities. In summary, each compartment held four containers, corresponding to the four culture media treatments. Seawater and culture media were replaced once a week for a month and a half, according to Romo and Paula (1995).

Growth measurements

We quantified tetrasporeling growth using digital images of the plants on the glass slides, which were obtained on six occasions over 45 days (days 10, 17, 24, 31, 38, 45) using a digital camera attached to a stereoscopic microscope. Images were processed using Image-Pro Plus, version 4.1. We took five photos in the center of each of the 36 slides, maintaining a uniform distance for each of the photographed areas. Using these photographs, we measured the diameter of all sporelings present in the image, analyzing a total of 3,600–4,600 sporelings per sample to obtain the average sporeling size.

Statistical analysis

A three-way Analysis of Variance (ANOVA) was used to evaluate differences in the sporeling growth under different nutrient and photon flux density treatments and given different time periods, as well as the interactions between these factors (Sokal and Rohlf 1979). When significant differences were found, we applied the Tukey test (Zar 1999) to evaluate which experimental treatment accounted for the observed differences (Sokal and Rohlf 1979; Zar 1999). Prior to the ANOVA, assumptions of a normal distribution and homogeneity of variance were evaluated using the Shapiro-Wilks test and Levene's tests, respectively (Sokal and Rohlf 1979; Zar 1999).

Results

Sporeling growth

During the 45 days of culturing, *S. crispata* tetrasporelings tended to increase steadily in size. This size increase was greater towards the end of the experiment, when we also observed differences in growth with the different nutrient media. However, these differences were not as evident under the different photon flux densities (Fig. 1).

The analysis of variance (Table 2) indicated significant differences in the *S. crispata* sporeling growth between the culture media ($p<0.0001$), under different photon flux densities ($p<0.0001$), and for different times ($p<0.0001$). We observed a significant interaction between the treatment factors ($p<0.0001$), indicating a synergistic effect of nutrients and light on growth over time (Table 2).

With respect to the different culture media, differences in the sporeling development were apparent in the first measurements, and the sporelings generally presented sustained, clear differences up to the penultimate measurement (day 38) with respect to the different treatments (Fig. 1). The cultivated sporelings in the control seawater treatment (i.e. no added nutrients) were smallest, reaching an average maximum size of 30.05±3.78 μm (mean ± SD) on day 45. The largest sporelings were those cultivated in media enriched with urea and ammonium nitrate, reaching average maximum sizes of 35.99±4.55 μm and 35.97± 4.44 μm, respectively, after 38 days of culture. Sporelings cultivated in sodium nitrate reached intermediate sizes, averaging maximum sizes of 33.28±4.17 μm after 38 days.

A post-hoc Tukey test (Table 3, Fig. 1) indicated that individuals in the seawater and sodium nitrate + mono-

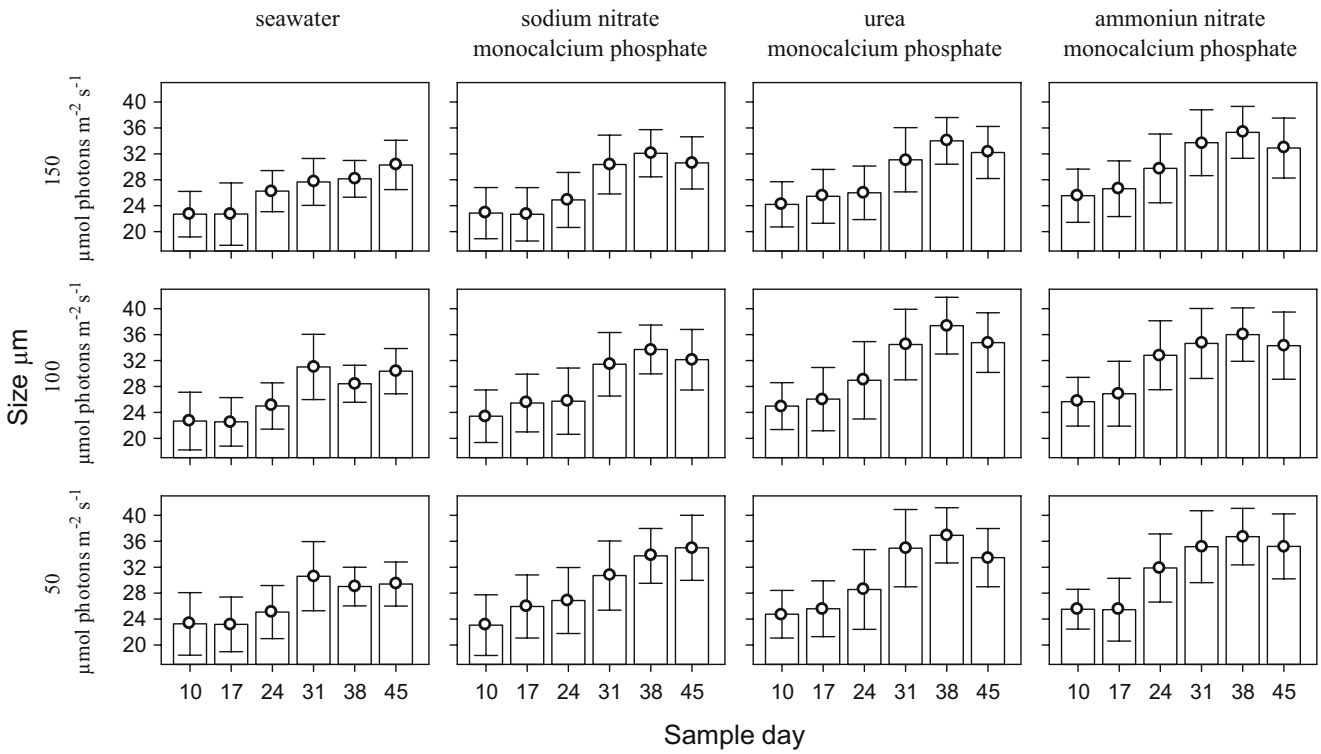

Fig. 1 Average size of *Sarcothalia crispata* sporelings grown under different photon flux density and in different cultura media; data are average values±S.D

calcium phosphate treatments were the smallest on average during almost the entire experimental period; they were significantly smaller than the sporelings cultivated in media enriched with urea or ammonium nitrate. There were no significant differences in the sizes of sporelings cultivated in the two media that most stimulated growth (urea + monocalcium phosphate and ammonium nitrate + monocalcium phosphate) for the first measurements (day 10) or the last two measurements (days 38 and 45). However, on three other occasions, the sporelings in ammonium nitrate enriched seawater (ammonium nitrate + monocalcium phosphate) were significantly larger than the urea enriched sporelings (urea + monocalcium phosphate), presenting average sizes of 25.56 µm±3.89, 31.17 µm±5.72 and 34.45 µm±5.63, respectively.

Table 2 Three-way analysis of variance for average size of *Sarcothalia crispata* sporelings with culture medium, photon flux density and time

Source of variation	SS	*df*	MS	*F*	*p*
Culture medium	91,556	3	30519	1,383	0.00001
Photon flux density	11,016	2	5508	250	0.00001
Time	402,396	5	80479	3647	0.00001
Interactions					
Medium × photon flux density	3,221	6	537	24	0.00001
Medium × time	22,687	15	1960	69	0.00001
Photon flux density × time	2,371	10	237	11	0.00001
Medium × photon flux density × time	9,564	30	319	14	0.00001

A visual inspection of germling growth under the different photon flux densities (Fig. 1) showed that all treatment groups increased in size over time, with differences between treatments beginning on day 17. Growth slowed on day 31 and tended to stabilize on the final two measurement dates (days 38 and 45; Fig. 1). Results from the Tukey test (Table 4) indicated that the greatest increase in growth occurred for sporelings submitted to the low (50 µmol photons m^{-2} s^{-1}) and medium (100 µmol photons m^{-2} s^{-1}) photon flux densities, as compared to the high photon flux density treatment (150 µmol photons m^{-2} s^{-1}); no significant differences were found between the low and medium photon flux density treatments. This pattern was observed throughout the entire experiment and became more evident over time. The largest sporelings were observed around 38 days of culture in the low and medium photon flux density treatments, averaging 34.10 µm±3.9 and 33.88 µm±4.0, respectively, whereas sporelings under the high photon flux density reached average values of 32.89 µm±3.9.

Combined effect of photon flux density and enriched seawater

Considering the effect of the different culture media with respect to the photon flux density (Table 5), the Tukey test

Table 3 Tukey test for differences in the growth of *S. crispata* sporelings grown under different combinations of enriched seawater on different sample days

Day	Culture medium						
	Seawater	Compared	Sodium nitrate + monocalcium phosphate	Compared	Urea + monocalcium phosphate	Compared	Ammonium nitrate + monocalcium phosphate
10	22.87	=	23.10	≠	24.63	≠	25.56
17	22.72	≠	24.61	≠	25.73	=	26.29
24	25.47	=	25.87	≠	27.93	≠	31.17
31	29.77	≠	30.88	≠	33.50	≠	34.45
38	28.46	≠	33.28	≠	35.98	=	35.97
45	30.05	≠	32.58	≠	33.59	=	34.16

revealed significant differences in growth between tetraspores deposited in seawater without added nutrients and those in enriched seawater (ammonium nitrate + monocalcium phosphate). The sporelings maintained under low and medium photon flux densities did not present significant differences in size, but were significantly larger than the sporelings maintained under the high photon flux density. The sporelings cultivated in the other two culture media (sodium nitrate + monocalcium phosphate, urea + monocalcium phosphate) also presented significant differences between the three photon flux density treatments (Table 5).

Considering the effect of the photon flux density relative to the different culture media (Table 6), we observed differences in the average sporeling size between all the enriched seawater treatments except for the sporelings maintained under medium light intensities and cultivated in seawater enriched with urea + monocalcium phosphate or ammonium nitrate + monocalcium phosphate; the average sizes were not significantly different for these treatment groups.

We observed a significant interaction of nutrient and photon flux density treatments on sporeling growth. The largest sporelings were observed in treatments with low and medium light intensities in seawater enriched with urea + monocalcium phosphate and ammonium nitrate + monocalcium phosphate (Tables 5 and 6).

Table 4 Tukey test for differences in the size of *S. crispata* sporelings grown under different treatments of photon flux density on different sample days

Day	Photon flux density (PAR) μmol photons m^{-2} s^{-1}				
	50	Compared	100	Compared	150
10	24.17	=	24.18	=	23.96
17	25.20	=	25.19	≠	24.53
24	28.22	=	27.74	≠	27.08
31	32.79	=	32.78	≠	30.86
38	34.43	=	33.70	=	32.56
45	33.43	=	32.74	=	31.44

Discussion

The most successful culture media for the development of *Iridaea*, under laboratory conditions, are enriched seawater, following the formula of Provasoli (McLachlan 1973) and employed by Infante and Candia (1988), and the Swm-3 medium modified by McLachlan (1973) and employed by Hannach and Santelices (1985) and Luxoro and Santelices (1989). For both *Iridea laminarioides* and *I. cilata*, good development of juvenile forms is favored by very complete nutrient media. A comparison done with *I. ciliata* revealed that the Provasoli medium promotes greater growth than that obtained with seawater enriched only with nitrate and orthophosphate, with respect to concentrations similar to the Erdschreiber medium (McLachlan 1973) and the addition of EDTA-Fe.

Nevertheless, today, the search for ways to reduce the culturing costs and times makes animal-based organic wastes and agricultural fertilizers a much more convenient option for obtaining nutritive solutions. Ambler et al. (1988) conducted experiments utilizing water with *Argopecten purpuratus* excrement as an additive for culturing *Gracilaria chilensis* in aquaria; they observed positive effects on growth. In contrast, Buschmann et al. (1994) and Martínez and Buschmann (1996) studied the effect of effluents from salmon tanks on biomass production and agar quality in *G. chilensis* cultures, but did not observe significant stimuli for either parameter of the alga. Yoneshigue-Braga and Neves (1981) studied the use of agricultural fertilizers for the culture of *Gracilaria* and other algae in tanks, finding that commercial preparations can be adequately used to fertilize algae, as previously proposed by authors such as Alveal et al. (1991, 1999a). The most-used fertilizers are ammonium sulfate (Tseng 1981; Friedlander 2001), urea, and pig manure or fermented bird guano (Lipkin 1985), but sodium nitrate and ammonium nitrate are also used (Buschmann et al. 2004). Furthermore, Alveal et al. (1991) indicated that concentrations of 0.06 g L^{-1} of sodium nitrate and 0.01 g L^{-1} of sodium phosphate are the most appropriate concentrations for the treatment of *G. chilensis* germlings.

Table 5 Tukey test for differences in the growh of *S. crispata* sporelings under different densities of photon flux for each type of culture medium

Culture medium	Photon flux density (PAR) μmol photons m^{-2} s^{-1}				
	50	Compared	100	Compared	150
Seawater	26.91	=	26.99	≠	26.43
Sodium nitrate + monocalcium phosphate	29.80	≠	29.01	≠	27.39
Urea + monocalcium phosphate	30.80	≠	31.56	≠	29.16
Ammonium nitrate + monocalcium phosphate	31.56	=	31.77	≠	30.55

The results obtained from this study indicate that all three nutrient solutions had positive effects on the tetraspore growth, although the magnitude of the effect varied between solutions. The most effective solutions were those containing a combination of urea-monocalcium phosphate and ammonium-monocalcium phosphate, differing partially from the results of Alveal et al. (1995), who evaluated the effect of the same nutrients on the microthalli of *Gracilaria* obtained from carpospores. These authors observed a greater increase in germling size when maintained in a solution of "Chilean nitrate" (sodium nitrate) + monocalcium phosphate, followed by those fertilized with a solution of ammonium nitrate + monocalcium phosphate; the smallest size increase occurred in germlings maintained in a solution of urea + monocalcium phosphate. Nevertheless, our results agree with the proposal by Santelices (1996) that, although algae can use organic nitrogen, particularly urea, they generally prefer ammonium to nitrate. Other authors, such as Haglund and Pedersén (1993), Lobban and Harrison (1994), and Chopin et al. (1990) have also indicated that algae probably conserve energy by using NH_4^+ since their cells must reduce NO_3^- to ammonium before it can be incorporated into amino acids.

With respect to the requirements of radiant energy, in general, the early phases of macroalgae development are well adapted to low light intensities since the reproductive structures liberated in winter must grow under the canopy of the adult algae (Gómez and Wiencke 1996; Hanelt and Nultsch 1997). This could explain the significantly lower growth of *S. crispata* tetraspores at 150 μmol photons m^{-2} s^{-1} as compared to the lower photon flux densities of 100 μmol photons m^{-2} s^{-1} and 50 μmol photons m^{-2} s^{-1} (Romo et al. 2001; Avila et al. 2003). These findings agree with those of Ugarte (1982), who found significant differences in the development of *Mazzaella laminarioides* tetraspores and carpospores under different irradiances. In the same study, after 5 days of culturing, the individuals developed from tetraspores grew faster at light intensities of 77 μmol photons m^{-2} s^{-1}, whereas individuals developed from carpospores grew better when cultivated at 55 μmol photons m^{-2} s^{-1}. In contrast, Avila et al. (1999a) did not observe differences between tetraspores and carpospores of *G. skottsbergii* grown at 5 and 10°C, using three irradiances (6, 18, and 25 μmol photons m^{-2} s^{-1}); the best results for both tetraspores and carpospores occurred at 10°C and a photon flux density of 6 μmol photons m^{-2} s^{-1}. Different studies conducted on *I. ciliata* have indicated different optimum ranges of photon flux density for the development of initial stages. Hannach and Santelices (1985) found, for example, that the optimal development of this species occurred at a photon flux density of 35–60 μmol photons m^{-2} s^{-1} (12 h:12 h light:dark photoperiod, temperature 15°C) for both gametophytes and tetrasporophytes. According to Romo and Alveal (1995), the photon flux density for the incubation of *Iridaea* sp. spores is restricted to moderate and low light intensities (12–60 μmol photons m^{-2} s^{-1}) in combination with temperatures of 10–20°C; at higher illumination values, the growth rate decreases and a photon flux density above 120 μmol photons m^{-2} s^{-1} is inhibitory. Avila et al. (2003) stated that the basic requirement for *S. crispata* cultivation at 15°C is an irradiance range of 30–50 μmol photons m^{-2} s^{-1}. On the other hand, for the same species under different laboratory conditions (16 h:8 h light:dark photoperiod, 15°C), the

Table 6 Tukey test for differences in the size of *S. crispata* sporelings grown in different culture media for each density of photon flux density

Photon flux density (μmol photon m^{-2} s^{-1})	Culture medium						
	Seawater	Compared	Sodium nitrate + monocalcium phosphate	Compared	Urea + monocalcium phosphate	Compared	Ammonium nitrate + phosphate monocalcium
50	26.91	≠	29.80	≠	30.80	≠	31.56
100	26.99	≠	29.01	≠	31.56	=	31.77
150	26.43	≠	27.39	≠	29.16	≠	30.55

greatest growth was observed at a photon flux density of 60 μmol photons m^{-2} s^{-1} (Romo et al. 2001).

A comparison of the results from different authors indicates a photon flux density range of 12–60 μmol photons m^{-2} s^{-1} for optimum growth in several red macroalgae. Nevertheless, all of the previously cited studies indicate that the different developmental stages require different temperatures, illumination, and photoperiods (Henkel 1952; Boalch 1961; Fries 1963). Differences may also be found between individuals from different populations of the same species, or between species that inhabit the same region but in areas with different light conditions (Gantt 1990).

In our study, the photon flux density that produced the greatest effect on sporeling development (50–100 μmol photons m^{-2} s^{-1}, 12 h:12 h light:dark photoperiod) was in the upper part of the range reported by earlier studies. Nevertheless, the temperature used in our study was approximately 8°C, which is several degrees cooler than the temperatures used by other researchers. This is an important point to consider, given that, in the Strait of Magellan, seawater temperatures are much lower than in the zones inhabited by the species used in previous studies.

In summary, the combined effect of enriched seawater solutions and photon flux density on the growth of *S. crispata* tetraspores was positive and synergistic. A significant interaction between different parameters in different species has been mentioned by authors such as Buschmann et al. (2004), in studies regarding the effects produced by nutrients (phosphorous, nitrogen, ammonia), photon flux density, and temperature in a culture of *Gigartina skottsbergii*. These researchers observed an interaction among these factors, which, jointly, produced up to 2% greater growth in this carrageenophytic algae. Furthermore, Navarro-Angulo and Robledo (1999) observed that a combination of two nutrients (nitrogen, phosphorous) produced a synergistic increase in the development of both carpospores and tetraspores of *Gracilaria cornea* (27±1.3°C, 12 h:12 h light:dark photoperiod, 100 μmol photons m^{-2} s^{-1}) with respect to the separate effects of each fertilizer. In addition, studies by Mansilla et al. (2002) clearly indicate an interaction between photoperiod and photon flux density in the development of tetraspores and carpospores of *Mazzaella laminarioides*.

The results from our study support the effect of differential stimulation on the growth of *S. crispata* sporelings as a consequence of different culture media prepared from agricultural fertilizers, combined with the use of different photon flux densities. Our results agree in general with those obtained by Romo et al. (2001) and Avila et al. (2003) for the same species. The combined effect of these variables produced a synergistic effect on sporeling development. We identified two culture media that were clearly responsible for stimulating germling growth (with no significant differences between media), as well as two photon flux density treatments that significantly increased the sporeling size. The best treatment combinations for germling growth in *S. crispata* correspond to a combination of seawater enriched with ammonium nitrate + monocalcium phosphate and urea + monocalcium phosphate, cultivated under low (50 μmol photons m^{-2} s^{-1}) or intermediate (100 μmol photons m^{-2} s^{-1}) photon flux density.

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