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Macrocystis pyrifera aquafarming: Production optimization of rope-seeded juvenile sporophytes

Carolina Camus, Alejandro H. Buschmann *

Centro i~mar, Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Los Lagos, Puerto Montt, Chile

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ABSTRACT

The use of kelp in different human activities has increased over recent decades and this demand is increasing the demand for cost-effective farming technologies. In the case of the giant kelp (*Macrocystis pyrifera*) there is a large accumulation of knowledge concerning the relevant information required for aquafarming that allowed the installation of a 20 ha pilot culture farm 5 years ago in southern Chile. However, this experience indicated that to achieve a production level above 200 tons per hectare per year it is necessary to optimize all the protocols starting from the hatchery level through to the growing phase in order to be cost effective. In this study we present information on optimizing the hatchery phase of *M. pyrifera* culture. The results show that it is possible to obtain 4 to 5 mm long *M. pyrifera* juvenile sporophytes attached to seeded rope in less than 45 days. To obtain this result, temperature should be 12 °C with a photon irradiance of 12 μmol m^{-2} s^{-1} and a photoperiod of 16:8, L:D. In addition, macro and micronutrient concentrations should be maintained as the traditional Provasoli culture medium indicates, and with aeration of 414 L h^{-1} to maintain water movement in the culture system.
Statement of relevance: Commercially hatchery kelp farming protocol development is presented.
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1. Introduction

The use of macroalgae in different human activities has increased in the last decade; as food ingredients, in cosmeceutical and pharmaceutical industries, extraction of phycocolloids and potentially for biofuel production (Hafting et al., 2015). Chile is one of the largest exporters of brown seaweeds harvested from natural populations including *Lessonia* spp. in northern Chile for alginate extraction, and *Macrocystis pyrifera* (Linnaeus) C. Agardh, used in abalone aquaculture in southern Chile (Buschmann et al., 2008; Vásquez et al., 2013). Seaweed aquafarming at a commercial scale in Chile remains restricted to the red alga *Gracilaria chilensis*, used for agar production, while brown algae have only been cultivated at experimental or pilot scales (Buschmann et al., 2014). Currently, due to the need to develop the economies of Chilean coastal communities, several government agencies are supporting research projects looking for alternative uses and ways of adding value to brown algae currently harvested (e.g. Wargacki et al., 2012; Camus et al., 2016; Ibañez and Cifuentes, 2013; Buschmann et al., 2014; Correa et al., 2016).

A number of previous attempts have been reported showing *Macrocystis* aquafarming in Chile (Westermeier et al., 2005, 2006, 2011; Gutiérrez et al., 2006; Macchiavello et al., 2010; Correa et al., 2016). All of which have shown promising results, achieving in 6 to 7 months a production that varied between 14 kg m^{-1} to 80 kg m^{-1} of culture line. However, this high variance in the production potential of *M. pyrifera* seems to be due to a variety of reasons such as site specific environmental conditions, genetics and genetic selection, fouling and grazers, as well as culture methods (Westermeier et al., 2005; Buschmann et al., 2014). Some pilot scale cultivation studies have suggested that productivity could potentially reach ca. 200 ton (wet) ha^{-1} yr^{-1} if proper environmental conditions, adequate control of pest organisms and two harvests can be achieved during a year (Buschmann et al., 2014). These previous studies have emphasized the cultivation methodologies and conditions for growing *Macrocystis* in the sea. However, no significant effort has yet been reported for improvements to the initial stages of this alga's cultivation at the hatchery level. In the hatchery phase, two principal methodologies have been described for producing juvenile sporophytes; free-floating plants (Westermeier et al., 2006) and rope-seeded (Gutiérrez et al., 2006; Macchiavello et al., 2010). On the one hand, free-floating kelp juveniles permit seeding with larger individuals that could out-compete potential epiflora and epifauna in open waters. However, the costs involved in their production are substantially higher as more time in the hatchery is needed to reach the seeding size (10–15 cm average), due to a larger hatchery capacity, higher costs due to light, increased water and nutrient needs, amongst other reasons. Furthermore, the attachment of free-floating kelp, increases labor costs as each individual requires attaching to the ropes for the final deployment in the sea. On the other hand, the rope-seeded plants required less time in the hatchery (35–40 day to reached 4–5 mm in length), a smaller hatchery, lower

* Corresponding author.
E-mail addresses: carolina.camus@ulagos.cl (C. Camus), abuschma@ulagos.cl (A.H. Buschmann).

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Table 1
Experimental factors tested on rope-seeded *Macrocystis pyrifera* plants at hatchery phase. N/P: nitrate and phosphate; M/V: micronutrients and vitamins. Temperature and photon irradiance of the different treatments during all the performed experiments was maintained within the ranges.

		Treatments			
Experiment	Factor	1	2	3	4
1	Temperature (°C)	T: 12*	T:(+) 25% = 15	T: 12*	T:(+) 25% = 15
	Photon irradiance (μmol m^{-2} s^{-1})	L: 12*	L: 12*	L:(+) 25% = 15	L:(+) 25% = 15
2	Photoperiod (Light:Dark hours)	12:12, L:D	16:8, L:D*	24:0, L:D	
3	Macronutrients concentration	(+) 25% N/P	N/P*	(−) 25% N/P	
4	Micronutrients concentration	(+) 25% M/V	M/V*	(−) 25% M/V	
5	Air flow rate (L h^{-1})	0	138	414*	

* Indicates standard environmental conditions for all the experimental treatments: temperature = 12 °C; photon irradiance; photoperiod 12:12, L:D; full Provasoli medium; and air flow = 138 L h^{-1}.

production costs, and the seeding can be industrialized by the use of seeding machines. However, the deployment of smaller sizes kelp increases the risks that they will be outcompeted by fouling organisms and they are also more susceptible to changes in environmental conditions (Buschmann et al., 2014). For these reasons, it is necessary to ensure that the juvenile sporophytes have the best physiological condition in order to reduce seeding failures.

A considerable amount of research exists concerning the physical and chemical requirements of *Macrocystis* and other kelp gametophytes for growth, reproduction and the production of sporophytes in both the natural environment and in laboratory culture experiments (Redmond et al., 2014; Carney and Edwards, 2010; Buschmann et al., 2004; Muñoz et al., 2004; Deysher and Dean, 1984; Hurd et al., 1996; Fram et al., 2008; Egan et al., 1989; Gerard, 1982; Jackson, 1977; Wheeler and North, 1980, 1981; Palacios and Mansilla, 2003; Westermeier et al., 2009; Westermeier et al., 2011; Westermeier et al., 2012). For *M. pyrifera*, the requirements of suitable temperatures, light at particular wavelengths, salinity, and the composition and quantity of nutrients are well known (Foster and Schiel, 2015). Despite the large number of studies, most of them only provide abiotic requirements needed to interpret the general variation in *Macrocystis* populations in nature, however, the cultivation of kelp microscopic phases needs to be carried at large-scale in order to support commercial farming. Therefore, a focus on the optimization of requirements needs to be established, with the objective of reducing costs by minimizing the amount of time and energy used for the production of the juvenile sporophytes. A production level system cannot simply follow the protocols developed for laboratory scale production, as it requires more cost-effective production models in terms of energy requirements (light, temperature control and aeration) and nutrient demands (type and amount of the chemicals

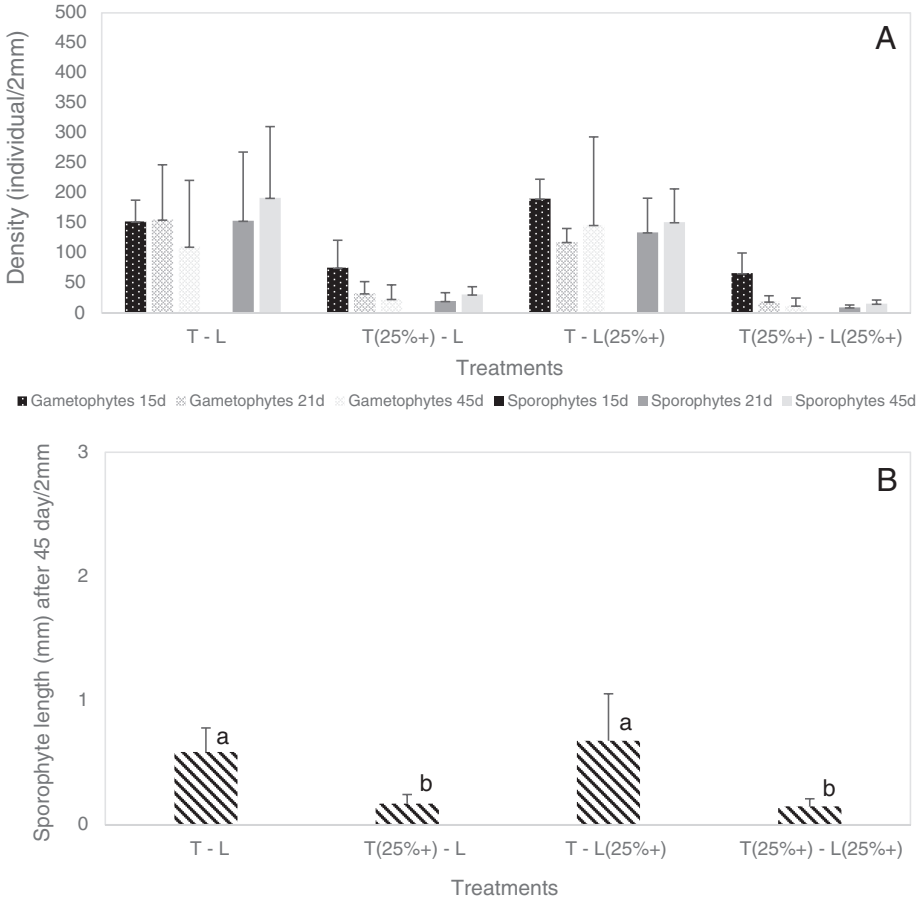

Fig. 1. A) Density of gametophytes and sporophytes of *Macrocystis pyrifera* under to four treatments of temperature and photon irradiance during 15, 21 and 45 days of culture. $T = 12$ °C, T(25%+) = 15 °C, L = 12 μmol m^{-2} s^{-1}, L(25%+) = 15 μmol m^{-2} s^{-1}. B) Sporophyte length after 45 days of culture under four treatments of temperature and irradiance. One-way ANOVA: The primary factor regulating the growth of kelp gametophytes is temperature $F_{(3,539)} = 214.76$; $P = 0.0001$.

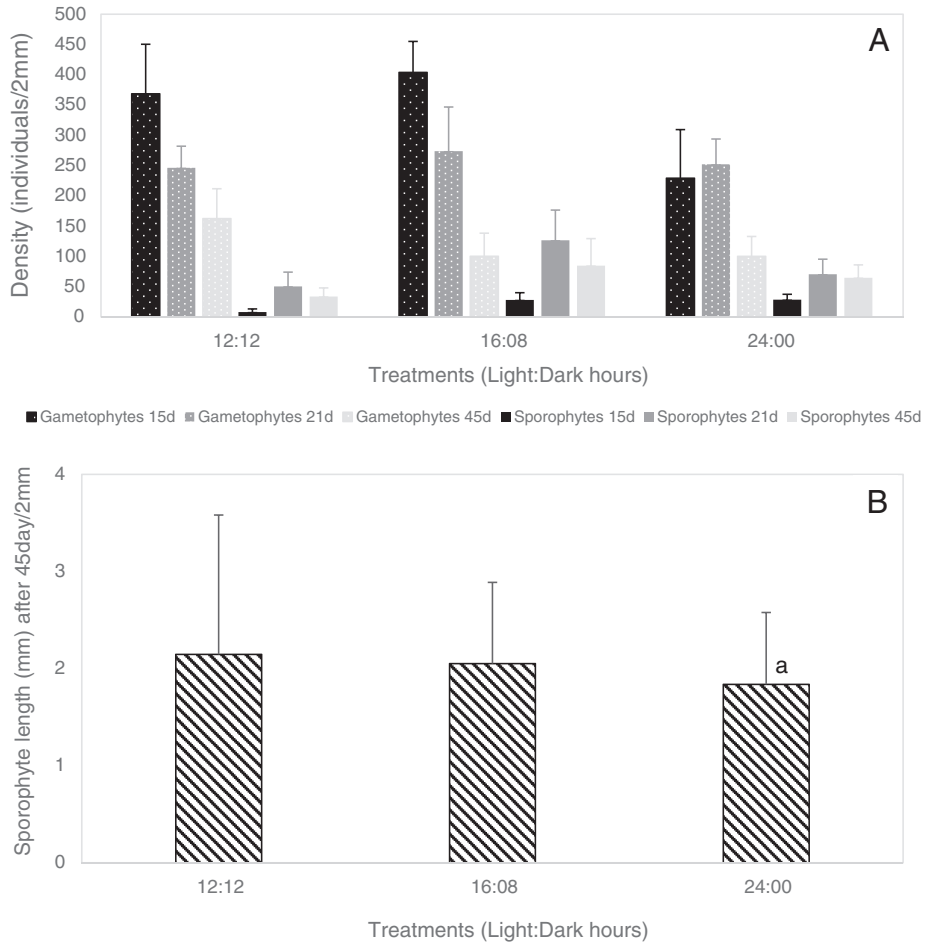

Fig. 2. A) Density of gametophytes and sporophytes of *Macrocystis pyrifera* submitted to three treatments of photoperiod during 15, 21 and 45 days of culture. B) Sporophyte length after 45 days of culture under three different photoperiods. One-way: ANOVA $F_{(2,404)} = 2.04$; $P = 0.108$.

used) amongst others. Previous work on kelps has demonstrated that temperature, photon irradiance, photoperiod, nutrients and water movement can affect the length and growth rates of the gametophytic and early sporophytic phases (Wheeler, 1980; Wheeler and North, 1981; Deysher and Dean, 1984; Muñoz et al., 2004; Palacios and Mansilla, 2003; Macchiavello et al., 2010; Egan et al., 1989; Kinlan et al., 2003; Carney and Edwards, 2010; Redmond et al., 2014). Importantly, these factors also exhibit strong interactions and for this reason the optimization process requires step by step development. The aim of this study was to optimize the growth of *Macrocystis*' juvenile sporophytes in a commercial-scale hatchery phase and to reduce the production costs involved. This was attempted by the experimental manipulation of temperature, photon irradiance, photoperiod, macro and micronutrients and aeration.

2. Materials and methods

Fertile sporophytes of *Macrocystis pyrifera* were collected at Faro Corona (southern Chile) and transported, within 3 h, in Styrofoam boxes to the CEACIMA hatchery (Centro de Investigación de Acuicultura y Ciencias del Mar, Universidad de Los Lagos). Once in the laboratory, sporophylls were selected and sporogenous tissues (sori) cut and soaked in fresh water with chlorine (5 mL of chlorine L^{-1} water) for 10 s. Then rinsed with fresh water and dried with paper towel. Then finally, packed in paper towels, cover with aluminum foil and stored for 12 h at 8–10 °C. After that period, 10 to 15 sori were placed in 1 L Erlenmeyer flasks with 500 mL of filtered (0.22 μm pore, Millipore) and sterilized seawater for 20–30 min for sporulation. Using a hemocytometer (Neubauer, Boecco) to determine the number of spores, regular samples were assessed until a final spore concentration of 40,000 spores mL^{-1} was obtained. The spore broth was then transferred to 10 L rectangular glass tanks (10 cm wide × 30 cm deep × 40 cm tall). Three PVC cylinders (25 cm tall and 50 mm in diameter) with 1.5 mm vinylon string tightly wrapped around them (see Merrill and Gillingham, 1991; Gutiérrez et al., 2006) were introduced into each of the 10 L containers to allow spore settlement. After 24 h, the PVC cylinders were removed and transferred to clean 10 L containers filled with filtered seawater enriched with Provasoli culture media (McLachlan, 1973). Five experiments were run in parallel to evaluate the following culture conditions: i) temperature and photon irradiance, ii) photoperiod, iii) macronutrient concentration of the Provasoli culture medium, iv) micronutrient and vitamin concentration of the Provasoli culture medium and v) air flow rate. Temperature was modified using a 50 W automatic heater (Whale VK-1000, Regent), irradiance was changed by modifying the number of fluorescent tubes (L15W/765, Cool Daylight, Osram), and the airflow rate was changed by increasing the number of air pumps (550R Plus, Sera) used. Table 1 describes the factors, levels and the factor interactions considered in each of the 5 experiments. Each experiment was replicated three times and the evaluations consisted of counting the number of gametophytes and sporophytes on three sections of string (2 mm in length each) at 15, 21 and 45 days of culture under a stereomicroscope at 8× magnification (SE2200, Ivens Microscope, China). Also at day 45, the length of 15 sporophytes of each section of string was measured by using a caliper (±0.1 mm). The temperature was monitored daily with an electronic digital thermometer (KW, China) and irradiance was measured inside the tank also on a

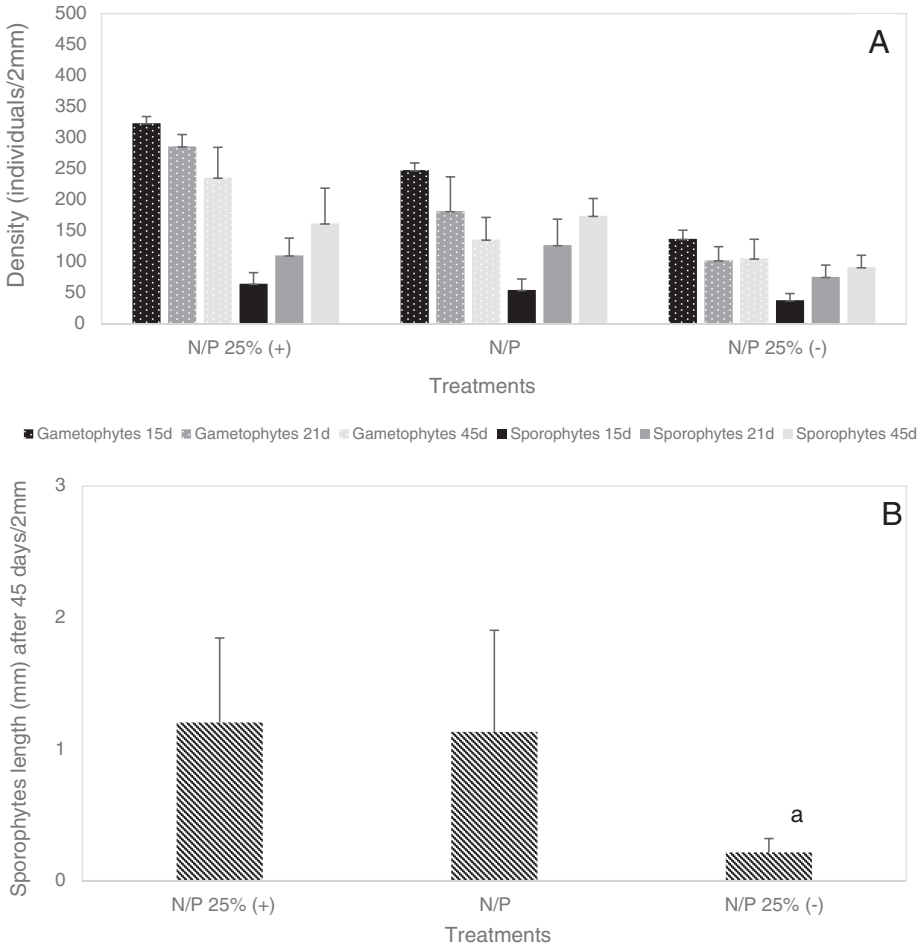

Fig. 3. A) Density of gametophytes and sporophytes of *Macrocystis pyrifera* submitted to three macronutrients concentrations during 15, 21 and 45 days of culture. B) Sporophyte length after 45 days of culture under three different macronutrients concentrations. One-way ANOVA: $F_{(2,404)} = 120.48$; $P < 0.0001$.

daily basis using a light meter (Li-Cor model LI 250A, US) with an underwater quantum sensor (Li-Cor, model LI-192, US). At the end of each experiment, the data was analyzed and the treatment with the best results was implemented in the following experiment.

For each of the five experiments, the experimental design fitted a two-way ANOVA with treatment and culture time as factors; statistical analyses were carried out with the Minitab 15 software. When data did not meet the assumptions of normality and homogeneity of variances, log or Box-Cox power transformations were used. If assumptions still were not met, a Kruskal-Wallis test was applied. For comparing sporophyte length between treatments, One-way ANOVA was used. If significant differences were detected a Tukey test was used to test a posteriori pairwise comparisons.

3. Results

The first experiment, evaluating temperature and photon irradiance, showed strong differences in the gametophyte density between treatments ($F_{(3,107)} = 101.95$; $P < 0.001$) and between culture period ($F_{(3,107)} = 26.60$; $P < 0.001$) (Fig. 1A). Treatment Temperature (T)-Light (L) and T-L(25%+) showed the highest densities of gametophytes per 2 mm of string. The interaction between treatments T-L and T-L(25%) after 21 days, was also significant ($F_{(6,107)} = 2.70$; $P = 0.018$). The production of sporophytes at day 21, increased significantly reaching more than 150 sporophytes 2 mm^{-1} in treatment T-L(25%+) ($H = 54.49$; $P < 0.001$) (Fig. 1A). Significant differences between culture period ($H = 1.66$; $P < 0.001$) were detected between treatments T-L and T-L(25%+) with higher densities at 21 and 45 days. After 45 days, there were significant differences ($F_{(3,539)} = 214.76$; $P < 0.001$) in sporophyte length, comparing treatments with high and standard temperatures, where 12.3 ± 0.2 °C produced sporophytes that were four times larger (Fig. 1B).

The second experiment tested the importance of the photoperiod, with temperature fixed at 12 °C and photon irradiance at 12 μmol m^{-2} s^{-1} (Table 1). Gametophytes developed under all photoperiod treatments, however there were significant differences between treatments ($F_{(2,80)} = 11.95$; $P < 0.001$) and culture period ($F_{(2,80)} = 97.39$; $P < 0.001$). The results indicated a lower level of development of gametophytes in the 24 h light treatment (Fig. 2A). Also, the interaction of treatments and time was significant ($F_{(2,80)} = 8.04$; $P < 0.001$), indicating that increasing the number of hours with light affects the development of gametophytes. Sporophyte densities (Fig. 2A) were significantly higher in the 16:8, L:D, 24:0, L:D ($F_{(2,404)} = 41.23$; $P < 0.001$) treatment when comparing 15 and 21 days, however no differences were observable after 45 days ($F_{(2,404)} = 75.86$; $P < 0.001$). As with gametophytes development, the interaction between the number of hours with light and time was significant ($F_{(2,404)} = 4.80$; $P = 0.019$). The length of the sporophytes after 45 days showed significant differences between treatments ($F_{(2,404)} = 3.07$; $P = 0.048$) with a 24 h of light resulting in delayed growth of sporophytes (Fig. 2B).

In the third experiment the nitrate and phosphate concentration of the Provasoli culture medium were experimentally manipulated, temperature and photon irradiance were the same as in experiment 2, and the photoperiod was fixed to 16:8, L:D (Table 1). Significant differences were observed in the production of gametophyte under the different treatments ($H = 53.36$; $P < 0.001$) and culture periods ($H =$

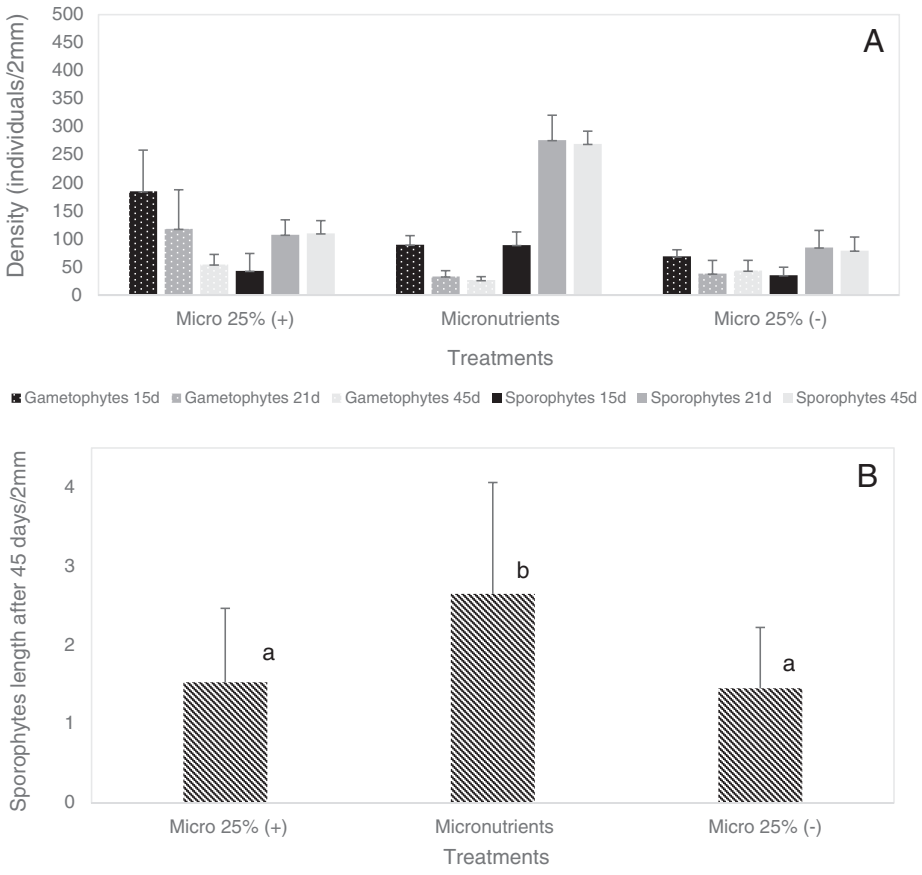

Fig. 4. A) Density of gametophytes and sporophytes of *Macrocystis pyrifera* submitted to three micronutrients and vitamins concentrations during 15, 21 and 45 days of culture. B) Sporophyte length after 45 days of culture under three different micronutrients concentrations. One-way ANOVA: $F_{(2,404)} = 52.46$; $P < 0.0001$.

13.04; $P = 0.001$), with higher macronutrients concentrations producing a higher number of gametophytes in all culture periods (Fig. 3A). However, sporophyte development increased significantly with the first two treatments ($F_{(2,80)} = 25.94$; $P < 0.001$) and during all the culture periods ($F_{(2,80)} = 77.05$; $P < 0.001$). Consistently, after 45 days, the length of the sporophytes were significantly ($F_{(2,404)} = 120.48$; $P = 0.001$) reduced in the treatment with the lowest macronutrient concentration (−25%) (Fig. 3B).

The fourth experiment tested variations in micronutrient and vitamin concentrations in the standard Provasoli culture medium (Table 1), with temperature, photon irradiance and photoperiod the same as in the third experiment. Gametophyte production was significantly higher in treatment with 25% higher micronutrients and vitamins concentrations ($F_{(2,80)} = 35.52$; $P < 0.001$) after 15 days ($F_{(2,80)} = 44.11$; $P < 0.001$). The interaction was also significant ($F_{(2,80)} = 3.78$; $P = 0.008$) (Fig. 4A). However, the production of sporophytes was higher at standard micronutrients and vitamins concentrations of Provasoli culture medium ($F_{(2,80)} = 91.45$; $P < 0.001$) after 21 and 45 days ($F_{(2,80)} = 86.83$; $P < 0.001$) (Fig. 4A). Consistently, sporophytes grown with standard nutrient and vitamins concentrations were significantly longer, reaching 2.65 ± 1.4 mm in length ($F_{(2,404)} = 52.46$; $P < 0.001$) (Fig. 4B).

The final experiment tested three different airflow rates (Table 1), keeping temperature, photon irradiance, photoperiod and macronutrients concentrations as in experiment 4, micronutrients and vitamins concentrations were maintained as in the standard Provasoli culture medium. The development of gametophytes at 3× ($F_{(2,80)} = 5.98$; $P = 0.004$) after 15 and 21 days was significantly higher ($F_{(2,80)} = 162.55$; $P < 0.001$) (Fig. 5A). The density of sporophytes at the higher intensity of air flow rate and 21 days was significantly higher ($F_{(2,80)} = 11.47$; $P < 0.001$ and $F_{(2,80)} = 62.03$; $P < 0.001$, respectively) (Fig. 5A). After 45 days, the length of the sporophytes was significantly longer at the two highest air flow rates (138–414 L h^{-1}) ($F_{(2,404)} = 19.59$; $P = 0.001$), reaching 3.35 ± 1.9 mm on average (Fig. 5B).

Temperature and photon irradiance of the different treatments during the experiment were maintained within ranges and presented as Supplementary material. (Figs. S1 and S2).

4. Discussion

The results obtained using the experiments indicate that the optimal protocol will generate a seeding rope with 2 to 4 mm long juvenile *M. pyrifera* sporophytes after 45 days in the hatchery (Fig. 6). The protocol requires a temperature of 12 °C a photon irradiance of 12 μmol m^{-2} s^{-1}, a photoperiod of 16:8, L:D, the macro- and micronutrient concentrations of the traditional Provasoli culture medium, and aeration of 414 L h^{-1}. Compared to previous protocols (described in Gutiérrez et al., 2006 and Correa et al., 2016) the time in the hatchery required to reach the seeding size was reduced from 60 to 45 days. The complexity of the protocol has also been reduced; by changing temperature from 9–10 °C to 12 °C, photon irradiance from 30–40 μmol m^{-2} s^{-1} to 12 μmol m^{-2} s^{-1}, and by maintaining the photoperiod at 16:8, L:D, for the complete cycle instead of changing it every week. Finally, the size that the plants obtain within 30–45 days has been increased from 2.85 mm (Gutiérrez et al., 2006) to an average length of 4 mm. The implications of these improvements impact *Macrocystis* aquafarming in at least two ways. First, from the economic point of view, the reduction in hatchery time and the simplification of the protocol means a considerable reduction in labor, supplies, and energy costs. Second, from a production point of view, shortening the hatchery phase means reducing the complete cycle of production, and even the possibility of two production cycles per year if, for example, the goal is to produce kelp for human

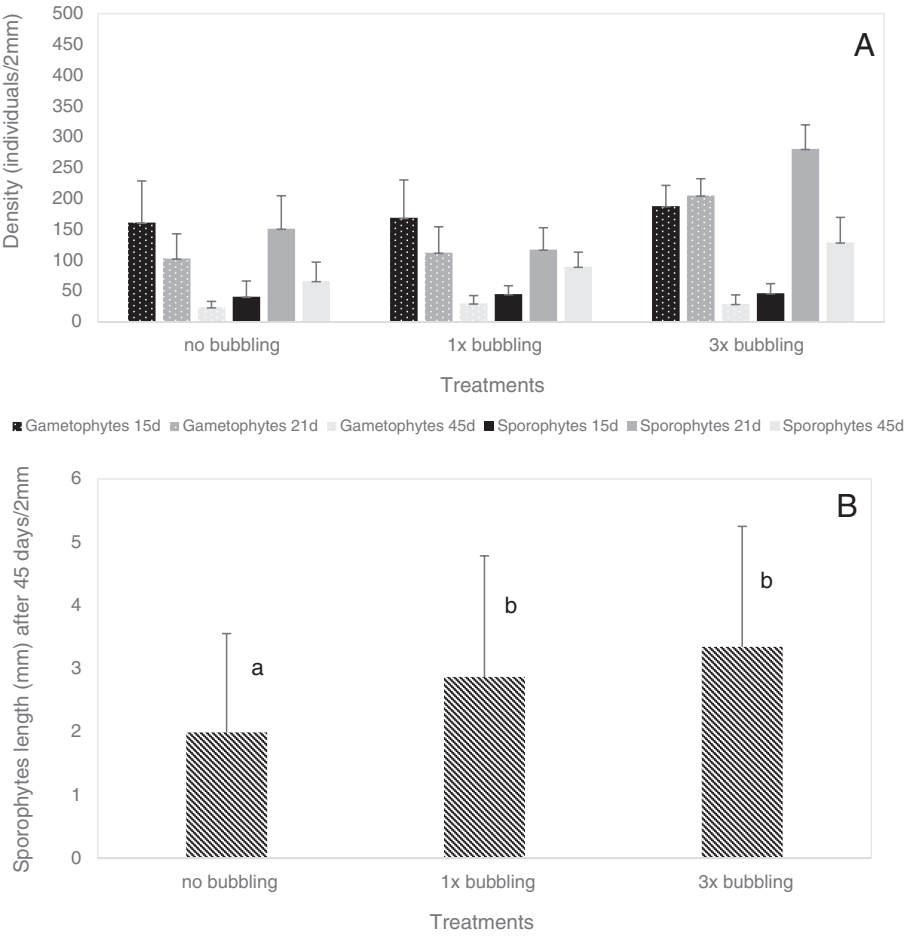

Fig. 5. A) Density of gametophytes and sporophytes of *Macrocystis pyrifera* submitted to three air flow rates during 15, 21 and 45 days of culture. B) Sporophyte length after 45 days of culture under three different airflow rates. One-way ANOVA: $F_{(2,404)} = 19.59$; $P < 0.0001$.

consumption, where plants can be harvested at the juvenile stages. If the goal is to produce biomass for energy purposes, the optimization will allow the producer to extend the open water culture phase by a month thus increasing the yield.

The first set of experiments with temperature and photon irradiance revealed that spore germination and development of gametophytes was higher at 12 °C independent of the photon irradiance used. The sporophytes grown at 12 °C treatments were longer than those grown at 15 °C. These results are consistent with other Laminariales; Lee and Brinkhuis (1988), as well as Egan et al. (1989), who reported that germination of *Saccharina latissima* and *Laminaria longricruris* gametophytes and juvenile sporophytes, respectively, were affected by higher temperatures (up to 20 °C), but with no effect of photon flux. They even suggest that germination generally requires low photon flux rates because higher rates could suppress the process. In *Laminaria ochroleuca*, the optimal development of female gametophytes was also independent of photon flux rate (Izquierdo et al., 2002). Furthermore, Lüning (1980, 1990) suggested that the ability of Laminariales, to maintain high germination rates under low light in the summer (higher temperatures) may be important because spores must attach to the

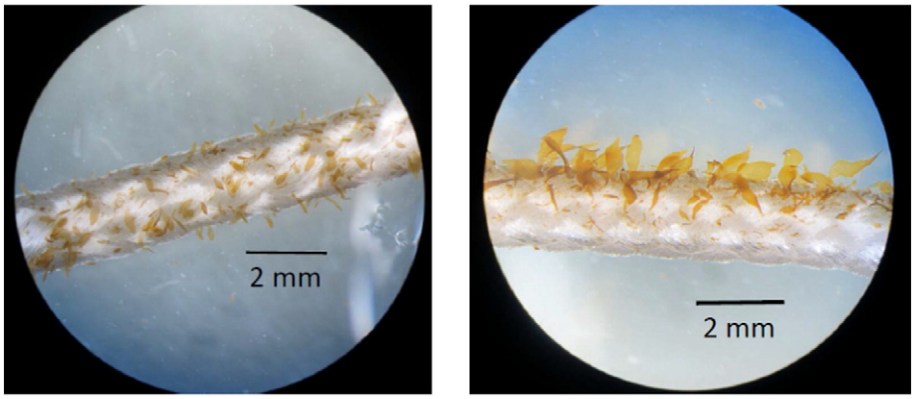

Fig. 6. Sporophytes attached to culture rope under the stereomicroscope.

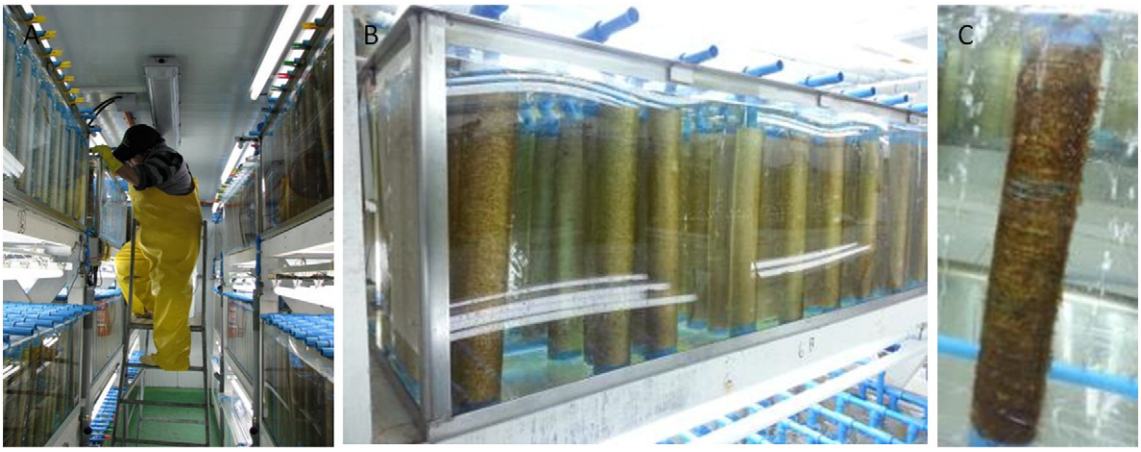

Fig. 7. Hatchery used to cultivate seeded kelp ropes. A) General view of the facility, showing culture tanks with seeded rope plants coiled around PVC tubes; B) details of a 400 L tank with *Macrocystis pyrifera* seeded juvenile kelp; and C) details of one cartridge fully covered by juvenile *M. pyrifera* sporophytes.

substrata at the bases of older plants and thus are subjected to some sedimentation and direct shading. In addition, gametophytes of *M. pyrifera* can survive over prolonged periods when kept under low irradiances (Ladah and Zertuche González, 2007). Specifically, for *M. pyrifera* in California, no recruitment or growth was possible in shallow water at the upper limit of its vertical distribution, suggesting that high PAR can inhibit the recruitment of the species (Graham, 1996). It is clear, that the primary factor regulating the growth of kelp gametophytes is temperature (Egan and Yarish, 1988; Egan et al., 1989; Yarish et al., 1990). According to our results, independent of the irradiance used, higher densities of gametophytes and length of sporophytes were achieved at 12 °C, which coincide with the tolerance of a cold - temperate distributed species.

Although temperature is involved in the reproductive cues, temperatures may undergo seasonal cycles. A more dependable seasonal cue is day length or photoperiod. Our second experiment, showed that 24 h of light (24:0, L:D) negatively affect the development of gametophytes resulting in lower densities of individuals and a decrease in the length of sporophytes. However, under long-day conditions (16:8, L:D) higher densities of gametophytes and longer sporophytes were obtained. According to Nelson (2005), for *Lessonia variegata*, a photoperiod of 15:9, L:D, and a temperature of 15 °C induce female gametogenesis (92%), however a short day (9:15, L:D) inhibits the process. They also observed a maximum growth of sporophytes at 15 °C and 15:9, L:D. In four other kelp species, Han and Kain (1996) concluded that a shorter daylength resulted in lower growth rates of gametophytes and sporophytes at both saturating and non-saturating irradiances. Also, *Undaria pinnatifida* gametophytes require a long daylength (16:8, L:D) for growth and short daylength (8:16 or 12:12, L:D) for sporophyte production (Choi et al., 2005). Therefore, our results agree with what has been reported for other Laminariales species and with the natural conditions found in the southern hemisphere, *Macrocystis pyrifera* mature during the summer and release their meiospores that have developed into newly formed gametophytes during the summer and autumn. This timing coincides with the long day (16:8, L:D) that produced the best results in our experiments. It is important to notice that this study supplied white fluorescent tubes (daylight), however it is well demonstrated that the percentage of fertile gametophytes and the production of sporophytes increase under blue fluorescent tubes (Lüning and Dring, 1972; Lüning and Dring, 1975; Wang et al., 2010). The implication that this change could have in time at hatchery still needs to be addressed.

The nutrient requirements of seaweeds are divided into three categories, macronutrients (e.g. N, P, C), micronutrients or trace elements (e.g. Fe, Zn, Cu, Mn, Mo) and vitamins (vitamin B_{12}, thiamine, and biotin) (Harrison and Hurd, 2001). Evidence that these nutrients, might limit algal growth comes from the fact that the concentrations of these elements in seawater vary considerably because of biological activity, and the concentrations of these elements in tissues are 10^4 to 10^5 greater than their concentrations in seawater (Lobban and Harrison, 1997). There is strong evidence that nitrogen limitation reduces growth rates of *Macrocystis pyrifera*, for example in California during the El Niño event (Dean and Jacobsen, 1986), in low-nitrogen environments at Santa Catalina Island (Gerard, 1982), or due to natural nutrient variation (Wheeler and North, 1981). These results are consistent with our results in the third and fourth experiments, where macronutrients and micronutrients/vitamins concentrations were tested. We found that the density of sporophytes and their length was greater in replicates with the same concentrations as used in Provasoli culture media, which is an enriched filtered natural seawater media. The macronutrients (P and N) are in a ratio of 16:1, N:P, which is generally the requirement for kelp species (Harrison and Berges, 2005), permitting the gametophytes and sporophytes to grow without limitation or excess. As we did already found a growth decrease by reducing 25% of the macronutrients we did not attempt to move further down reducing the macronutrients.

Water motion can affect the uptake of nutrients and carbon dioxide by reducing the diffusion boundary layer around the algal surface (Hurd, 2000; Harrison and Hurd, 2001). In our experiments, at the higher airflow rate (414 L h^{-1}), we observed a higher density of individuals and longer sporophytes. This was expected, as higher nutrient diffusion rates are observed at higher water velocities (Hurd et al., 1996; North et al., 1986). The effect is so important, that the morphology of *Macrocystis* blades change in response, depending upon whether the area is exposed or protected: narrow and heavily corrugated blades are found in exposed areas, and thin blades with few surface corrugations and large undulations along their edges are found in protected areas (Hurd et al., 1996).

Nowadays, the requirements for *Macrocystis* as raw material are increasing as the demands of abalone aquaculture and alginate producers had increase. Nonetheless, culture production satisfies only a small proportion of the demand, with the bulk being sourced from natural kelp beds. In order to change this scenario, the improvement in hatchery protocols is a key step together with the development of open water *Macrocystis* culture. Together these two developments can advance the kelp industry in Chile. In our study, a pilot hatchery (Fig. 7) was used, that contains 24 tanks of 400 L each, and has the capacity to produce sufficient seeded rope plants to seed 5–6 ha of culture every cycle. The reduction in time in reaching seeding size, the simplification of the protocol, and the larger size of the sporophytes allows a facility

like the one describe here to produce sporelings for 15–18 ha during the seeding season which takes place between June and August in the southern hemisphere.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2016.10.010.

References

Buschmann, A.H., Vásquez, J.A., Osorio, P., Reyes, E., Filún, L., Hernández-González, M.C., Vega, A., 2004. The effect of water movement, temperature and salinity on abundance and reproductive patterns of *Macrocystis* spp. (Phaeophyta) at different latitudes in Chile. Mar. Biol. 145, 849–862.

Buschmann, A.H., Varela, D.A., Hernández-González, M.C., 2008. Seaweed future cultivation in Chile: perspectives and challenges. Int. J. Environ. Pollut. 33, 432–456.

Buschmann, A.H., Prescott, S., Potin, P., Faugeron, S., Vásquez, J.A., Camus, C., Infante, J., Hernández-González, M.C., Gutierrez, A., Varela, D.A., 2014. The status of kelp exploitation and marine agronomy, with emphasis on *Macrocystis pyrifera*, in Chile. Adv. Bot. Res. 71, 161–188.

Camus, C., Ballerino, P., Delgado, R., Olivera-Nappa, A., Leyton, C., Buschmann, A.H., 2016. Scaling up bioethanol production from the farmed brown macroalgae *Macrocystis pyrifera* in Chile. Biofuels Bioprod. Biorefin. http://dx.doi.org/10.1002/bbb.1708.

Carney, L.T., Edwards, M.S., 2010. Role of nutrient fluctuations and delayed development in gametophyte reproduction by *Macrocystis pyrifera* (Phaeophyceae) in southern California. J. Phycol. 46, 987–996.

Choi, H.G., Kim, Y.S., Lee, S.J., Park, E.J., Nam, K.W., 2005. Effects of daylength, irradiance and settlement density on the growth and reproduction of *Undaria pinnatifida* gametophytes. J. Appl. Phycol. 17, 423–430.

Correa, T., Gutiérrez, A., Flores, R., Buschmann, A.H., Cornejo, P., Bucarey, C., 2016. Production and economic assessment of giant kelp *Macrocystis pyrifera* cultivation for abalone feed in the south of Chile. Aquac. Res. 47, 698–707.

Dean, T.A., Jacobsen, F.R., 1986. Nutrient-limited growth of juvenile kelp, *Macrocystis pyrifera*, during the 1982–1984 "El Niño" in southern California. Mar. Biol. 90, 597–601.

Deysher, L.E., Dean, T.A., 1984. Critical irradiance levels and the interactive effects of quantum irradiance and dose on gametogenesis in the giant kelp *Macrocystis pyrifera*. J. Phycol. 20, 520–524.

Egan, B., Yarish, C., 1988. The distribution of the genus *Laminaria* (Phaeophyta) at its southern limit in the western Atlantic Ocean. Bot. Mar. 31, 155–161.

Egan, B., Vlasto, A., Yarish, C., 1989. Seasonal acclimation to temperature and light in *Laminaria longicruris* de la Pyl. (Phaeophyta). J. Exp. Mar. Biol. Ecol. 129, 1–16.

Foster, M.S., Schiel, D.R., 2015. The Biology and Ecology of Giant Kelp Forests. University of California Press, Oakland, CA.

Fram, J.P., Stewart, H.L., Brzezinski, M.A., Gaylord, B., Reed, D.C., Williams, S.L., MacIntyre, S., 2008. Physical pathways and utilization of nitrate supply to the giant kelp, *Macrocystis pyrifera*. Limnol. Oceanogr. 53, 1589–1603.

Gerard, V.A., 1982. In situ water motion and nutrient uptake by the giant kelp *Macrocystis pyrifera*. Mar. Biol. 69, 51–54.

Graham, M.H., 1996. Effect of high irradiance on recruitment of the giant kelp *Macrocystis* (Phaeophyta) in shallow water. J. Phycol. 32, 903–906.

Gutiérrez, A., Correa, T., Muñoz, V., Santibañez, A., Marcos, R., Caceres, C., Buschmann, A.H., 2006. Farming of the giant kelp *Macrocystis pyrifera* in southern Chile for development of novel food product. J. Appl. Phycol. 18, 259–267.

Hafting, J.T., Craigie, J.S., Stengel, D.B., Loureiro, R.R., Buschmann, A.H., Yarish, C., Edwards, M.D., Critchley, A.T., 2015. Prospects and challenges for industrial production of seaweed bioactives. J. Phycol. 51, 821–837.

Han, T., Kain, J.M., 1996. Effect of photon irradiance and photoperiod on young sporophytes of four species of the Laminariales. Eur. J. Phycol. 31, 233–240.

Harrison, P.J., Berges, J.A., 2005. Marine culture media. In: Andersen, R.A. (Ed.), Algal Culturing Techniques. Elsevier Academic Press, California, pp. 21–34.

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah. Biol. Mar. 42, 71–82.

Hurd, C.L., 2000. Water motion, marine macroalgal physiology, and production. J. Phycol. 36, 453–472.

Hurd, C.L., Harrison, P.J., Druehl, L.D., 1996. Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave-sheltered and exposed sites. Mar. Biol. 126, 205–214.

Ibañez, E., Cifuentes, A., 2013. Benefits of using algae as natural sources of functional ingredients. J. Sci. Food Agric. 93, 703–709.

Izquierdo, J.L., Pérez-Ruzafa, I.M., Gallardo, T., 2002. Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. Helgol. Mar. Res. 55, 285–292.

Jackson, G.A., 1977. Nutrients and production of giant kelp, *Macrocystis pyrifera*, off southern California. Limnol. Oceanogr. 22, 979–995.

Kinlan, B.P., Graham, M.H., Sala, E., Dayton, P.K., 2003. Arrested development of giant kelp (*Macrocystis pyrifera*, Phaeophyceae) embryonic sporophytes: a mechanism for delayed recruitment in perennial kelps. J. Phycol. 39, 47–57.

Ladah, L.B., Zertuche González, J.M., 2007. Survival of microscopic stages of a perennial kelp (*Macrocystis pyrifera*) from the center and the southern extreme of its range in the Northern Hemisphere after exposure to simulated El Niño stress. Mar. Biol. 152, 677–686.

Lee, J.A., Brinkhuis, B.H., 1988. Seasonal light and temperature interaction effects on development of *Laminaria saccharina* (Phaeophyta) gametophytes and juvenile sporophytes. J. Phycol. 24, 181–191.

Lobban, C.S., Harrison, P.J., 1997. Seaweed Ecology and Physiology. Cambridge University Press, UK.

Lüning, K., 1980. Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). J. Phycol. 16, 1–15.

Lüning, K., 1990. Seaweeds – their environment, biogeography, and ecophysiology. In: Yarish, C., Kirkman, H. (Eds.), Meeresbotanik: Verbreitung, Okophysilogie und Nutzung der marinen Makroalgen by Klaus Lüning. John Wiley and Sons, Inc. New York, p. 527.

Lüning, K., Dring, M.J., 1972. Reproduction induced by blue light in female gametophytes of *Laminaria saccharina*. Planta 104, 252–256.

Lüning, K., Dring, M.J., 1975. Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. Mar. Biol. 29, 195–200.

Macchiavello, J., Araya, E., Bulboa, C., 2010. Production of *Macrocystis pyrifera* (Laminariales: Phaeophyceae) in northern Chile on spore-based culture. J. Appl. Phycol. 22, 691–697.

McLachlan, J., 1973. Growth media-marine. Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, Cambridge.

Merrill, J.E., Gillingham, D.M., 1991. Bull Kelp Cultivation Handbook. National Oceanic and Atmospheric Administration, Washington, D.C., p. 70.

Muñoz, V., Hernández-González, M.C., Buschmann, A.H., Graham, M.H., Vásquez, J.A., 2004. Variability in per capita oogonia and sporophyte production from giant kelp, gametophytes (*Macrocystis pyrifera*, Phaeophyceae). Rev. Chil. Hist. Nat. 77, 639–647.

Nelson, W.A., 2005. Life history and growth in culture of the endemic New Zealand kelp *Lessonia variegata* J. Agardh in response to differing regimes of temperature, photoperiod and light. J. Appl. Phycol. 17, 23–28.

North, W.J., Jackson, G.A., Manley, S.L., 1986. *Macrocystis* and its environment, knowns and unknowns. Aquat. Bot. 26, 9–26.

Palacios, M., Mansilla, A., 2003. Desarrollo de gametófitos y esporófitos de *Macrocystis pyrifera* (L.) C. Agardh (Laminariales: Lessoniaceae) de la región de Magallanes en condiciones de laboratorio. 31. Anales Instituto Patagonia, pp. 43–53.

Redmond, S., Green, L., Yarish, C., Kim, L., Neefus, C., 2014. New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01; R/A 38, p. 93 (Available from: http://digitalcommons.uconn.edu/seagrant_weedcult/1/(accessed 16.08.09)).

Vásquez, J.A., Zuñiga-Jara, S., Tala, F., Piaget, N., Rodríguez, D.C., Alonso, J.M., 2013. Economic valuation of kelp forests in northern Chile: values of goods and services of the ecosystem. J. Appl. Phycol. 26, 791–801.

Wang, W., Sun, X., Wang, G., Xu, P., Wang, X., Lin, Z., Wng, F., 2010. Effect of blue light on indoor seedling culture of *Saccharina japonica* (Phaeophyta). J. Appl. Phycol. 22, 737–744.

Wargacki, A.J., Leonard, E., Win, M.N., Regitsky, D.D., Santos, C.N.S., Kim, P.B., Cooper, S.R., Raisner, R.M., Herman, A., Sivitz, A.B., Lakshmanaswamy, A., Kashiyama, Y., Baker, D., Yoshikuni, Y., 2012. An engineered microbial platform form direct biofuel production from brown macroalgae. Science 335, 308–313.

Westermeier, R., Patiño, D., Piel, M.I., Müller, D.L., 2005. Manual de cultivo del alga parda *Macrocystis pyrifera* (Huiro), Chile. Proyecto FONDEF D0011144. Universidad Austral de Chile, Chile.

Westermeier, R., Patiño, D., Piel, M.I., Maier, I., Mueller, D.G., 2006. A new approach to kelp mariculture in Chile: production of free-floating sporophyte seedling from gametophyte culture of *Lessonia trabeculata* and *Macrocystis pyrifera*. Aquac. Res. 37, 164–171.

Westermeier, R., Patiño, D.J., Müller, H., Müller, D.G., 2009. Towards domestication of giant kelp (*Macrocystis pyrifera*) in Chile: selection of haploid parent genotypes, outbreeding, and heterosis. J. Appl. Phycol. 22, 357–361.

Westermeier, R., Patiño, D.J., Murua, P., Müller, D.G., 2011. *Macrocystis* mariculture in Chile: growth performance of heterosis genotype constructs under field conditions. J. Appl. Phycol. 23, 819–825.

Westermeier, Murua, P., Patiño, D.J., Muñoz, L., Ruiz, A., Müller, D.G., 2012. Variations of chemical composition and energy content in natural and genetically defined cultivars of *Macrocystis* from Chile. J. Appl. Phycol. 24, 1191–1201.

Wheeler, W.N., 1980. Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. Mar. Biol. 56, 103–110.

Wheeler, W.N., North, W.J., 1980. Effect of nitrogen supply on nitrogen content and growth rates of juvenile *Macrocystis pyrifera* sporophytes. J. Phycol. 16, 577–582.

Wheeler, P.A., North, W.J., 1981. Nitrogen supply, tissue composition and frond growth rates for *Macrocystis pyrifera* off the coast of southern California. Mar. Biol. 64, 59–69.

Yarish, C., Brinkhuis, B.H., Egan, B., Garcia-Esquivel, Z., 1990. Morphological and physiological bases for *Laminaria* selection protocols in Long Island Sound aquaculture. In: Yarish, C., Penniman, C.A., van Patten, M. (Eds.), Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation. The Connecticut Sea Grant College, Groton, CT, pp. 53–94.