Overview of 3 year precommercial seafarming of Macrocystis pyrifera along the Chilean coast

Alejandro Buschmann

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Overview of 3 year precommercial seafarming of *Macrocystis pyrifera* along the Chilean coast

Carolina Camus[1], Javier Infante[2] and Alejandro H. Buschmann[1]

1 Centre for Biotechnology and Bioengineering (CeBiB) & Centro i~mar, Universidad de Los Lagos, Puerto Montt, Chile
2 Patagonia Seaweeds SpA, Puerto Montt, Chile

Correspondence
Alejandro H. Buschmann, Centre for Biotechnology and Bioengineering (CeBiB) & Centro i~mar, Universidad de Los Lagos, Camino Chinquihue Km 6, Puerto Montt, Chile. Email: abuschma@ulagos.cl

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Abstract

Chile is one of the main producers of seaweeds in the world; however, most of the production comes from harvesting natural beds and only 2.4% from cultures, dominated by the agarophyte *Gracilaria chilensis*. One of the most exploited resources is the giant kelp *Macrocystis pyrifera*, which is sold fresh for abalone feed and dry for alginate extraction. Recently, new possible markets are developing for this species, for example human consumption and biofuel/chemical production that could increase the demand and justify the development of a commercial cultivation system. The objective of this work was to present the recent development of the seafarming of *M. pyrifera* in Chile, focusing on the fundamental determinants of productivity in cultivated systems and the identification of the binding constraints to productivity. Three experimental plots (up to 21 Ha) were designed and deployed in three study areas (Caldera in northern Chile and Quenac and Ancud in southern Chile) to test different environmental conditions. During a period of 3 years, sporophytes produced in an indoor hatchery were deployed monthly, at different densities, and followed until harvest. Environmental parameters and biomass were monitored on a monthly basis. Our findings demonstrate the feasibility of *Macrocystis* seafarming on a large scale in Chile. Important differences in yield were observed between the study areas associated with either environmental physical or biological factors, such as the presence of herbivores. Our best production cycle reached 124 WMT Ha^{-1} month $^{-1}$ in southern Chile, and the worst, less than 20 WMT Ha^{-1} $month^{-1}$ in northern Chile. Finally, some direct and indirect constrains were encountered, including seeding season and depth, and the presence of pests and diseases, that are discussed.

Key words: aquaculture, Chile, giant kelp, *M. pyrifera*, suspended cultivation.

Introduction

Today, the global utilization of products obtained from macroalgae is a multibillion dollar industry, with Asia being the main supplier, especially for species used as food (Ibañez & Cifuentes 2013; Kraan 2013; Evans & Critchley 2014; Murphy *et al.* 2014). On a global basis, seaweed aquaculture production attained 27.3 million tonnes on a weight basis and US$5.6 billion on a monetary basis (FAO 2016). Currently, macroalgae uses include human and animal foods, fertilizers, cosmetics ingredients and phycocolloids. Worldwide, up to 221 species of macroalgae are exploited by people and 66% of these are used as food (Milledge *et al.* 2014). However, the majority of algal biomass comes from a relatively small number of species within five genera: *Saccharina*, *Undaria*, *Porphyra*, *Eucheuma* and *Gracilaria*, where cultivated species represent the 76% of the total tonnage of landings of macroalgae (Milledge *et al.* 2014). In order to fulfil the requirements of the above-mentioned industries, seaweeds must be produced at higher yields and lower costs. Macroalgae had been harvested for centuries, and the world production of farmed seaweeds is expanding at 8% per year in the last decade, with output more than doubling from 2012 to 2014 (FAO 2016). Current practices of kelp cultivation in Asia, and mainly in China, have suffered a rapid evolution due to technological developments such as artificial seedling rearing and floating raft cultivation (Pereira & Yarish 2008; Titlyanov & Titlyanova 2010; Liu *et al.* 2014). Also, they have increased their production by focusing on the development of elite cultivars of important species, for example *Undaria* variety 'Haibao No1' (Shan *et al.* 2016), *Saccharina* variety 'Huangguan No1' (Liu *et al.* 2014) and 'Dongfang 2, Dongfang 3, Dongfang 6' (Li *et al.* 2007, 2008, 2016) with a production primarily oriented to human consumption, which includes specific texture of blades, taste and form (Shan *et al.* 2016; Zhao *et al.* 2016).

There are two main approaches to cultivate macroalgae: one is based on the sexual reproduction of the various species and the other on the ability of some species to propagate vegetatively (Santelices 1999; Kraan 2013). In kelp (brown algae), cultivation involves a reproductive cycle, with alternations between large sporophytes and microscopic gametophytes, two generations with quite different forms. The mature sporophyte releases spores that germinate and grow into microscopic gametophytes. The gametophytes became fertile, releasing sperm and oogonia that join to form embryonic sporophytes that develop into large ones (Graham & Wilcox 2000). Main difficulties of kelp cultivation are the management of sporophyte grow-out in the open ocean, as the transition from spores to embryonic sporophytes takes place on land-based facilities with careful control of cultivation variables (temperature, light and nutrients). The high costs involved in these early stages can be absorbed if the seaweed is sold as food or other high-value products; otherwise, the production is too expensive or unless the productivity and the scale can make cultivation profitable (Correa *et al.* 2016). Because of all the above-mentioned reasons, any future success of macroalgae farming will be dependent on achieving an optimized, energy-efficient hatchery and open water farming stages.

Chile is one of the main producers of seaweed in the world; however, most of the production comes from harvesting natural stocks (97.6%) and only 2.4% from culture farms (Servicio Nacional de Aduanas de Chile, 2016). One of the most exploited resources is the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh or 'huiro', which inhabits the Chilean coast from Arica to the Magallanes region, covering more than 4000 kilometres of coast (Hoffmann & Santelices 1997). The principal markets are as fresh biomass for abalone feed (Correa *et al.* 2016), as dry biomass for alginate extraction (Indergaard & Østgaard 1991), as potential food (Gutierrez *et al.* 2006) and as biomass to produce plant growth enhancers (Buschmann *et al.* 2008). Recently, new possible markets are under development for *M. pyrifera*, like human consumption (mini-seavegetables, www.elrepuertero.cl) and biofuel/chemical production (Song *et al.* 2015; Suutari *et al.* 2015). This could increase the demand for biomass (Wargacki *et al.* 2012; Enquist-Newman *et al.* 2014) and justify the development of a cultivation system specific for the giant kelp. However, for developing the cultivation of *M. pyrifera*, we need to develop a reliable technology and cultivation strategy in order to achieve profitability (Correa *et al.* 2016; Zuñiga-Jara *et al.* 2016).

Many of the techniques developed for the commercial-scale cultivation of *Saccharina's* species (Chen 2004; Peteiro *et al.* 2016a) have been transferred to the cultivation of *Macrocystis* (Gutierrez *et al.* 2006; Correa *et al.* 2016), due to their life history similarities. However, certain differences still need to be considered. The giant kelp possesses aerocysts in the basal portion of each lamina, which are structures filled with gas that confer positive buoyancy to the sporophytes, making more complex the engineering of the culture structure. Furthermore, unlike for *Saccharina* cultivation where the objective is the production of high-quality sporophytes for human consumption, the goal of giant kelp aquaculture is to produce a sustainable source of biomass for extracts, meaning high-density culture systems in which the individual quality is less relevant.

The aim of this review was to transfer our understanding of the fundamental determinants of *Macrocystis pyrifera* productivity in cultivated systems in Chile, and to identify the binding constraints to productivity. We present the information gathered along a 3 year cultivation period in which we had one pilot scale farm (>20 ha) and two other experimental farms of less than 1 ha to test for spatial variability along the Chilean coast. The results from this study are summarized in this overview allowing us to recommend the most effective giant kelp farming strategies and methodologies to produce high biomass per unit area over an annual production cycle. Two main issues were addressed: (i) environmental determinants of productivity: specifically light, nutrients and water motion; and (ii) optimization of productivity that can be improved in two dimensions – space and time. Due to the precommercial-scale approach used in this study, the scientific rigour was not always possible as it is in small scales. However, the results provide a general idea of the success and failures, making possible to transfer the acquired knowledge of *Macrocystis* seafarming in Chile and abroad.

Methods

Study sites and environmental conditions

Suspended farming of *M. pyrifera* was conducted from 2010 to 2013 at Quenac, Ancud and Caldera, Chile (Fig. 1). Of the three sites, Quenac was the main culture centre, with a total of 21 ha; at Ancud and Caldera, the culture system occupies 0.5 ha. Site-specific conditions are described in Table 1. At each site, water samples for the analysis of

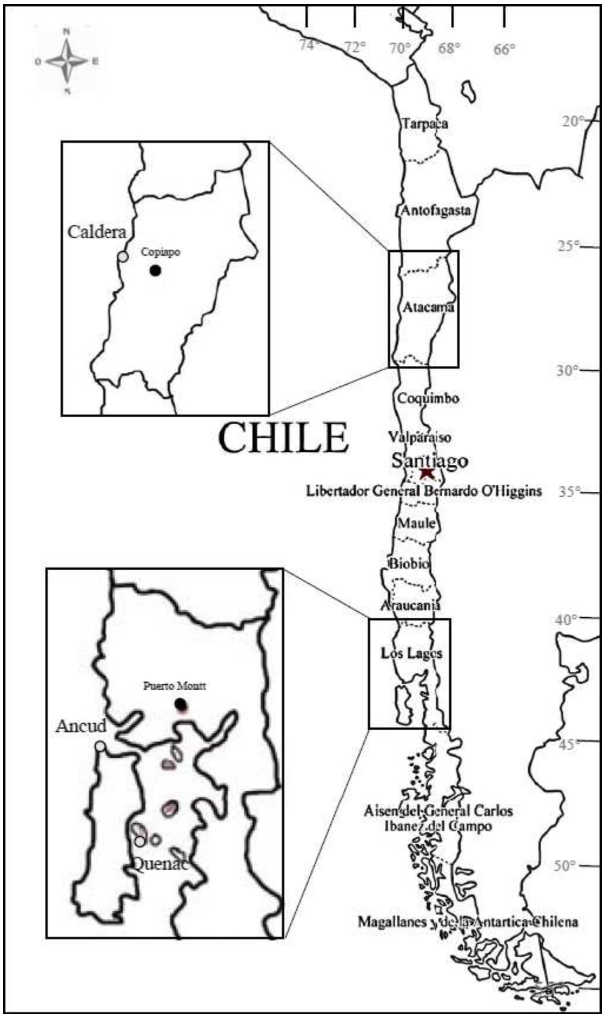

Figure 1 Map of Chile, indicating experimental plot locations.

nutrients (nitrate and phosphate) were collected using a Niskin bottle, and environmental parameters (pH, dissolved oxygen (DO), salinity and total dissolved solids (TDS)) using a Pro Plus Multiparameter Instrument (YSI, OH, USA) were registered monthly at 0, 3, 7 and 10 m at Quenac, 0, 7 and 10 m at Caldera and 0, 4 and 7 m at Ancud. Water clarity, by means of a Secchi disc, was monitored at monthly intervals. Water temperature was registered every half an hour by means of an Onset Pendant temperature data logger (Tidbit v2, Hobo), installed at different depths (surface, 3, 7, 10, 15 and 20 m).

Experimental seedling production

To provide the suspended culture centres with young sporophytes, two different methodologies were developed: free-floating sporophytes (following Westermeier *et al.* 2006 protocol) and rope-seeded sporophytes (following Gutierrez *et al.* 2006 protocol and optimized following Camus & Buschmann 2017) (Fig. 2). In brief, to produce free-floating sporophytes, clean sporogenous tissues (sori) of *M. pyrifera* were placed into 250 mL plastic bags (Ziplock ™) with filtered natural sea water supplemented with Provasoli culture medium (McLachlan 1973). The plastic bags were exposed to a photon flux density of 35–50 μmol m^{-2} s^{-1} (L15W/765, fluorescent tubes cool daylight, Osram) measured with a flat quantum sensor (LI-192 SA, Li-Cor, US) and a light meter (LI-250A, Li-Cor, US), 12L:12D photoperiod, a temperature of 10–12°C. After 3 weeks of development, the juvenile sporophytes (±8–10 mm) were transferred to indoor 400 L tanks supplied with air. Culture medium was changed weekly. Finally, the sporophytes were transferred to outdoor 1000 L tanks supplied with air and filtered sea water (300, 50 and 20 μm sequential filters) supplemented with commercial fertilizer (15% nitrogen and 14% potassium, Salitre potásico, Anasac S.A., Chile) and daily irradiance average of 66 μmol m^{-2} s^{-1} (taken between 8AM and 18 PM), until they reached seeding size (8–10 cm in length). For seeding operation, the sporophytes were transported to the culture centre in stereofoam boxes. Once they

Table 1 Environmental characteristics of experimental plot locations

	Quenac farm	Ancud farm	Caldera farm
Location	South	South	North
Location depth	60 m	5 to 10 m	40 m
Culture depth	4 m	4 m	5 and 10 m
Sea bottom	Sand	Sand	Sand
Temperature range	7.9–15.5°C	10.1–14.6°C	12.0–19.6°C
Average salinity	32.56 psu	32.80 psu	34.13 psu
Water clarity – Secchi disc	9.86 m	4.98 m	7.29 m
Light	Seasonally overcast	Seasonally overcast	High light
Wave exposure	Offshore, exposure to deeper water mixing	Near shore, coastal exposure	Near shore, coastal exposure
Aquaculture interaction	Salmon and mussel culture	Mussel culture	Pectinid culture
Presence of kelp natural beds	Yes (year-round)	Yes (year-round)	Yes (year-round)

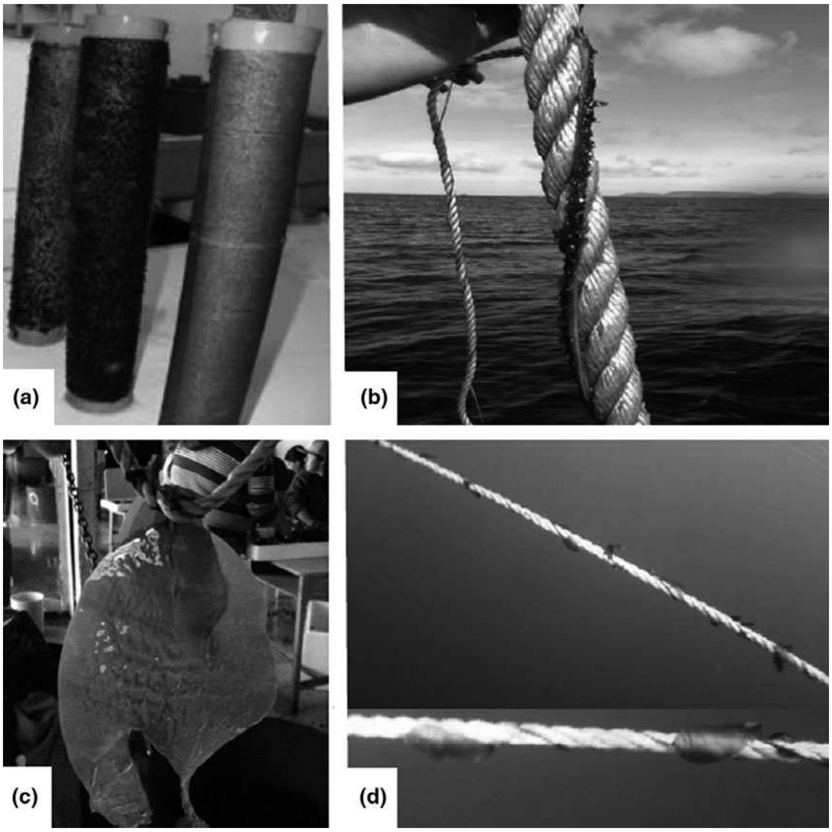

Figure 2 System of sporophyte seeding. (a) Rope-seeded sporophytes, (b) rope-seeded sporophytes just seeded, (c) free-floating sporophytes, (d) free-floating sporophytes seeded.

arrived, in land each sporophyte was inserted in pieces of 6 mm string, 15 cm long. Then, the piece of string with the sporophyte was inserted in the culture line (12 mm) in the desired density. Finally, the seeded ropes were deployed in the open water farm.

To produce rope-seeded sporophytes, zoospores from mature sporophytes were released from sori of *M. pyrifera* that had been cleaned and brushed with iodine. Sori were dried in a moist chamber at 10°C overnight wrapped in paper towel and aluminium foil. The sporulation process consisted in placing the sori in cold (10°C) filtered (0.2 μm) water to stimulate spore release. Water samples from spore broth were taken every 10 min to evaluate quantity and motility of the spores. Once the desired number of spores was obtained, seeding took place by pouring the spore solution into 15 L basins containing several PVC cartridges wrapped with 60 metres of 2 mm vinylon string. After 12 h, the cartridges were arranged vertically in 400 L tanks and were exposed to a photon flux density of 35–50 μmol m^{-2} s^{-1}, temperature of 10 $\pm$ 2°C, photoperiod of 16L:8D and aeration. The sea water was filtered and supplemented with commercial fertilizer. Approximately 2 months after seeding, the sporophytes reached an average length of 4–5 mm and cartridges were transported to the culture centre in the ocean.

Culture centre design

The *Macrocystis* culture centre was designed as a cross-linked suspended (floating) system of horizontal lines placed at 4 m depth forming 1 ha quadrants (Fig. 3). The external frame of each hectare was built with a 38 mm diameter nylon rope, 100 m long. Each hectare had either 49, 65 or 99 culture lines of 12 mm diameter (2 m, 1.5 m and 1 m line separation, respectively) depending on the density trial. Culture lines were used for attaching the *Macrocystis* seeded lines.

Experimental design, sampling and harvesting techniques

At the Quenac farming centre, juvenile sporophytes were seeded monthly during two production cycles on continuous years, in 2011 and 2012. At Ancud, four seeding events took place on June, July, October and November of 2011, whereas in Caldera, the events were on September and November of 2011 and February and June of 2012.

Biomass sampling of each experiment was performed monthly and involved collecting five randomly selected samples of 1 m of seeded rope each, on each hectare. Each 1 m sample was processed in order to obtain the total wet weight (Kg), the number of sporophytes per metre and the

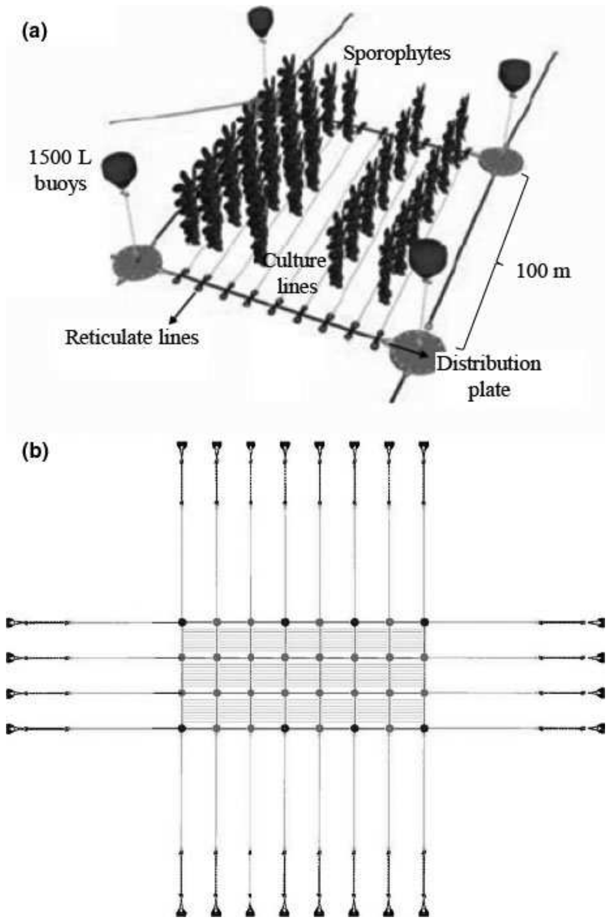

Figure 3 (a) Representative 3-D scheme of the cross-linked suspended culture system. (b) Layout of Quenac seafarm.

individual sporophyte wet weights (Kg). With these data, the biomass yield was determined as wet tons per hectare (wet Ton Ha^{-1}).

Algal biomass yields of any species depend strongly on the stocking density, temperature (Davison 1991) and light (Franklin & Forster 1997) and nutrients availability (Harrison & Hurd 2001). Considering these factors, the following variables were tested:

- Sporophyte density – high initial seedling density in the system could restrict growth rates due to light and nutrient competition. On the other hand, at low density, algae can have high growth, but the yield will be low due to the low stocking density. Sporophyte density is a variable controlled through the number of sporophytes seeded per linear metre of culture rope, but using rope-seeded sporophyte we were not able to control spacing and density control is only achievable modifying the line spacing.
- Depth adjustment – light availability and temperature have a direct inverse relationship to depth, and nutrients can have a positive correlation with depth, but also interact with sporophyte density, as a larger biomass allocated to the canopy will decrease the light transmittance especially if our intention is to produce a canopy forming kelp like *Macrocystis pyrifera*. Because of the difficulty in changing the depth for a complete large-scale suspended culture unit, small experimental units were deployed. Depth adjustments were tested by means of metallic structures of 5 × 5 m, provided with five lines of 5 m each and with a 1 m spacing between lines. Two metallic structures were deployed at 1.5, 3 and 5 m depth during summer and autumn.
- Kelp seeding system – Free-floating and rope-seeded sporophytes were produced at the hatchery. Different hectares were seeded with free-floating sporophytes with a density of three or six sporophytes per metre, and rope-seeded sporophytes were seeded using a seeding machine that coiled the seeded rope onto the long line maintaining a constant velocity in order to obtain a constant density of sporophytes along the long line.
- Variety control – variety development using either traditional breeding techniques or the hybrid clone method (Zhang *et al.* 2008) can be used to improve yield and/or other agronomic characters. For free-floating sporophytes, four different varieties were considered, produced using the Westermeier *et al.*'s (2006) protocol. Parental sporophytes were collected in four different sites located at the Chiloé area, and four different mixtures of their spores produce four varieties denominated types A, B, C and D. Free-floating sporophytes were seeded manually at a density of three sporophytes per linear metre of culture rope and deployed at Quenac from June to December in order to evaluate their productivity (wet Ton Ha^{-1}).

Statistical analysis

Comparison between biomass data was made using one-way ANOVA in the statistical software Minitab 17 (Minitab Inc., State College, PA, USA). If significant differences were detected, a *post hoc* Tukey test was used to test *a posteriori* pairwise comparisons. When data did not meet the assumptions of normality and homogeneity of variances, root transformation was used. If assumptions still were not meet, a nonparametric Kruskal–Wallis test was used.

Results

Environmental data revealed striking differences between the three selected *Macrocystis* farm locations. Average water temperature at Quenac showed fluctuations between 9° and 13°C (data logger recorded episodic events of higher temperature that are not observed in the average values, Fig. 4a) with a clear increase during summer months

(Fig. 4a) that coincide with the decrease in nitrate concentration in the area (Fig. 4b). Phosphate concentrations were relatively constant along the years, with minor fluctuations (Fig. 4c). During mid-autumn and winter, water clarity increased, which coincides with a decreased irradiance (Fig. 4d). Ancud, despite being in the Chiloé Island area, showed slight warmer waters and no major nutrient fluctuations, appearing to be a more stable environment (Table 2). At the third farm, Caldera, located in the north of the Chilean coast, a higher water temperature was recorded reaching even sublethal temperatures during summer, but with minor fluctuations in concentrations of nutrients (Table 2). The main differences between Ancud–Caldera and Quenac are due to the position of the farms (nearshore versus offshore, respectively), and the presence of salmon farms that maintained a higher nutrient concentration during the year (11.8 ± 7.1 μM of nitrate at Quenac, vs 6.0 ± 4.7 μM and 5.3 ± 4.6 μM at Caldera and Ancud, respectively). Minimum concentrations of nitrate also varied during 2012 and 2013 because the first-year salmon farms nearby were not active (see Fig. 4b).

Strong differences in productivity were obtained between the three aquafarms. In Ancud, after the four seeding events carried out in June, July, October and November 2011, the maximum biomass was achieved in 5, 7, 5 and 4 months, respectively (Fig. 5). In terms of yield, it varied between 5.5 and 8.8 wet ton Ha^{-1} $month^{-1}$ (Fig. 5). The first three seeding events did not show significant differences, but were significantly different from the spring (November) seeding trial ($H = 40.63$; $P > 0.0001$) (Fig. 5b). At Ancud, the location depth ranged between 5 and 10 m and the culture depth was 4 m; this situation facilitated the presence of sea urchins (*Loxechinus albus*) and an herbivorous crab (*Taliepus* spp.) on the culture lines and increased the biomass losses during all culture periods (Table 3).

The seafarm located at Caldera, northern Chile, showed the worst results in terms of biomass yield (Fig. 6). Higher water temperature and a high density of bryozoans (*Membranipora* sp.) covering the blades affected sporophyte growth. The seeding event of September 2011 in 2 months reached 17.5 wet ton Ha^{-1} and then the biomass started to decline, mostly because of bryozoans. The *Membranipora*

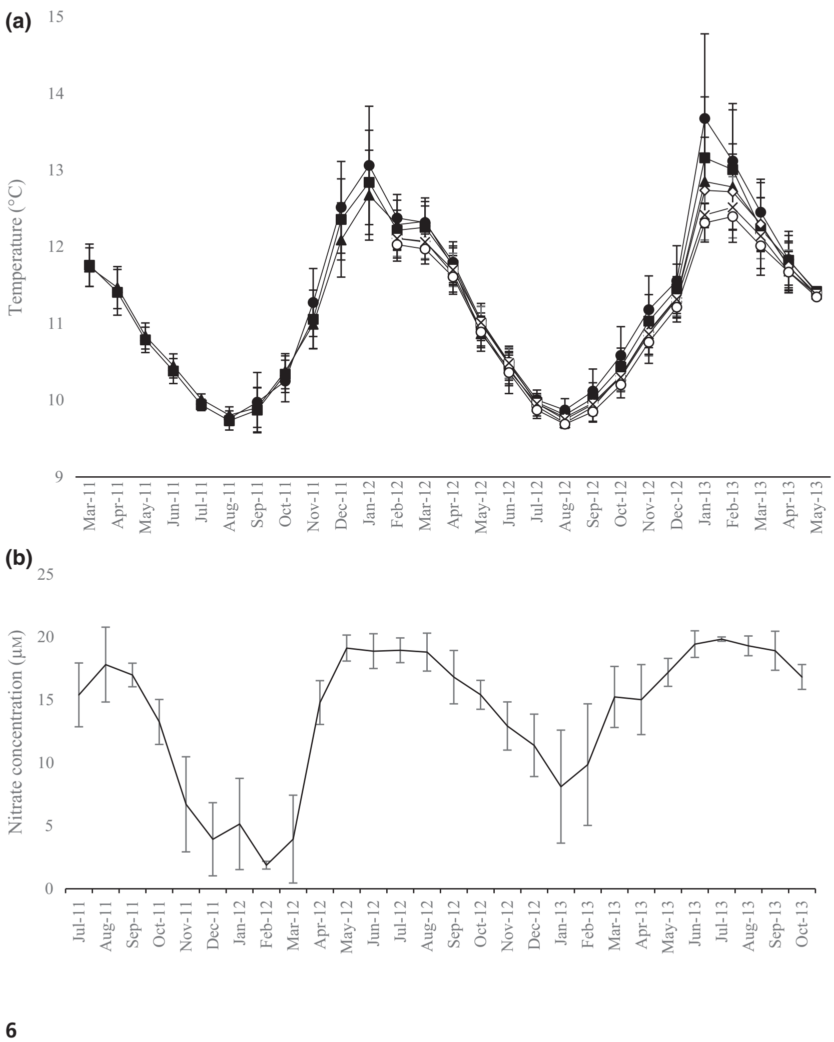

Figure 4 Environmental parameters at Quenac: (a) temperature (°C); (b) nitrate concentration (μM); (c) phosphate concentration (μM); (d) water clarity measured using a Secchi disc (m). (a): (●) Sup; (■) 3 m; (▲) 7 m; (◊) 10 m; (×) 15 m; (○) 20 m.

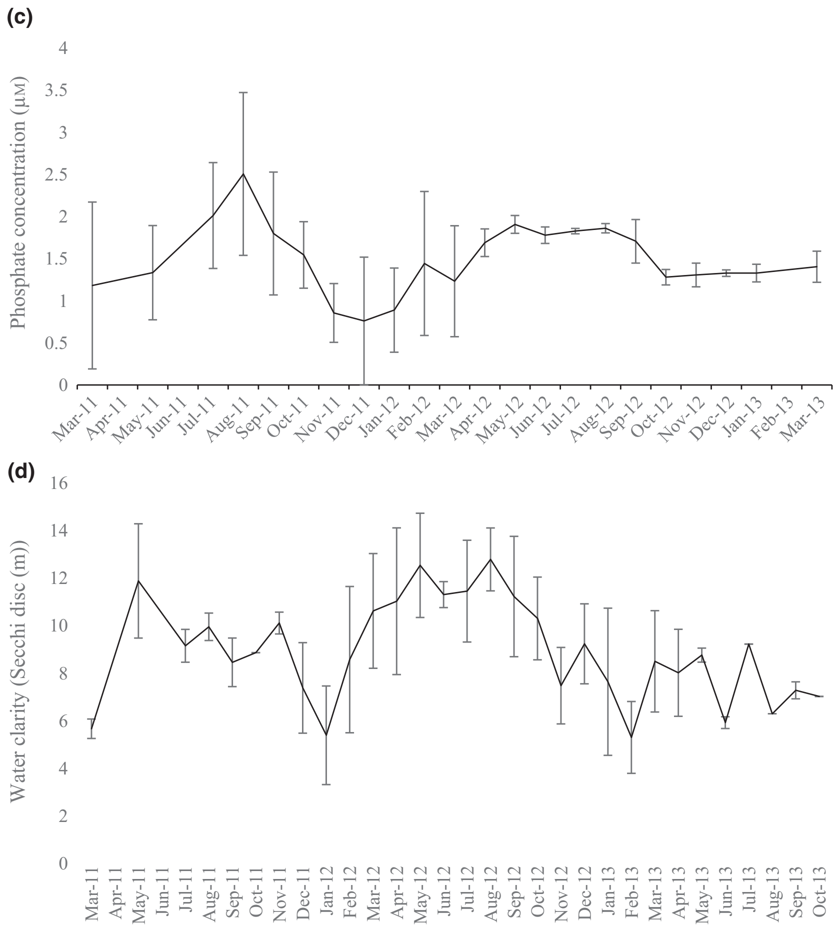

Figure 4 Continued.

Table 2 Environmental parameters at Ancud and Caldera aquafarm, measured at different depths

Parameters	Ancud			Caldera		
Depth	0 m	4 m	7 m	0 m	7 m	10 m
Average temperature (°C)	12.17 ± 2.08 (10.11–14.56)	11.89 ± 2.08 (10.13–14.51)	12.24 ± 2.08 (10.52–14.42)	15.55 ± 2.22 (13.1–19)	14.22 ± 1.22 (12.6–17.4)	14.09 ± 2.22 (12–18.3)
Average nitrate concentration (μM)	4.45 ± 4.50 (0.35–14)	6.54 ± 4.51 (0–28.22)	4.58 ± 3.16 (0.21–14)	6.06 ± 5.36 (0.21–26.79)	5.94 ± 4.46 (0.21–15)	6.14 ± 4.55 (0.21–14.88)
Average phosphate concentration (μM)	1.05 ± 0.76 (0–3.06)	1.28 ± 0.71 (0–2.95)	1.06 ± 0.55 (0–2.73)	1.67 ± 1.18 (0–8.03)	1.59 ± 0.70 (0–3.26)	1.69 ± 0.80 (0–3.47)
Water clarity (Secchi disc)		2.35–6.60 m			3–14 m	

frond colonization was also observed during the other culture periods. In terms of biomass yield, significant differences were observed between the different seeding periods ($F_{(3,156)} = 62.58$; $P < 0.0001$), with the seeding of early spring showing the best results (8.75 wet ton Ha^{-1} $month^{-1}$; Fig. 6).

At Quenac, the biomass yields obtained by far exceeded the ones obtained at Ancud and Caldera, obtaining the kelp maximum yields after 5 to 6 month in culture (Fig. 7). Winter and mid-spring seedings delivered significantly higher yields ($F_{(10,104)} = 3.78$; $P > 0.0001$), reaching values up to 124 wet ton Ha^{-1} in 5 months of culture (Fig. 7b). When seeding was carried out during late spring and summer, the kelp yields were lower as expected because young sporophytes cannot develop in an environment with high temperatures and low nutrient concentrations (Fig. 4). In addition to the environmental factors, at Quenac, when seedings took place in the summer, an amphipod (*Peramphitoe femorata*) was recorded and its density started increasing rapidly, reaching an

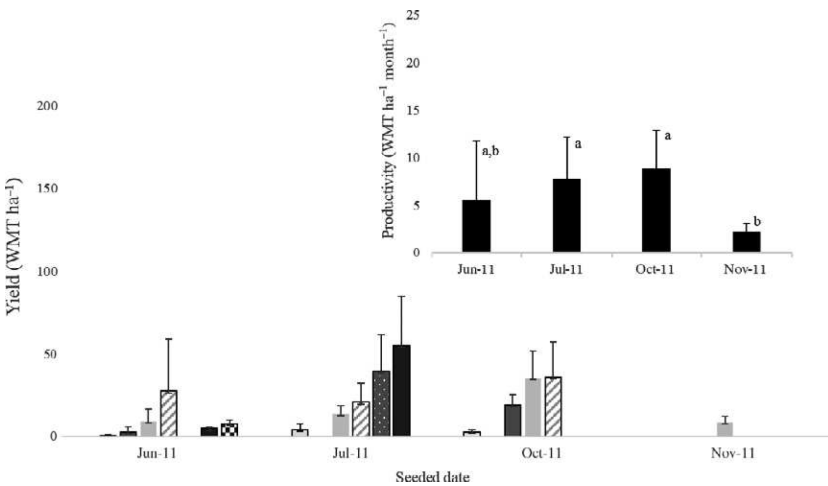

Figure 5 Yield (WMT Ha^{-1}) at Ancud. Four seeding events were performed on 2011. Within each seeding event, monthly biomass sampling was performed (represented in different colours). Upright plot showing biomass productivity (WMT Ha^{-1} month^{-1}) at Ancud. (□) 1 month; (□) 2 month; (■) 3 month; (□) 4 month; (▨) 5 month; (▩) 6 month; (■) 7 month; (▦) 8 month.

Table 3 Critical points detected at each culture site

Critical points	Quenac	Ancud	Caldera
Fouling	–	–	*Membranipora* sp. – Bryozoans
Predation	*Peramphitoe femorata* – Amphipods	*Loxechinus albus* – Sea urchin	–
Nutrients	Input of salmon excretion products	Input of mussel excretion products	Input of pectinid excretion products
Water movement	Strong tidal currents	Strong tidal currents	Wave protected bay with sporadic storms

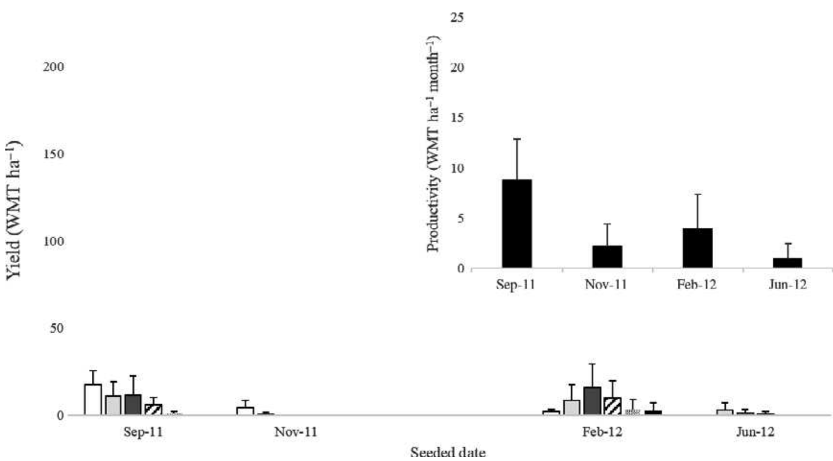

Figure 6 Yield (WMT Ha^{-1}) at Caldera. Two seeding events were performed on 2011 and two on 2012. Within each seeding event, monthly biomass sampling was performed (represented in different colours). Upright plot showing biomass productivity (WMT Ha^{-1} month^{-1}) at Caldera. (□) 2 month; (□) 3 month; (■) 4 month; (▨) 5 month; (▩) 6 month; (■) 7 month.

average of 400 individuals Kg^{-1} of wet sporophyte during March (Carolina Camus, unpublished data). The consequence of the amphipod population increase was a complete loss of biomass, because of the damage caused by the amphipod to the sporophytes. New seedings were attempted during the presence of the amphipod (on March, April and May), but the results were discouraging. Due to that event, a contingency plan was applied; the kelp farm at Quenac was completely harvested and left for 60 days without new seeding to ensure the mortality of amphipods due to starvation. On August 2012, the seeding events started again reaching comparable results with 2011 (Fig. 7b). In terms of productivity, the best period for deploying *M. pyrifera* to the system was June to September. During this period, sporophytes can grow on winter and mid-spring seasons, where concentrations of nutrients are higher, water temperatures lower and water clarity is high allowing light penetration to the system (Fig. 4). Figure 8 shows how Quenac farm looks during a 4 month period showing a sustained growth of the sporophytes during the culture period.

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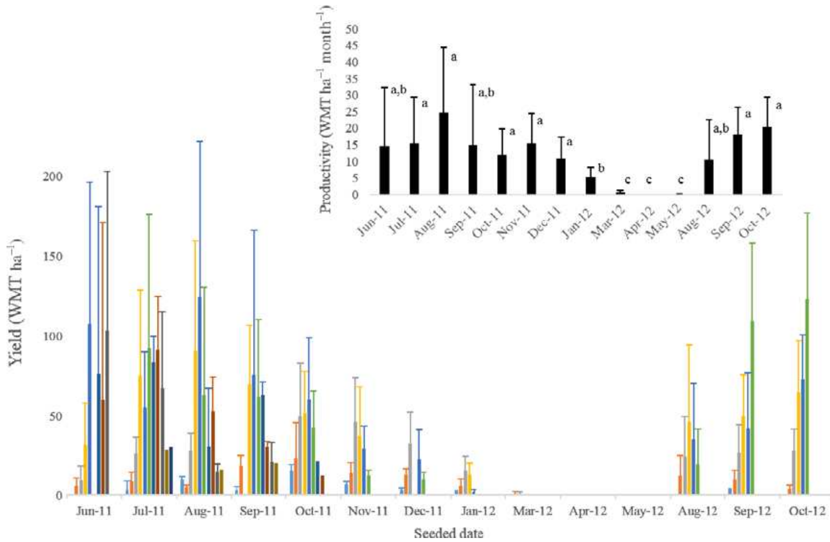

Figure 7 Yield (WMT Ha^{-1}) at Quenac. Seven seeding events were performed during 2011 and seven during 2012. Within each seeding event, monthly biomass sampling was performed (represented in different colours). Upright plot showing biomass productivity (WMT Ha^{-1} month^{-1}) at Quenac. (■) 1 month; (■) 2 month; (■) 3 month; (■) 4 month; (■) 5 month; (■) 6 month; (■) 7 month; (■) 8 month; (■) 9 month; (■) 10 month; (■) 11 month.

The first production cycle allowed us to compare the performance of *Macrocystis* in three different suspended systems, which showed strong differences in biomass yield, associated mainly with environmental factors (nutrient concentrations) and biological factors (predation, fouling). With this information in mind, the second objective, optimization of productivity, was decided to be carried out in the Quenac farm only. The first variable tested was sporophyte density controlled through the number of sporophytes seeded per linear metre of culture rope. The results showed no significant differences between the densities tested, three and six sporophytes m^{-1} seeding during the same month ($F_{(10,149)}$ = 1.94; P = 0.05) (Fig. 9), which indicates that the seeding effort should not be larger than three kelp individuals per metre of culture line.

Sporophytes seeded during the summer at different depths showed poor development, reaching on average 4.27 ± 2.7 Kg after 4 months of cultivation (Fig. 10a). No significant differences were observed between depths each month (January $F_{(2,36)}$ = 0.58, P = 0.564; February $F_{(2,37)}$ = 0.04, P = 0.964; March $F_{(2,41)}$ = 1.33, P = 0.277). During autumn (April), no differences were observed between depths (Fig. 10b). During the rest of the months, differences between depth were found between 1.5 and 5 m (May $F_{(2,29)}$ = 9.40, P = 0.001; June $F_{(2,29)}$ = 4.06, P = 0.029; August H = 10.13, P = 0.006; September $F_{(2,19)}$ = 23.04, P < 0.001).

The third variable tested was the seeding system. The free-floating sporophytes seeded at density of three sporophytes per metre were compared with rope-seeded sporophytes with a density of approximately 25 sporophytes mm^{-1}. No differences were observed between both systems ($F_{(7,39)}$ = 1.06, P = 0.408) (Fig. 11). Finally, four different varieties were seeded on different months, which were produced through free-floating method. Productivity obtained for each variety did not show a clear pattern; however, significant differences were observed ($F_{(10,104)}$ = 3.78, P < 0.0001) (Fig. 12). Variety A showed higher productivity when seeded in July and August; variety B in October; variety C in August; and variety D in September, indicating that the maximum biomass not only depends on environmental conditions, but also on the genetic pool.

Discussion

The aim of this review was to transfer our general understanding of the fundamental determinants of *M. pyrifera* productivity in suspended cultivated systems and to identify the binding constraints to productivity following 3 years of cultivation practices on the Chilean coast. Our results demonstrated the feasibility of culturing *M. pyrifera* in suspended systems along the coast of the south-eastern Pacific in a precommercial scale. Strong differences in productivity were observed between different localities associated with environmental and biological factors. In southern Chile, Quenac and Ancud farms showed significant differences mostly due to the lower nutrient concentrations found in Ancud, despite being located near a mussel farm, and the shallowness of the site allowed invertebrates to prey on the *Macrocystis* sporophytes, impacting seaweed biomass. In turn, Quenac farm, which is located near salmon cultures, received higher nutrient concentrations and had productivity averaging 20 wet ton Ha^{-1} month^{-1}, when the kelp seeding took place during the winter months. In comparison, Ancud reached in average 6.1 wet ton Ha^{-1} month^{-1}, and Caldera, located in the north of Chile, showed lower productivity results (average of 4

Figure 8 Photographic record of the seeded kelps during 4 months of growth of *Macrocystis pyrifera* at the Quenac farm.

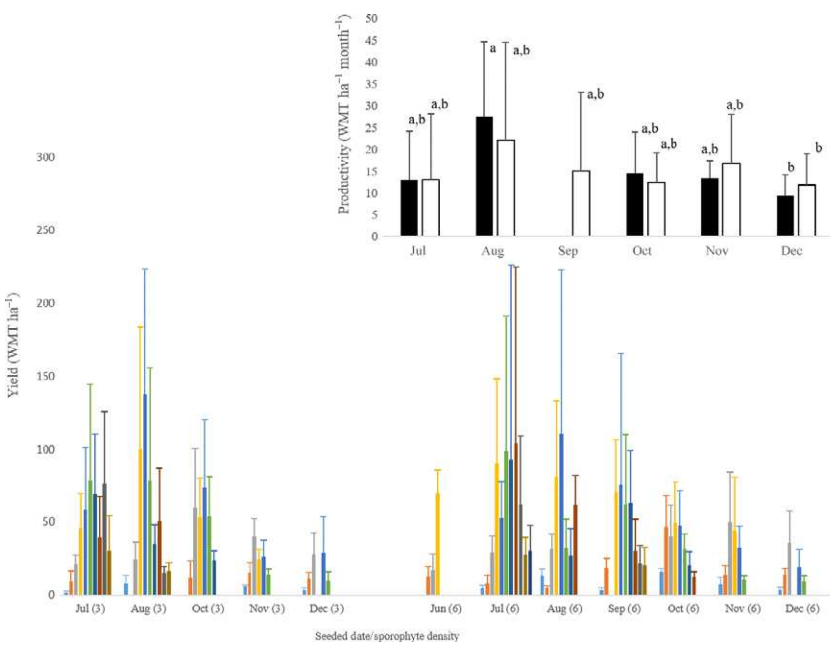

Figure 9 Yield at different kelp densities (three and six sporophytes per linear metre) at Quenac seafarm. Seeding events considering a density of three sporophytes per metre at the left of the plot and six sporophytes per metre at the right. Bars of each seeding event represent the monthly biomass samplings. Upright plot showing mean biomass productivity (WMT Ha^{-1} month^{-1}) at different densities at Quenac. ( ) 1 month; ( ) 2 month; ( ) 3 month; ( ) 4 month; ( ) 5 month; ( ) 6 month; ( ) 7 month; ( ) 8 month; ( ) 9 month; ( ) 10 month; ( ) 11 month.

wet ton Ha^{-1} month^{-1}), mostly because of the presence of bryozoan *Membranipora* covering the blades and high sea water temperature episodes during summer, which in turn affect the growth and survival of the kelp.

In the southern Pacific coast, few authors have attempted to cultivate *M. pyrifera* mostly in experimental trials (Gutierrez *et al.* 2006; Macchiavello *et al.* 2010; Westermeier *et al.* 2011; Correa *et al.* 2016). The results obtained

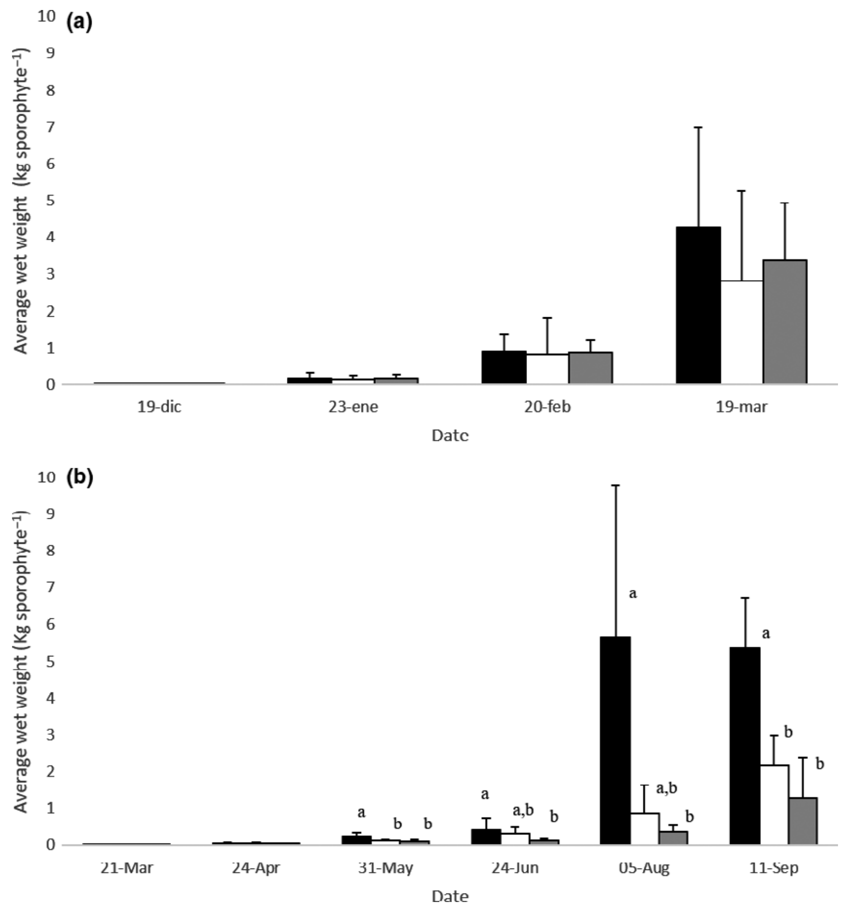

Figure 10 Average wet weight/sporophyte seeded at different depths (1.5, 3 and 5 metres) at Quenac seafarm during (a) summer and (b) autumn seedings. (■) 1.5 m; (□) 3 m; (■) 5 m.

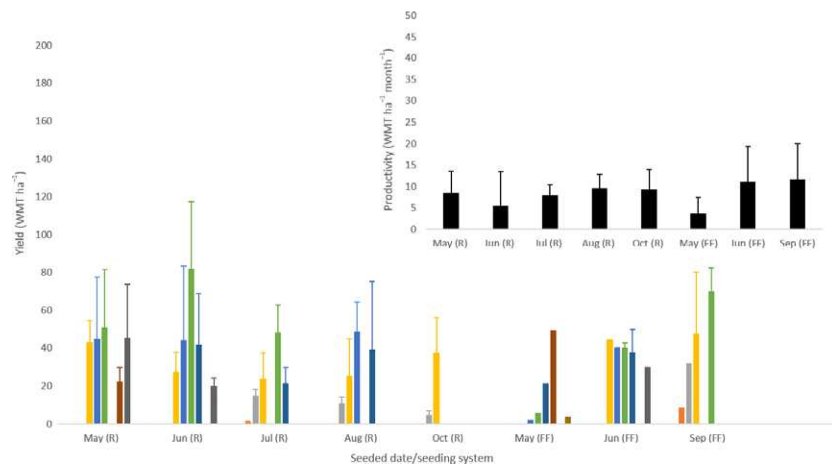

Figure 11 Yield of free-floating sporophytes (FF) and rope-seeded sporophytes (R) at Quenac seafarm. Upright plot showing mean productivity (WMT Ha^{-1} month^{-1}) of free-floating and rope-seeded sporophytes at Quenac. (■) 1 month; (■) 2 month; (■) 3 month; (■) 4 month; (■) 5 month; (■) 6 month; (■) 7 month; (■) 8 month; (■) 9 month; (■) 10 month.

varied between 14.4 Kg m^{-1} (Gutierrez *et al.* 2006) and 66 Kg m^{-1} (Westermeier *et al.* 2011) in southern Chile using rope-seeded and free-floating methods, respectively. In northern Chile, the results reached 22 Kg m^{-1} using rope-seeded method (Macchiavello *et al.* 2010). The results obtained in our 21 Ha farm are equivalent to approximately 15 Kg m^{-1}, which is lower than the values reported, but it is difficult to compare the results obtained in a reduced number of long lines with several seeded hectares.

Another determinant of the differences observed in productivity is water movement, as it has a direct effect on growth and production, such as increasing the uptake of nutrients and carbon dioxide by reducing the diffusion boundary layer around the algal surface (Hurd 2000;

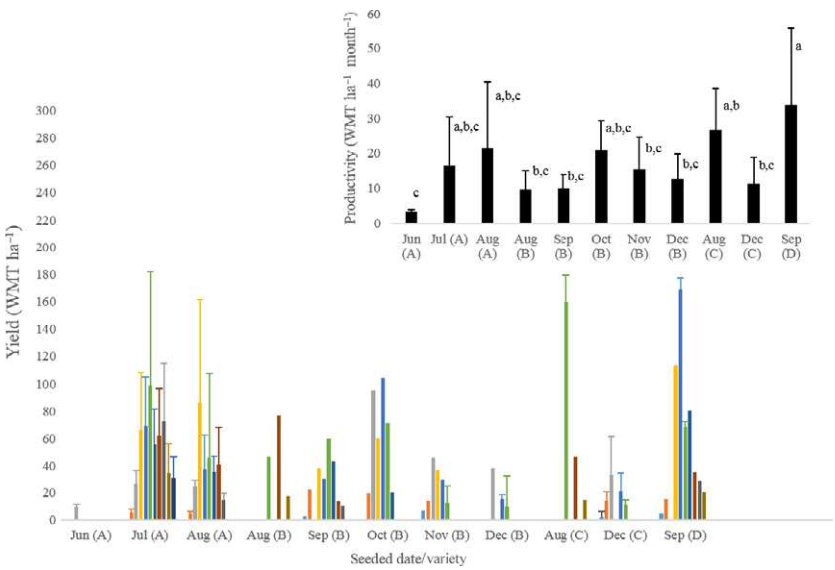

Figure 12 Yield of four different varieties of sporophytes (a, b, c and d) at Quenac seafarm. Upright plot showing mean productivity (WMT Ha^{-1} month^{-1}) of different kelp varieties seeded at Quenac. (■) 1 month; (■) 2 month; (■) 3 month; (■) 4 month; (■) 5 month; (■) 6 month; (■) 7 month; (■) 8 month; (■) 9 month; (■) 10 month; (■) 11 month.

Peteiro & Freire 2013). Quenac is located offshore and with high exposure (average water velocity of 20 cm s^{-1}) in comparison with Ancud and Caldera (7 cm s^{-1} and 4 cm s^{-1}, respectively), which agrees with the productivities obtained at each site. The same effect has been reported for the kelp *S. latissima* in the bay of Galicia (Peteiro & Freire 2013) and for *Undaria pinnatifida* in Galicia and Japan (Nanba *et al.* 2011; Peteiro & Freire 2011). For both species, the authors observed higher biomass yield and substantiality, and difference in morphology when culturing in exposed versus sheltered areas. It seems to be an important factor to take into consideration when selecting the cultivation site.

Some constrains were encountered when trying to increase the yields of the cultures. Seeding season is one of the most important factors affecting yields. It was clearly demonstrated that the winter seeding months (during June, July and August) provide better results than the summer (January, February, March) using either free-floating sporophytes or rope-seeded sporophytes. Both kelp seeding systems tend to self-achieve a final density of three sporophytes per linear metre. Also importantly to note is the depth of culture lines: the ranges showing the best development of sporophytes were found to be at 1.5–3 m during winter and 3–5 m during summer months. If it is not possible to move the infrastructure, it is proposed to maintain the culture lines at a 4 m depth. The management of the culture lines should not be underestimated because it is a constant during the complete culture cycle. The first 2 months after seeding, the system remains stable; however when aerocysts development starts, the buoyancy changes abruptly from slightly negative to positive, pulling the system upwards. Caution must be taken during harvesting season when sporophytes complete their life cycle and start losing the blades; the high biomass will pull down the system, which can cause it to collapse and sink. All of these changes in culture depth must be controlled through adding weight or buoys to avoid changing the culture conditions, which in turn affect the yield.

Another determinant of the productivity is the development of cultivars of economic seaweeds (Patwary & van der Meer 1992; Robinson *et al.* 2013; Charrier *et al.* 2015). In brown and red seaweed, several examples of successful cultivar have been reported (e.g. *Saccharina* Dongfang No. 2, 3 and 6 (Li *et al.* 2007, 2008, 2016), *Undaria* Haibao No. 1 (Shan *et al.* 2016), *Pyropia yezoensis* (Niwa *et al.* 2008)) that show an improvement in biomass production, disease resistance and chemical composition. In these 3 year trials, we tested four different varieties in the open ocean cultivation, and the results show a tendency where three varieties (A, C and D) reached better results in terms of productivity (wet Ton Ha^{-1} month^{-1}). During the same period, several varieties, produced through massive cloning of selected gametophytes and hybridization, were seeded at Quenac and results in biomass and chemical composition were significantly different (Camus, C., Faugeron, S. & Buschmann, A.H., unpublished manuscript); these authors also report strong differences between varieties in growth rate at hatchery and open ocean conditions. In addition, significant differences were observed in carbohydrate and protein contents, as reported by Westermeier *et al.* (2012) who found that protein and lipids were 20% higher than in natural populations.

Finally, an indirect constraint that could affect the productivity of the culture is the presence of diseases or pests. Natural kelp populations of *M. pyrifera* in the south-eastern Pacific coast are normally infected by the alga *Laminariocolax macrocystis* (Burkhardt & Peters 1998), and no bacterial disease has yet been described in Chilean kelps. In the Quenac seafarm, no significant symptoms including deformation were observed in the tissue over a period of 3 years (see Buschmann *et al.* 2014a). However, high number of the amphipod *Peramphitoe femorata* called 'kelp curler' were recorded mainly during the summer and mid-autumn months, producing a significant damage to the sporophytes due to their feeding behaviour (see Thiel & Hinojosa 2010 for more details). Contingency plans must be developed in order to control the population growth of this amphipod, as the artificial culture condition farms are installed at 40–60 m depth where the natural enemies of the amphipod are not present. Similarly, epiphytism is major problem for other seaweed cultures, and few solution has been encountered. In the kelps *S. latissima* and *U. pinnatifida*, epiphytic organisms such as hydroids, copepods, algae, crustaceans and bryozoans have been reported, degrading the biomass and breaking the lamina (Førde *et al.* 2016, Peteiro & Freire 2013). The authors suggested that to diminish the damage, culturing should take place in exposed areas and deeper depth to avoid higher coverage of hydroids and crustaceans, and bryozoans, respectively.

No negative externalities from the seafarming were detected in our study period, as evidenced by Buschmann *et al.* (2014a,b), but more experimental studies are needed to fully understand the possible environmental effects of large-scale seaweed aquaculture (impact of biomass losses, modification in bacterial communities or nutrient uptake, among others). In southern Chile, *M. pyrifera* wild populations in protected areas show a clear annual cycle, becoming completely decimated during autumn (Buschmann *et al.* 2004), contributing to detritus flow between habitats. In other regions, erosion, fragmentation and dislodgment of complete sporophytes have been quantified (Yoshikawa *et al.* 2001; de Bettignies *et al.* 2013), concluding that water movement (velocity), low supply of inorganic nitrogen, ageing and reproductive status of the seaweeds are between the most important factors. Usually, exported kelp detritus can provide a significant resource subsidy and enhance secondary production in communities ranging from tens of metres to hundreds of kilometres from the source of production (Krumhansl & Scheibling 2012), but detachment of whole fronds could end up accumulating in nearby shores, with the consequent negative impact to activities as tourism or general discomfort to local communities dwelling the seashore. On the other hand, several positive externalities have been described, such as CO_2 sequestration, uptake of nutrient excess, among others (Jacquin *et al.* 2014).

During these farming trials, no attempt to evaluate harvesting and transportation of the biomass was made; however, mowing experiments were performed to evaluate the regeneration capacity of *M. pyrifera*, as mowing is a strategy to harvest natural kelp beds in California by Kelco (Briand 1991, FAO 2003). Mowing is performed by means of a vessel equipped with cutters that approaches the seaweed bed, cuts the stipes about one metre below the surface and the forward motion of the vessel forces the seaweed onto the moving belt. In our culture system, it was not possible to use this kind of harvesting technology and we manually carried out our cutting kelp trials. The results show that under these kelp farming conditions, the algae did not regrow, as did the Californian giant kelp. On the Chilean coast, *Macrocystis* sporophytes survive up to 1 year and have a few fronds per sporophyte (Buschmann *et al.* 2014a,b) in comparison with Northern Hemisphere giant kelp that can live up to 4–7 years and reach up to 400 fronds per *pyrifera*-form sporophyte (Graham *et al.* 2007). These relevant differences explain our results, as it was demonstrated in Californian giant kelp that the long lifespan is due to a process of continuous turnover of fronds after a year of growth coupled with a progressive senescence of the fronds and blades (Rodriguez *et al.* 2013). Those sporophytes have several fronds that allow them to survive a couple of years, but the Chilean giant kelp in the wild and in culture have fewer fronds (1–2) growing at the same time; once they reach maturity, they senesce and do not resume growth (Buschmann *et al.* 2006). In addition, as our floating culture system is based on seeding the juvenile sporophytes at only 3 to 4 m depths to induce a high growth during the late winter and early spring, the canopy biomass formed occupies in relative terms mostly the first metre of the water column. This situation does not allow cutting the algae to allow the individuals to resume growth after a partial harvest.

From an economic point of view, the type of kelp seeding system did not affect the biomass yields significantly as was initially expected (rope-seeded sporophytes mean more sporophytes per linear metre in comparison with free-floating sporophytes seeded at three or six sporophytes m^{-1}), although the selection of either system will affect the production costs. To produce both types of sporophytes, a hatchery facility is needed, but rope-seeded sporophytes need less nursery time, space and water pumping. For deployment, the rope-seeded system could be optimized through seeding machines reducing the cost of the operation in comparison with manually seeding free-floating sporophytes. In addition, optimization of environmental parameters at the hatchery stage (light, nutrients, temperature, photoperiod and aeration) must be taken into consideration to reduce the culture time at the hatchery and associated economic costs (see Camus & Buschmann, 2017). Finally, the infrastructure and its maintenance through the culture period is the variable with the greatest influence of the total cost of *M. pyrifera* farmings. This conclusion is supported by Correa *et al.* (2016), who through a production cost projection for a 10 ha commercial kelp farm showed that 34.4% and 24.4% of the annual costs were due to the culture system and support equipment, respectively. According to the same authors, assuming a market price of US$78 wet ton^{-1} and a culture area of 30 ha, profitability is achieved and recovery of capital invested occurs from year 1 of production.

Conclusion

Macrocystis seafarming is a reality and offers high biomass productivity in the southern Chilean coast. The results highlight the importance of site selection in terms of depth, concentration of nutrients and currents. The rest of the variables involved in the development of the culture could be controlled once optimized to produce a stable biomass year after year. Despite these achievements, several challenges need to be addressed in order to create a sustained industry, legislation created specifically for macroalgae; conservation of the farmed species (germplasm); strain selection programmes; development of new massive plantlet production independent of collecting reproductive material every cycle; disease research; research on environmental impacts of large-scale cultures; added value to the farmed species, among others.

An industry based on processing of cultivated macroalgae demands a stable and predictable delivery of biomass with defined qualities if the goal is to extract or produce bioactive compound, functional food, nutraceutical, cosmeceutical, pharmaceutical products, as is the tendency (see Gupta & Abu-Ghannam 2011; Holdt & Kraan 2011; Freitas *et al.* 2012; Andrade *et al.* 2013; Hafting *et al.* 2015). Major breakthroughs are necessary in the area of harvesting, storage and distribution of the biomass in order to maintain their contents intact and avoid decomposition. But the sustained increase in cultivated areas must be accompanied by a proper legislation that drives the development of the industry while protecting the natural kelp beds to avoid other environmental issues associated with uncontrolled exploitation (Buschmann *et al.* 2013). The development of a seaweed industry must take into consideration the coastal communities with the intensification of the exploitation of the resource (review Frangoudes 2011 for the evolution of seaweed management regimes in four countries). Initiatives like the proposal to develop a Seaweed Standard by the Marine Stewardship Council (MSC) and Aquaculture Stewardship Council (ASC) move on that route because they recognized the importance of having a standard that rewards those harvesting seaweed sustainably as well as providing a benchmark for improvement. The basis of the standard lies on five core principles: (i) sustainable wild populations, (ii) environmental impacts, (iii) effective management, (iv) social responsibility and (v) community relations and interaction (MSC web page, www.msc.org, ASC web page, www.asc-aqua.org).

Finally, research on seaweed domestication is required. As highlighted in Valero M, Guillemin ML, Destombe C, Jacquemin B, Gachon CMM, Badis Y (unpublished data), the initial steps for the cultivation of any species imply conscious or unconscious selection of trait of interest. Due to the lack of knowledge about the environmental and genetic drivers of phenotypic diversity, effect such as reduced genetic diversity of cultivated stands and impact on life history traits could lead to a greater susceptibility to pathogens or even to a reduction in productivity. Therefore, understanding the predomestication processes, which traits and which environmental factors of the cultivation system are involved, may allow maximizing the process itself and may be a cornerstone for future sustainability of seaweed aquaculture (Loureiro *et al.* 2015, Valero *et al.* in press). There is an urgent need to develop macroalgal germplasms, as it has been proposed for the giant kelp (Barrento *et al.* 2016), what has been done with commercial kelps in Asia (Pang *et al.* 1997; Shan *et al.* 2016; Zhao *et al.* 2016), and it is beginning to be developed in Europe (Peteiro & Freire 2014; Peteiro *et al.* 2016). These initiatives will allow the conservation of the genetic diversity of the species selected for domestication.

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