Production of Macrocystis pyrifera (Laminariales; Phaeophyceae) in northern Chile on spore-based culture

Article *in* Journal of Applied Phycology · December 2010

DOI: 10.1007/s10811-010-9508-8

CITATIONS
40

READS
626

3 authors, including:

Cristian Bulboa
Universidad Andrés Bello
34 PUBLICATIONS **816** CITATIONS

SEE PROFILE

J Appl Phycol (2010) 22:691–697
DOI 10.1007/s10811-010-9508-8

Production of *Macrocystis pyrifera* (Laminariales; Phaeophyceae) in northern Chile on spore-based culture

Juan Macchiavello · Evelyn Araya · Cristian Bulboa

Received: 13 August 2009 / Revised and accepted: 28 January 2010 / Published online: 24 February 2010

Abstract Since the establishment of abalone farming, there has been an increase in the demand for *Macrocystis* as a food source. Therefore, the pressure on natural stock has also augmented and the sustainability of the actual harvesting practices has been questioned. In this article, an attempt to farm *Macrocystis pyrifera* by zoospores in northern Chile is described; initially under laboratory conditions and subsequently by cultivation in the sea. The experiments were executed during 1 year and two different cultivation methodologies were used: a direct and an indirect method. A maximum frond length of 175 cm was reached and 22 kg m^{-1} of rope was produced after 120 to 150 days of cultivation in the sea. The algae grew under both methodologies, and no differences in algal length and biomass were detected between the two cultivation systems. However, the direct culture method can be recommended for productive and practical reasons.

Keywords Abalone · Chile · Kelps · *Macrocystis* · Mariculture · Seaweeds

J. Macchiavello · E. Araya · C. Bulboa
Departamento de Biología Marina,
Universidad Católica del Norte,
Larrondo 1281,
Coquimbo, Chile

J. Macchiavello · C. Bulboa (✉)
(CIDTA) Centro de Investigación y Desarrollo Tecnológico en Algas,
Casilla 117,
Coquimbo, Chile
e-mail: crbulboa@ucn.cl

Introduction

In recent years, since the establishment of abalone cultivation in Chile, there has been a strong demand for fresh seaweed, mainly *Macrocystis*, in order to feed the abalone. Currently, in Chile, both the red abalone (*Haliotis rufescens*) and the Japanese abalone (*Haliotis discus hannai*) are being cultivated. Vásquez et al. (2006) estimated that these cultivations require an average of 500 t fresh algae per month. Moreover, this amount is continuously growing, and it has been roughly calculated that close to 1,000 t of abalone will be produced by 2010. This would require 100,000 t of brown algae. The greatest challenge for this industry is to obtain fresh algae for feeding the abalone, which has become one of the greatest limiting factors (Viana et al. 1993; Corazani and Illanes 1998; Camus 2005). Until now, the algal supply has been collected from natural kelps populations which are extracted in large volumes. This means that the collection area needs to be expanded as the algae supply is becoming exhausted near the abalone cultivation centers (Pizarro 2003), which already is the case in northern Chile (Bulboa, C. personal observation).

In northern Chile, *Macrocystis pyrifera* form small beds from intertidal to 15 m in depth, characterized by low abundance and fragmented distribution (Vásquez et al. 2006). The overexploitation of *M. pyrifera* beds may have severe consequences for the environment because these species, as well as *Lessonia trabeculata*, are very important structural species in their respective communities (Vásquez et al. 2001), harboring a high diversity of organisms and generating particular conditions in their local environments.

In order to feed the abalone, the supply of brown algae cannot be maintained only by the exploitation of natural populations since this practice does not ensure a constant stock. This is due to: (1) natural biological processes such as the seasonal variation in the abundance of these resources (Vega et al. 2005), (2) harvest activities without considering management and sustainability aspects, and (3) the imminent legal restriction regulations for accessing and harvesting kelps in Chile. Taking this scenario into account, the development of *M. pyrifera* aquaculture is being adopted as a complement to abalone cultivation to prevent its collapse due to lack of food. In Chile, several attempts to cultivate brown algae such as *M. pyrifera* (Gutiérrez et al. 2006; Westermeier et al. 2006) and *L. trabeculata* (Edding and Tala 2003; Westermeier et al. 2006) have been made. However, in the northern part of Chile, quantitative reports about *M. pyrifera* cultivation have not been considered yet. In this study, two different methods to farm these species in northern Chile are evaluated. The results from both culture systems are compared on algae length and biomass production, and practical considerations for implementing each system are made.

Material and methods

Collection of sporophylls and sporulation

Fertile sporophylls of *Macrocystis pyrifera* (L.) C. Ag. were collected in March of 2007 from "Totoralillo Sur" (32°01′ 11″ S; Fig. 1) and transported in wet insolated containers (10±2°C) to the Marine Botany Laboratory at the Universidad Católica del Norte in Coquimbo (29°57′56″ S). The parental sporophytes had a typical morphology of *Macrocystis* beds of northern Chile, being 2 m long with large and narrow fronds.

The sporophylls were cleaned with sterilized seawater to remove debris, other algae, and small invertebrates. Clean sporophylls were kept out of the water in plastic trays in low light conditions (approx. 10 µmol photons m^{-2} s^{-1}) for about 2 to 3 h. After this, ten sporophylls were placed into plastic trays with 1,000 mL of sterilized seawater for 1 h. Small aliquots were taken to estimate zoospore concentration by means of a Neubauer hemocytometer, and the final concentrations were adjusted to 30.000 zoospores mL^{-1}. Zoospore suspension was decanted through a funnel in order to remove any impurities, and it was used for in vitro cultivation.

In vitro cultivation

Glass aquaria of 20 L, divided into two independent compartments of 10 L each, were used. In each compartment, four PVC cylinders of 60 mm in diameter were placed in a vertical position as previously noted by Gutiérrez et al. (2006). To each cylinder, 15 m of polypropylene rope (3 mm ø) was attached. One liter of zoospore solution was added to each aquarium compartment. During the first 78 h, the aquariums remained without aeration and under controlled conditions of temperature (15±1°C), of photon flux density (50± 10 µmol photons m^{-2} s^{-1}), provided by two fluorescent lights (40 W), and of photoperiod of 12:12 h (L/D) in order to allow spore settlement. After this period, the first change of seawater and culture media, enriching the seawater with a solution of von Stosch medium (Oliveira et al. 1995), was carried out. The cultivation conditions, described above, were the same, but with permanent aeration. The second change of enriched seawater was on the 15th day of cultivation, and then the aquarium content was renewed weekly. GeO_2 (3 µg L^{-1}) was added when diatoms were observed. The cultures were kept in the laboratory for

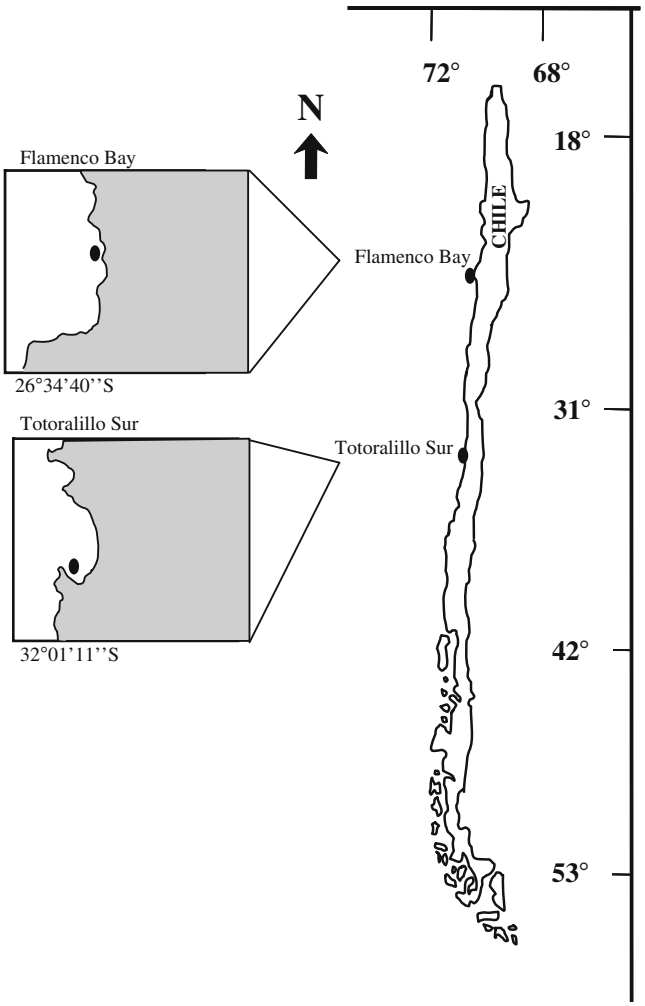

Fig. 1 Geographical location of the sites used for the collection of sporophylls (Totoralillo Sur) and for cultivation (Flamenco Bay)

2 months until sporophytes reached an approximate length size of 5±1 mm and a density of 40±18 fronds cm^{-1}.

Transfer and cultivation in the sea

The experiments took place at Flamenco Bay, 390 km north of Coquimbo, under the authority of Cultivos Marinos Flamenco S.A. Company (26°34′40″ S; Fig. 1). Transfers of juvenile sporophytes to the sea were conducted by two methods. For the first (indirect) method, the initial cultivated ropes (3 mm ø) were tied around 2-m nylon support lines (10 mm ø), as mentioned by Gutiérrez et al. (2006) for the same species in southern Chile. For the second (direct) method, the initial 3-mm ø cultivated ropes were divided into units of 2 m in length and placed directly into the sea without a nylon support line. For both types of cultivation, the ropes were placed vertically along the surface of the sea, each separated by 1 m, in an arrangement similar to a long-line cultivation system. The ropes were transferred to the sea monthly from May (autumn) to October (spring) 2007. Every month, 20 ropes were prepared and transferred to the long-line system for each type of cultivation (indirect and direct). Every 30 days, all ropes were retrieved from the sea and weighed individually (wet weight) in order to record the total biomass (kg m^{-1}; n=20). In addition, one individual sporophyte was randomly selected from each rope (n=20) and the maximum length (cm) recorded. The final lengths and total produced biomass were analyzed separately using two-way ANOVA in order to evaluate the effects of both systems and the time of cultivation (month of seeding). Prior to the analyses, the data were tested for normality and homoscedasticity. Any differences were then examined using the Tukey's post hoc test.

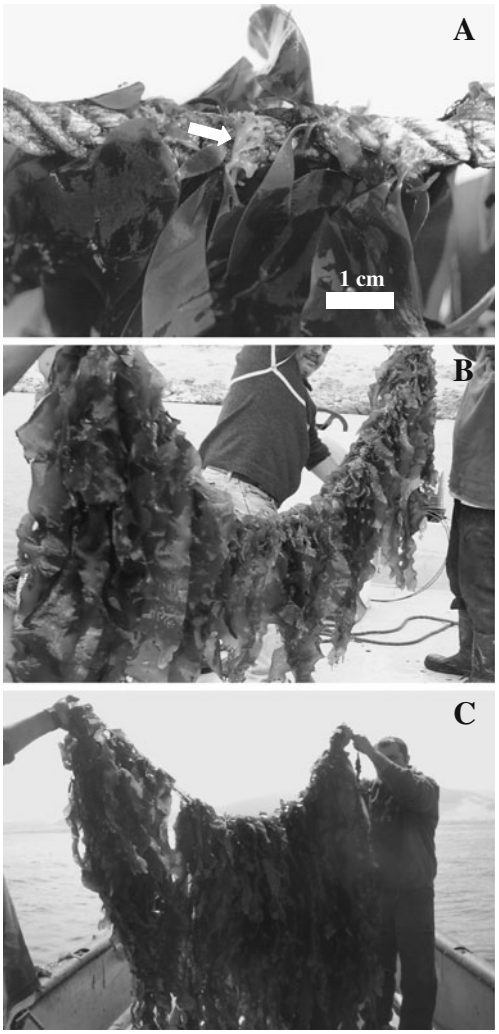

Fig. 2 **a** Algae produced on ropes, adhered by means of basal disks (*white arrow*). **b**, **c** Cultivation ropes after 60 and 120 days at sea, respectively

Results

The sporophytes grew in both culture systems during the whole study period, although there were differences in final size and biomass. The thalli, developed from both the direct and indirect method, were attached to the ropes through basal disks from which the fronds originated (Fig. 2). However, it was possible to observe that the basal disks were bigger, at least double in size, for the indirect compared to the direct cultivation method. No epiphytes or biofouling were seen during autumn and winter months; however, this situation changed during spring, starting in October, when the bryozoan *Membranipora isabelleana* became frequent on the distal portions of the fronds. At the end of the experiments, detachment of algae was scarce and the culture ropes were densely covered with sporophytes.

A maximum frond length of *M. pyrifera* was reached after 120 to 150 days of cultivation in the sea (Fig. 3). Values varied with the time of the year (p<0.01), with a maximum of 175 cm, obtained when ropes were placed into the sea in May (Fig. 3). From May to September, the fronds had a final length over 100 cm after 120 to 150 days. When the ropes were transplanted in October, the fronds fouled and poor growth was registered (Fig. 3). There were no differences (p>0.01) in the final length of the fronds between both methodologies (Table 1).

Maximum biomass was obtained after 120 to 150 days of cultivation in the sea (Fig. 4). Similar to sporophyte length, the accumulation of biomass varied with the season (Table 1). A maximum of 22 kg $m^{-1}$of rope was observed after 120 days of cultivation in September (Fig. 4). There were no significant differences in biomass between both culture methodologies. However, there was a first-order

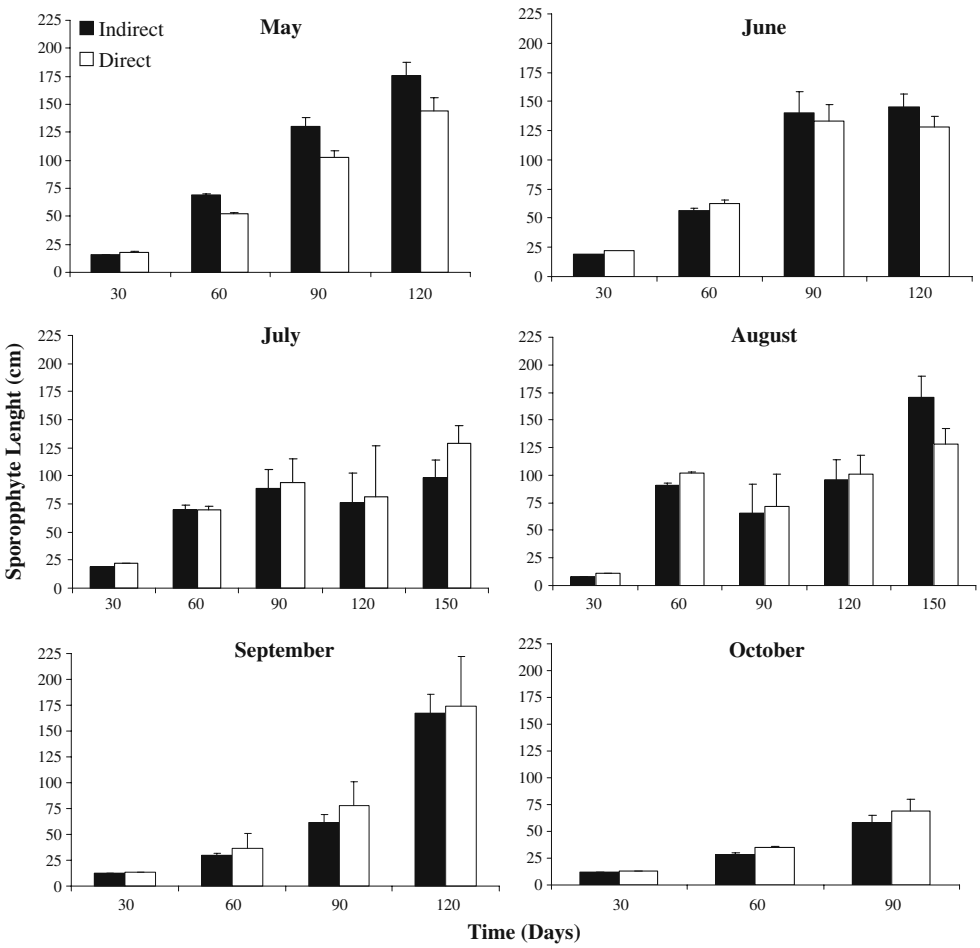

Fig. 3 Individual length (cm) of sporophytes of *M. pyrifera* produced in the laboratory and transferred into the sea, beginning in May 2007, under two different cultivation systems (direct and indirect). *Bars* Mean ± SD

interaction between month of seeding and methods (direct and indirect system; $p<0.01$), reaching a higher biomass in the indirect system for cultivations that were transplanted in May and June. On the other hand, with the direct method, larger biomass was obtained for cultivations transplanted in July and onwards (Fig. 4).

Increase in biomass was lower in spring, especially in October and November (Fig. 4), periods when fouling was higher.

Discussion

The results demonstrate that the growth of *M. pyrifera* is viable using both direct and indirect culture methodologies. These results are promising and can promote the commercial development of this activity in northern Chile. However, it should be taken into account that in spring (October to December), production decreased and the incorporation of new culture ropes was restricted by fouling development.

The short period in the laboratory and the fast sporophyte development in the sea would allow successive harvests after a period of 4 to 5 months. Studies with *M. pyrifera* indicate that it is possible to have an annual harvest for this species. However, these results were obtained, principally in southern Chile and are closely associated with the environmental conditions of that region (Gutiérrez et al. 2006; Westermeier et al. 2006).

Our results showed that with both methodologies, sporophytes reached a maximum frond length of 175 cm. These results are lower than found by Westermeier et al.

Table 1 Two-way ANOVA for length (cm) and biomass (kg m^{-1}) of *M. pyrifera* cultured at different months of seeding and using different culture methods (direct and indirect systems)

	Length				Biomass			
	df	MS	*F*	*p*	*df*	MS	*F*	*p*
M	5	60,173.726	53.827	<0.001*	5	926.264	241.399	<0.001*
CM	1	6,625.504	5.926	0.021	1	0.938	0.244	0.621
M × CM	5	8,251.673	7.381	<0.001*	5	301.957	78.694	<0.001*

M month, *CM* culture method
*$p<0.01$

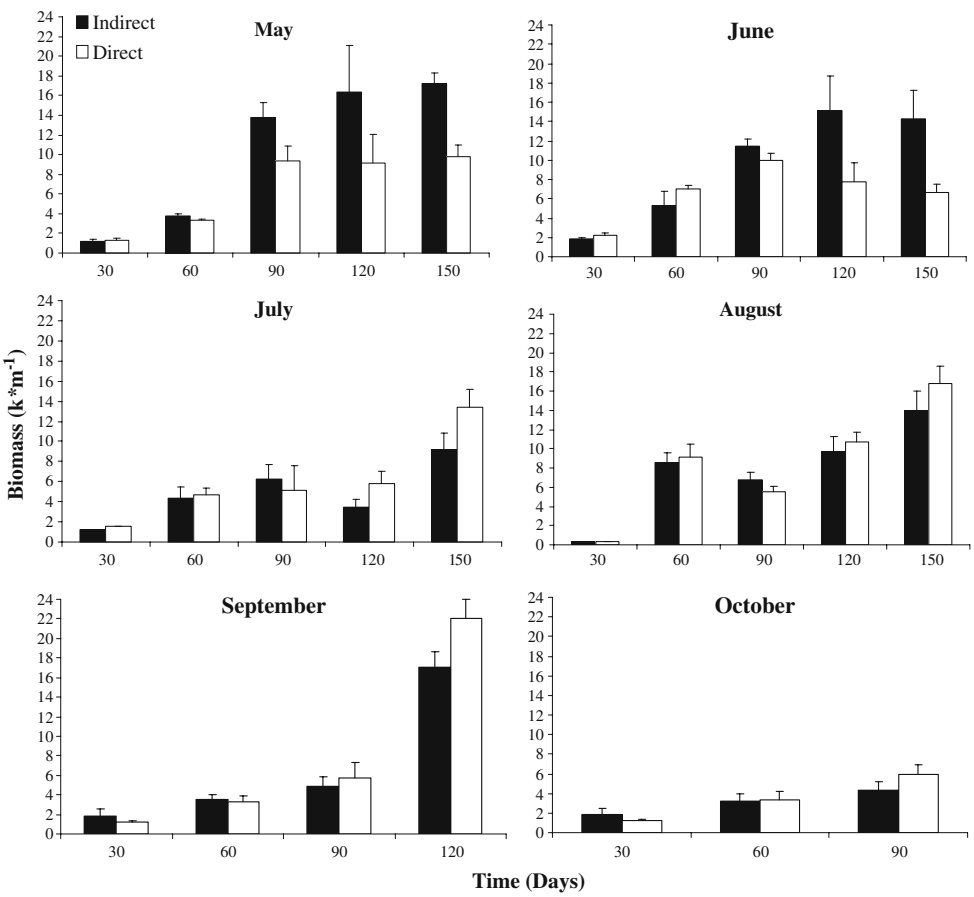

Fig. 4 Biomass (kg m^{-1}) of sporophytes of *M. pyrifera* produced in the laboratory and transferred into the sea, beginning in May 2007, under two different cultivation systems (direct and indirect). *Bars* Mean ± SD

(2006) in southern Chile experiments, working with a low density culture system where fronds grew up to 14 m. Our results could be explained by the high density culture system used where a large number of individuals grew in a reduced space (rope), with probably high competition for light and nutrients. However, our results could also be related to the origin of the seeds used since sporophylls were selected from a northern Chilean bed where algae have typical morphological characteristics (*M. integrifolia* shape), being generally smaller than *Macrocystis* from southern Chile.

Moreover, length results could be a very important tool for fisherman's communities. These communities have started to cultivate *Macrocystis* in northern Chile, and they sometimes do not have the possibility of weighing the algae at the cultivation site, which often are located in isolated places. In this way, length can be a good criterion to decide when is the right time to harvest. Thus, we recommended harvesting the total accumulated biomass when the sporophytes reach over 150 cm.

Our results of biomass yield between 13 and 22 kg m^{-1} are similar to those of Gutiérrez et al. (2006) using a similar culture strategy for *M. pyrifera* (14 kg m^{-1}) in southern Chile. It is important to note that the biomass did not vary with the methodology used. On the one hand, for practical and economic reasons, we suggest that the direct method would be more convenient since it requires less effort and manipulation of the cultivation and does not require a support rope, which is the case for indirect cultures. Additionally, it is important to point out that during the experiment, it was possible to observe no detachment of the sporophytes from direct cultures, but instead achieving a good kelp attachment to the system.

On the other hand, the indirect method provides a greater surface area for sporophyte adhesion, resulting in larger basal holdfasts, which increase the final weight of sporophytes. However, this does not represent an advantage since the holdfast is not used as food for the abalone. These results may also explain the larger biomass obtained from the cultures transferred to the sea in May and June for the indirect method.

Previous attempts to cultivate kelps in Chile indicated that *L. trabeculata* (Edding and Tala 2003) and *M. pyrifera* (Gutiérrez et al. 2006; Westermeier et al. 2006) needed a period longer than 10 months. Our results demonstrate that culture of *M. pyrifera* can be done in a period of 4 to 5 months at sea, delaying the complete process to 6 or 7 months since the cultures require 2 months in the laboratory in order to reach a sporophytes size which is large enough to be transferred into the sea. On the one

hand, this situation indicates the possibility to maintain a permanent year-round cultivation program. On the other hand, the shortest time period for reaching an adequate biomass is highly desired for this type of cultivation since periods of cultivation are very extensive and increase the possibilities of loss of production. This can be due to fouling (Edding and Tala 2003), as well as the exposure to harmful seasonal climatic events such as storms (Seymour et al. 1989), the increase in temperature, or the decrease in nutrient availability during the summer (North et al. 1986). However, as mentioned by Vásquez (2008), the culture of *Macrocystis* would be independent from unpredictable environmental catastrophic fluctuations such as El Niño, which could produce alterations in natural beds of kelp and inclusive their local extinction (Vásquez et al. 2006).

In January, a biomass of 22 kg m^{-1} was recorded from cultivations placed into the sea in September. Placing new cultures in the sea during spring (beginning in October) is not recommended since most of the small sporophytes did not survive, and those that did showed little growth and low production (6 kg m^{-1}). These poor biomass production results can be attributed to an increase in water temperature that may reach up to 20°C (Zúñiga and Acuña 2002) because this species is highly sensitive to temperature, preferring low temperature for developing microscopic sporophytes (Buschmann et al. 2004). Another reason may be due to its sensitivity to higher irradiances (over 500 µmol photons m^{-2} s^{-1} at sea surface) during summer, which can increase sporophyte mortality during its early stages of development (Bulboa, unpublished data). Moreover, during spring, there is the fouling caused by *M. isabelleana*, which rapidly covers the small sporophytes as previously has been reported in natural environments of *M. integrifolia* by Hurd et al. (1994) and Hurd (2000).

Previous experiments, with horizontal cultivation systems at 1- to 5-m depth and with similar conditions in culture time and locality, showed lower biomass production (7 kg m^{-1}; Macchiavello, unpublished data) than this study. Thus, vertical cultures are better, at least in areas with low turbidity, such as the study area. However, both strategies must be tested for each cultivation area because changes in water quality may be found. Nevertheless, from our results, the vertical cultivation technique is recommended in order to farm *M. pyrifera* in northern Chile, whereas horizontal cultures along the ocean surface have shown to be highly productive in southern Chile (Gutiérrez et al. 2006; Westermeier et al. 2006) where low light penetration prevents the realization of vertical cultures.

Considering the biomass yield obtained from sea cultures started in September, in long-line systems of 100 m, distributed over the sea every 5 m from one another, a production of 134 t ha^{-1} $year^{-1}$ can be estimated. This biomass production could be improved both by the selection of strains and by simple modifications of the system, such as managing the sporophyte density in the cultures or such as increasing the vertical culture rope length from 1 to 2 m. In addition, the use of selected sporophytes, originated by crossing strains from different populations of Chilean *Macrocystis*, is a very important tool, as already demonstrated by Westermeier et al. (2007), in order to control and improve production characteristics in commercial farming.

Acknowledgments We are grateful to the staff of Cultivos Marinos Flamenco Ltda. and Edwin Martínez for field assistance and technical support. We also thank Eurico C. Oliveira and Fadia Tala for discussions and comments made on the manuscript

References

Buschmann A, Vásquez J, Osorio P, Reyes E, Filún L, Hernández-González M, 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

Camus P (2005) Introducción de especies en ambientes marinos Chilenos: no sólo exóticas, no siempre evidentes. Rev Chil Hist Nat 78:155–159

Corazani D, Illanes J (1998) Growth of juvenile abalone, *Haliotis discus hannai* Ino 1953 and *Haliotis rufescens* Swaison 1822, fed with different diets. J Shellf Res 17:663–666

Edding M, Tala F (2003) Develoment of techniques for the cultivation of *Lessonia trabeculata* Villouta et Santelices (Phaeophyceae: Laminariales) in Chile. Aquaculture Res 34:507–515

Gutiérrez A, Correal T, Santibáñez A, Marcos R, Caceres C, Buschmann A (2006) Farming of the giant Kelp *Macrocystis pyrifera* in southern Chile for development of novel food products. J Appl Phycol 98:259–267

Hurd C (2000) Water motion, marine macroalgal ecophysiology and production. J Phycol 36:453–472

Hurd C, Durante K, Chia F, Harrison P (1994) Effect of bryozoan colonization of inorganic nitrogen acquisition by the kelps *Agarum fimbriatum* and *Macrocystis integrifolia*. Mar Biol 121:167–173

North W, Jackson G, Manley S (1986) *Macrocystis* and its environment, knowns and unknowns. Aquat Bot 26:9–26

Oliveira E, Paula E, Plastino E, Petti R (1995) Metodologias para cultivo no axénico de macroalgas marinas in vitro. In: Alveal K, Ferrario M, Oliveira E, Sar E (eds) Manual de métodos ficológicos. Universidad de Concepción, Concepción, pp 429–447

Pizarro C (2003) Evaluación de una técnica de ensilado para el alga Macrocystis pyrifera y observación de su consumo por parte del abalón rojo (Haliotis rufescens). Tesis de Licenciatura en Ciencias de la Acuacultura, Universidad Católica de Temuco, Chile

Seymour R, Tegner M, Dayton P, Parnell P (1989) Storm wave induced mortality of giant kelp, *Macrocystis pyrifera*, in southern California. Estuarine Coast Shelf Science 28:277–292

Viana T, López L, Salas A (1993) Diet development for juvenile abalone *Haliotis fulgens*. Aquaculture 117:149–156

Vásquez J (2008) Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. J Appl Phycol 20:457–467

Vásquez J, Fonck E, Vega M (2001) Comunidades submareales rocosas dominadas por macroalgas en el norte de Chile: diversidad, abundancia y variabilidad temporal. In: Alveal K (ed) Sustentabilidad de la biodiversidad. Universidad de Concepción, Concepción, Chile, pp 351–366

Vásquez J, Vega M, Buschmann A (2006) Long term studies on El Niño–La Niña in northern Chile: effects on the structure and organization of subtidal kelp assemblages. J Appl Phycol 18:505–519

Vega A, Vásquez J, Buschmann A (2005) Population biology of the subtidal kelps *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales, Phaeophyceae) in a upwelling ecosystem of northern Chile: interannual variability and El Niño 1997–1998. Rev Chil Hist Nat 78:33–50

Westermeier R, Patiño D, Piel M, Maier I, Mueller D (2006) A new approach to Kelp mariculture in Chile: production of free-floating sporopyte seedlings from gametophyte cultures of *Lessonia trabeculata* and *Macrocystis pyrifera*. Aquaculture Res 37:164–171

Westermeier R, Patiño D, Müller D, Dieter G (2007) Sexual compatibility and hybrid formation between the giant kelp species *Macrocystis pyrifera* and *M. integrifolia* (Laminariales, Phaeophyceae) in Chile. J Appl Phycol 19:215–221

Zúñiga S, Acuña E (2002) Informe final estudio identificación y caracterización de las oportunidades en el sector acuícola de la Región de Atacama. Universidad Católica del Norte, Coquimbo