Open-sea cultivation using the transplanting method in the kelp *Saccharina latissima*

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Abstract *Saccharina latissima* is an economically and ecologically important native kelp. As its limited supply from wild stock cannot meet increasing current and future demands, methods for its cultivation in the ocean need to be developed. This kelp is now beginning to be farmed off the Atlantic coast of Spain using a regular method similar to the "forced cultivation" technique used with Asian kelps (kombu). Its cultivation is also a growing enterprise in other European countries. In this study, the open-sea farming of *S. latissima* using the transplanting method is tested on a commercial-scale. This cultivation method has not been studied with kelp species outside Asian waters. The tested method includes the following steps: indoor production of seedlings, pre-culture in greenhouse tanks and open-sea cultivation by transplanting young fronds. Results demonstrate that open-sea cultivation using transplanted young fronds is a technically and biologically viable method. The total yield obtained (8.3 kg fresh m^{-1} rope equivalent to 45.6 ton fresh ha^{-1} farm) is satisfactory considering the low densities of transplanted fronds (25–30 fronds m^{-1} rope). Moreover, these values are comparable to those reported in previous cultivations with this species, as well as in the farming of similar kelps. The transplanting method used in conjunction with the regular cultivation method has valuable practical applications for the commercial farming of *S. latissima*.

Keywords Cultivation method · Kelp mariculture · *Saccharina latissima* · Transplanting method · Yield

Introduction

Seaweeds are important natural stocks used for a large number of commercial purposes. *Laminaria* sensu lato (i.e., *Laminaria* and *Saccharina*) includes an economically and ecologically important group of kelp species which are used as food for humans and as a source of alginates for a wide variety of industrial applications. These kelps also support ecological services as ecosystem engineers, as they provide habitat and resources for associated fauna and flora, acting as foundation species of temperate coastal ecosystems (e.g., see review by Bartsch et al. 2008).

Laminaria and *Saccharina*, commercially known as kombu, were traditionally collected from wild stocks; however, nowadays this practice has been replaced to a great extent by sea farming. World aquaculture production of kombu, as in other cultured seaweeds, currently accounts for more than 95 percent of total production (FAO 2012). Kombu mariculture has significantly contributed to increasing production to meet commercial demands and, in turn, conserve natural resources from overharvesting. Kombu cultivation techniques have already been well developed in Japan and China where kombu, particularly *Saccharina japonica*, is cultivated on a large scale (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders (Kawashima 1984; Sanbonsuga 1984; Tseng 1987; Kawashima 1993). The so-called "forced cultivation" method is the most widely-used technique in Asian countries, because the culture period in the sea is reduced, resulting in lower costs for farmers (see review by Kawashima 1984, 1993). In "forced cultivation", seedlings (i.e., sporophyte phase) are transferred to the sea after being produced indoors. Another cultivation method often used in conjunction with "forced cultivation" is the transplanting method, in which young fronds are transferred to the sea, allowing delayed outplanting. Although promising, the transplanting cultivation method has not been assayed for European kelps to date.

Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, (formerly *Laminaria saccharina* (Linnaeus) Lamouroux) is a native kelp in the European Atlantic and is currently intended for direct human consumption (e.g., Peteiro and Freire 2013). It also has many other applications such as animal feed for aquaculture (e.g., Kelly et al. 2001; Troell et al. 2006), feedstock for biofuel production (e.g., Adams et al. 2009; Kraan 2013), and a species in integrated multi-trophic aquaculture (e.g., Subandar et al. 1993; Sanderson et al. 2012). Sugar kelp (the commercial name for *S. latissima*) is collected from natural stands in several European countries (e.g., Tasende and Rodríguez González 2003). However, natural stocks are limited and, populations around Atlantic European coasts have declined drastically in recent years (Pehlke and Bartsch 2008; Andersen et al. 2011; Bekkby and Moy 2011). Among other factors, the decline of the sugar kelp forest could be the result of the increase in sea temperature due to climate change (see Bekkby and Moy 2011). Therefore, the commercial collecting of *S. latissima* (even a small harvest) is considered environmentally undesirable, as biomass removals may compromise the reproductive output, recruitment and population size of kelp species (e.g., Vásquez and Santelices 1990; Thompson et al. 2010) and thus the integrity of coastal ecosystems.

The sea farming of *S. latissima* to increase stocks for commercial use is currently a growing enterprise in Europe (e.g., Forbord et al. 2012; Sanderson et al. 2012; Peteiro and Freire 2013). Open-sea cultivation has been tested in the Atlantic off the coast of Spain (Peteiro et al. 2006; Cremades et al. 2007; Peteiro and Freire 2009, 2011, 2012a, 2013), the United Kingdom (Kain (Jones) et al. 1990; Sanderson et al. 2012), Russia (Chugaynova and Gorennikov 1996), Germany (Buck and Buchholz 2004, 2005) and Norway (Forbord et al. 2012). In fact,

sugar kelp is now beginning to be cultivated on a small commercial-scale off the Atlantic coast of Spain. The cultivation method used is similar to the "forced cultivation" method applied in Asia (Kawashima 1984, 1993). However, this regular method experiences occasional problems during the first few months of culture (late autumn and early winter), such as major storms, torrential rains or fish grazing (C. Peteiro, personal observation). These events can cause important damage, often leading to the complete loss of the culture. Previous cultivation trials with *S. latissima* have assayed the later outplantings of seedlings, but they were not fully successful, because the favourable growing season for this species is very short off the southern European Atlantic coast (Spain) (Peteiro and Freire 2009).

In an attempt to overcome these limitations, we assayed for the first time in Europe the transplanting of young fronds for *S. latissima* cultivation. We hypothesized that transplanting could be a valuable method for the commercial cultivation of *S. Latissima*, as found for other kombu species in Asia (see above). Moreover, there is little scientific information available on kombu cultivation using the transplanting method. To test our hypothesis, we evaluated biomass yield and mean growth obtained in a commercial-scale cultivation trial of *S. latissima* using the method of transplanting young fronds off the Atlantic coast of northern Spain. The tested method included the following steps: indoor seedling production, pre-culture in greenhouse tanks, and transplanting young fronds for open-sea cultivation.

Material and methods

Indoor production of seedlings

S. latissima seedlings were produced on seed strings from gametophyte stock cultures (germplasm storage) under controlled environmental conditions using the modified methodology developed by Pérez et al. (1984, 1990, 1992) and outlined in Salinas et al. (2006), Peteiro and Freire (2009) and Salinas (2011). Gametophyte stocks were kept vegetatively in free-living cultures in 1–2 L bottles with filtered bubbling aeration at a temperature of 10°C, an irradiance (PAR) of 10–20 µmol photons m^{-2} s^{-1} and a 12:12 h light:dark photoperiod using daylight fluorescent tubes (Sylvania, USA). Photosynthetically active radiation (PAR) was determined with a spherical quantum sensor (Model LI-193SA; Li-Cor Biosciences, USA) connected to a photometer (Model LI-250A). Sterilized seawater enriched with 4 ml L^{-1} Provasoli solution without the addition of vitamins (e.g., Pérez et al. 1990) was used as the culture medium, to which 2.5 mg L^{-1} germanium oxide (GeO_2) and occasionally 50 mg L^{-1} gentamicin (or related antibiotic) were added to inhibit the growth of diatoms and protozoa. The culture medium was renewed approximately every 15 days.

Free-living gametophyte filaments (sex ratio 1:1; female:male) were fragmented with a homogenizer. Gametophyte fragments were then sprayed onto soft nylon string wound on frames (so-called collectors) using compressed air. The frames (12 cm width × 12 cm depth × 47 cm height) were constructed of stainless steel rods, allowed water circulation and promoted lighting efficiency. Forty meters of string were wound around each stainless steel frame at intervals of about 2 mm. Prior to the assay, the string (2 mm diameter), which was made from 4 braided strands of polyamide, was subjected to the following treatment: The string was boiled for 1 h and then washed until waterproofing agents were removed. After drying, it was sanded on a bench grinder with fine grinding wheels to obtain fibers of frayed string. The string was then heated using a hot air gun. This treatment

was applied to enhance the microstructure and absorption of the string in order to increase gametophyte attachment and lower the detachment rate.

After gametophyte fixation, the collectors were placed in rectangular culture tanks (1400 L) with transparent side walls under controlled conditions of air-bubbling agitation, temperature and illumination. Water temperature was controlled within ±0.1 °C using a submersible water heater and a refrigerated water cooler placed in the storage tanks and connected by a feedback temperature control loop to a stainless steel heat exchange coil immersed in each tank. Culture tanks were illuminated by aquastar and coralstar fluorescent tubes (Sylvania, USA). Agitation by air-bubbling was provided through a series of air pipes located across of the bottom of the culture tanks and supplied by a filtered electrical air blower. Air flow was adjusted in each tank by a control valve to obtain the required aeration intensity.Tanks were filled with sterilized seawater enriched with 17.5 mg L^{-1} of $NaNO_3$, 0.6 mg L^{-1} of NaH_2PO_4 and 0.2 mg L^{-1} of Fe^{+3}-chelate (Jisaquel© Fe), to which 2.5 mg L^{-1} germanium oxide (GeO_2) and 30 mg L^{-1} gentamicin (or related antibiotic) were occasionally added. To sterilize the seawater, it was chlorinated with 1.5 mg Cl_2 L^{-1} from commercial bleach (NaClO) and then dechlorinated using sodium thiosulphate ($Na_2S_2O_3$) and vigorous aeration.

Female and male gametophytes were induced to sexual maturation and oogamous sexual reproduction to produce zygotes that grow into young sporophytes. Gametogenesis induction and zygote formation were triggered by a quantum dose of blue light and temperature. Coralstar fluorescent tubes achieve blue light conditions (about 1/2 quantum dose of light) and emit light with a wavelength of around 450–460 nm. Required culture conditions were maintained for 7 days at 14°C under an irradiance (PAR) of 60 µmol photons m^{-1} s^{-1} and a 12:12 h light: dark cycle. Light intensity was measured on the collector in the seawater tanks using the spherical quantum sensor.

About 7 days after zygote formation, water temperature and irradiance (PAR) were gradually increased to 17°C and 100 µmol photons m^{-1} s^{-1}, respectively, to improve seedling growth. Coralstar fluorescent tubes were then replaced by daylight fluorescent tubes. Air flow was adjusted to fine air bubbles to prevent gametophytes from detaching from the string, and it was gradually raised after zygote formation to improve rhizoid seedling development at the end of the culture period. Culture medium was renewed after 15 days of culture and approximately weekly thereafter. After about 35 days of culture, seedlings reached 2–4 mm in length.

Pre-culture in greenhouse tanks

S. latissima seedlings (i.e., young sporophytes) were detached from the strings and transferred to indoor greenhouse tanks, where they were cultured from 10 October 2005 to 13 March 2006. Sporophytes were first cultured in circular tanks with a surface area of 0.5 m^2 and a water depth of 1 m (500 L water volume) until they reached about 5 cm length. Then, they were then transferred to rectangular tanks with a surface area of 4.5 m^2 and a water depth of 0.7 m (3000 L water volume) until they were approximately 50 cm long.

A central air blower aerated the 500 L tanks through air pipes in the middle of the bottom, while aeration in the 3000 L tanks was supplied through a series of air pipes located along one shorter side of the tank bottom to allow better circulation for the larger size of sporophytes. Culture in greenhouse tanks was carried out under solar irradiance with natural day length and ambient temperature. Illumination was controlled by covering the tanks with shading sheets to prevent exposure to high irradiance that could damage the sporophytes. Underwater

irradiance (PAR) was regulated to about 100 µmol photons m^{-2} s^{-1} until sporophytes reached a length of about 10 cm (23 November 2005), after which they were exposed to direct sunlight. Mean irradiance in the tanks varied between 160 and 373 µmol photons m^{-2} s^{-1}, with a maximum value of 746 µmol photons m^{-2} s^{-1}. Irradiance (PAR) was sporadically measured in the centre of the tank on a sunny day. Throughout the culture period, ambient seawater temperature ranged from 9.5°C in December to 16.6°C in March. Seawater temperature was recorded daily every 4 hours by a data logger (StowAway Tidbit®, Onset Computer Corporation, USA). Tanks were filled with filtered seawater enriched with nitrate, phosphate and iron-chelate. Concentrations of 4 mg L^{-1} of $NaNO_3$, 0.5 mg L^{-1} of NaH_2PO_4 and 0.2 mg L^{-1} Fe^{+3}-chelate were added to the seawater until sporophytes reached a length of 10 cm, after which 8 mg L^{-1} of $NaNO_3$, 1 mg L^{-1} of NaH_2PO_4 and 0.2 mg L^{-1} of Fe^{+3}-chelate (Jisaquel© Fe) were added. Seawater was renewed (100% of volume) every week, and tanks were brushed clean when necessary.

Initial biomass density in tanks was 0.8 kg m^{-3}, and *S. latissima* seedling length was about 2–4 mm long. Biomass density was generally maintained at the target yield (4–5 kg m^{-3}) by periodically harvesting the extra biomass. When most of the young fronds had reached a length of about 40 cm, they were transplanted to the culture ropes.

Open-sea cultivation by the transplanting method

Clusters of young *S. latissima* fronds were fixed to 7 ropes using rubber anchor-bands (anchor type). Each cluster consisted of 5–6 fronds. Clusters were spaced every 20 cm along the 20 m long rope (i.e., 5 clusters m^{-1} rope, 25–30 fronds m^{-1} rope). Details of how the young fronds were fixed to the culture ropes are shown in Fig. 1. Before transplanting to ropes, the length and fresh weight of 100 randomly-selected fronds were recorded, and all specimens for each rope were weighed collectively.

Ropes were then deployed to the floating raft farm. Outplanting took place on 14–15 March 2006, and open-sea cultivation continued until 26–27 June 2006 (i.e., 106 days). The open-sea cultivation experiment was conducted in a small inlet adjacent to Mataleñas beach, outside the Bay of Santander (Santander, Cantabria), on the Atlantic coast of northern Spain, Bay of Biscay (43º29′ N, 3º47′ W; Fig. 2). The site is open to ocean swell conditions with episodes of wave surge which often occur in late autumn and early winter.

A commercial-scale floating raft with long-line culture ropes (Fig. 3) was used for the experiment. The culture raft consisted of a frame (30×20 m long ropes) buoyed at a depth of 4 m and tied to anchor ropes with a sinker at mid-length. The anchor ropes were supported by poles fixed to the sandy bottom at a depth of 20 m. The 20 m long culture ropes were fastened to the frame horizontally and spaced at a distance of approximately 2 m.

Biomass yield and growth measurements

Biomass yield was calculated as average fresh weight (FW) from harvested ropes (n=7 ropes of 20 m long) and was expressed per meter of culture rope. Prior to weighing, fronds were hung to drain excess water. Total biomass yield per hectare farm was also determined from the fresh weight sum of all culture ropes. The harvested ropes occupied an area of 240 m^2 on the culture raft which was designed for commercial scale

production. Measurements were expressed per unit length (m) of culture rope and per unit area farm for comparative purposes.

Frond length and weight (stipe plus blade) were measured for 100 individuals randomly collected from the culture ropes. Growth rates of transplanted fronds were estimated as length and wet weight increase per day, according to the equation for absolute growth rate (AGR; Hunt 1982).

Environmental conditions of culture site

Temperature and salinity were recorded approximately every week at a 5 m depth in the vicinity of the farm site (43°30′ N, 3°47′ W) using a seabird CTD (Model SBE-25, USA) attached to a rosette sampler (Model SBE-32SC) equipped with Niskin bottles. Nutrient concentration (μmol L^{-1}= μg-at L^{-1}= μM) was measured in the water samples by automated colorimetric analyses (Technicon Autoanalyzer, USA), as described in Grasshoff et al. (1983). These data were recorded by IEO (Instituto Español de Oceanografía) in the framework of the project Radiales (http://www.seriestemporales-ieo.net/) as part of an oceanographic time-series monitoring program. Solar radiation data were obtained from the Meteorological Observatory of Santander (AEMET, Spanish State Meteorological Agency, Station coordinates: 43º29' N, 3º48' W). Irradiance data (W m^{-2}) were converted to photon fluence rate (μmol photons m^{-2} s^{-1}) using the conversion factor (1 W m^{-2} ~ 4.2 μmol photons m^{-2} s^{-1}) described in Lüning (1990). Light attenuation at the farm site was calculated by measuring irradiance from the surface to a depth of 6 m at 1m-depth intervals using the sensor described above to estimate underwater irradiance. Day length (number of day light hours) was calculated using online-photoperiod calculator V 1.94L (author: J. Lammi, © 1996–2008, available at http://www.sci.fi/~benefon/sol.html).

Results and discussion

This study provides a new approach based on a commercial-scale experiment using the transplanting method to improve the open-sea cultivation of *S. latissima* outside Asian waters. Until now, the regular cultivation used for this species off the Atlantic coast of Spain (e.g., Peteiro and Freire 2009, 2013) is similar to the "forced cultivation" of kombu species in Asia (Kawashima 1984, 1993). The transplanting method optimized in this study is an alternative to the later cultivation of *S. latissima* in the case of occasional damage to regular cultivation in the sea (e.g., major storms, torrential rains or fish grazing). A diagram of the major seasonal stages of cultivation using the transplanting method is shown and compared to regular cultivation in Fig. 4.

Although the transplanting method is often used to culture kombu in Japan (Kawashima 1984), no data on biomass yield are available. In this study in the Atlantic waters of northern Spain, we obtained a mean yield of 7.8 kg fresh m^{-1} rope (Table 1). This value is comparable to that reported for cultivation trials with *S. latissima* elsewhere (e.g., Druehl et al. 1988) and for the commercial cultivation of *Saccharina japonica* (e.g., Mairh et al. 1991). However, it is lower than the mean yield obtained by Cremades et al. (2007) and Peteiro and Freire (2013) in Galicia (northwest Spain) when using the regular cultivation method with *S. latissima* (see Fig. 4). In these studies, productivity was 12–16 fresh kg m^{-1} rope with a density of 400–700 fronds m^{-1} rope. However, we should consider the low density culture of 25–30 fronds m^{-1} rope used here. Yield values could easily be improved by increasing the density of transplanted fronds on the culture ropes. In this experiment, total biomass

yield per unit culture area was 45.6 ton fresh ha^{-1} farm (Table 1), which could be considered a good value according to previous studies (Peteiro and Freire 2013). In our study, this value was determined from the fresh weight sum of all culture ropes on the raft designed on a commercial scale (240 m^2). The production obtained per unit culture area is also associated with the culture design, and different farm configurations could result in higher or lower densities on the culture ropes.

The growth rate found here, based on the change in length (0.72 cm d^{-1} in Table 2), was also lower than that reported in previous studies in Spain by Cremades et al. (2007) and Peteiro and Freire (2009) off Galician coasts (1–1.5 cm d^{-1}). This may be due to less favourable environmental conditions for the growth of *S. latissima* sporophytes on Cantabrian coasts, where there are warmer temperatures and less nutrient-rich conditions than in the Galician upwelling region (e.g., Gorostiaga et al. 2004 and references therein). Indeed, there is a biotic boundary for cold-temperate seaweeds between Galician and Cantabrian coasts in the Bay of Biscay in Northern Spain (see Fig. 2 for locations) (Sauvageau 1897; van den Hoek and Donze 1966; Gorostiaga et al. 2004; Bárbara et al. 2005). For example, cold temperate species such as *S. latissima* are absent on most eastern coasts of northern Spain (Cantabria and Basque Country) (Fig. 2, see map 1 for *S. latissima* distribution). Nevertheless, our study shows that cultivation areas for *S. latissima* sporophytes can be extended further west into the warm waters of the Bay of Biscay. This region is within the species distribution range in European waters, but *S. latissima* does not grow naturally here. This may be because environmental conditions are not suitable enough for the species to complete its life history (van den Hoek 1982; Breeman 1988; van den Hoek et al. 1996; Müller et al. 2009).

Environmental conditions (summarized in Table 3) during sea cultivation were, however, generally within the growth range for *S. latissima* sporophytes (see Peteiro and Freire 2013 for details). Optimal seawater temperature for the growth of *S. latissima* sporophytes is between 10°C and 15°C (Fortes and Lüning 1980; Bolton and Lüning 1982; Lüning and Freshwater 1988). In fact, sporophyte growth slows down, when temperature exceeds about 16°C (e.g., Brinkhuis et al. 1983; Lee and Brinkhuis 1986). In our experiment, this temperature was reached in May. Moreover, although nitrogen concentrations did not reach the saturation level (10 μM) reported for the growth of *S. latissima* sporophytes (Chapman et al. 1978), measured values provided the necessary requirements for growth. We should highlight that harvest time coincided with the onset of summer, when severe nutrient depletion starts (<0.1 μM for nitrates and nitrites in July) and seawater temperature increases (>20 °C from July). Harvesting is recommended under these conditions, since most sporophytes do not survive until summer, and those that do show little growth and low production (Peteiro et al. 2006). Other studies have found that the decline in the survival of *S. latissima* sporophytes at the southern limit of its distribution is due to the negative effects of nitrogen limitation and heat stress (Lee and Brinkhuis 1986; Gerard 1997). Therefore, the lower growth of sporophytes found here could be attributed to warmer temperatures and less nutrient-rich conditions compared to other regions.

With regard to sea cultivation, the culture raft presented in Fig. 3, and in particular the anchor type used (fixed poles), withstood the exposed sites with rough weather conditions of this study. Under typical open-ocean swell conditions, the concrete blocks traditionally employed (e.g., Peteiro and Freire 2012b, 2013) to securely moor the floating raft may be washed ashore during storms, as observed in previous cultivation trials in this location (JM Salinas, personal communication). Special attention was paid to the possible natural reattachment of frond holdfasts on the culture rope, as well as the survivorship of transplanted fronds. The method of attaching

young fronds, ranging from 35 cm to 62 cm in length, on ropes using rubber anchor-bands was also successful. Indeed, culture ropes were almost always covered with the initial cluster density (i.e., 5 clusters m^{-1} rope, 25–30 fronds m^{-1} rope); and no noticeable detachments of transplanted fronds or clusters were observed at the end of the experiment. Furthermore, new holdfasts grew and re-fixed the fronds to the culture rope. Similar results have been described in the commercial cultivation of other Asian kelp species, thereby indicating that kombu is able to attach to ropes and other substrata in the sea (Kawashima 1984). This ability appears to be more pronounced in winter and spring when meristem activity increases (Kawashima 1984), which coincides with the transplanting time in our study. This capacity to develop new holdfasts from transplanted *S. latissima* fronds can be used not only in commercial cultivation, but also to restore areas where this kelp species has disappeared. Recent studies have found that *S. latissima* populations have disappeared or declined dramatically around the European Atlantic (Pehlke and Bartsch 2008; Andersen et al. 2011; Bekkby and Moy 2011). In the near future, the absence of *S. latissima* from coastal areas may evoke interest in restoring populations, taking advantage of the capacity of transplanted fronds to regenerate holdfasts and re-attach to the substratum. In fact, restoration by transplanting young fronds has already been used in some kelp species as a potential tool for environmental mitigation purposes (Hernandez-Carmona et al. 2000; Carney et al. 2005; Correa et al. 2006; Hasegawa and Unno 2010).

With regard to the production of young culture fronds, young *S. latissima* sporophytes grew well in the greenhouse tanks. The average length of sporophytes increased from 2–4 mm on 10 October 2005 (early autumn) to 35–62 cm on 13 March 2006 (late winter) after approximately 4 months of culture. No epiphytes or damage (e.g., breakage of the blade) were observed in the fronds during tank culture. The regulation of biomass density at 4–5 kg m^{-3} was essential for 10–15 cm long fronds to ensure uniform light exposure (to prevent frond overlap and mutual shading), to facilitate the availability of nutrients and carbon dioxide, as well as to prevent fronds from tangling together and forming biomass balls settling in the corners of the tank. However, the indoor production of young fronds might not always be necessary, since the fronds obtained by manual thinning in regular sea cultivation could be used in the transplanting method (Fig. 4). The thinning of excessive fronds in kombu farming is a common practice in mid-winter in Asian waters to accelerate growth and improve the quality of fronds for human consumption (Kawashima 1984, 1993). This practice could also be used in North Atlantic waters, as *S. latissima* grows densely on the culture rope (Cremades et al. 2007; Peteiro and Freire 2013). Therefore, the transplanting method for *S. latissima* could be combined with the thinning of fronds in regular cultivation (see Fig. 4). In this way, the high production costs of indoor tank culture (Titlyanov and Titlyanova 2010) would be reduced, while increasing yield quality in regular cultivation (Kawashima 1984, 1993).

Conclusions

This study demonstrates that the open-sea cultivation of *S. latissima* by transplanting young fronds is a technically and biologically viable method, which obtains reasonably good growth and productivity. A remarkable advantage of this method is the possibility of later sea cultivation, which is of great interest when used in conjunction with regular cultivation, a method often employed in northern Spain (e.g., Peteiro and Freire 2009, 2013). Thus, the transplanting method would have great practical applications for the commercial cultivation of *S. latissima* and other related kelps species in Spain and other potential farming regions. In

general, the development of mariculture techniques has contributed both to increasing production and conserving natural resources from overharvesting. This is of particular interest in northern Spain, the southern limit of the distribution of many cold-temperate species such as kelps (e.g., Lüning 1990). In this area, kelp resources are very limited, and natural stocks have been threatened by growing pressure due to an increase in the demand for edible species. Moreover, the commercial take (harvest) of seaweeds in northern Spain is subject to minimal regulation, and seaweed resource management is not based on the bio-ecological features of each target species (information obtained from Xunta Galicia, available at http://www.pescadegalicia.com). Under this scenario, mariculture should be developed in parallel to management and conservation strategies for *S. latissima* and other commercial kelps.

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Table 1. Biomass yield of cultivation by transplanting of young fronds of *Saccharina latissima* after the cultivation period in the sea (n = 7 culture ropes of 20 m length)

Transplanting (14–15 March 2006)	Harvesting (26–27 June 2006)	
Fresh yield per length rope (kg fresh m^{-1} rope)	Fresh yield per length rope (kg fresh m^{-1} rope)	Fresh yield per hectare farm* (ton fresh ha^{-1} farm)
2.1 ± 0.2 (1.8–2.5)	7.8 ± 1.1 (6.2–8.8)	45.6

Data expressed as mean ± standard deviation, minimum–maximum are shown in parentheses when applicable

* sum of the fresh weight of all culture ropes in an area of 240 m^2

Table 2. Absolute growth rate (mean ± standard deviation) of transplanted fronds of *Saccharina latissima*, estimated as length and wet weight increase per day after the cultivation period in the sea (*n*=100 fronds)

Transplanting (14–15 March 2006)		Harvesting (26–27 June 2006)		Absolute growth rate (AGR)	
Lengh (cm)	Fresh weight (g)	Length (cm)	Fresh weight (g)	AGR_{length} (cm d^{-1})	AGR_{weight} (g d^{-1})
44 ± 8 (35–62)	22 ± 4 (12–35)	120 ± 8 (101–147)	86 ± 6 (76–99)	0.72 ± 0.12	0.61 ± 0.07

Data expressed as mean ± standard deviation, minimum–maximum are shown in parentheses with the exception of yield per hectare

Table 3. Environmental conditions during the cultivation period (14 March to 27 June 2006) at a site adjacent to the farm location in Santander on the Cantabrian coast (northern Spain).

Parameter		Values
Temperature (ºC)		13.2 ± 2.2 (11.1–16.2)
Irradiance (μmol m^{-2} s^{-1})		223 ± 80 (0–746)
Photoperiod (h light: h dark)		13:11 (11:13–15:9)
Nutrients (μM)	Nitrate	4.9 ± 4.2 (0.50–9.05)
	Nitrite	0.25 ± 0.1 (0.08–0.38)
	Phosphate	0.26 ± 0.2 (0.09–0.43)
Salinity (psu)		35.2 ± 0.6 (34.4–35.6)

Data expressed as mean ± standard deviation, minimum–maximum shown in parentheses.

Fig. 1. Fixation of young *Saccharina latissima* fronds to culture ropes by rubber bands (not drawn to scale)

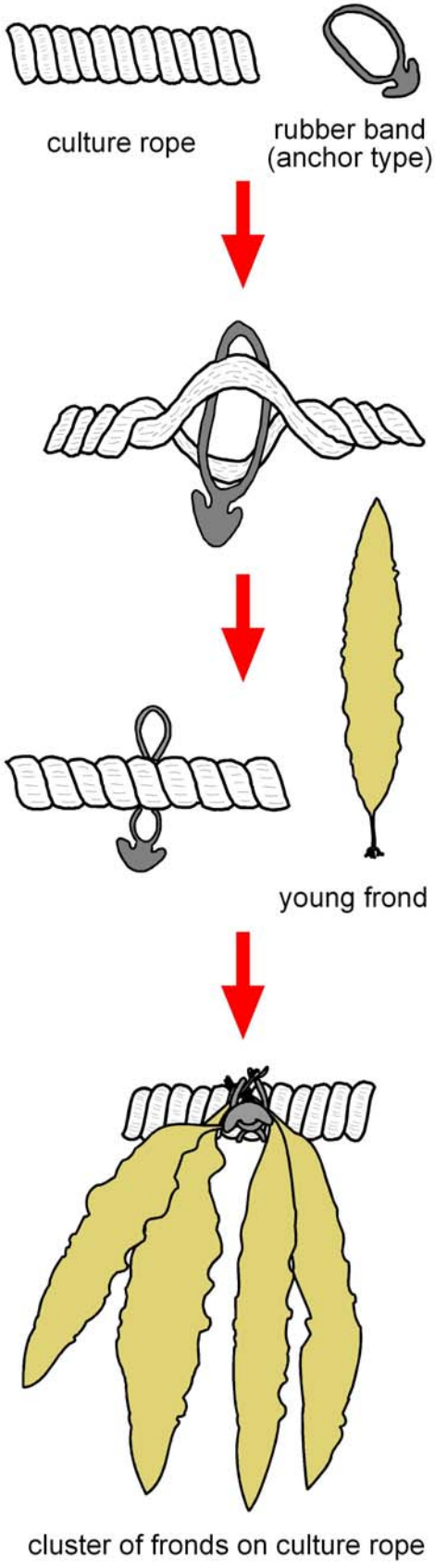

Fig. 2. Culture site of *Saccharina latissima* in Mataleñas, outside the Bay of Santander on the Cantabrian coast, northern Spain. *S. latissima* distribution is indicated by a red line along the coast of the Iberian Penninsula and the southern European Atlantic (map 1), obtained from Lüning (1990), Pérez-Ruzafa et al. (2003) and Müller et al. (2009)

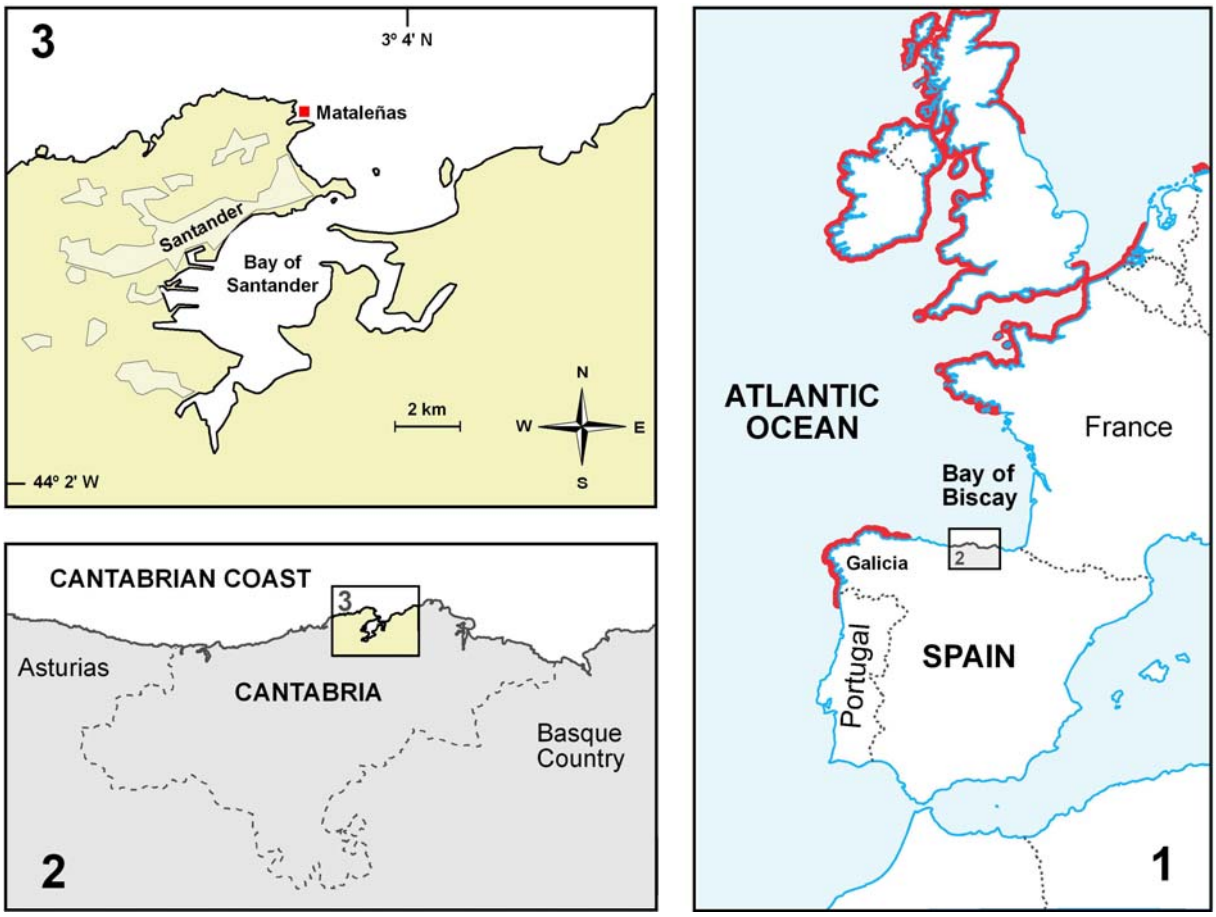

Fig. 3. Design of the floating raft with long-line rope culture for the open-sea cultivation of *Saccharina latissima*

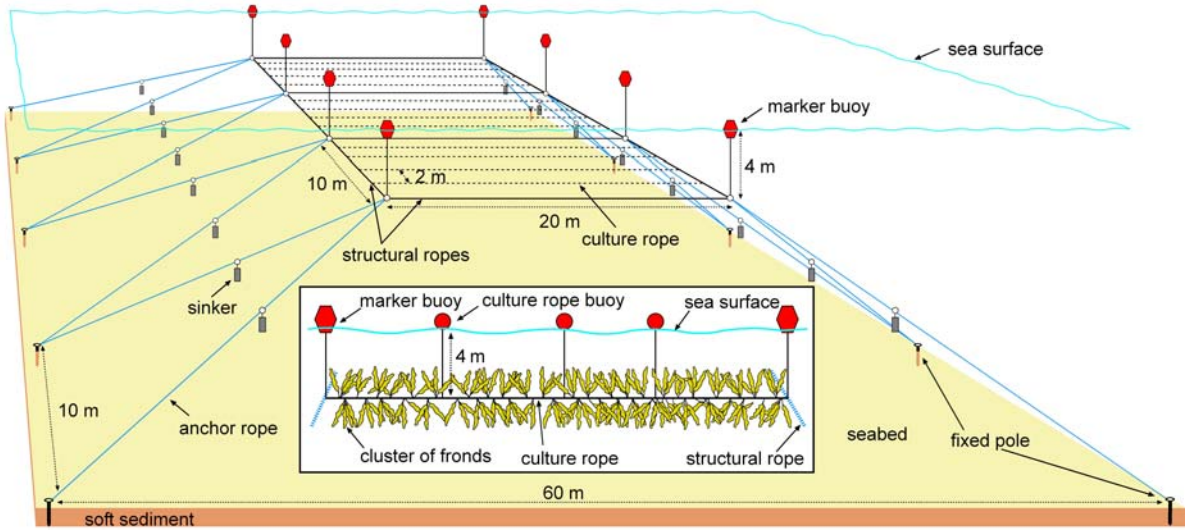

Fig. 4. Major seasonal stages in the cultivation method of *Saccharina latissima*: cultivation by transplanting (as proposed in this study) vs. regular cultivation (as practiced off the Atlantic coast of Spain).
Arrows indicate the duration (from October to June) of each stage. In the regular cultivation method, the flexible period of dates (the window for each stage) is represented by dashed arrows and comprises the time between the early period (the earliest dates when each stage could start or finish) and the late period (the latest dates when each stage could start or finish).