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Open sea cultivation of *Palmaria palmata* (Rhodophyta) on the northern Spanish coast

Brezo Martínez [a,*], Rosa M. Viejo [a], Jose M. Rico [a], Ruth H. Rødde [b], Vanesa A. Faes [a], Jesús Oliveros [a], Dámaso Álvarez [a]

[a] *Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, c/ Catedrático Rodrigo Uría s/n, E-33071 Oviedo, Spain*
[b] *Department of Biotechnology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway*

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Abstract

The aim of this study was to adapt the techniques of rope culture to the cultivation of the edible seaweed *Palmaria palmata* on the northern Spanish coast. Vertical rope rafts were installed in two locations. Fronds were attached to 4-mm polyethylene ropes that were suspended from a steel frame secured in position using weights and buoys. In the first two trials (April to August 1999) we investigated the effects of the outplanting season (spring–summer), and length of the cultivation period (number of weeks) on the growth of *P. palmata*. Cultivation in autumn and winter was not performed due to the rough sea conditions. The following three trials (April to August 2000) aimed to test the effects on the growth and quality (i.e. N content) of the fronds of other additional factors: cultivation technique (fronds inside mesh bags versus directly inserted into ropes), use of marginal proliferations as source stock (versus field material), different stocking densities, and addition of nutrients versus no enrichment. Maximum observed growth during the best cultivation season in spring was about 14% of the initial fresh weight per day (about 0.7 g FW). The growth of cultivated fronds was noticeably greater than the growth of field individuals and four weeks was a suitable period for cultivation. The bag method was better than inserting the fronds into ropes due to the avoidance of frond loss, and enhancement of the quality. Appropriate stocking density was very important when using bags since the growth tended to decrease with increasing number of fronds per bag. The artificial nutrient enrichment also enhanced the quality of the fronds in two locations, and the growth in one site (with lower seawater nutrient concentration). Nutrient enhanced fronds grew at a rate similar to that observed one month and a half earlier when nutrient concentration was higher. In Spain the stock of *P. palmata* is limited. However, the marginal proliferations grew at a similar rate than field material partially solving this limitation. Results from these trials suggest the potential to aquaculture *P. palmata* in northern Spain.

Keywords: Bag cultivation; Fertilizer; Mariculture; *Palmaria palmata*; Rope cultivation

1. Introduction

Humans have carried out the culture of seaweeds for hundreds of years and it is well developed in several Asian countries (Perez, 1992; Ohno and Critchley, 1993; Lobban and Harrison, 1997; Neori et al., 2004).

* Corresponding author. Present address: Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos. c/ Tulipán s/n, Móstoles, E-28933, Madrid, Spain. Tel.: +34 914888102; fax: +34 916647490.
E-mail address: brezo.martinez@urjc.es (B. Martínez).

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Nowadays, cultured seaweeds represent most of the seaweed production, which is about 10 million tons fresh weight (FW) worldwide (Lüning and Pang, 2003). However, the use of seaweeds as food and its cultivation in western countries is by far less developed (Browne, 2001). *Palmaria palmata* (Linnaeus) Kuntze is probably the most popular seaweed used as food in North America and Europe, and has been also used for feeding abalone in hatcheries (Morgan et al., 1980a; Irvine and Guiry, 1995; Le Gall et al., 2004). This species, commonly known as "dulse" in the northern Europe, is a good source of dietary requirements, rich in vitamins and minerals, proteins, and fibre (Morgan et al., 1980b; Lahaye et al., 1993). *P. palmata* is commercially harvested and sold in dry form by several food selling companies in Canada, USA, Ireland, and UK (Chopin, 1998; Browne, 2001). The development of the mariculture of this species has been suggested to have high potential in Canada and in the USA (Chopin, 1998; Cheney, 1999). Cultivation in small tanks (8 l) received large attention in the 1980s in Nova Scotia (Morgan et al., 1980a; Morgan and Simpson, 1981a,b,c). Recently, cultivation in larger tanks has been developed with fronds collected from Roscoff (France), and open sea cultivation has been tested in Northern Ireland (Browne, 2001; Pang and Lüning, 2004).

We summarize in this paper the results from the first open sea cultivation of *P. palmata* on the northern Spanish coast. The cultivation technique was modified from rafts culture methods developed for *Laminaria japonica* Areschoug mass cultivation in China, and from *P. palmata* experimental cultivation in Northern Ireland (FAO, 1989, 2001, 2003; Perez, 1992; Ohno and Critchley, 1993; Browne, 2001). The aim of our study was to adapt the known techniques of vertical rope raft culture to suit the local conditions of the coast of Asturias (northern Spain). For this purpose, we investigated the effects of several factors on the growth and quality (i.e. N content) of the fronds during five successive cultivation trials.

The two first trials aimed to test the best outplanting season given that field *P. palmata* showed their highest growth rates during spring (Faes and Viejo, 2003). The optimum length of the cultivation period (number of weeks) was also tested. High growth during the first weeks followed by reduced growth has been observed in cultures of *Gigartina atropurpurea* J. Agardh and other red seaweeds (McNeill et al., 2003).

In the next trial the cultivation of fronds inside mesh bags was compared to the cultivation of fronds directly inserted into ropes following the more traditional vertical rope culture techniques (FAO, 1989, 2001, 2003; Perez, 1992; Ohno and Critchley, 1993). The coast of Asturias is exposed to waves with few moderately sheltered sites. The use of mesh bags in such conditions has been shown to prevent the detachment of algal material (Ask and Azanza, 2002; Reddy et al., 2003).

The efficiency of an alternative method to the collection of field material for culturing was tested in the fourth trial. The ultimate objective was the reduction of harvest pressure on the natural populations. *P. palmata* develops marginal proliferations that were collected from the material harvested in the third trial and used as source stock. Pruning methods are common in algal cultivation procedures to assure the sustainability of the cultivation system (Melo et al., 1991; Perez, 1992; Ohno and Critchley, 1993; Lobban and Harrison, 1997; McNeill et al., 2003).

The third and fourth trials also tested the hypothesis that increasing the density on ropes, or increasing the number of fronds inside mesh bags, would cause a decrease in growth due to the reduction of light and nutrients (Browne, 2001).

Finally, in the fifth trial we tested the effect of an artificial fertilizer on frond growth and quality. In the studied area, the growth of *P. palmata* fronds from the field populations is limited by very low nutrient concentrations in late summer (Martínez and Rico, 2002; Faes and Viejo, 2003). Nutrient enrichment treatments have been shown to prevent growth and quality decay in cultures from other geographic areas (FAO, 1989, 2001, 2003; Ask and Azanza, 2002; Tseng, 2004).

These experiments represented a pioneer activity in Spain since algae are not traditionally consumed nor cultured in this country (Juanes and Sosa, 1998).

2. Material and methods

2.1. Sites

The *P. palmata* culture rafts were placed in two locations along the coast of Asturias (northern Spain): *Ensenada de Arnao* (43°33′ N, 7°01′ W) and *Concha de Artedo* (43°34′ N, 6°12′ W). The *Ensenada de Arnao* (Arnao) is a bay at the eastern side of the mouth of an embayment about 5 km long (*Ría de Ribadeo*). The hydrography and dynamics of the *Ría de Ribadeo* are dominated by tidal and wave forcing. The site where the rafts were set is subjected to a significant outflow current caused by the wave breaking at the west side (Piedracoba et al., in press). The *Concha de Artedo* (Artedo) is a bay dominated by wave forcing due to its reduced size (about 1.5 km long). The seasonal variations of major environmental factors in Artedo have been published elsewhere (Martínez and Rico, 2002). Temperature records from the temperature monitoring program currently undergoing by the *Ecology Department* of the *University of Oviedo* reveal that the temperature regime in Arnao is similar to the seasonal variation measured at Artedo and summarized in Martínez and Rico (2002). These sites were selected because they are moderately sheltered to the dominant wave regimes, providing some protection to the culture units. They have the suitable water depth for accommodating the floating rafts, and are easily accessible by boat. Both represent the best potential sites for the installation of commercial seaweed farms in the coast of Asturias.

2.2. Cultivation procedure

A total of 3–5 culture ropes (depending on the trial) were secured to a steel bar (1 m long). The bar was suspended 1m below the sea surface from two buoys and fixed in position on the seabed by two concrete anchor weights (40 kg each). Four-millimeter polyethylene ropes were used for growing the fronds; each one was 3 m in length. A second steel bar at the bottom kept all ropes in place avoiding rope twisting. Three independent floating rafts separated 3 m and sited in parallel were adopted as the cultivation unit (Fig. 1). One cultivation unit was used per locality in 1999 and two units separated around 50 m were set up in 2000.

P. palmata fronds collected subtidally at Artedo were used as source stock material. When inserting the fronds was not possible the same day of collection, fronds were kept submersed in the sea inside a wide mesh bag (1 cm width) less than 24 h. Only fronds free from epiphytes and dark-red in colour (healthy-looking fronds) were selected for cultivation. Many fronds were ramified showing small marginal proliferations at the time of collection.

2.3. Cultivation experiments in 1999

The effect of the outplanting season (spring–summer), and length of the cultivation period (number of weeks) were analysed during 1999 in two locations (Artedo–Arnao). One trial started in April (spring culture) and a second trial in June (summer culture). Trials in autumn and winter were not performed due to rough sea conditions that are common at these seasons in the studied area. A culture period of approximately three months was used. The initial mean length of the fronds was 14.8 cm (±0.16 SE, $n=969$). Frond length and numbers were monitored weekly or fortnightly. Single fronds were inserted in the lay of the ropes by opening the twist and pushing the fronds through the lay (see Kain (Jones), 1991). Fifteen vertical ropes were seeded and hung from the three floating rafts installed at each locality (five ropes per raft). All ropes in each cultivation unit were seeded with a mean density of 12–15 fronds/m of rope in spring and 22–25 in summer.

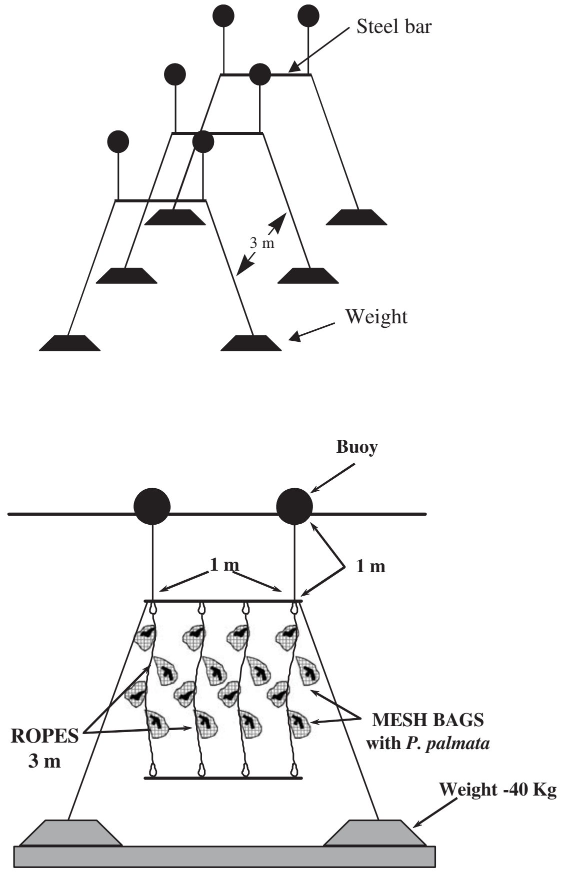

Fig. 1. Vertical rope culture rafts adapted for the local conditions and experiments done. Each unit consisted of three floating rafts.

2.4. Cultivation experiments in 2000

The following three trials were set up in the spring and summer of 2000. The fronds were only attached to the bottom meter of each rope between 3 and 4 m depth. This reduced the amount of material needed for the experiments, and thus the demand of individuals from natural populations. The deepest meter was chosen because individuals from filed populations growing underneath kelp canopies showed higher nutritional value and quality (i.e. N and protein content) than those growing in high light conditions (Martínez and Rico, unpublished data).

The third cultivation trial was done in Artedo (April–May) and examined the effects of cultivation procedure (fronds inserted into ropes at density of 30/m, and compared to fronds placed inside bags with 10 fronds/bag and 3 bags/m), and frond density (10, 30 and 60 fronds/m of rope) on the growth rate. The bags were made of a 25 × 30 cm polyethylene net 4 mm in width, which filtered out 10% of the incident light. The effect of using bags in the quality of the fronds was also tested. In subsequent experiments only the bag procedure was used as a consequence of the results of this experiment.

In the fourth trial (Artedo, May–June) the growth and quality of the marginal proliferations collected from the material cultivated in the third trial were compared to that from small fronds collected in the field at a density of 10/bag. Small marginal proliferations (<7 cm in length) were excised at their base from the edge of bigger fronds. The effect of the different density of fronds inside bags (10, 20 and 30 fronds/bag and 3 bags/m) on the growth was also tested.

The fifth experiment (July–August) tested the combined effect of *Locality* (two locations: Artedo versus Arnao) and *Enrichment* (control versus nutrient enhanced units) on the growth and quality of the fronds. A pair of fishnet stockings, each containing 250 g of a controlled release fertilizer (*Multicote 4 months*, 20N : 10P : 20K, Haifa Chemicals Ltd., Haifa Bay, Israel), was attached to half of the ropes close to each bag. All the bags contained 10 fronds both in the nutrient enhanced and control treatments.

To compare frond growth among trials run during spring and summer, bags with a density of 10 fronds/bag were included in the three cultivation trials done during 2000.

In all trials, the ropes were randomly located in the two units available. However, in the last trial (effect of *Locality* and *Enrichment*) all the nutrient enhanced ropes were set together in a single cultivation unit per locality to avoid an undesirable enrichment of the water around the control ropes. Therefore, the effects of the addition of fertilizer may be confounded with possible spatial differences in this trial. However, previous study in Artedo showed no spatial changes in nitrogen (N) and phosphorus (P) inorganic concentration according to the very low values of these compounds in summer (Martínez and Rico, unpublished data).

At the start of the experiments in 2000, the initial mean fresh weight per frond was 0.73 g (±0.02 SE, $n = 47$) without significant differences among treatments, locations or periods. The number and weight (FW) of fronds per meter of rope (or per bag) were recorded at the beginning and the end of each experiment. The mean increase in FW (final–initial FW) per frond per day was calculated in order to make comparisons among trials, and densities. Additionally, in the third trial the lengths of the fronds were also measured. Mean initial length in this trial was 7 cm (±0.3 SE, $n = 55$). The experiments were run for 23 to 33 days.

2.5. Biochemical analyses

Nutrient contents of fronds from different cultivation procedures: bags versus fronds inserted into ropes (third trial), origin of the fronds (newly collected versus marginal proliferations; fourth trial), and *Locality* and *Enrichment* treatments (fifth trial), were recorded in the experiments of 2000 to characterize the quality and nutritional value of the fronds. Two samples per treatment were analysed in the third and fourth trials, and 10 samples in the fifth trial. Biochemical analyses were performed after drying the samples in a circulation dryer at temperatures below 40 °C, and grinding the dried sample in a hammer mill with a 2 mm sieve. Thallus N content was measured using a CNH Elemental Analyser (Perkin Elmer Analytical Instruments, Shelton). P extraction from thallus was performed by alkaline persulphate digestion (Ameel et al., 1993), and phosphate was measured as described in Koroleff (1983).

To further characterize the dietary value and physiological status of the fronds cultured by the two different methods (bags and fronds inserted into ropes), ash and main carbohydrates content (xylan, glucose and floridoside) were analysed in two samples from the third trial. Thallus ash content was determined by heating the samples to 530 °C for 18 h (Larsen, 1978). For carbohydrate extraction dry algal powder was subjected to pre-hydrolysis with 80% sulphuric acid (18 h, 4 °C) prior to hydrolysis by 1 M sulphuric acid (4 h, 100 °C) according to Haug and Larsen (1962). The quantitative monosaccharide composition was determined by gas chromatography (Chaplin, 1982), and new extinction coefficients were calculated. The concentrations of xylan, a dietary fibre, and glucose were determined according to methods in Dubois et al. (1956). Floridoside was extracted in boiling water for 4 h and then determined by the method of Dubois et al. (1956) after a new extinction factor was calculated by gas chromatography.

2.6. Seawater nutrient concentrations

N and P levels in seawater were monitored during the fifth trial (2000) near the nutrient enhanced and the control ropes. A total of two samples per treatment and locality at 4 different dates were taken. Seawater concentrations of nitrate, nitrite, and orthophosphate were measured as described in Koroleff (1983) and ammonium as outlined in Álvarez (1993) using a Technicon II Autoanalyzer (Industrial Method no. 158-71 W/A, Ireland). All the inorganic N forms showed parallel trends during the experiment thus their concentration values were summed to calculate total N.

2.7. Statistical analyses

The initial length of fronds varied between locations in experiments of 1999. Mean frond length was compared between sites after four weeks of cultivation using Analysis of Covariance (ANCOVA), with initial length as covariate. Mean increases in FW per frond in experiments of 2000 were analysed using uni- or bifactorial Analysis of Variance (ANOVAs). *Locality* was considered a fixed factor. Analyses of total N and P in seawater and of thallus constituents were also done using multi- and unifactorial ANOVAs. In ANOVAs involving bag cultivation procedures an additional factor, *Rope*, was nested in the combination of main treatments. Cochran's test was used to test for heterogeneity of variances. When indicated data were transformed to homogenize variances (see Tables). Student–Newman–Keul (SNK) tests were used to discriminate among different treatments after significant *F*-tests. All tests were done with SPSS (11.0.1) for Windows.

3. Results

3.1. Cultivation experiments in 1999

Mean length of cultivated fronds increased in 1999 during the first trial (spring), and at the beginning of the second trial (summer) (Fig. 2). The increase was especially marked in the first four weeks of culture in spring. During this time interval, the growth was similar between locations (ANCOVA for differences between locations in final mean length of fronds, $F_{1,290}=0.13$, $p=0.713$). Fronds in Arnao grew for a longer period than in Artedo during the spring experiment. Plants from both sites were bleached and covered by epiphytes at the end of the cultivation

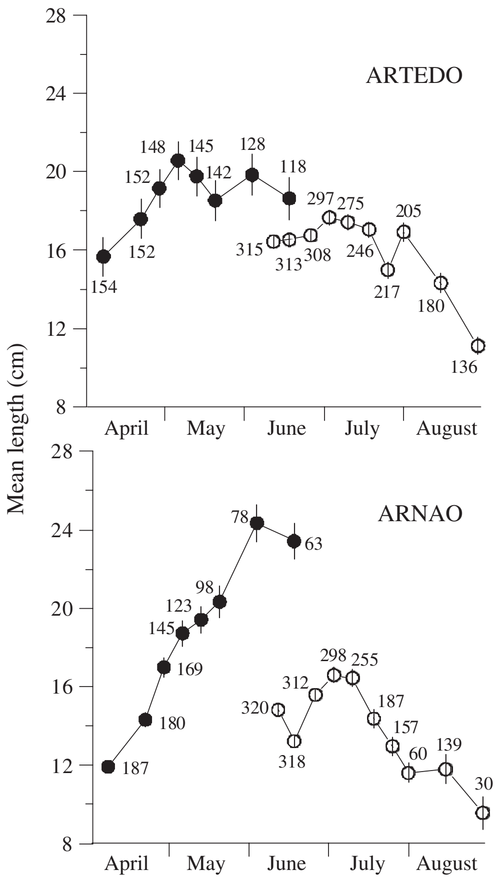

Fig. 2. Temporal changes in frond length of *Palmaria palmata* inserted into ropes in 1999 at Artedo and Arnao (first and second trials). Closed symbols indicated the first trial (spring) and open symbols indicate the second trial (summer). Error bars represent standard error, numbers indicate total number of fronds (n) inserted into 15 vertical ropes.

period, and there were considerable losses of fronds attached to ropes from the beginning of the experiments. In Arnao, 76.1% of all fronds in spring and 90.6% in summer were lost. Losses in Artedo were lower (22.4% in spring and 56.5% in summer).

3.2. Cultivation experiments in 2000

In the third trial, fronds cultured inside bags, and those directly inserted into ropes at different densities, showed similar growth rates (Fig. 3, one-way ANOVA, $F_{3,12}=1.80$, $p=0.201$). Mean daily frond growth was 103.7 mg FW (±7.49 SE, $n=16$). This corresponded to a daily increase of about 14% of the mean initial weight of the fronds. Fronds cultured inside bags during this trial showed a final mean length of 21 cm (±1.5 SE, $n=10$). The density of fronds directly inserted into ropes varied during the

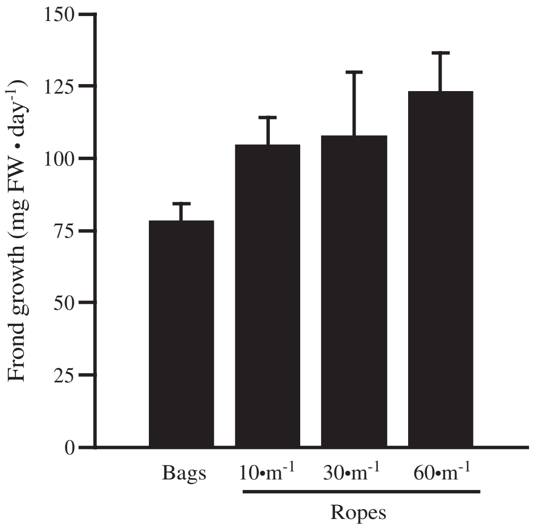

Fig. 3. Mean daily frond growth of *Palmaria palmata* cultivated inside bags and inserted into ropes at three densities in the third trial (April–May 2000). Error bars represent standard error ($n=4$).

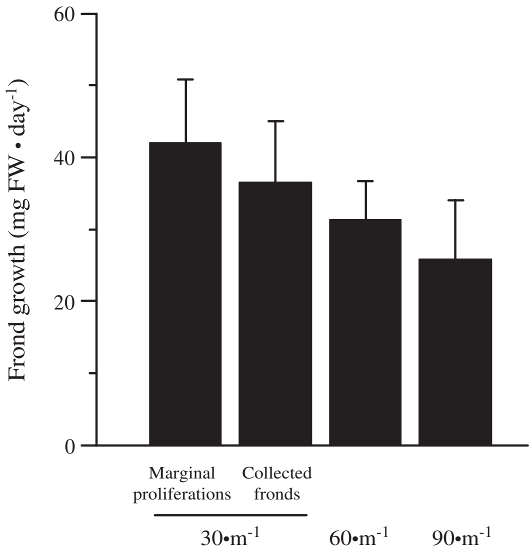

Fig. 4. Mean daily frond growth of *Palmaria palmata* cultivated inside bags during the forth trial (May–June 2000). Fronds were collected from the field and grown at three densities or marginal proliferations excised from harvested fronds and grown at one density. Error bars represent standard error ($n=9$).

experiment due to losses. The proportion of frond losses was similar at all densities used (one-way ANOVA, $F_{2,9}=1.89$, $p=0.207$), with an overall mean of 28% (±21.5 SE, $n=12$) of the initial number of fronds. Moreover, fronds directly inserted into ropes bleached by the end of the experiment independently of the cultivation density used. This was associated with significant differences in the physiological status of the fronds. Floridoside content was higher in fronds directly inserted into ropes than in fronds within bags (Table 1). The opposite trend was detected for N content. Low glucose content was measured and no significant differences were found in this component or in P content, xylan, and ash between cultivation methods.

In the forth experiment a trend was observed for frond growth to decrease with increased frond density inside bags (Fig. 4). Moreover, marginal proliferations tended to grow more than newly collected fronds at any density (nested ANOVA, $F_{3,8}=3.90$, $p=0.054$). Significant differences in thallus N and P content were not observed (one-way ANOVAs, overall mean % DW; nitrogen: $F_{3,4}=0.70$, $p=0.601$, 1.1±0.06 SE; phosphorous: $F_{3,4}=1.36$, $p=0.375$, 0.1±0.01 SE; $n=8$).

In the enrichment experiment (fifth trial during July–August), N and P in seawater around control and nutrient enhanced ropes varied among locations and dates (ANOVAs, significant interaction *Enrichment×Locality×Date*, $F_{3,16}=31.37$, $p=6.19\times10^{-7}$ for total N; $F_{3,16}=14.47$, $p=8.04\times10^{-5}$ for orthophosphate). A significant increase in total N around nutrient enhanced ropes was detected at Arnao in the first and last dates and at Artedo in the first and second dates. Differences in orthophosphate between

Table 1
Analyses of variance for differences in N, P, floridoside, xylan, glucose, and ash content in fronds cultivated inside bags or inserted into ropes in the third trial (April–May 2000)

	df	MS source	MS residual	*F*	*p*	Mean±SE % DW, *n*=2		
						Bags	Ropes	Overall
N	1,2	0.374	0.015	25.142	0.038	2.25±0.3	1.28±0.1	–
P	1,2	0.003	0.001	2.473	0.256	–	–	0.183±0.03
Floridoside	1,2	136.890	0.530	258.283	0.004	14.50±0.7	26.20±0.2	–
Xylan	1,2	2.723	3.262	0.834	0.457	–	–	27.37±1.0
Glucose	1,2	4.000	0.565	7.080	0.117	–	–	4.75±0.8
Ash	1,2	124.769	16.544	7.542	0.111	–	–	25.74±4.2

Variances were homogeneous.

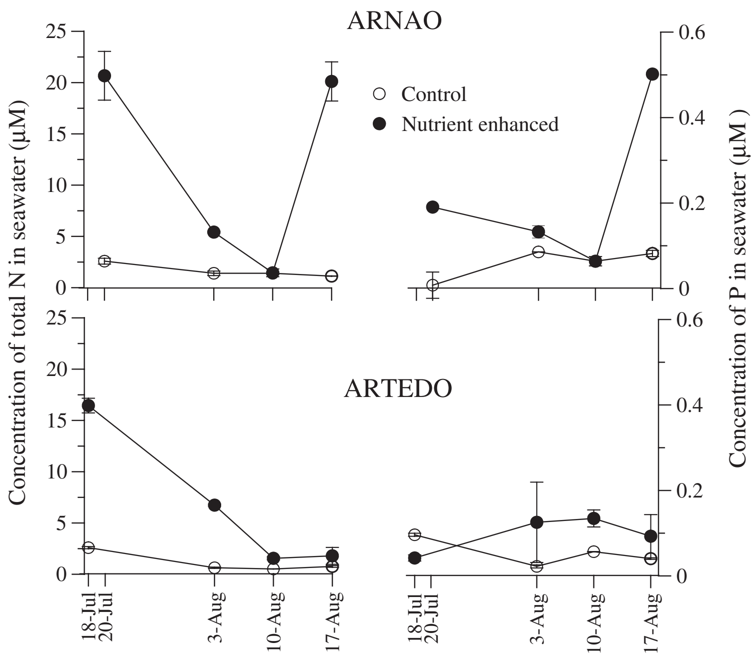

Fig. 5. Changes in levels of total N and P through time around culture rafts of *Palmaria palmata* during the fifth trial (July–August 2000) at Arnao and Artedo. Closed and open symbols indicate nutrient enhanced or control cultures, respectively. Error bars represent standard error ($n=2$).

treatments were detected at Arnao in the last date (Fig. 5). These data support that inorganic nutrients were released from the fertilizer pellets. When only controls were compared, total N was higher in Arnao than in Artedo in 2 out of 4 sampling dates (Table 2, SNK test). P levels were also higher at Arnao in 2 out of 4 sampling dates, and at Artedo in 1 date. Frond growth was affected by the combined effect of *Locality* and *Enrichment* treatments (Table 3, Fig. 6A). Fronds on nutrient enhanced ropes in Artedo had higher growth rates compared to those on control ropes, whereas no significant differences were found between treatments at Arnao. Higher N content was observed in nutrient enhanced fronds from both sites. In addition, fronds from Arnao had higher N content than those from Artedo irrespective of the treatment (Table 4, Fig. 6B). P content was higher in fronds from Arnao than from Artedo but no differences were observed in response to the addition of nutrients within locations (Table 4, Fig. 6C).

Growth was highest at the beginning of the spring (April–May 2000), and declined towards July–August in Artedo (Fig. 7, ANOVA, $F_{3,12}=52.13$, $p=3.7\times10^{-7}$). Frond growth in nutrient enhanced bags in July–August

Table 2
Analyses of variance of differences in (a) total N and (b) orthophosphate in seawater around control ropes in the different locations (Artedo and Arnao) and sampling dates (experiment of July–August 2000)

Source	*df*	(a) N in seawater			(b) P in seawater		
		Mean square	*F*	*p*	Mean square	*F*	*p*
Locality	1	0.876	3.330	0.165	1.41×10^{-4}	0.031	0.871
Date	3	3.001	72.860	0.001	0.83×10^{-4}	0.290	0.831
Locality×*Date*	3	0.263	6.374	0.016	44.90×10^{-4}	15.676	0.001
Residual	8	0.041			2.87×10^{-4}		
SNK tests		Dates 2, 3: Arnao > Artedo			Date 1: Artedo > Arnao		
		Dates 1, 4: Arnao ≈ Artedo			Dates 2, 4: Arnao > Artedo		
					Date 3: Arnao ≈ Artedo		

Mean values differing between locations are indicated (from SNK tests). Variances were homogeneous.
≈ non-significant differences at $p<0.05$.
> significantly higher at $p<0.05$.

Table 3
Analysis of variance of the effect of *Locality* and *Enrichment* on the daily frond growth (experiment of July–August 2000)

Source	Mean square	*df*	*F*	*p*
Locality	0.023	1	2.62×10^{-5}	0.996
Enrichment	407.450	1	0.471	0.498
Locality × *Enrichment*	864.861	1	12.199	0.002
Pooled Residual	70.897	27[a]		

The effect of Rope (nested in the interaction *Locality* × *Enrichment*) was not significant ($p > 0.25$) and it was thus pooled with Residual (Winer et al., 1991).
[a] In order to homogenize variances, one data was replaced by the mean of the group and 1 *df* subtracted from Residual.

was similar to that in control bags in the previous period (April–May).

4. Discussion

Spring was the optimal season for mariculture of *P. palmata* in northern Spain. Spring is frequently the best cultivation season for seaweeds from diverse geographic areas (e.g. McNeill et al., 2003). The individuals cultivated, irrespective of the method used, reached their annual maximal growth in May 1999 and 2000. Parallel growth trends were observed in individuals from a close population (Faes and Viejo, 2003). *P. palmata* is a pseudoperennial seaweed that shows its maximal growth during spring; growth is reduced during summer and ceases in August in the studied area (Faes and Viejo, 2003). A small proportion of the thallus persisted over autumn and winter, but showed no evidence of growth and thus cultivation at these seasons could hardly proceed. Moreover empty rafts were left and damaged by winter and autumn storms that are common in the studied area.

Growth of cultured fronds was noticeably greater than values from natural populations. The elongation of fronds at the beginning of spring 2000 (April–May) was about 14 cm showing a final mean length of about 21 cm. This elongation was noticeably greater than the increase of about 3.5 cm showed by field individuals during the same period (Faes and Viejo, 2003). At this time of the year, the daily weight increase (about 14% initial FW) was among growth values observed in tank cultures in previous experiments (Morgan et al., 1980a; Morgan and Simpson, 1981a,b,c; Pang and Lüning, 2004). However, growth rates were higher in fronds cultured in open sea in Strangford Lough, Northern Ireland (Browne, 2001). The vicinity of the Spanish cultures to the southern distributional limit of *P. palmata* may explain this difference. Spanish populations typically show smaller individuals probably due to their peripheral nature (Kain, 1986; Irvine and Guiry, 1995). This pattern has also been observed in other macroalgae (Carter and Prince, 1988; Bengtsson, 1993).

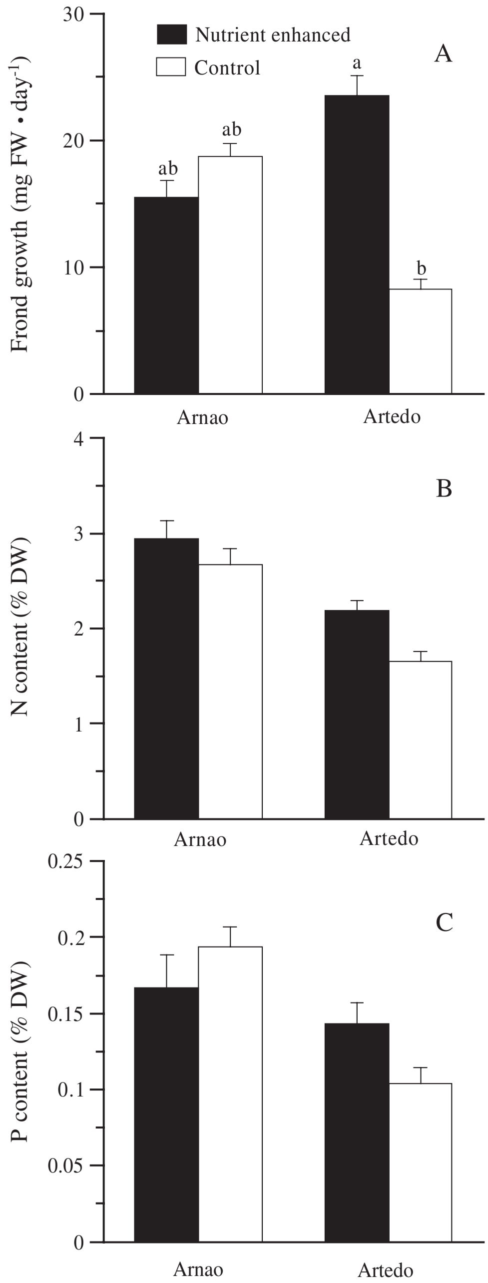

Fig. 6. Differences in daily frond growth (A), N content (B), and P content (C), in *Palmaria palmata* cultivated inside bags during the fifth trial (July–August 2000) at Arnao and Artedo. Solid bars indicated nutrient enhancement and open bars indicated control fronds. Error bars represent standard error ($n = 8$ for daily frond growth, $n = 10$ for N or P). In A) lower case letters denote significantly different mean values ($p < 0.05$; Student–Newman–Keul test after significant F-test).

Table 4
Analysis of variance of the effect of *Locality* and *Enrichment* treatment on (a) the N and (b) P content of fronds (experiment of July–August 2000)

Source	*df*	(a) Thallus N content[a]			(b) Thallus P content[b]		
		Mean square	*F*	*p*	Mean square	*F*	*p*
Locality	1	0.009	39.497	0.001	0.042	23.220	0.001
Enrichment	1	0.002	9.052	0.005	0.002	1.102	0.301
Locality × *Enrichment*	1	0.0003	1.502	0.228	0.006	3.490	0.070
Residual	36	0.0002			0.002		

[a] Data were arcsine transformed to homogenize variances.
[b] In order to homogenize variances, one data was replaced by the mean of the group and 1 *df* subtracted from Residual.

Four weeks was a suitable period for cultivation. Growth was severely reduced if fronds were cultured over a longer period particularly at one of the studied sites (Artedo), and several problems, such as frequent frond loss, bleaching, and coverage by epiphytes, increased with the length of rope cultivation in both locations. A period of 4 weeks also produced the highest sustained yields in open sea cultivation in Strangford Lough and in tank culture (Morgan et al., 1980a; Browne, 2001). Maximal growth rates were also observed in the thirty days after pruning and transplantation in many seaweeds (e.g. McNeill et al., 2003).

The growth of the fronds cultured inside bags and inserted into ropes was similar, but the bag method solved the problem of frond loss. Very often when fronds were passed through the ropes the tissue underneath the rope bleached and eventually broke. Especially in Arnao, where an outflow current is present (Piedracoba et al., in press), entire fronds were also detached from the rope. Wave force in both sites, and the wave and tidal driven currents in Arnao accelerated frond losses. The bag method is particularly recommended in the exposed conditions that prevail along the northern Spanish coast. This procedure also provided more consistent yields as it avoided the problem of frond detachment and lessened the impact of grazing, both problems also associated with rope culture of other species (Ask and Azanza, 2002; Reddy et al., 2003).

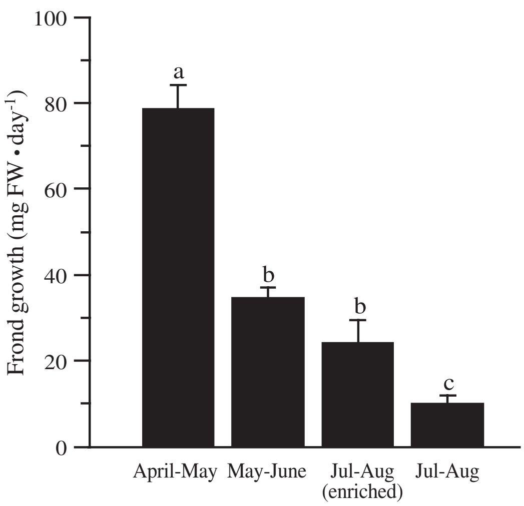

Fig. 7. Mean daily frond growth of *Palmaria palmata* cultivated inside bags through time in the trials done during spring and summer 2000 in Artedo (nutrient enhanced or not nutrient enhanced in July–August). Error bars represent standard error ($n = 4$). Lower case letters denote significantly different mean values ($p < 0.05$; Student–Newman–Keul test after significant F-test).

Moreover, the use of bags enhanced the dietary value of the harvested material increasing the N content of fronds. In *P. palmata* the protein pool represents up to 93% of total cell N, thus higher N content of fronds was related to higher protein and food quality (Martínez and Rico, 2002). Furthermore, fronds cultured inside bags exhibited lower floridoside content. Higher incident light in the fronds inserted into ropes may explain these differences. Storage of carbohydrates (such as floridoside) was activated to benefit from higher light conditions (Meng and Srivastava, 1993). This response is well known in macroalgae (Neish et al., 1977; McGlathery and Pedersen, 1999; Lüning and Pang, 2003) and particularly in this species (Morgan and Simpson, 1981a; Martínez and Rico, 2002), and is commonly associated with a decrease in the N content of the thallus as shown in this study (Neish et al., 1977; McGlathery and Pedersen, 1999). In addition to increased yield and quality, the use of bags reduced the personnel required particularly during outplanting due to the easier manipulation.

Appropriate stocking density was very important when using bags. The growth rate tended to decrease with increasing number of fronds per bag. Reduced turbulence, and thus lower nutrient supply, and lower irradiance due to self-shading of the fronds explained similar results in Strangford Lough (Browne, 2001). Lower growth rates with increasing density were also measured in cultures of *Chondrus crispus* Stackhouse inside cylindrical mesh containers (Zertuche-González et al., 2001).

Nutrient enrichment at the end of summer enhanced growth at one of the studied sites. In Artedo, nutrient enhanced fronds in July–August grew at a rate similar to fronds cultured one month and a half earlier. A resin-coated fertilizer, of the same type used in this research, was shown to improve *Gelidium* cultivation in the sea (Melo et al., 1991). In situ nutrient enrichment methods have been extensively used in cultivation of Laminarial and Euchematoid species, and have caused the fast development of kelp farming in oligotrophic Chinese coastal areas (FAO, 1989, 2001, 2003; Ask and Azanza, 2002; Tseng, 2004). Our results suggested that the addition of nutrients would allow the cultivation of *P. palmata* during summer in locations not receiving high nutrient inputs such as Artedo in this study. Fronds also showed higher N in response to the higher seawater nutrient levels due to the enrichment irrespective of the location. The fertilizer increased the quality of the harvested material slowing down the trend of decreasing N content as the cultivation proceeded (source material collected in April: mean % DW: 4.3 ±0.42 SE, $n=2$). Field individuals also showed this natural trend in response to the nutrient starving conditions during summer (Martínez and Rico, 2002). In spite of the increase in quality, the growth of fronds from Arnao was not enhanced in response to the addition of nutrients. In Arnao growth was not nutrient limited probably due to the higher nutrient concentration associated with the estuary and with the nutrient load from large oyster and clam farms operating in this location. Similar growth responses have been observed in bays with high nutrient loading, which are traditionally selected for extensive *L. japonica* cultivation and do not require fertilizer additives (FAO, 1989). Cultivation of macroalgae in such areas may prevent harmful effects from eutrophication, balancing the negative effects of animal cultivation (FAO, 1989; Fei and Tseng, 2003; Fei, 2004). The integration of *P. palmata* cultivation with the bivalves' cultivation in Arnao would reduce potential eutrophication effects. *P. palmata* has been mentioned as a good candidate for such integrated cultivation systems (Chopin et al., 2001; Lüning and Pang, 2003; Neori et al., 2004; Pang and Lüning, 2004).

As pointed out by Waaland (1978), the major requirement for sustained marine algal cultivation is the availability of sufficient source stock. *P. palmata* is patchily distributed and populations are isolated along the Spanish coastline (Faes and Viejo, 2003). The marginal proliferations were suitable source material for the following cultivation trial. Similar procedures are common in macroalgal farming on a commercial scale. In many species the individuals can be pruned back to seedling size to allow a second harvest (Melo et al., 1991; Perez, 1992; Ohno and Critchley, 1993; Lobban and Harrison, 1997; McNeill et al., 2003). The use of marginal proliferations would partially solve the limitation of source material but research is needed to assure the sustainability of the cultivation system. Le Gall et al. (2004) suggested the possibility of seeding the culture ropes with spores of *P. palmata* and then transferring seeded ropes to the sea for cultivation.

Results from these trials suggest the potential to aquaculture *P. palmata* in northern Spain. Cultivation methods should be preferred to harvesting natural populations since the natural stock is limited. To this end, preliminary techniques developed from these trials have been transferred to a new company. This company has installed vertical rope rafts in one of the locations used in this research. Fronds are being cultivated during four weeks inside mesh bags similar to those used in this study.

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