J. Phycol. **43,** 90–100 (2007)

DOI: 10.1111/j.1529-8817.2006.00300.x

SEASONAL GROWTH AND PHENOTYPIC VARIATION IN *PORPHYRA LINEARIS* (RHODOPHYTA) POPULATIONS ON THE WEST COAST OF IRELAND[1]

Elena Varela-Álvarez[2]

Department of Botany and Martin Ryan Institute, National University of Ireland, Galway, Ireland

Dagmar B. Stengel[3] *and Michael D. Guiry*

Department of Botany and Martin Ryan Institute, National University of Ireland, Galway, Ireland

The phenology and seasonal growth of *Porphyra linearis* Grev. were investigated in two morphologically dissimilar populations from the west coast of Ireland. Thallus size and reproductive status of individuals were monitored monthly between June 1997 and June 1998. Both populations exhibited a similar phenology: gametophyte stages appeared on the shore in October, with spermatangial and zygotosporangial sori appearing the following February; the gametophyte stage began to degenerate in April and had disappeared completely by June. However, significant differences in growth and reproduction in the field and in cultures of plants from the two populations were observed. Thallus length and width of individuals from one population were significantly longer throughout the sample period, and reproduction and sporulation occurred 1 month earlier. Also, *in situ* relative growth rates (RGRs) of plants differed significantly and were correlated with different climatic factors (sunshine, day length, irradiance, rainfall, seawater temperature, and intertidal temperatures), suggesting that plants were affected by two different microhabitats. At one site, blades were more exposed to wave action, sunshine, and extreme minimum temperatures, while at the other site, blades were more protected in winter, spring, and early summer. In culture, RGRs of blades from the second site were higher than RGRs of blades from the first site under short days, corroborating the field results and suggesting a degree of phenotypic differentiation between the two populations. However, there were no sequence divergences of the RUBISCO spacer between strains of the two *P. linearis* populations.

Key index words: **Bangiales; conchospore; growth rates; North Atlantic; phenology; phenotype; *Porphyra*; seasonality; RUBISCO**

The genus *Porphyra* was first established by C. Agardh in 1824, and presently there are 267 species names in the AlgaeBase species data base, of which 113 are flagged as current (Guiry and Guiry 2006). These species generally occur intertidally or in the shallow subtidal attached to rocks and other seaweeds. *Porphyra* (known commonly as nori in Japan, zicai in China, and purple laver in Great Britain) is a major source of food for humans and is the most valuable seaweed grown by mariculture in the world today (Hanisak 1998). Several species of *Porphyra* occur along the North Atlantic coast of Europe, but none of these are currently grown under artificial conditions (Varela-Álvarez et al. 1999). If nori cultivation were to develop in Europe, one of the native species should be used. *Porphyra linearis* is a common winter alga in the high littoral and spray zones of northern Atlantic coasts (Bird 1973) and is considered a superior species with a pleasant flavor (McLachlan et al. 1971) and a higher protein content than other *Porphyra* spp. (McGregor 1992).

Previous studies on the life history and phenology of putative *P. linearis* were conducted in Nova Scotia by Bird et al. (1972, Bird 1973) and in Port Erin by McGregor (1992). Bird et al. (1972, Bird 1973) observed that the first appearance of thalli in the supralittoral zone approximately coincided with a sea temperature of 13°C, and a major release of conchospores in culture was produced at this temperature. By contrast, Varela-Álvarez et al. (2004) observed that for Irish isolates of *P. linearis* in culture, optimal conditions for conchocelis growth and conchospore production were 20°C and long days (16:8 light:dark [L:D]), suggesting that intertidal conditions in summer are ideal for the growth of this phase in the wild.

Investigations of the phenology and population dynamics of red seaweeds, and in particular of the genus *Porphyra*, are difficult to carry out because of difficulties in labeling plants in the field and the generally small population sizes. Kornmann and Sahling (1991) described the morphology and life history of species of *Porphyra* in Helgoland, Germany. *Porphyra* blades exhibited phenological differences within a species as a result of gradients of physical factors and variation over time (Conway 1964a, b, Mumford 1976, Boney 1978, Hawkes 1978, Griffin et al. 1999a, b, López-Vivas and Riosmena-Rodriguez 2000, Broom

[1]Received 2 December 2005. Accepted 29 August 2006.
[2]Present address: CCMAR-FCMA, Centre of Marine Science, Faculty of Marine Science, University of the Algarve, Campus Gambelas, P-8005-139 Faro, Portugal. E-mail evarela@ualg.pt.
[3]Author for correspondence: e-mail dagmar.stengel@nuigalway.ie.

et al. 2002). Other studies on the field occurrence of *Porphyra* species have been obtained from general collections made over time (Lindstrom and Cole 1992, Nelson 1993, Nelson et al. 2001, West et al. 2005). Two recent publications (Zertuche-Gónzalez et al. 2004, Hwang et al. 2005) report on the seasonality of growth of *Porphyra perforata* J. Agardh and *Porphyra kuniedae* Kurogi; however, there was no seasonality in the blade phase of *Porphyra dioica* J. Brodie et L. M. Irvine 1997 (Holmes and Brodie 2004).

In the present study, the phenology, growth, and reproduction of two populations of *P. linearis* in Ireland were studied in relation to environmental factors in order to identify populations or ecotypes most suitable for mariculture. The impact of environmental factors (sunshine, day length, irradiance, rainfall, seawater temperature, intertidal temperatures) on the growth of *P. linearis* at two different sites in Galway Bay was studied, and the development of potential ecotypes or phenotypes in *P. linearis* on the west coast of Ireland is discussed. Distribution of length classes and variation in growth rates in relation to different shore positions are reported. In addition, spore arrangement and cell thickness were assessed, and RGRs of blades in culture were compared with observations of seasonality in the field to determine whether the differences in growth and reproduction observed *in situ* were reflected in the micromorphology of the blades.

The intergenetic RUBISCO spacer was useful in the differentiation between species of *Porphyra* (Brodie et al. 1996, 1998); *P. dioica* (as *Porphyra laciniata* C. Agardh) was distinguished from *Porphyra purpurea* (Roth) C. Agardh using this technique, and morphological characters confirmed it as a distinct species (Brodie and Irvine 1997). Here, direct sequencing of the RUBISCO spacer and coding adjacent regions from isolates of both populations at Salthill, Galway Bay, Ireland, was performed to assess the genetic homogeneity of plants from the two locations.

MATERIALS AND METHODS

Study sites, field collections, and environmental measurements. The phenology, reproduction, and seasonal growth of two intertidal populations of *P. linearis* were investigated on a monthly basis at Salthill, Galway Bay, Ireland, between June 1997 and June 1998. Plants from the first population grew on a sloping granitic platform and on boulders facing the open Atlantic at Salthill, Galway Bay (Site 1; 53°15.447′ N, 0.09°04.672′ W). The second population was located at a wider and more gently sloping granitic platform (Site 2; 53°15.458′ N, 0.09°04.996′ W). At Site 1, plants grew both on the platform and on boulders, but at Site 2, plants grew only on the platform. Generally, Site 1 was observed to be more exposed to wave action than Site 2.

At both sites, three squares (~15 cm × 15 cm) were placed haphazardly within the area where the populations occurred between June 1997 and June 1998 at monthly intervals, and all plants within the three squares were removed (Molenaar et al. 1996). Plants were brought to the laboratory, herbarium specimens were made, and the relative growth rates (RGRs; difference in plant lengths between collections) were calculated. The following parameters for each plant were recorded: length, condition (plants with actively growing blades or bearing some degenerating blades), life history phase (juvenile, sterile adult male, or female gametophyte, presence of zygotosporangia), and reproductive status (young, mature, or spore-releasing reproductive structures). RGRs ($RGR = \ln (N_t/N_o)$ day^{-1}; N_t, final length; N_o, initial length) were calculated for each collection interval and are expressed as percent per day.

Daily water and air temperature were recorded at hourly intervals for 2 years (1997 and 1998) at both sites using Hugrun temperature Seamon miniprobes (MarineTalk, North Vancouver, BC, Canada) in collaboration with the Fisheries Research Centre of the Marine Institute, Dublin, Ireland. Data for day length and hourly irradiances were obtained from Dunsink Observatory, Castleknock, Dublin. Daily sunrise and sunset data and rainfall (amount and duration) were obtained from Met Éireann (Irish Meteorological Service), which records information at Shannon Airport, Co. Clare, and from Mace Head, Co. Galway.

Spearman's rank correlation coefficient (r_s) was used to test for significance of relationships between environment factors and growth rates, $r_s = 1 - [(6 \sum d^2)/(n^3 - n)]$, where n is the number of units in a sample, d the difference between ranks, and 6 a constant in the formula.

Data were separated into four categories representing four seasons based on the reproductive phenology observed in the field. These seasonal categories were defined as autumn (September 15, 1997, to November 7, 1997), winter (November 8, 1997, to March 4, 1998), spring (March 5, 1998, to April 4, 1998), and early summer (April 5, 1998, to May 12, 1998). Correlation coefficients between RGR and climatic data were calculated for the four seasons separately, and together for the growth period (autumn and winter, September 15, 1997, to March 4, 1998) and the sporulation period (spring and early summer, March 5, 1998, to May 12, 1998). For the correlation analyses, the following climatic factors were used: sunshine (h); day length (h); sunshine/day length (or number of sunshine hours divided by hours of day length per day); irradiance ($J \cdot cm^{-2}$); rainfall duration (h); rainfall amount (mm); seawater temperature (°C); and maximum, average, and minimum intertidal temperatures (°C).

Morphological studies. Samples were collected for morphological investigation at the two sites at Salthill, Galway Bay. Morphological observations (color, shape, reproduction, frond thickness, zygotospore, and spermatangial diameter) were made on both herbarium specimens and on fresh material from both sites using a microscope and an ocular micrometer. At least 100 cells from thalli from each site were measured, and cell size, diameter, and arrangement were recorded. Sections were cut by hand. The formulae for zygotosporangial and spermatangial packets were calculated following Hus (1902).

Laboratory experiments. The combined effects of temperature and day length on growth rates in blades from the two sites were studied in laboratory experiments. At the beginning of the growing season (October 1998) when plants became visible at the two sites at Salthill, juvenile blades were collected, about 0.5–1 cm in length at Site 1 and 2–2.5 cm at Site 2, and transported to the laboratory. Blades were then cleaned and immersed in von Stosch-enriched seawater medium, modified according to Guiry and Cunningham (1984). Cultures were established in square petri dishes of 10 cm × 10 cm divided into 20 compartments. Per treatment, 20 individual whole blades from Site 1, and 20 pieces of thalli from Site 2 (disks of 2 cm in diameter, as blades from Site 2 were already too large to fit into the compartments) were used. Blades from Site 1 were grown at 10°C and 20°C at different day lengths (8:16 L:D, 16:8 L:D); plants from Site 2 were only tested at 10°C as they did not survive at 20°C. All cultures were incubated at an irradiance of 10 μmol pho-

tons $\cdot m^{-2} \cdot s^{-1}$. After 21 days, the blade area (mm^2) was assessed using an interfaced digital image processor (NIH Image 1.60 for Macintosh; http://rsb.info.nih.gov/nih-image/). RGRs were calculated, and differences between the levels of each factor were analyzed by 95% confidence limits.

Sample preservation, DNA extraction, PCR amplification for direct sequencing. Several specimens were collected from both sites at Salthill, Galway Bay; dried at room temperature; and then placed in silica gel. The DNA was extracted using the LiCl extraction protocol described by Hong et al. (1992) as modified by van Oppen et al. (1995). Material from each sample has been deposited at the Phycological Herbarium, Martin Ryan Institute, NUI, Galway (GALW). A region of approximately 333 bp encompassing the 3′ region of the *rbc*L gene, the RUBISCO spacer, and the 5′ region of the *rbc*S gene were PCR amplified using the primers complementary to the 3′ end of the *rbc*L (5′TGTG GACCTCTACAAACAGC3′) and the 5′ end of *rbc*S (5′CCCC ATAGTTCCCAAT3′) (Maggs et al. 1992). Reactions were incubated in a thermal cycler (PCR Hybrid Omn-E) as follows: 1 cycle of 95°C for 3 min (denaturation), 30 cycles of 96°C for 1 min, 50°C for 2 min, and 74°C for 2 min. The PCR mixtures were prepared with variations in the DNA concentration (10^0, 10^{-1}, 10^{-2}, 10^{-3}). Samples of DNA were amplified by PCR with each reaction mixture containing 22.5 µL 10 × buffer, 22.9 µL 10 × dNTP (Sigma-Aldrich, Dublin, Ireland), 13.5 µL $MgCl_2$, and 2.25 µL of each oligonucleotide primer). The final volume was adjusted to 100 µL with sterilized distilled water. The template DNA and Taq polymerase (Sigma-Aldrich), 1.12 µL per reaction, were always added to the mixture last to reduce the chance of contaminating other PCR components. The amplified DNA fragments were purified by the gel extraction method (Volgestein and Gillespie 1979), using the Agarose Gel DNA Extraction Kit (Roche Diagnostics GmbH., Mannheim, Germany) in accordance with the manufacturer's instructions. Double-stranded PCR products were sent for sequencing to MWG BioTech (Milton Keynes, UK). All fragments were sequenced completely on both strands.

RESULTS

In situ *growth*. The frequency distribution of length classes of the two *P. linearis* populations showed a clear seasonal pattern (Fig. 1). Both populations had a similar phenology: gametophytic fronds appeared in October, with spermatangial and zygotosporangial sori developing the following January in the population at Site 1, and in February in the population at Site 2. Gametophytes began to degenerate in April and had disappeared completely by June. From May to September, blades were absent from the shore. Conchocelis-phase plants were never found in the field.

In both populations, maximum thallus lengths (Site 1: 30 cm, Site 2: 37 cm) were observed in January (Fig. 2a). Between January and May 1998, the period when plants sporulated and degenerated, the median length class decreased to 3 cm at both sites. Thallus widths were the highest in March (Site 1: 3.5 cm, Site 2: 5.1 cm; Fig. 2b), but the increase in size was the greatest between October and January at both sites. After January, the average width remained almost constant. The increase in both mean thallus length and width at Site 2 was always greater, and individuals from Site 2 were significantly longer (two-way ANOVA, $P<0.00001$, $F=41.0563$; df = 7) and wider (two-way ANOVA, $P<0.00001$, $F=41.0563$; df = 7) throughout the sampling period.

Reproduction and sporulation. Some differences in reproduction between specimens from the two sites were apparent (Fig. 3). Although juvenile gametophytes began to appear from October to January at both sites, male gametophytes started to appear in late November at Site 1, and in late October at Site 2. Female gametophytes appeared in January at Site 1, but at Site 2 in late November. At Site 1, juveniles were still appearing in February, but at Site 2 no further juveniles appeared after early January.

The release of zygotospores and spermatia was first observed at Site 2. Spermatangial release occurred slightly earlier than zygotosporangial release. Gametophytes at both sites became sterile in late March and completely disappeared by May from Site 2, and by June from Site 1. The conchocelis phase of *P. linearis* was searched for at both sites at different times of the year. Stones and barnacles were examined exhaustively in both locations and adjacent areas, but no conchocelis or conchosporangia filaments were found.

Climatic factors and RGRs. Seasonal variations in daily average seawater temperature and day length in relation to the main life-history events of *P. linearis* in the field are shown in Fig. 4. The maximum seawater temperatures (18.7°C) occurred in August, and the minimum seawater temperatures (3.25°C) in January. The maximum day length occurred in July (16:8 L:D), and the minimum (7:17 L:D) in December. At both sites, day length intersected the seawater temperature line at two points; these intersects occurred after January (when zygotospore release took place in the field) and after June (when conchospore release probably took place at Salthill).

The relative length growth rates of blades from the two locations in the field are represented in Fig. 5. During the growth period (from October 6, 1997 to March 4, 1997, during which plants were mainly vegetative and increased in length and width), the values of RGRs reached maximum values at both sites, while during the sporulation period (from March 4, 1997, to May 12, 1998, during which thalli were reproductive and decreased in length, due to sporulation), the values were always negative. The average RGRs were significantly higher at Site 2 than at Site 1 during the growth and/or the sporulation period. During the sporulation period, average RGRs were also negative at both sites (Table 1).

At both sites, significant positive correlations were determined between growth rates and climatic factors at different seasons (Table 2). At Site 1, RGR presented a positive correlation with the relationship sunshine/day length and with minimum average intertidal temperature in winter, and with rainfall in early summer. At Site 2, RGRs were significantly correlated with irradiance in winter, intertidal temperature in spring, and day length in autumn.

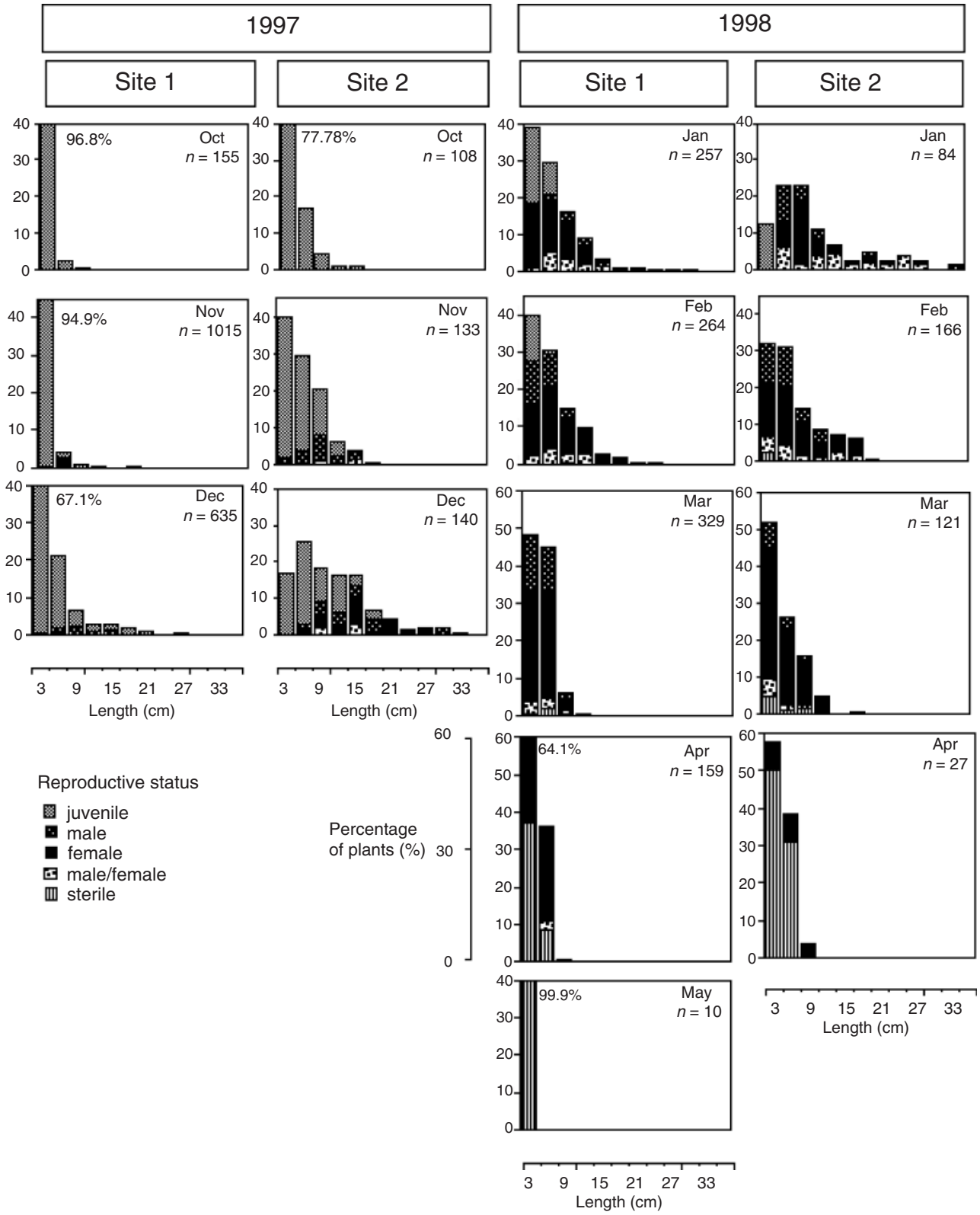

FIG. 1. Monthly size class distribution of *Porphyra linearis* at Salthill, Galway Bay, Ireland, between September 1997 and May 1998. Size classes: 3 = 1–3 cm, 9 = 6–9 cm. Percent values given in some graphs refer to the highest values of scale.

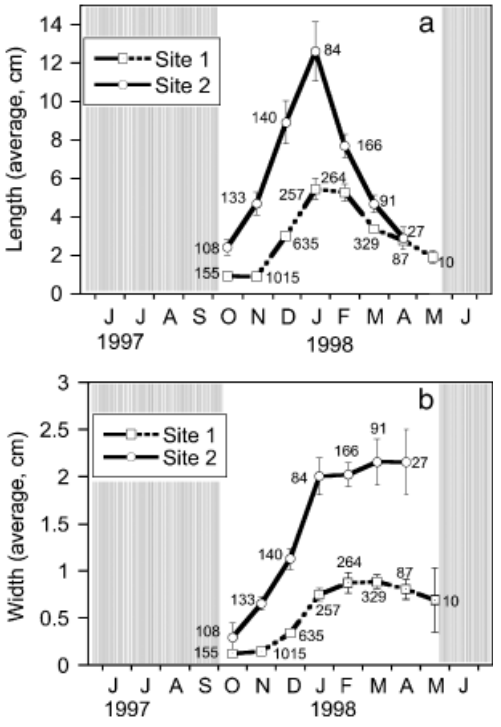

FIG. 2. Average length (a) and width (b) of *Porphyra linearis* at Sites 1 and 2 between September 1997 and May 1998. Error bars: 95% confidence limits; values on graphs = sample size, *n*.

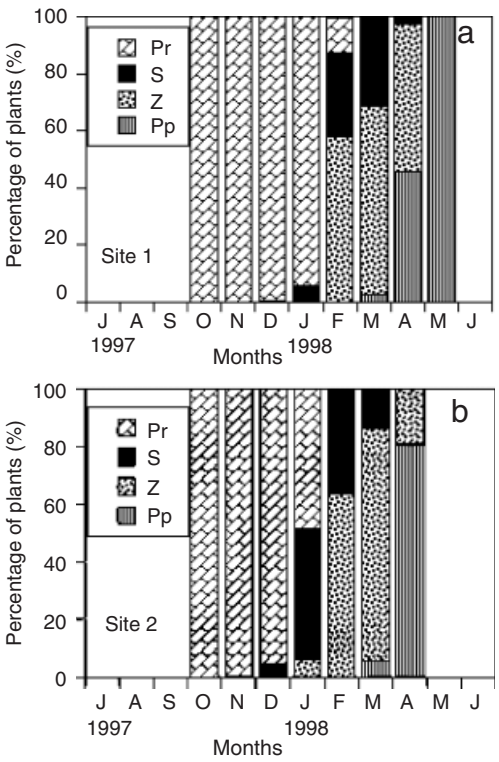

FIG. 3. Seasonality of sporulation of *Porphyra linearis* at Site 1 (a) and Site 2 (b). Pp, postreproductive; Pr, prereproductive; S, spermatangia; Z, zygotosporangia.

Morphological characters. No differences were observed between plants from the two sites in surface or in transverse view. Generally, *P. linearis* at Salthill was light red-brown to reddish in color. It had a linear frond typically pear shaped at the base and then more or less parallel sided. Blades were relatively thin, with a frond thickness of 36–74 μm. Reproductive bodies were present at the margin, with a male sorus with a pale yellow edge and a female sorus with a red edge on the base. Most fronds were dioecious, but some were monoecious with a distinct separation between male and female sori (Fig. 6). This distinct separation could be in the form of a straight line that traverses the full plant or a line in the form of a *V* in the center of the plant (Fig. 6c). Plants were monostromatic with a vegetative cell thickness of 32–36 μm for both populations. The transverse section of female thalli showed tiers of four spores in mature sporangia (Fig. 7). The absence of visible trichogynes suggests that sexual fusion may not be occurring. This agrees with Kornmann and Sahling (1991), who described plants from Helgoland, Germany, that only formed asexual sporangia. The putative zygotospore diameter was about 12.0 μm, and that of spermatia 4.5 μm. Zygotospore arrangement appeared as packets of 16 ($2 \times 2 \times 4$), and spermatangial arrangement as packets of 64 ($2 \times 4 \times 8$). No differences were observed among thalli of the two populations in surface view.

Growth rates of thalli in culture. When RGRs of thalli from the two sites were compared in culture under short-day conditions, thalli from Site 2 grew significantly faster ($P<0.05$) than thalli from Site 1 (Fig. 8). Under long-day conditions, thalli from Site 1 grew significantly faster ($P<0.05$) than thalli from Site 2, and the highest value in RGR was recorded ($0.059\ mm^2 \cdot day^{-1}$). Small thalli grew faster in culture under long-day conditions (Fig. 8).

Sequencing of the RUBISCO spacer and coding adjacent regions. The PCR amplification of the *rbc*L–*rbc*S spacer and flanking coding regions was obtained and yielded a product of 333–358 bp for all isolates (Table 3). Identical sequences were produced for the four isolates of *P. linearis* from Ireland.

DISCUSSION

Most species of *Porphyra* investigated exhibit a seasonal recruitment pattern, in which annual gametophyte recruitment occurs either in spring–summer or autumn–winter, leading to distinct annual summer or winter gametophyte populations (Bird 1973, Dickson and Waaland 1985, Ávila et al. 1986, Waaland et al. 1990, Griffin 1999a, b, López-Vivas and Riosmena-Rodriguez 2000, Holmes and Brodie 2004). Phenological and morphological differences between *Porphyra* populations have been related to temperature and day length (Shimizu 1983, López-Vivas

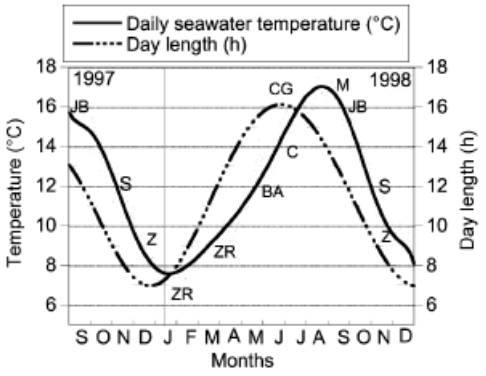

FIG. 4. Daily seawater temperature and day length at Salthill (Galway Bay, Ireland) shore from September 1997 to December 1998 in relation to the significant events in the life history of *Porphyra linearis*. BA, blades are absent; C, conchocelis phase; CG, conchosporangia; JB, juvenile blades; M, meiosis; S, spermatia; Z, zygostosporangia; ZR, zygotospores release. BA, JB, S, Z, and ZR are the only phases of *P. linearis* found in the field.

and Riosmena-Rodriguez 2000). Our study confirms the annual nature of *P. linearis* populations, with a clear seasonal growth pattern revealed by a distinct change in the frequency distribution of length classes from September to May. Thallus lengths and widths in algae from both sites differed throughout the four seasons and during both growth and sporulation periods. Both reproduction and sporulation occurred a month earlier in the population at Site 2.

There was a strong seasonal dependency of growth and phenology on environmental factors at both sites. Significant correlations between RGRs and climatic data (light, temperature, and rainfall) were determined for different periods and seasons at both sites. Generally, RGRs correlated most significantly with temperature. Between November and December, RGRs at Site 1 were highly significantly, positively correlated with minimum intertidal temperatures. In winter, freezing conditions may occur due to minimum temperatures occurring during low tide; and because of their position on the rocks, algae were subject to a high degree of general physical stress, in particular thermal and desiccation stress. It is likely that a combination of physical damage from wave action, freezing, and desiccation stress would reduce growth in algae at Site 1, while growth was positively influenced by sunshine, as indicated by the positive relationship between sunshine/day length and RGR at Site 1 in autumn and winter.

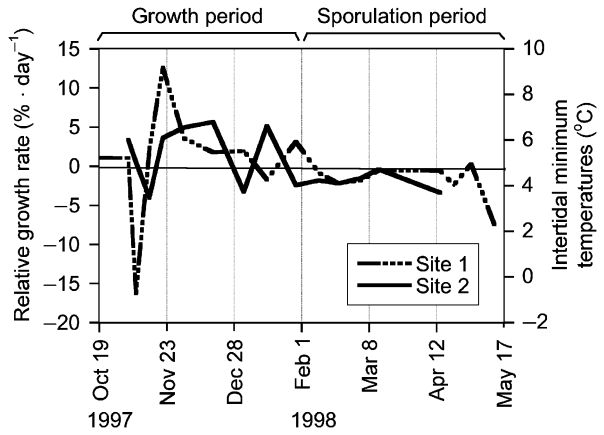

FIG. 5. Seasonal variation in daily relative growth rate (RGR) of *Porphyra linearis* at Sites 1 and 2 versus intertidal minimum temperature during the growth period (autumn and winter, from October 6, 1997, to March 4, 1998) and the sporulation period (spring and early summer, from February 21, 1998, to May 12, 1998).

TABLE 1. Average relative growth rates for the growth and sporulation period at both sites (average ± SE).

Average growth rates (% · day^{-1})	Site 1	Site 2
Growth period	0.540 ± 0.156	0.866 ± 0.250
Sporulation period	− 2.078 ± 0.849	− 1.761 ± 1.020

At Site 2, growth was affected negatively by increasing temperatures in spring and summer, and it is possible that thermal stress and desiccation in plants at Site 2 could induce fragmentation following sporulation, and therefore blades at Site 2 disappeared earlier from the shore. In spring and early summer, RGRs were positively correlated with rainfall at Site 1, and the position of the rocks may have allowed the plants to retain moisture for longer than plants at Site 2.

The RGRs of plants from Site 2 were higher than that of algae from Site 1 under short-day conditions or during winter in the field. Under long-day conditions in culture, algae from Site 2 (large thalli) grew faster than algae from Site 1 (small thalli). *In situ*, there was a positive effect of sunshine and day length at Site 1 at the beginning of the growing season in autumn, when days were still longer, and this also may explain their higher growth rates in culture under long-day conditions.

Porphyra linearis from both sites at Salthill, Co. Galway, exhibited the same micromorphology, spore arrangements, cell layers, and thickness, and they did not vary between the two populations. The morphological characters were similar to those described by Kornmann and Sahling (1991) and the original description by Greville (1830) based on material collected in April and May at Peakhead, near Sidmouth in South Devon, England. For this study, the holotype of *P. linearis* kept at the Royal Botanical Garden, Edinburgh, was examined and carefully compared with the protologue (or original description) of *P. linearis* (Greville 1830). This helped to confirm that the algae from both populations at Salthill were identical to the original algae collected by Greville. This was also corroborated by chromosome and molecular studies. Varela-Álvarez et al. (2005) observed no differences in chromosome number in several populations of *P. linearis* from Ireland, including blades of *P. linearis* from the two sites

TABLE 2. Significant Spearman's rank correlation coefficients ($P<0.05$) of average daily growth rates of blades of *Porphyra linearis* at Salthill, Galway Bay, Ireland, at Sites 1 and 2 during four seasons.

	Autumn (df = 1)	Winter (df = 9)	Spring (df = 4)	Early summer (df = 1)
Site 1	Sunshine/day length 0.999	Minimum temperature (°C) 0.881 Intertidal temperature (°C) 0.819	Irradiance ($J \cdot cm^{-2}$) 0.999	Rainfall (mm) 0.996
Site 2	Day length (h) 0.994	Irradiance ($J \cdot cm^{-2}$) −0.602	Intertidal temp (°C) −0.991	—

Climatic factors include: sunshine/day length, intertidal temperature (maximum, minimum, and average), day length, irradiance, and rainfall. The seasonal categories are defined as autumn (October 6, 1997 to November 7, 1997), winter (November 8, 1997 to April 3, 1998), spring (March 5, 1998 to April 4, 1998), and early summer (April 5, 1998 to March 12, 1998). df = *n*–1, degrees of freedom.

studied here, which was $n = 4$ in all the Irish populations. In addition, PCR amplification of the RUBISCO spacer (*rbc*L–*rbc*S) and coding adjacent regions were produced for isolates of *P. linearis* from all over the world, which clearly showed that *P. linearis* is not a homogenous entity as currently conceived (Varela-Álvarez 2002). Nevertheless, identical sequences were obtained including isolates from both populations at Salthill (Table 3), indicating clearly that *P. linearis* from Salthill is genetically homogenous with respect to the region sequenced. The significant phenological differences that existed between the two populations of *P. linearis* at Salthill indicate that morphological plasticity in *P. linearis* populations existed at the two sites, seemingly in accordance with the two different microhabitats; this was also supported by laboratory cultures.

The life history of *P. linearis* in Galway Bay at Salthill generally corresponds to that previously described by Bird (1973) for this species in Nova Scotia, Canada, but

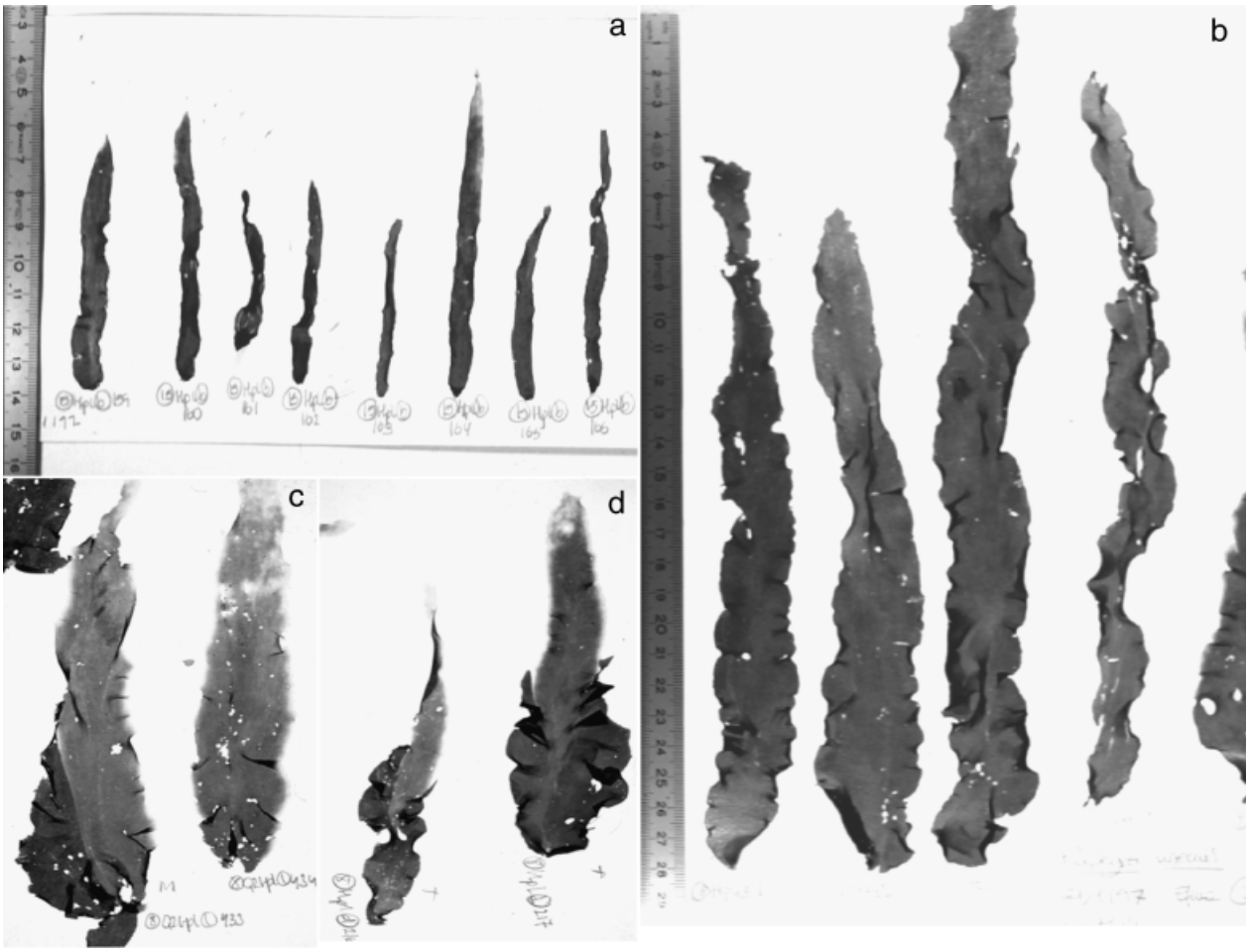

FIG. 6. *Porphyra linearis* collected at Salthill, Co. Galway, Ireland (GALW). (a) Fronds collected at Site 1, in February 1998, GALW 011331. (b) Fronds collected at Site 2, GALW 011338 in February 1998. (c, d) Monoecious herbarium specimens of *P. linearis*, GALW 011338, GALW 011332.

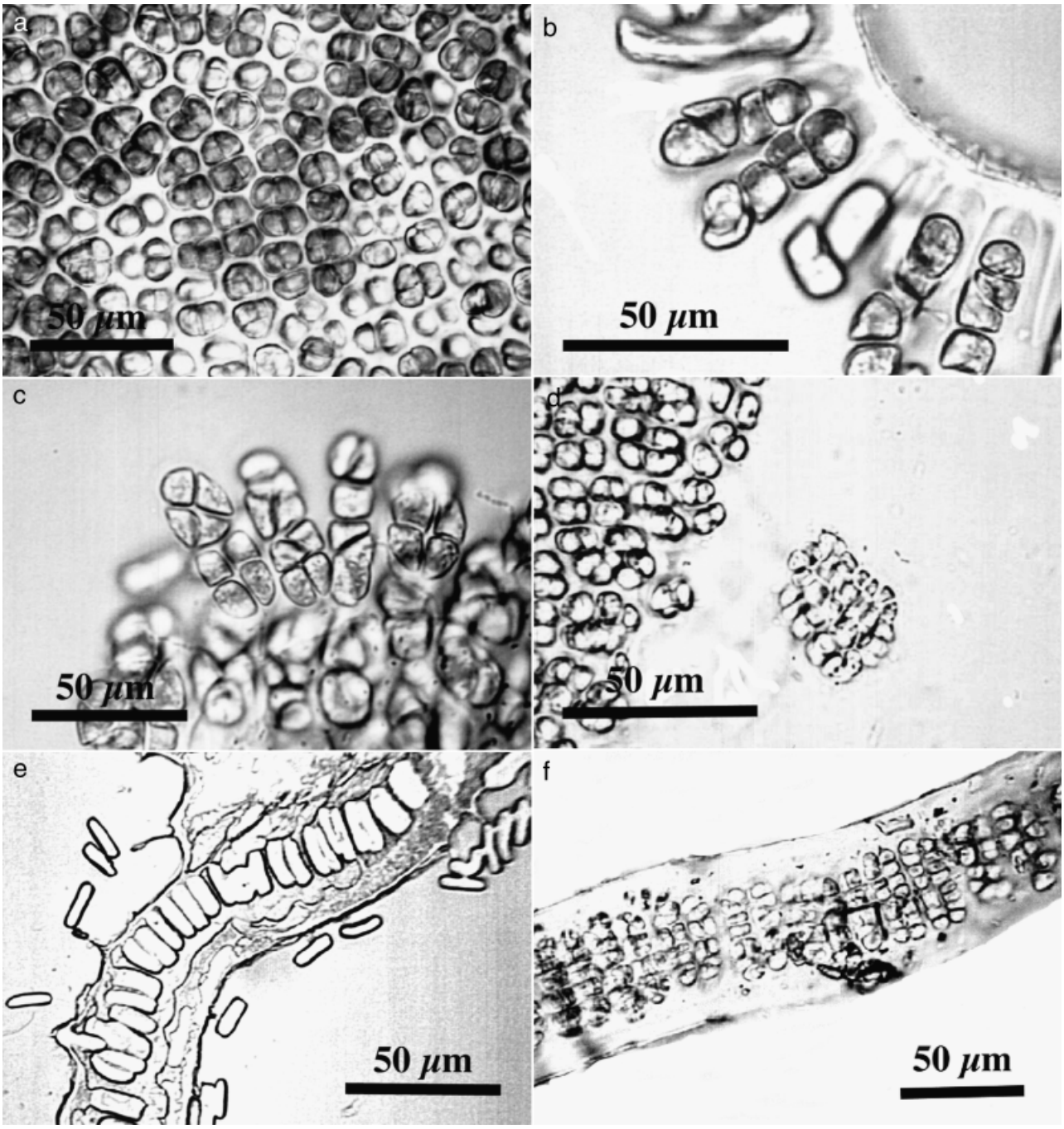

FIG. 7. (a) Reproductive features of *Porphyra linearis* from Salthill, Co. Galway, Ireland. The surface view of zygotospores is in packets of four. (b) Transverse view of the zygotosporangial packets. (c) Zygotosporangial packets. (d) Zygotosporangial packets of 64 cells. (e) Transverse section of vegetative cells, one cell thick. (f) Transverse view of the zygotosporangial packets.

there were also some important differences. Previous reports of appearance of thalli of *P. linearis* in the supralittoral zone approximately coincided with a sea temperature of 13°C in Nova Scotia (Bird 1973). By contrast, in the present study, leafy thalli appeared in the field at a sea temperature of 15°C. Also, Varela-Álvarez et al. (2004) showed that optimal conditions for conchocelis growth and conchospore production in *P. linearis* from Ireland in culture are 20°C and long days (16:8 L:D).

Optimum temperatures for growth of marine species determined in the laboratory are frequently higher than those recorded in the sea (Dring 1982). In fact, in Galway Bay, the seawater temperature in winter never reaches 20°C, but algae at Site 1 could still grow at 20°C in culture (data not shown). Recent evidence from

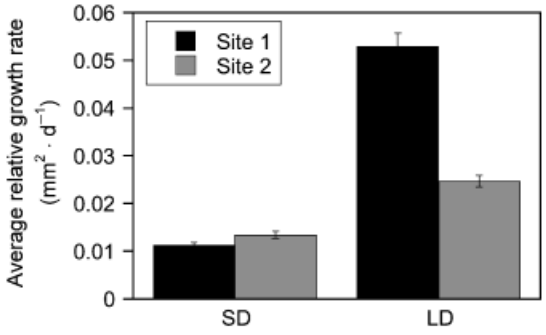

FIG. 8. Average relative growth rate ($mm^2 \cdot day^{-1}$) at 10°C, 8:16 (light:dark [L:D]) of blades in culture 21 days, of *Porphyra linearis* from Sites 1 and 2, at Salthill, Galway Bay, Ireland. $n = 20$, error bars represent 95% confidence limits.

SSU sequences of different strains of *P. linearis* from the western North Atlantic suggests an intraspecific variation between 0% and 3.5% (Klein et al. 2003). Also, chromosome studies indicate that *P. linearis*, as it is currently recognized, consists of two morphologically similar species (Klein et al. 2003, Varela-Álvarez et al. 2004, 2005), which may explain the discrepancies in the critical temperature for conchospores release in *P. linearis* strains on both sides of the Atlantic. Ecotypic differentiations within seaweed species have been previously observed in populations of a range of seaweed species (Gerard 1988, Innes 1988, Bäck et al. 1992, Rueness and Kornfeldt 1992, Molenaar et al. 1996, Yoshida et al. 2004). Temperature ecotypes occurring in some tropical to warm temperature species possibly evolved through isolation of regional populations caused by climatic or other barriers during glaciations (Pakker et al. 1995). Here, the influence of the two microhabitats was created mainly by the different growth positions of the blades at the two sites. Even if important morphological, phenotypic, and ecophysiological differences existed between two populations of *P. linearis*, due to microclimatic conditions, evidence of ecotypic differentiation between the two different sites seems to be limited due to the lack of differences in micromorphology, chromosome number (Varela-Álvarez et al. 2005), or genetic differences. On the other hand, cultures of blades from both sites under the same conditions resulted in different RGRs. The presence of genetic differences at a different locus between the two potential subpopulations may provide some evidence of ecotypic differentiation.

The results from this study are important for potential commercialization, as algae from Site 2 had unusually large sizes and higher growth rates and would therefore be particularly suitable for use in aquaculture in Ireland. Similar phenotypic selection has previously led to the discovery of new, faster-growing strains of commercial seaweeds (Neish and Fox 1971, Doty and Álvarez 1975, Doty 1978, Waaland 1978, Ryther et al. 1979, Cheney et al. 1981, Hansen 1984).

Major differences between American and European isolates of *P. linearis* from both sides of the Atlantic can be found in chromosome number (Müller et al. 2001, Klein et al. 2003, Varela-Álvarez et al. 2004), conchospore release temperature, and in different DNA sequences at a different locus (Müller et al. 2001, Klein et al. 2003, Varela-Álvarez et al. unpublished data). Revision of the *P. linearis* complex, including isolates from both sides of the Atlantic, using molecular methods is urgently required.

TABLE 3. Collection locations and GenBank accession numbers of *Porphyra linearis* RUBISCO intergenic spacer and coding adjacent regions (*rbc*L–*rbc*S genes) of four Irish isolates sequenced for this study.

Species	Location	Herbarium (GALW)	GenBank accession number
P. linearis	Site 1, Salthill, Co. Galway	011327	DQ837583
P. linearis	Site 1, Salthill, Co. Galway	011328	DQ837594
P. linearis	Site 2, Salthill, Co. Galway	011331	DQ837595
P. linearis	Site 2, Salthill, Co. Galway	011333	DQ837596

We are grateful to Padraic Cooke and Jim Morrissey for their help with the collections and transportation to the shore and the Marine Institute in Dublin, particularly Terry McMahon, for the loan of the field thermometers. We also thank Lynne McIvor, Stefan Kraan, and Vincent Laize for their help with the production, analysis, and submission of sequences to GenBank, and Liam Cronin for his comments on the manuscript. Part of this investigation was presented at the International Workshop "The Biology of *Porphyra*" funded by the European Commission, COST Action 49. This study was supported by a TMR Marie Curie Intraeuropean Research Fellowship (GT960697, 1997–1998); a postgraduate fellowship from the Faculty of Science, National University of Ireland, Galway (1999–2001); and a Galway County Council postgraduate fellowship (2001) to E. Varela-Álvarez.

Ávila, M., Santelices, B. & McLachlan, J. 1986. Photoperiod and temperature regulation of the life history of *Porphyra columbina* (Rhodophyta, Bangiales) from central Chile. *Can. J. Bot.* 64:1867–72.

Bäck, S., Collins, J. C. & Russell, G. 1992. Comparative ecophysiology of Baltic and Atlantic *Fucus vesiculosus*. *Mar. Ecol. Prog. Ser.* 84:71–82.

Bird, C. J. 1973. Aspects of the life history and ecology of *Porphyra linearis* (Bangiales, Rhodophyceae) in nature. *Can. J. Bot.* 51:2371–9.

Bird, C. J., Chen, L. C.-M. & McLachlan, J. 1972. The culture of *Porphyra linearis* (Bangiales, Rhodophyceae). *Can. J. Bot.* 50:1859–63.

Boney, A. D. 1978. Survival and growth of alpha-spores of *Porphyra schizophylla* Hollenberg (Rhodophyta, Bangiophyceae). *J. Exp. Mar. Biol. Ecol.* 35:27–9.

Brodie, J., Hayes, P. K., Barker, G. L. & Bartsch, I. 1998. A reappraisal of *Porphyra* and *Bangia* (Bangiophycidae, Rhodophyta) in the Northeast Atlantic based on the *rbc*L–*rbc*S intergenic spacer. *J. Phycol.* 34:1069–74.

Brodie, J., Hayes, P. K., Barker, G. L. & Irvine, L. M. 1996. Molecular and morphological characters distinguishing two *Porphyra* species. *Eur. J. Phycol.* 31:303–8.

Brodie, J. & Irvine, L. M. 1997. A comparison of *Porphyra dioica* sp. nov. and *P. purpurea* (Roth) C. Ag. (Rhodophyta, Bangiophycidae) in Europe. *Cryptogam. Algol.* 18:283–93.

Broom, J. E., Nelson, W. A., Yarish, C., Jones, W. A., Aguilar Rosas, R. & Aguilar-Rosas, L. E. 2002. A reassessment of the taxonomic status of *Porphyra suborbiculata, Porphyra carolinensis* and

Porphyra lilliputiana (Bangiales, Rhodophyta) based on molecular and morphological data. *Eur. J. Phycol.* 37:227–37.
Cheney, D., Mathieson, A. & Schubert, D. 1981. The application of genetic improvement techniques to seaweed cultivation: I. Strain selection in the carragenophyte *Chondrus crispus*. *Proc. Int. Seaweed Symp.* 10:567–9.
Conway, E. 1964a. Autecological studies of the genus *Porphyra* I. The species found in Britain. *Br. Phycol. Bull.* 2:342–8.
Conway, E. 1964b. Autecological studies of the genus *Porphyra*. II. *Porphyra umbilicalis* (L.). J. Ag. *Br. Phycol. Bull.* 2:349–63.
Dickson, L. G. & Waaland, J. R. 1985. *Porphyra nereocystis*: a dual-daylength seaweed. *Planta* 165:548–53.
Doty, M. S. 1978. *Eucheuma* – current marine agronomy. *In* Krauss, R. [Ed.] *The Marine Plant Biomass of the Pacific Northwest Coast*. Oregon State University Press, Corvallis, pp. 203–14.
Doty, M. S. & Álvarez, V. B. 1975. Status, problems, advances and economics of *Eucheuma* farms. *Mar. Technol. Soc. J.* 9:30–5.
Dring, M. J. 1982. *The Biology of Marine Plants*. Arnold, London, 199 pp.
Gerard, V. A. 1988. Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. *Mar. Biol.* 97:25–36.
Greville, R. K. 1830. *Algae Britannicae*. Baldwin and Cradock, London.
Guiry, M. D. & Cunningham, E. M. 1984. Photoperiodic and temperature responses in the reproduction of north-eastern Atlantic *Gigartina acicularis* (Rhodophyta: Gigartinales). *Phycologia* 23:357–67.
Guiry, M. D. & Guiry, G. M. 2006. *AlgaeBase version 4.2*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org.
Griffin, N. J., Bolton, J. & Anderson, R. J. 1999a. Distribution and population dynamics of *Porphyra* (Bangiales, Rhodophyta) in the southern Western Cape, South Africa. *J. Appl. Phycol.* 11:429–36.
Griffin, N. J., Bolton, J. J. & Anderson, R. J. 1999b. The effects of a simulated harvest on *Porphyra* (Bangiales, Rhodophyta) in South Africa. *Hydrobiologia* 399:183–9.
Hanisak, M. D. 1998. Seaweed cultivation: global trends. *World Aquaculture* 29:18–21.
Hansen, J. E. 1984. Strains selection and physiology in the development of *Gracilaria* mariculture. *Proc. Int. Seaweed Symp.* 11:89–94.
Hawkes, M. W. 1978. Sexual reproduction in *Porphyra gardneri* (Smith et Hollenberg) Hawkes (Bangiales, Rhodophyta). *Phycologia* 17:329–53.
Holmes, M. J. & Brodie, J. 2004. Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, UK. *Phycologia* 43:176–88.
Hong, Y.-K., Coury, D. A., Polne-Fuller, M. & Bibor, A. 1992. Lithium chloride extraction of DNA from the seaweed *Porphyra perforata* (Rhodophyta). *J. Phycol.* 28:217–20.
Hus, H. T. A. 1902. An account of the species of *Porphyra* found on the Pacific Coast of North America. *Proc. Calif. Acad. Sci. 3rd. Ser. (Bot.)* 2:173–236.
Hwang, M. S., Kim, J. K., Sim, D. S., Oh, Y. S. & Choi, H. G. 2005. Growth and reproduction of *Porphyra kuniedae* Kurogi (Bangiales, Rhodophyta) from Korea. *Key. Eng. Mater.* 279: 569–76.
Innes, D. J. 1988. Genetic differentiation in the intertidal zone in populations of the *Enteromorpha linza* (Ulvales, Chlorophyta). *Mar. Biol.* 97:9–16.
Klein, A. S., Mathieson, A. C., Neefus, C. D., Cain, D. F., Taylor, H. A., Teasdale, A. L., West, E. J., Hehrre, J., Brodie, J., Yarish, C. & Wallace, A. L. 2003. Identification of north-western Atlantic *Porphyra* (Bangiaceae, Bangiales) based on sequence variation in nuclear SSU and plastid rbcL genes. *Phycologia* 42: 109–22.
Kornmann, P. & Sahling, P.-H. 1991. The *Porphyra* species of Helgoland (Bangiales, Rhodophyta). *Helgol. Wiss. Meeresunters.* 45:1–38.
Lindstrom, S. C. & Cole, K. M. 1992. Relationships between some North Atlantic and North Pacific species of *Porphyra* (Bangiales, Rhodophyta) evidence from isozymes, morphology and chromosomes. *Can. J. Bot.* 70:2066–75.
López-Vivas, J. M. & Riosmena-Rodriguez, R. 2000. Phenology of *Porphyra pendula* (Bangiales, Rhodophyta) in the south-western Gulf of California, Mexico. *J. Phycol.* 36 (Suppl.): 45.
Maggs, C. A., Douglas, S. E., Fenety, J. & Bird, C. J. 1992. A molecular and morphological analysis of the *Gymnogongrus devoniensis* (Rhodophyta) complex in the North Atlantic. *J. Phycol.* 28:214–32.
McGregor, B. J. 1992. Aspects of the biology of *Porphyra* (Bangiales, Rhodophyta) of the Isle of Man. Ph.D. thesis, University of Liverpool, UK, 204 pp.
McLachlan, J., Craigie, J. S. & Chen, L. C.-M. 1971. *Porphyra linearis* Greville – an edible species of nori from Nova Scotia. *Proc. Int. Seaweed. Symp.* 7:473–6.
Molenaar, F. J., Breeman, A. & Venekamp, L. A. M. 1996. Ecotypic variation in *Cystoclonium purpureum* (Rhodophyta) synchronizes life history events in different regions. *J. Phycol.* 32:516–25.
Müller, K. M., Cannone, J. J., Gutell, R. R. & Sheath, R. G. 2001. A structural and phylogenetic analysis of the group IC1 intron in the order Bangiales (Rhodophyta). *Mol. Biol. Evol.* 18: 1654–67.
Mumford, T. F. Jr. 1976. Observations on the distribution and seasonal occurrence of *Porphyra schizophylla* Hollenberg, *Porphyra torta* Krishnamurthy, and *Porphyra brumalis* sp. nov. (Rhodophyta, Bangiales). *Syesis* 8:321–32.
Neish, A. C. & Fox, C. H. 1971. Green house experiments on the vegetative propagation of *Chondrus crispus* (Irish Moss). Technical Report of the Atlantic Regional Laboratory, National Research Laboratory, National Research Council of Canada, Halifax, 35 pp.
Nelson, W. A. 1993. Epiphytic species of *Porphyra* (Bangiales, Rhodophyta) from New Zealand. *Bot. Mar.* 36:525–34.
Nelson, W. A., Broom, J. E. & Farr, T. J. 2001. Four new species of *Porphyra* (Bangiales, Rhodophyta) from the New Zealand region described using traditional characters and the 18S rDNA sequence data. *Cryptogam. Algol.* 22:263–84.
Pakker, H., Breeman, A. M., Prud'homme van Reine, W. F. & van den Hoek, C. 1995. A comparative study of temperature response of Caribbean seaweeds from different biogeographic groups. *J. Phycol.* 31:499–507.
Rueness, J. & Kornfeldt, R. A. 1992. Ecotypic differentiation in salinity responses of *Ceramium strictum* (Rhodophyta) from Scandinavian waters. *Sarsia* 77:207–12.
Shimizu, T. 1983. Taxonomic studies on *Porphyra variegata* (Kjellman) Hus and *P. tenuitasa* Fukuhara (Bangiales, Rhodophyta). *Jpn. J. Phycol.* 31:229–37.
van Oppen, M. J. H., Olsen, J. L. & Stam, W. Y. 1995. Genetic variation within and among North Atlantic and Baltic populations of the benthic alga *Phycodrys rubens* (Rhodophyta). *Eur. J. Phycol.* 30:251–60.
Varela-Álvarez, E. 2002. Phenology, life history and genetics of *Porphyra linearis* Greville, a candidate for nori mariculture in Europe. Ph.D. thesis, National University of Ireland, Galway.
Varela-Álvarez, E., Guiry, M. D. & Kelly, B. 1999. Cultivo, genética y fenología de *Porphyra linearis* Greville, candidata para la futura industria del Nori en Europa. *In* Bárbara Criado, I. [Ed.] *Algas. Boletín Informativo de la Sociedad Española de Ficología*. Vol. 21. Universidad Complutense de Madrid, Madrid, pp. 5–9.
Varela-Álvarez, E., Stengel, D. B. & Guiry, . M. D. 2004. The use of image processing in assessing conchocelis growth and conchospore production in *Porphyra linearis*. *Phycologia* 43:282–7.
Varela-Álvarez, E., Stengel, D. B., Rindi, F. & Guiry, M. D. 2005. Alternation of nuclear phases and chromosome numbers in *Porphyra linearis* (Bangiales, Rhodophyta) from western Ireland and Maine, USA. *Phycologia* 44:61–5.
Volgestein, B. & Gillespie, D. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. U. S. A.* 76:615–9.
Waaland, J. R. 1978. Growth and strain selection in *Gigartina exasperata* (Florideophyceae). *Proc. Int. Seaweed Symp.* 9:241–8.
Waaland, J. R., Dickson, L. G. & Duffiedl, E. C. S. 1990. Conchospore production and seasonal occurrence of some *Porphyra*

species (Bangiales, Rhodophyta) in Washington state. *Hydrobiologia* 204/205:453–9.

West, A. L., Mathieson, A. C., Klein, A. S., Neefus, C. D. & Bray, T. L. 2005. Molecular ecological studies of New England species of *Porphyra* (Rhodophyta, Bangiales). *Nova Hedwigia* 80: 1–24.

Yoshida, T., Ajiksaka, T., Noro, T. & Horiguchi, T. 2004. Species of the genus *Sargassum* subgenus *Schizophycus*. *In* Abbott, I. A. & McDermid, K. J. [Eds.] *Taxonomy of Economic Seaweeds with Reference to the Pacific and Other Locations*. Vol. 9. Hawaii Sea Grant College Program, Honolulu, Hawaii, pp. 93–106.

Zertuche-González, J. A., Pacheco-Ruiz, I., Cabello-Pasini, A., Chee-Barragan, A., Guzman, J. M., Gálvez, A., Arroyo, E. & Yarish, C. 2004. *In situ* growth and reproduction of *Porphyra perforata* in the Pacific coast of Baja California. *J. Phycol.* 36 (Suppl.): 72.