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The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta)

Rui Pereira [a,b,c,*], Charles Yarish [c], Isabel Sousa-Pinto [a,b]

[a] *CIIMAR, Centre for Marine and Environmental Research, Rua dos Bragas 289. 4050-123 Porto, Portugal*
[b] *Botany Department, Science Faculty of the University of Porto, Rua do Campo Alegre, 1191. 4150 Porto, Portugal*
[c] *University of Connecticut, Department of Ecology and Evolutionary Biology. One University Place, Stamford, CT 06901-2315, USA*

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Abstract

The optimal conditions for growth of *Porphyra dioica* gametophytes were investigated in the laboratory, focusing on bioremediation potential. *Porphyra dioica* is one of the most common *Porphyra* species along the northern coast of Portugal and can be found year-round. The influence of stocking density and photon flux density (PFD) on the growth, production and nutrient removal was tested. Maximum growth rates, up to 33% per day, were recorded with 0.1 g fw l^{-1} at 150 and 250 μmol photons m^{-2} s^{-1}. Growth rate decreased significantly with increasing stocking density. Productivity (g fw $week^{-1}$) had an inverse trend, with more production at the higher stocking densities. At 150 μmol m^{-2} s^{-1} and with 1.5 g fw l^{-1}, 1.4 g fw $week^{-1}$ were produced. At this PFD, there was no significant difference in production between 0.6 to 1.5 g fw l^{-1}. Nitrogen (N) content of the seaweeds decreased with increasing stocking densities and PFDs. The maximum N removal was recorded at 150 μmol m^{-2} s^{-1}, with 1.5 g fw l^{-1} stocking density (1.67 mg N day^{-1}). However, the N removed by thalli at 50 μmol photons m^{-2} s^{-1} was statistically equal to that at 150 and 250 μmol photons m^{-2} s^{-1}, at a stocking density of 1.0 g fw l^{-1}. The influence of temperature and photoperiod on growth and reproduction was also assessed. Growth rates of *P. dioica* were significantly affected by temperature and photoperiod. In this experiment (with 0.3 g fw l^{-1} stocking density), the highest growth rate, 27.5% fw day^{-1}, was recorded at 15 °C and 16:$\overline{8}$, L:$\overline{D}$. Male thalli started to release spermatia 21 days after the beginning of the experiment, in temperatures from 10 to 20 °C and with 10, 12 and 16 h of day length. Unfertilized female-like thalli were observed at 10 to 20 °C, under all photoperiods tested. Growth of these thalli declined after 4 weeks. By then, formation of young bladelets in the basal portion of these thalli was observed. After 7 weeks all biomass produced was solely due to these vegetatively propagated young thalli, growing 22.4% to 26.1% day^{-1}. The results of this study showed that *P. dioica* appears to be a candidate as a nutrient scrubber in integrated aquaculture systems.

Keywords: Integrated aquaculture; *Porphyra*; Nutrient uptake; Nitrogen content; Bioremediation

* Corresponding author. CIIMAR, Centre for Marine and Environmental Research, Rua dos Bragas 289. 4050-123 Porto, Portugal. Tel.: +351 223401835; fax: +351 223390608.
E-mail address: rpereira@cimar.org (R. Pereira).

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1. Introduction

Porphyra dioica Brodie and Irvine is one of at least 140 species of *Porphyra* described worldwide (Yoshida et al., 1997; Silva, 1999). In Portugal it is one of at least 5 species described (Ardré, 1970; South and Titley, 1986; Brodie and Irvine, 1997) and the most common in the North of Portugal, together with *P. umbilicalis* (L.) Kützing. In Portugal, *P. dioica* can be found at least from Moledo (in the North) to Buarcos (in the centre, near Figueira da Foz). Although its exact distribution is unknown, Brodie and Irvine (1997) consider that this species is widespread in the northeastern Atlantic. *Porphyra dioica* inhabits the intertidal zone of rocky beaches throughout the year, with higher densities in late winter and spring months (Pereira et al., 2004). The life cycle of *P. dioica* is biphasic and heteromorphic, with a foliose haploid gametophyte, male and female on separated fronds, and a filamentous diploid sporophyte. The sporophyte phase is commonly referred as the conchocelis phase (Drew, 1954). *Porphyra* is one of the world's most important maricultured seaweeds (FAO, 2003). Cultivation is extensively done in China, Japan and Korea. The value of nori, the commercial name for *Porphyra*, was estimated, in 2001, at 1.2 billion US$ (FAO, 2003). Despite this economic importance, all production relies in the few species being used in those countries, mainly *P. yezoensis*, *P. tenera* and *P. haitanensis* (Oohusa, 1993; Fei et al., 1998; Kito and Kawamura, 1999). Besides being used for direct human consumption, *Porphyra* can also be used as a source of r-phycoerythrin, a red pigment used as a dye in immunofluorescence reactions (Mumford and Miura, 1988; Fleurence, 1999). Due to its high surface/volume ratio, *Porphyra* is a fast growing species, capable of rapid assimilation of nutrients, namely nitrogen and phosphorus (Neori et al., 2004). This, together with the mentioned economic value, makes this genus one of the most promising for bioremediation purposes and integrated aquaculture (Chopin et al., 1999, 2001; Kraemer and Yarish, 1999; McVey et al., 2002; Carmona et al., 2006-this issue).

In the last decade, there has been a growing interest of western countries in this genus. Until now, most studies have been conducted mainly to describe the life cycles of different species, Bird (1973), Avila et al. (1986), Waaland et al. (1987), Sidirelli-Wolf (1992), Candia et al. (1999) and Stekoll et al. (1999) are just a few examples. More recently, a few studies have dealt with physiological aspects focused on the macroscopic gametophytes (Figueroa et al., 1995; Hafting, 1999a; Kraemer and Yarish, 1999; Katz et al., 2000; Conitz et al., 2001; Orfanidis, 2001). Simultaneously, recent molecular studies have reported on the wide cryptic molecular variation in the genus (Broom et al., 2002; Neefus et al., 2002; Klein et al., 2003). In the United States, after an unsuccessful attempt to introduce *P. yezoensis*, a research program is now being focused on the "domestication" of native northeastern American species by Yarish et al. (1998, 1999, 2001). To our knowledge, there are not any published studies with *Porphyra* focusing, simultaneously, on the cultivation conditions that are relevant for aquaculture application and its influences on the bioremediation potential and tissue properties.

The objective of this study was to focus on factors that are relevant in the development of the algal component of an integrated aquaculture system along the Portuguese coast, i.e.: purposes (biomass production and/or nutrient removal), stocking densities, photon flux densities, temperatures and photoperiod. Simultaneously, we were also interested in understanding how those factors influence the quality of the biomass (its carbon, nitrogen and pigment contents) and the reproduction of the gametophytes of *P. dioica.*

2. Material and methods

2.1. Photon flux density and stocking density

The conchocelis cultures were isolated and induced to form gametophytes at 15°C and 8–16, L:$\overline{D}$, as described in our previous paper (Pereira et al., 2004). All the thalli were originated from conchocelis culture PD2-1, a strain cloned from a single zygotospore and maintained in culture at the Marine Biotechnology Laboratory, University of Connecticut at Stamford, USA. The strain PD2-1 was originated from a specimen collected in Praia da Luz, Porto, Portugal (41°10′N, 8°58′W). The blades used in the experiment were between 1 and 3 cm long and 2 weeks old. The experiment was carried out at 15 °C and neutral day ($12:\overline{12}$, $L:\overline{D}$). A combination of 5 stocking densities, 0.1, 0.3, 0.6, 1.0 and 1.5 g fresh weight l^{-1} and 3 photon flux densities (PFD), 50, 150 and 250 μmol photons m^{-2} s^{-1} were tested. Three replicates per condition were used. Blades were grown in 1 l flasks with Von Stoch's modified enriched seawater (VSE) (Ott, 1965). The medium was gently aerated. The culture medium had approximately 500 μM NO_3^-, as the source of nitrogen. Twice a week, at 3 and 4 day intervals, the medium was renewed, biomass was recorded, as fresh weight (fw), and the density was reduced back to the initial stocking density. To determine the fresh weight

the algae were blotted dry before weighing. The excess material was kept for tissue analysis. Samples from the incubated media were analyzed for inorganic N and P by the Environmental Research Institute, University of Connecticut, using a Four Channel Auto Analyzer equipped with High-Sensitivity Seawater Cartridges (Lachat—QuikChem AE Ion Analyzer).

Samples for carbon (C) and nitrogen (N) analysis were taken prior to the experiments and at the end. Dry weight (dw) was measured after drying the samples for 48 h at 50 °C. Dried samples were ground by using an automatic grinder (Model MM200, Retsch, Haan, Germany) and total C and N content were determined in triplicates for each replicate sample, with a Perkin-Elmer Series II 2400 CHNS/O Analyzer, Wellesley, MA. For pigment analysis the algae were immediately frozen. All the thalli were previously acclimated for 1 week to the temperature, photoperiod and different PFDs used. The experiment lasted 3 weeks.

2.2. Temperature and photoperiod

Based on the results of the experiment described above, a stocking density of 0.3 g fw l^{-1} and a PFD of 150 μmol photons m^{-2} s^{-1} were used in all the conditions of the temperature and photoperiod experiment. A combination of 2 temperatures 10 and 15 °C and four photoperiods, 8–$\overline{16}$, 10–$\overline{14}$, 12–$\overline{12}$ and 16–$\overline{8}$, L:$\overline{D}$, were tested. Additionally, two extreme temperatures, 5 and 20 °C were tested with 12–$\overline{12}$, L:$\overline{D}$. Three replicates were used in all the conditions. Blades were grown in 1 l flasks with VSE, as described in the previous experiment. Twice each week, at 3 and 4 day intervals, the medium was renewed, biomass was recorded, as fresh weight (fw), and the density was reduced back to the initial stocking density. All the thalli were previously acclimated, during 1 week, to 150 μmol m^{-2} s^{-1} and to the different temperatures and photoperiods used for the experiment.

Samples from the incubated media were analyzed for inorganic N and P by the Environmental Research Institute, University of Connecticut, using a Four Channel Auto Analyzer equipped with High-Sensitivity Seawater Cartridges (Lachat—QuikChem AE Ion Analyzer).

Tissue samples for carbon (C) and nitrogen (N) analysis were taken prior to the experiments and at the end and processed as described in the first experiment.

2.3. Pigment analysis

The material collected during the experiments was kept frozen at −20 °C. The analysis of phycobiliproteins (phycoerythrin and phycocyanin) concentrations was performed in aqueous crude extracts, following the method described by Beer and Eshel (1985) with some modifications. Samples of 0.03 to 0.1 g fw of tissue were ground, using mortar and pestle, with 5 ml of 0.1 M phosphate buffer (pH 6.8) and sand. The extraction was done in the cold, in dim light, and the extracts were kept in the dark at 4 °C overnight. The extracts were centrifuged at 10,000 *g* for 20 min and the supernatant used for determinations of phycobiliprotein (PBP) concentration. Light absorption was measured using a Perkin Elmer, spectrophotometer (UV/VIS spectrophotometer Lambda 20, Perkin Elmer Analytical Division of EG and G, Wellesley, MA). Concentrations of phycoerythrin (PE) and phycocyanin (PC) were calculated using the formulas described by Beer and Eshel (1985).

For Chl *a* analysis we followed a procedure adapted from Lobban and Chapman (1988). The tissue pellet (collected from the PBP extraction) was analysed by the following procedure. A few milligrams of $MgCO_3$ were added and the material was ground in 4 ml of 90% acetone. After centrifuging for 20 min. (at 10,000 *g*) the supernatant was collected and the pellet was used to repeat the extraction procedure. The two extraction volumes were combined and the absorbance at 665 nm was measured using a Perkin Elmer spectrophotometer (UV/VIS spectrophotometer Lambda 20, Perkin Elmer Analytical Division of EG and G, Wellesley, MA).

2.4. Statistical analysis

For all the treatments, three independent replicates were analyzed, and means and standard deviations were calculated. Differences among treatments were tested for significance using two-way ANOVA. Multiple post-hoc comparisons among means were tested by the SNK test. Data that did not comply with normality or equal variance were transformed (log x or $x^{1/2}$). In all cases, the null hypothesis was rejected at the 5% significance level, according to Sokal and Rholf (1995).

3. Results

3.1. Photon flux density and stocking density experiment

The gametophytes of *P. dioica* grow faster at lower stocking densities and higher PFD (Fig. 1A). The highest mean growth rate, over 3 weeks, was 33.6% fw day^{-1}, recorded at 250 μmol photons m^{-2} s^{-1} and 0.1 g fw l^{-1} stocking density. This value was very close

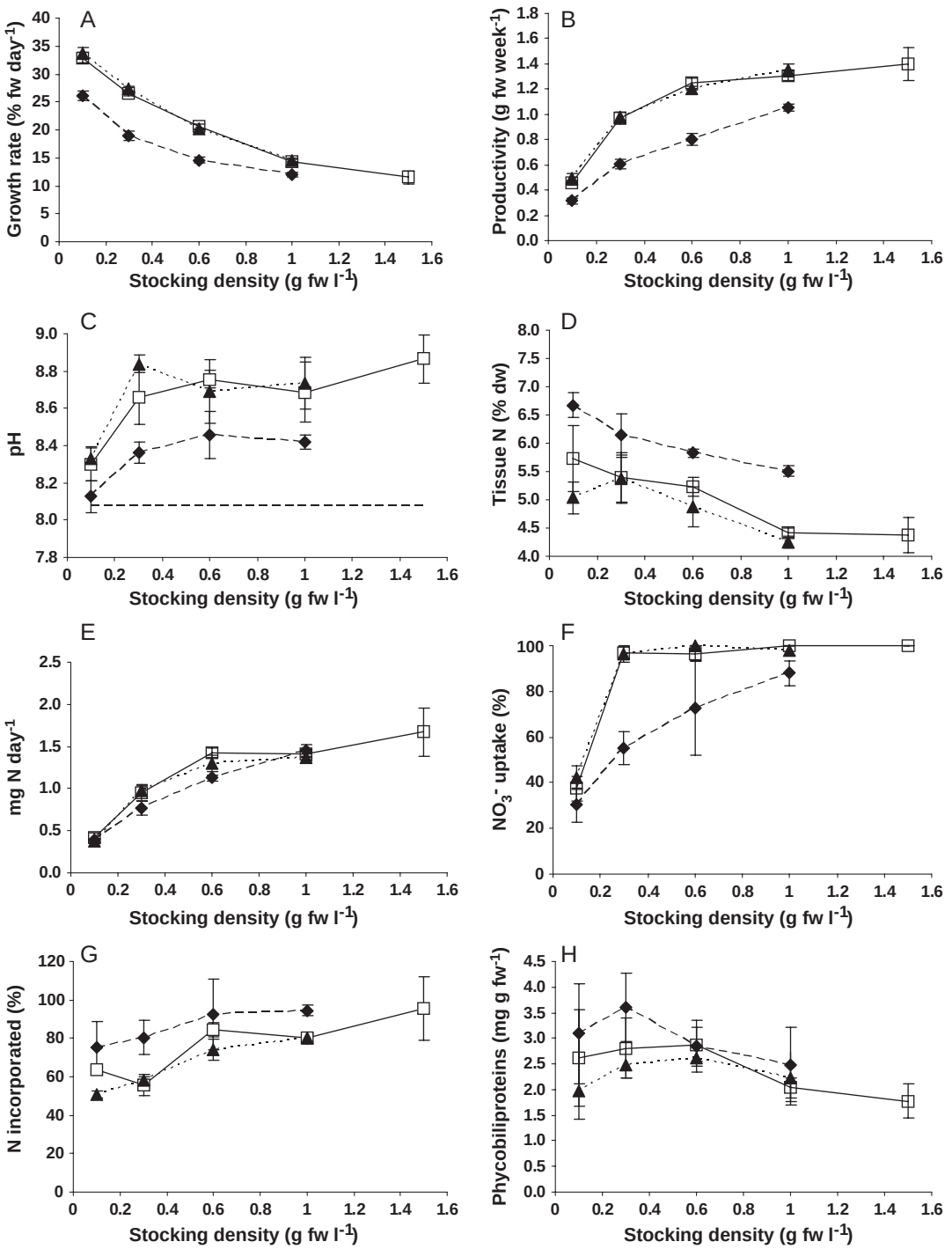

Fig. 1. Effects of stocking density and PFD, (♦) 50, (□) 150 and (▲) 250 μmol photons m^{-2} s^{-1}, on the gametophytes of *P. dioica* cultivated at 15 °C and 12 : $\overline{12}$, L : $\bar{D}$, with 500 μM NO_3^- as a source of nitrogen. Symbols represent the average of 3 replicates with correspondent standard deviation. A) growth rate; B) productivity; C) variation of the pH of the culture medium, after 4 days, the horizontal dashed line closer to the *xx* axis represents the initial pH; D) nitrogen content of the gametophytes; E) potential nitrogen removal; F) uptake percentage of NO_3^- from the culture medium, after 4 days; G) percentage of N removed from the water that was incorporated into new tissue; H) phycobiliprotein content.

to the one obtained at 150 μmol photons m^{-2} s^{-1} and 0.1 g fw l^{-1} stocking density, 32.9% fw day^{-1}. The ANOVA showed significant differences among stocking densities and among PFD ($P<0.001$) as well as an interaction between the two factors ($P<0.001$). In all stocking densities the Student Newman Keuls (SNK) test showed significant differences between 50 and 150 and between 50 and 250 μmol photons m^{-2} s^{-1} ($P<0.01$). No significant differences between the two highest light levels were detected. On the other hand, the SNK also showed that, within each light level, all the stocking densities are significantly different from each other ($P<0.01$).

If we consider the results in terms of productivity, the shape of the curve is inverted and the highest biomass production was obtained with the higher stocking densities and higher PFDs (Fig. 1B). A stocking density of 1.5 g fw l^{-1} yielded, on average, 1.40 g fw per week, at 150 μmol m^{-2} s^{-1}. A stocking density of 1.0 g fw l^{-1}, at 250 μmol m^{-2} s^{-1}, yielded 1.35 g fw per week. The ANOVA indicated that the differences among stocking density and PFD were significant ($P<0.001$). There was also a significant interaction between the two factors ($P<0.001$). The SNK showed that, in all stocking densities, the lower productivity at 50 μmol photons m^{-2} s^{-1} was significantly different from that at 150 and 250 μmol photons m^{-2} s^{-1} ($P<0.01$). Again, as seen for growth rate, there was no significant difference between the two highest PFDs. Within each light level, the results of the SNK test are different from those observed for growth rate. At 50 μmol photons m^{-2} s^{-1}, the productivity increased significantly ($P<0.01$) with the increase of the stocking density. The same was observed for 250 μmol photons m^{-2} s^{-1}, although the productivity seemed to stabilize between 0.6 and 1.0 g fw l^{-1} of stocking density. At 150 μmol photons m^{-2} s^{-1} the productivity curve also reached a maximum after 0.6 g fw l^{-1} of stocking density. In fact, at 150 μmol photons m^{-2} s^{-1}, there was no significant difference ($P>0.05$) between the productivity at 0.6, 1.0 and 1.5 g fw l^{-1} of stocking density. The differences between these and the lower stocking densities were significant ($P<0.01$).

The pH of the culture medium increased, as expected, after the presence of the thalli. This variation was highest at 150 and 250 μmol photons m^{-2} s^{-1}, and at higher stocking densities (Fig. 1C). There was no control of the pH in the cultures during this work. CO_2 was provided by the continuous aeration of the flasks with normal air.

The nitrogen content decreased at all PFDs tested, at increased stocking densities (Fig. 1D). The nitrogen content was always higher at 50 μmol photons m^{-2} s^{-1}. The highest nitrogen percentage, 6.67% dry weight (dw), was recorded at 50 μmol photons m^{-2} s^{-1} and 0.1 g fw l^{-1} of stocking density. The lowest, 4.24% dw, was recorded at 250 μmol photons m^{-2} s^{-1} and 1.0 g fw l^{-1} of stocking density. Combining the results of growth rate with the nitrogen content we also estimated the potential nitrogen removal, in mg of nitrogen per day (mg N day^{-1}), of the thalli grown in the different conditions (Fig. 1E).

The potential nitrogen removal increased significantly ($P<0.01$) with the increasing stocking densities, in all PFDs tested (Fig. 1E). The higher nitrogen removal, 1.67 mg N day^{-1}, was achieved at 150 μmol photons m^{-2} s^{-1} with 1.5 g fw l^{-1} of stocking density. At 50 μmol photons m^{-2} s^{-1} the increase in nitrogen removed was significantly different between each stocking density. At 150 and 250 μmol photons m^{-2} s^{-1} the potential nitrogen removal increased significantly from 0.1 to 0.3 and to 0.6 g fw l^{-1}. Above the 0.6 g fw l^{-1} of stocking density the nitrogen removed was statistically equal ($P>0.05$) to that at 1.0 and even at 1.5 g fw l^{-1}. The highest stocking density was tested only at 150 μmol photons m^{-2} s^{-1}. Nitrogen removed by thalli at 50 μmol photons m^{-2} s^{-1} was statistically equal to that at 150 and 250 μmol photons m^{-2} s^{-1}, when a stocking density of 1.0 g fw l^{-1} was used.

The carbon content of the gametophytes (data not shown) increased, slowly but consistently, with the increasing stocking density only at 50 μmol photons m^{-2} s^{-1}, from 35.3% to 37.7% dw. At 150 and 250 μmol photons m^{-2} s^{-1} there was no defined trend. The carbon content was always higher, varying from 38% to 39.9% dw, at the two highest PFDs than at 50 μmol photons m^{-2} s^{-1}. On the other hand, the carbon removal by the gametophytes was significantly influenced by stocking density and the photon flux density. Within each PFD, carbon removal increased significantly ($P<0.05$) with an increase in stocking density. The highest value, 14.7 mg C day^{-1}, was recorded at 250 μmol photons m^{-2} s^{-1} and 1.5 g fw l^{-1} stocking density. The comparison within each stocking density showed that 150 and 250 μmol photons m^{-2} s^{-1} promoted significantly higher removal of carbon ($P<0.05$). There was no difference between these two PFDs.

The highest percentages of nitrate removed from the medium were recorded at the two highest PFDs, for all stocking densities (Fig. 1F). The nitrate available in the culture medium was completely removed (i.e. 99.9%) in the flasks with 1.0 g fw l^{-1} or higher stocking densities at 150 μmol photons m^{-2} s^{-1}. At 0.6 g fw l^{-1} the percentage of NO_3^- removed from the medium was also

higher than 99.9% at 250 μmol photons m^{-2} s^{-1} and around 96.5% at 150 μmol photons m^{-2} s^{-1}. At 0.3 g fw l^{-1}, 97.0% and 96.1% of the available nitrate was removed at 150 and 250 μmol photons m^{-2} s^{-1}, respectively.

At the lowest PFD (50 μmol photons m^{-2} s^{-1}) the percentage of nitrate removed ranged from 30.2% to 88.0%, with 0.1 and 1.0 g fw l^{-1} stocking densities, respectively.

The phosphate uptake (data not shown) presented a similar pattern, with the difference that the percentage uptake was not so high. The highest uptake percentage, 97.7% was recorded at 1.5 g fw l^{-1} and at 150 μmol photons m^{-2} s^{-1}. At 0.3 g fw l^{-1} the percentage was around 87% for the higher PFDs.

Not all the nitrogen removed from the water was incorporated into new tissue (Fig. 1G). When we compare the ratio (N in new tissue):(N disappearing from the medium), the higher percentages of N incorporation, around 95%, were observed in the cultures with 1.5 g fw l^{-1} and 1.0 g fw l^{-1} stocking density, at 150 and 50 μmol $m^{-2}s^{-1}$, respectively (Fig. 1G). In the cultures with 0.6 g fw l^{-1} at the higher PFDs and with 1.0 g fw l^{-1} (all PFDs), approximately 400 μmol of nitrogen were incorporated in new tissue. With 0.3 g fw l^{-1}, also at the higher PFDs, around 275 μmol of nitrogen were incorporated in new tissue. At the lower PFD, 50 μmol photons m^{-2} s^{-1}, the amount of nitrogen incorporated into new tissue was smaller except for the cultures with 1.0 g fw l^{-1}. In these conditions, although the thalli removed 88% of the available N (Fig. 1F), they incorporated 95% of that amount (Fig. 1G). For all stocking densities, the amount of nitrogen incorporated into new tissue was closer to the amount removed from the medium at 50 μmol photons m^{-2} s^{-1} than at the higher PFDs. In Fig. 1G, the linear regression slopes increase with increasing photon flux density. In other words, the influence of the stocking density on the percentage of N incorporated is higher in the higher photon flux densities.

Except for the thalli in cultures with 0.6 mg fw l^{-1}, the PBP content was always higher in the lower PFD (Fig. 1H). The percentages of PE and PC in the total of PBP remained considerably stable, between 60% and 70% PE and between 30% and 40% PC. The average percentages, of all the conditions, were 64.1% and 35.9% (±4.67) for PE and PC, respectively. Nonetheless, for all stocking densities, the percentage of PE was always higher at the lowest PFD.

The PE content of the thalli was significantly influenced by PFD ($P<0.01$). On average, the thalli at 50 μmol photons m^{-2} s^{-1} had 2.1 mg PE g fw^{-1}, while those at 150 and 250 μmol photons m^{-2} s^{-1} had 1.6 and 1.4 mg PE g fw^{-1}, respectively. The SNK test showed significantly higher PE content in the thalli at 50 μmol photons m^{-2} s^{-1} and no difference between the two higher light levels. There were no significant differences between stocking densities and no interaction between the two factors.

3.2. Temperature and photoperiod experiment

The objective of this experiment was also to assess the influence of the photoperiod on reproduction. In previous experiments, at 15 °C and $12:\overline{12}$, L:$\overline{D}$, we observed male thalli releasing spermatangia 14 to 17 days after the beginning of the experiment. Some of these thalli were less than 10 cm long. This was not, however, a generalized event. After 25 days in culture there were still some male thalli that were not releasing spermatia and grew to 30 cm long. These results were confirmed by this experiment. Fourteen days after the beginning of the experiment (21 considering the acclimation week) we observed male thalli at 10, 15 and 20 °C and at $10:\overline{14}$, $12:\overline{12}$ and $16:\overline{8}$, L:$\overline{D}$. The male thalli were removed whenever detected. The idea was to verify for how long the thalli would keep growing if there was no fertilization. Female-like thalli were first observed 18 days after the beginning of the experiment. Female-like thalli were so-called because they presented the typical brick-red margins but were not releasing zygotospores. In fact, when observed under the microscope, the marginal zones were not even clearly differentiated (Fig. 2A). These thalli were observed at 10, 15 and 20 °C and in all photoperiods. The female-like thalli never released any kind of spores. After the fourth week we noticed that these female-like thalli had a thicker texture and did not look healthy. Besides, in flasks with only these thalli, growth rate decreased.

Another very interesting result was the observation of young blades forming in the basal parts of old thalli. This was first noticed after the fourth week, in all photoperiods, at 10 and 15 °C but also at 20 °C, $12:\overline{12}$, $L:\overline{D}$ (Fig. 2B). These young thalli were responsible for growth rates obtained after the 7th week. By then almost all initial thalli had been removed, either because they were male thalli releasing spores, or because they were old female-like thalli, not growing much or with negative growth. At 15 °C, weeks 7 to 10, growth rates averaged from 22.4% to 26.1% fw day^{-1} without significant differences between the four photoperiods. Although the thalli kept growing at a constant level throughout the 10 weeks, we decided to use data

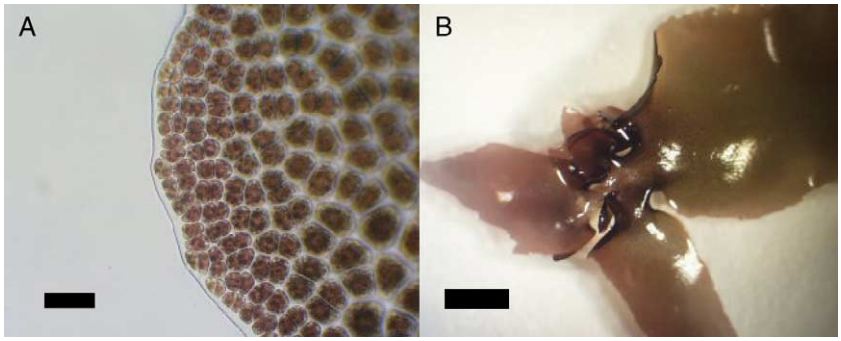

Fig. 2. Blades of *P. dioica* in culture. A) detail of the margin of a female-like thallus, there were no reproductive cells differentiated or any kind of spores released, scale bar equal to 25 μm; B) bladelets formed in the basal parts of older thalli, scale bar equal to 2.5 mm.

corresponding to the average of 4 weeks in culture for the statistical analysis.

The analysis of variance showed that growth rate of the gametophytes of *P. dioica* was significantly affected by temperature and photoperiod ($P<0.05$). There was also a significant interaction between the two factors. The highest growth rate, 27.5% fw day^{-1} was recorded at 15 °C and 16:$\overline{8}$, L:$\overline{\text{D}}$ (Fig. 3A). According with the SNK test, this value was significantly different ($P<0.01$) from the growth rate at 15 °C, 12:$\overline{12}$ and 10:$\overline{14}$, L:$\overline{\text{D}}$, 25.2 and 25.2% fw day^{-1}, respectively. The SNK also showed that there was no significant difference between 12:$\overline{12}$ and 10:$\overline{14}$, L:$\overline{\text{D}}$.

At 10 °C, growth rate at 12:$\overline{12}$ and 16:$\overline{8}$, L:$\overline{\text{D}}$, was significantly higher than that at 8:$\overline{16}$ and 10:$\overline{14}$, L:$\overline{\text{D}}$ ($P<0.01$). There was no significant difference within each pair.

In all photoperiods, the growth rate was significantly higher at 15 °C than at 10 °C ($P<0.01$).

At 12:$\overline{12}$, L:$\overline{\text{D}}$, where more temperatures were tested, growth rate was significantly higher at 15 °C ($P<0.01$) than in other temperatures. There was no significant difference between 10 and 20 °C, but these had, in turn, significantly higher growth rates than at 5 °C.

In terms of nitrogen in the tissue, there was no significant influence of the different photoperiods at 10 and 15 °C ($P>0.05$). There was also no significant difference between these two temperatures ($P>0.05$). The ANOVA performed with growth rate data from the different temperatures tested under 12:$\overline{12}$, L:$\overline{\text{D}}$, also showed that there are no differences from 5 to 20 °C. The highest content of nitrogen in the tissue was recorded for the thalli grown at 10 °C and 12:$\overline{12}$, L:$\overline{\text{D}}$, with 5.7% dw (Fig. 3B).

The nitrogen removal capacity of the thalli was higher at 15 °C and 16:$\overline{8}$, L:$\overline{\text{D}}$, with 1.12 mg N day^{-1} (Fig. 3B). This value was significantly higher than those at other photoperiods and temperatures. At 10 °C, the nitrogen removal capacity was not significantly influenced by the photoperiod. Comparing the four temperatures tested in 12:$\overline{12}$, L:$\overline{\text{D}}$, the ANOVA showed significantly lower nitrogen removal at 5 °C. There was no significant difference between 20, 15 and 10 °C.

The carbon content of the gametophytes of *P. dioica* was higher on thalli at 20 °C and 12:$\overline{12}$, L:$\overline{\text{D}}$, with 39.8% dw. This value was not significantly higher than that of the thalli at 10 and 5 °C and the same photoperiod (Fig. 3D). On the other hand, the carbon content of the thalli at 15 °C was significantly lower. In fact, at 15 °C the carbon content decreased significantly with increasing day length, from 8 to 10 h per day ($P<0.01$). Only at 8:$\overline{16}$, L:$\overline{\text{D}}$, the carbon content was higher at 15 than at 10 °C.

The PBP and chlorophyll *a* contents were not significantly influenced by the temperatures or photoperiods tested. The highest value of PBP, 6.3 mg PBP g fw^{-1}, was recorded at 10 °C and 12:$\overline{12}$, L:$\overline{\text{D}}$. This corresponded to 3.54 and 2.77 mg g fw^{-1}, of PE and PC, respectively. For chlorophyll *a*, the maximum, recorded at 15 °C and 16:$\overline{8}$, L:$\overline{\text{D}}$, was 2.68 mg g fw^{-1}. At 15 °C, the phycoerythrin and phycocyanin content showed a slight tendency to decrease with an increase in day length. The Chl *a* did not follow this tendency. In terms of PE and PC fractions, there was a higher percentage of PE in thalli at 5 °C and 12:$\overline{12}$, L:$\overline{\text{D}}$, when compared to all the others. At these conditions, PE and PC represented 74.4% and 25.6% (±1.07) of the total PBP content, respectively. The average for all other conditions was 58.2% and 41.8% (±3.29), for PE and PC, respectively.

4. Discussion

The effects of the temperature on the growth rate showed that *P. dioica* is well adapted to the water

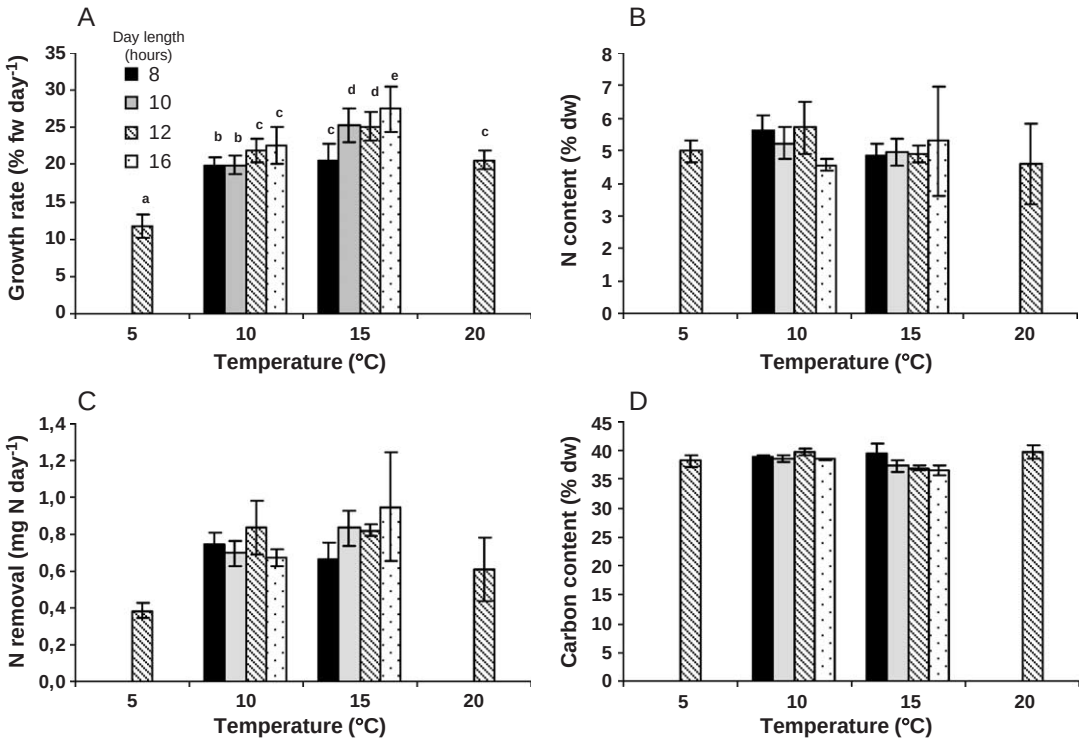

Fig. 3. Effects of different temperatures and photoperiods on the gametophytes of *P. dioica*. The legend in the figure represents day length (hours). Other conditions controlled included PFD, 150 μmol photons m^{-2} s^{-1}, stocking density, 0.3 g fw l^{-1}, and N availability, 500 μM NO_3^-. The bars represent the average of 3 replicates, during 4 weeks, with the correspondent standard deviation. A) growth rate, the different letters over the bars represent significant differences; B) nitrogen content of the gametophytes; C) nitrogen removal capacity; D) carbon content of the gametophytes.

temperatures in its natural habitat. The water temperature in the North of Portugal ranges roughly from 11 to 21 °C (Anonymous, 2004) and *P. dioica* is most abundant at 15 °C (Pereira et al., 2004). Although the statistical analysis detected significantly higher growth at 15 °C and 16 : $\overline{8}$, L : $\overline{D}$, the results obtained in other conditions should be noted. *P. dioica* was able to achieve growth rates over 20% fw day^{-1} from 10 to 20 °C.

The results of the "temperature and photoperiod experiment" confirmed that thalli have a limited life span at least in laboratory cultures. Males reproduce at all photoperiods between 10–20 °C after 14 days in culture (21 days considering the acclimation week or approximately 42 days considering the time since conchospore release). Female-like thalli also stopped growing, approximately after 28 days in culture, even without fertilization and loss of tissue by sporulation. The formation of young blades from the basal parts of adult thalli can explain the presence of *P. dioica* thalli throughout the year along the North Portuguese Coast, a question mentioned in our previous communication (Pereira et al., 2004). On the other hand, the life span of *P. dioica* gametophytes in the wild remains to be determined.

The effects of photoperiod on the growth rate were expected. We knew from previous experiments (authors personal observations) that the gametophytes were able to grow in photoperiods with 8 to 16 h of light per day. Other authors have shown that the production is closely related to the number of hours of light (Bidwell et al., 1984). In *Chondrus crispus*, net productivity in tank culture is a linear function of irradiance over temperatures of 10–20 °C when nutrients are not limiting (Craigie, 1990). A similar relation was found for *P. dioica* in this study, where growth rates increased with increasing day length. It is interesting to note also the influence of the stocking density on biomass production. Higher yields may be obtained at higher stocking densities and higher PFDs. At these PFDs and with these stocking densities production is not light limited.

Nitrogen limitation is unlikely to occur at 500 μM NO_3^- if pulsed every 3–4 days, except at the highest stocking density. With a stocking density of 1.5 g fw l^{-1} and at 150 μmol photons m^{-2} s^{-1}, with a biomass production of 1.39 g fw per week, the thalli would remove from the water 1.67 mg N per day. This amounts to a N removal of 5.0 to 6.69 mg N per each nutrient pulse (3–4 days), corresponding to an uptake of 358 to

478 μmol of NO_3^- (72% to 95% of the N available in the medium). At lower stocking densities the amount of N incorporated into new tissue after 4 days is lower. Comparing between stocking densities at 150 μmol photons m^{-2} s^{-1}, with 0.1, 0.3, 0.6 and 1.0 g fw l^{-1} stocking densities, 119, 271, 408 and 402 μmol of nitrogen, respectively, were incorporated in new tissue. This corresponds, in the same order, to approximately 64%, 56%, 85% and 80% of the nitrogen removed from the medium (see Fig. 1G). In other words, although the thalli were removing more than 92% of the available N (except the ones with 0.1 g fw l^{-1}), they were incorporating into new tissue only a fraction of this nitrogen. When we compare stocking densities within the other two PFDs the trend is similar. However, at 50 μmol photons m^{-2} s^{-1} the percentage of N incorporated into the tissue is always higher for all stocking densities when compared to the higher PFDs. The higher N content of these thalli agrees with these results. It seems that although the thalli could remove an enormous amount of N from the water, they were not able to metabolize all that and incorporate it into new tissue. This is a very important question if this alga is to be considered for bioremediation purposes. In this case, the uptake efficiency cannot be determined simply by water analysis and quantification of the percentage of N removed from the water. Tyler et al. (1994) reported for the first time the release of dissolved organic nitrogen (DON) by *Ulva lactuca* during active growth. Release of free amino acids (FAA) was measured before in studies with phytoplankton (Collos et al., 1992; Bronk and Ward, 1999). On the other hand, Naldi and Wheeler (2002), using ^{15}N isotopes, observed very little release of DON by *Ulva fenestrata* and *Gracilaria pacifica*. Naldi and Wheeler (2002) suggested that the release of DON is more likely to be due to degradation of detritus than from live algal tissue. In the case of our study there was no detritus present in the system. What happened to that missing nitrogen fraction is a question that remains to be answered. Further studies are being planned to determine the N uptake and N incorporation kinetics and the N saturation levels for this species.

Another limiting factor could be CO_2 limitation (Craigie and Shacklock, 1995). The measurements taken revealed that the pH changes to a maximum of 8.8 (average of the replicates) after 4 days at stocking densities of 0.3 and 1.5 g fw l^{-1}. A similar change was observed in all the conditions where biomass production was higher. In other words, pH changed as much in cultures with 1.5 g fw l^{-1} as in those with 0.6 and even 0.3 g fw l^{-1}. We do not know, at this point, if *P. dioica* is capable of using HCO_3^- as a source of carbon. Bicarbonate utilization has been suggested for *P. leucosticta* (Mercado et al., 1997), *P. umbilicalis* (Maberly, 1990) and, although with a limited capacity, for *P. linearis* (Israel et al., 1999). On the other hand, those authors also agree that species restricted to utilizing CO_2 did not increase pH above 9.0. At this pH, CO_2 levels would account for only 0.06% or less of the inorganic carbon available in the seawater (Beer and Eshel, 1983). This seemed to be the case for *P. dioica*. If *P. dioica* is not able to use bicarbonate then we must consider that CO_2 limitation could have occurred. In this case, we must also note that carbon limitation is likely to occur in stocking densities as low as 0.3 g fw l^{-1} if pH is not controlled. On the other hand, this can also mean that growth rate and, consequently, productivity can be improved by adding inorganic carbon to the culture medium. Gao et al. (1991) reported that photosynthesis and growth rate of *P. yezoensis* was enhanced when the cultures were aerated with CO_2.

Porphyra dioica is characterized by rapid growth and nutrient assimilation. The results confirm what is predicted by the functional-form model (Hanisak et al., 1990). However, like those authors have explained, production of a species with this flat blade morphology is not sustainable for significant periods of time. Large portion of the thalli may become reproductive, entire cultures can sporulate and be lost in a short time period. For this reason, one characteristic of *Porphyra* cultivation is the need for a constant supply of zygotospores. Each crop, every year, has to start from a conchocelis culture that is induced to form conchosporangia and to release conchospores at a desired time. In other words, there must be a fairly complex operating system behind the profitable gametophyte culture. This cultivation system is usually labor and cost intensive and this is one of the reasons why this industry has not been established in developed countries, where labour costs are higher. In an effort to reduce the costs of *Porphyra* cultivation and make the all process simpler, several studies have focused on vegetative propagation techniques of the gametophytes, bypassing the need for the sporophyte culture (e.g. Chen, 1997; Notoya, 1999; Hafting, 1999b). Our efforts have revealed that *P. dioica* is capable of regenerating new blades from the basal portions of adult blades. It becomes even more interesting to note that such phenomena happened in temperatures from 10–20 °C (and, at least from 10–15 °C, in all photoperiods tested). The growth rate of these new blades varied between 22.4% and 26.1% day^{-1} and was similar to the initial blades and similar in all photoperiods. The applications of these observations are easy to understand in light of what was previously said.

This would, at least theoretically, allow a continuous culture of gametophytes for a land-based integrated recirculating system, bypassing the need of a periodic "seeding" procedure, and the maintenance of conchocelis cultures. These clonal cultures would be less costly and simpler to maintain.

Neori et al. (2004) have already pointed out the limitations of the use of bacterial biofilters for the treatment of intensive aquaculture waters. According to those authors, it is the added cost that prevented bacterial-based intensive aquaculture technologies from producing large quantities of fish at competitive prices. Neori et al. (2004) also described the advantages of the use of seaweed-based bioremediation. There isn't, however, a global solution. Land-based integrated aquaculture systems are dynamic, changing according to such variables as location, season, species and social environment (Little and Muir, 1987; Edwards, 1994). If integrated aquaculture is to become a common practice, there is a need to find different species that fit different animal aquaculture conditions, or, even better, to find species with satisfactory performances in a wide range of culture conditions. Having this in perspective, this study is one of the few of this kind dealing with a northern Atlantic species.

The wide range of temperature and photoperiod in which *P. dioica* is able to grow above 20% day^{-1} is also an interesting feature. If this genus is acceptable for biofiltration of land-based finfish aquaculture, flexibility will be important. Using eurythermal species provides an advantage for year round aquaculture. Other important aspects are the nutrient removal efficiency of *P. dioica* and the quality of its biomass (e.g. protein, FAA and pigments content). Recently, Troell et al. (2003) published a comprehensive review of the major studies done within an integrated aquaculture perspective. The results obtained for N uptake in our work compare well with ones referred by those authors. The figure we obtained for N removal is similar to the productivity figure. At 150 and 250 μmol photons m^{-2} s^{-1} the N removal data are close to each other above 0.6 g fw l^{-1} stocking density, inclusive (see Fig. 1E). Again there was no difference between 150 and 250 at 0.6 and 1.0 g fw l^{-1} stocking density. The main difference is that thalli grown at 50 μmol photons m^{-2} s^{-1} have a nitrogen removal capacity similar to that of the thalli grown at higher light levels. At 0.1 and 1.0 g fw l^{-1} stocking density this capacity is statistically equal ($P>0.05$). These results might come as a surprise because of the significantly lower growth rates and productivity of the thalli at 50 μmol photons m^{-2} s^{-1}. The differences on growth rates are balanced by their higher nitrogen content, especially at higher stocking densities. An important pool of nitrogen in the tissue are the phycobiliprotein pigments, namely PE. The PE content at the lower light level is 21.7% and 30.4% higher than at 150 and 250 μmol photons m^{-2} s^{-1}, respectively. This effect (increase of the photosynthetic pigments with lower PFDs) has been reported as photoacclimation (Falkoswki and LaRoche, 1991). The values obtained in this study are similar to those reported for other *Porphyra* species. Conitz et al. (2001) detected 1.80 to 3.67 mg g dw^{-1} in young gametophytes of *P. linearis* grown at 90–110 μmol photons m^{-2} s^{-1}, $8:\overline{16}$, $L:\overline{D}$ although these authors used less nitrate (88 μM). Carmona et al. (2006-this issue) reported 3.25 (±0.35) mg PE g^{-1} fw, for *Porphyra purpurea*, the highest for the several species tested in their study. However, that higher PE content we observed at 50 μmol photons m^{-2} s^{-1} might not be sufficient to explain the equivalent N removal at this PFD, when compared to the N removal at 150 and 250 μmol photons m^{-2} s^{-1}, where productivity is significantly higher. Another important pool of N in the tissue are the FAA (Bird et al., 1982; Naldi and Wheeler, 1999). Peinado et al. (2004) also showed an accumulation of micosporine-like amino acids (MAA) in response to high ammonium concentrations. The effects of the culture conditions on the FAA and MAA quantity and quality in *P. dioica* needs to be investigated. Pedersen et al. (submitted for publication) showed that FAA is positively correlated with the total N content in *P. perforata*, *P. suborbiculata* and *P. leucosticta* Type C (sensu Neefus et al., 2000). A good example of the importance of the FAA content on the cultivated *Porphyra* is the work of Niwa et al. (2003). Those authors crossed a "high FAA content" green mutant with a "high growth rate" wild type *Porphyra* to obtain F_1 blades with both characteristics. Genetic improvement is another way of increasing the quality of the cultivated *Porphyra*, the other being the manipulation of the culture conditions.

Overall, this work shows that the gametophytes of *P. dioica* can sustain a high growth rate and productivity over a range of temperature, photoperiod and light conditions. The optimal conditions can be adapted to the objective of the culture. Stocking density of the culture is important in terms of biomass production. In combination with light, these factors influence the biochemical properties of the thalli. If the purpose of the culture is to obtain biomass with higher N content (>6% dw), for instance for phycoerythrin extraction, stocking densities of 0.3 and 0.1 g fw l^{-1} and 50 μmol photons m^{-2} s^{-1} should be used. If the focus is on biomass production and maximum nutrient assimilation, higher

stocking densities and PFDs should be used. This difference is relevant if we think in terms of a bioremediation application. Chopin et al. (2001) pointed out that the concepts of nutrient uptake efficiency and nutrient uptake rates must be distinguished. Neori et al. (1996) showed a way to combine both results using seaweed biofilters integrated with intensive fish aquaculture. Their system consisted of fish tanks-seaweed tanks water recirculation system plus a polishing tank with seaweeds. Higher uptake efficiency in the polishing tank was achieved by reducing the water influx.

To extrapolate the potential N-removal results obtained in this study into a bigger scale, we will consider that: approximately 600 g of N are released per day by ton of fish (data from a 50 fish pond, 36 m^3 each, turbot and sea bass farm, as described by Matos et al., 2006-this issue); in a 1 m^3 seaweed tank (1 m^2 surface area), we could have 1.5 kg of *P. dioica* in culture, capable of removing 1.67 g N per day. The area needed to remove 50% of the N effluent would be 179 m^2 of *P. dioica* culture. We believe that at this scale the performance of *P. dioica* can be improved. Under these tank cultivation conditions, *P. dioica* would have more nutrients and CO_2 available, due to the constant water flux. This would also allow to experiment with higher stocking densities, possibly increasing nutrient removal.

In conclusion, this study demonstrates that *P. dioica* should be considered for bioremediation of aquaculture effluents. Being a fast grower and able to assimilate nutrients rapidly, it may be expected to have higher growth rates when nutrients are higher and continuously supplied. The growth rates obtained, the N removal potential and the possibility of vegetative propagation are promising features for land-based integrated aquaculture systems. These results are conclusive at the lab-scale, and the transfer of the methodology to an integrated aquaculture system should be considered.

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References

Anonymous, 2004. Water temperature data in Leixões, data from January 2003 to January 2004, available on the Instituto Hidrográfico web site at http://www.hidrografico.pt/wwwbd/boias/grafico.asp.

Ardré, F., 1970. Contribution a l'étude des algues marines du Portugal. I-La Flore. Portugaliae Acta Biologica (B), vol. X, n°s 1/4. Coimbra Editora, Limitada, Portugal, pp. 50–53.

Avila, M., Santelices, B., McLachlan, J., 1986. Photoperiod and temperature regulation of the life history or *Porphyra columbina* (Rhodophyta, Bangiales) from central Chile. Canadian Journal of Botany 64, 1867–1872.

Beer, S., Eshel, A., 1983. Photosynthesis of *Ulva* sp. II. Utilization of CO_2 and HCO_3^- when submerged. Journal of Experimental Marine Biology and Ecology 70, 99–106.

Beer, S., Eshel, A., 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Australian Journal of Marine Freshwater Research 36, 785–792.

Bidwell, R.G.S., Lloyd, N.D.H., McLachlan, J., 1984. The performance of *Chondrus crispus* (Irish moss) in laboratory simulations of environments in different locations. Proceedings of the International Seaweed Symposium 11, pp. 292–294;
McLachlan, J., Bidwell, R.G.S., Smith, R.G., Moseley, C.M., 1984. Cultivation strategies for seaweeds. Actas II Congresso Algas Marinas Chilenas, pp. 47–62.

Bird, C.J., 1973. Aspects of the life history and ecology of *Porphyra linearis* (Bangiales, Rhodophyceae) in nature. Canadian Journal of Botany 51, 2371–2379.

Bird, K.T., Habig, C., DeBusk, T., 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). Journal of Phycology 18, 344–348.

Brodie, J., Irvine, L.M., 1997. A comparison of *Porphyra dioica sp. nov.* and *P. purpurea* (Roth) C. Ag. (Rhodophyta, Bangiophycidae) in Europe. Cryptogamie. Algologie 18 (3), 283–297.

Bronk, D.A., Ward, B.B., 1999. Gross and net nitrogen uptake and DON release in the euphotic zone of Monterey Bay, California. Limnology and Oceanography 44, 573–585.

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 morphlogical data. European Journal of Phycology 37, 227–235.

Candia, A., Lindstrom, S., Reyes, E., 1999. *Porphyra sp.* (Bangiales, Rhodophyta): reproduction and life form. Hydrobiologia 398/399, 115–119.

Carmona, R., Kraemer, G.P., Yarish, C., 2006-this issue. Exploring Northeast American and Asian species of *Porphyra* for use in an Integrated Finfish-Algal Aquaculture System. doi:10.1016/j.aquaculture.2005.11.049.

Chen, L.C.-M., 1997. *Porphyra* cell suspension cultures, novel biotechnological approach for the production of Hoshi-nori. Natural History Research. Special issue 3, 111–121.

Chopin, T., Yarish, C., Wilkes, R., Belyea, E., Lu, S., Mathieson, A., 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. Journal of Applied Phycology 11, 463–472.

Chopin, T., Buschman, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture

systems: a key towards sustainability. Journal of Phycology 37, 975–986.

Collos, Y., Döhler, G., Biermann, I., 1992. Production of dissolved organic nitrogen during uptake of nitrate by *Synedra planctonica*: implications for estimates of new productions in the ocean. Journal of Plankton Research 14, 1025–1029.

Conitz, J.M., Fagen, R., Lindstrom, S.C., Plumley, F.G., Stekoll, M., 2001. Growth and pigmentation of juvenile *Porphyra torta* (Rhodophyta) gametophytes in response to nitrate, salinity and inorganic carbon. Journal of Applied Phycology 13, 423–431.

Craigie, J.S., 1990. Irish moss cultivation: some reflections. In: Yarish, C., Penniman, C.A., Van Patten, P. (Eds.), Economically Important Plants of the Atlantic: Their Biology and Cultivation. Groton, Connecticut Sea Grant College Program, USA, pp. 37–52.

Craigie, J.S., Shacklock, P.F., 1995. Culture of Irish moss, In: Boghen, A.D. (Ed.), Cold-Water Aquaculture in Atlantic Canada, 2nd edition. Can. Inst. Res. Reg. Devel. University of Moncton, Moncton, N. B., pp. 363–390.

Drew, K.M., 1954. Studies in the Bangioidea. III. The life-history of *Porphyra umbilicalis* (L.) Kütz. *var. laciniata* (Light.). J. Ag. Annals of Botany, N.S., vol. XVIII, No. 7, pp. 183–209.

Edwards, P., 1994. A systems approach for the promotion of integrated aquaculture. Paper Presented at Integrated Fish Farming International Workshop, 11–15 October 1994, Wuxi, People's Republic of China;
Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 1994. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, pp. 361–391.

Falkoswki, P.G., LaRoche, J., 1991. Acclimation to spectral irradiance in algae. Journal of Phycology 27, 8–14.Lobban, Harrison (Eds.), 1994. Seaweed Ecology and Physiology. Cambridge University Press, Cambridge, UK.

FAO 2003 yearbook, Fishery statistics, Aquaculture production 2001. Vol 92/2. Formerly published as: FAO Fisheries Circular N.815 "Aquaculture Production Statistics" in http://www.fao.org/fi/statist/statist.asp (18/09/2003).

Fei, X.G., Lu, S., Bao, Y., Wilkes, R., Yarish, C., 1998. Seaweed cultivation in China. World Aquaculture 29 (4), 22–24.

Figueroa, F.L., Aguilera, J., Jiménez, C., Vergara, J.J., Robles, M.D., Niell, C.X., 1995. Growth, pigment synthesis and nitrogen assimilation in the red alga *Porphyra* sp. (Bangiales, Rhodophyta) under blue and red light. Scientia Marina 59 (1), 9–20.

Fleurence, J., 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science & Technology 10, 25–28.

Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., Kiyohara, M., 1991. Enhanced growth of the red alga *Porphyra yezoensis* Ueda in High CO_2 concentrations. Journal of Applied Phycology 3, 355–362.

Hafting, J.T., 1999a. Effect of tissue nitrogen and phosphorus quota on growth of *Porphyra yezoensis* blades in suspension cultures. Hydrobiologia 398/399, 305–314.

Hafting, J.T., 1999b. A novel technique for propagation of *Porphyra yezoensis* Ueda blades in suspension cultures via monospores. Journal of Applied Phycology 11, 361–367.

Hanisak, M.D., Littler, M.M., Littler, D.S., 1990. Application of the functional-form model to the culture of seaweeds. Hydrobiologia 204/205, 73–77.

Israel, A., Katz, S., Dubinsky, Z., Merril, J.E., Friedlander, M., 1999. Photosynthetic inorganic carbon utilization and growth of *Porphyra linearis* (Rhodophyta). Journal of Applied Phycology 11, 447–453.

Katz, S., Kizner, Z., Dubinsky, Z., Friedlander, M., 2000. Responses of *Porphyra linearis* (Rhodophyta) to environmental factors under controlled culture conditions. Journal of Applied Phycology 12, 535–542.

Kito, H., Kawamura, Y., 1999. The cultivation of *Porphyra* (nori) in Japan. World Aquaculture 30 (2), 35–39.

Klein, A.S., Mathieson, A.C., Neefus, C.D., Cain, D.F., Taylor, H.A., Teasdale, B.W., West, A.L., Hehre, E.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 *rbc*L genes. Phycologia 42 (2), 109–122.

Kraemer, G., Yarish, C., 1999. A preliminary comparison of the mariculture potential of *Porphyra purpurea* and *Porphyra umbilicalis*. Journal of Applied Phycology 11, 473–477.

Little, D., Muir, J., 1987. A Guide to Integrated Warm Water Aquaculture. Institute of Aquaculture Publications, University of Stirling, Stirling, p. 238;
Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G. P., Halling, C., Shpigel, M., Yarish, C., 1987. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, pp. 361–391.

Lobban, C.S., Chapman, 1988. Experimental Ecology, A Laboratory Manual, pp. 134–140.

Maberly, S.C., 1990. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. Journal of Phycology 26, 439–449.

Matos, J., Costa, S., Rodriguez, A., Pereira, R., Sousa Pinto, I., 2006-this issue. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. doi:10.1016/j.aquaculture.2005.11.047.

McVey, J., Stickney, R.R., Yarish, C., Chopin, T., 2002. Aquatic polyculture and balanced ecosystem management: new paradigms for seafood production. In: Stickney, R.R., McVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, New York, USA. 391 pp.

Mercado, J.M., Niell, F.X., Figueroa, F.L., 1997. Regulation of the mechanism for HCO_3^- use by the inorganic carbon level in *Porphyra leucosticta* Thur. in Le Jolis (Rhodophyta). Planta 201, 319–325.

Mumford, T.F., Miura, A., 1988. *Porphyra* as food: cultivation and economics. In: Lembi, C.A., Waland Jr. (Eds.), Algae and Human Affairs. Cambridge University Press, London, pp. 87–117.

Naldi, M., Wheeler, P., 1999. Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. Journal of Phycology 35, 70–77.

Naldi, M., Wheeler, P., 2002. ^{15}N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparison of net nutrient disappearance, release of ammonium and nitrate, and ^{15}N accumulation in algal tissue. Journal of Phycology 38, 135–144.

Neefus, C.D., Mathieson, A.C., Yarish, C., Klein, A., West, A., Teasdale, B., Hehre, E.J., 2000. Five cryptic species of Porphyra from the Northwest Atlantic. Journal of Phycology 36 (suppl.).

Neefus, C.D., Mathieson, A.C., Klein, A.S., Teasdale, B., Bray, T., Yarish, C., 2002. *Porphyra birdiae sp. nov.* (Bangiales, Rhodophyta): a new species from the Northwest Atlantic. Algae 17 (4), 203–216.

Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Dvir, O., Zuber, D., Ucko, M., Angel, D., Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. Aquaculture 14, 183–199.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.

Niwa, K., Furuita, H., Aruga, Y., 2003. Free amino acid contents of the gametophytic blades from the green mutant conchocelis and the heterozygous conchocelis in *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta). Journal of Applied Phycology 15, 407–413.

Notoya, M., 1999. "Seed" production of *Porphyra* spp. by tissue culture. Journal of Applied Phycology 11, 105–110.

Oohusa, T., 1993. The cultivation of *Porphyra* "Nori". In: Ohno, M. & Chritchley, A.T. (Eds.), Seaweed Cultivation and Marine Ranching. University of Witwatersrand, Johannesburg, South Africa.

Orfanidis, S., 2001. Culture studies of *Porphyra leucosticta* (Bangiales, Rhodophyta) from the Gulf of Thessaloniki, Greece. Botanica Marina 44, 533–539.

Ott, F.D., 1965. Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. Virginia Journal of Science 16, 205–218.

Pedersen, A., Kraemer, G., Hariskov, S., Yarish, C., submitted for publication. Free amino acids, Tot C, Tot N and phycobiliproteins content in *Porphyra* spp. and its response during short term uptake of nitrate.

Peinado, N.K., Díaz, R.T.A., Figueroa, F.L., Helbling, E.W., 2004. Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. Journal of Phycology 40, 248–259.

Pereira, R., Sousa-Pinto, I., Yarish, C., 2004. Field and culture studies of the life history of *Porphyra dioica* (Bangiales, Rhodophyta) from Portugal. Phycologia 43 (6), 756–767.

Sidirelli-Wolf, M., 1992. The influence of temperature, irradiance and photoperiod on the reproductive life history of *Porphyra leucostica* (Bangiales, Rhodophyta) in laboratory culture. Botanica Marina 35, 251–257.

Silva, P.C., *Porphyra* from the *Index nominum algarum*. http://ucjeps.berkeley.edu/rlmoe/porphyra.html. Univ. Herbarium, UC Berkeley. Rev: 12/20/99.

Sokal, R.R., Rholf, F.J., 1995. Biometry: The Principles and Practices of Statistics in Biological Research, 3rd ed. W.H. Freeman, New York. 887 pp.

South, G.R., Titley, I., 1986. A checklist and Distribution Index of the Benthic Marine Alga of the North Atlantic Ocean. Huntsman Marine Laboratory and British Museum (Natural History). London.

Stekoll, M.S., Lin, R., Lindstrom, S.C., 1999. *Porphyra* cultivation in Alaska: conchocelis growth of three indigenous species. Hydrobiologia 398/399, 291–297.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N., Yarish, C., 2003. Integrated mariculture: asking the right questions. Aquaculture 226, 69–90.

Tyler, A.C., McGlathery, K.J., Anderson, I.C., 1994. Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. Estuarine, Coastal and Shelf Science 53, 155–168.

Waaland, J.R., Dickson, L.G., Duffield, E.C.S., Carrier, J.E., 1987. Conchocelis growth and photoperiodic control of conchospore release in *Porphyra torta* (Rhodophyta). Journal of Phycology 23, 399–406.

Yarish, C., Wilkes, R., Chopin, T., Fei, X.G., Mathieson, A.C., Klein, A.S., Neefus, C.D., Mitman, G.G., Levine, I., 1998. Domestication of indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America. World Aquaculture 29 (4), 26–29 (55).

Yarish, C., Chopin, T., Wilkes, R., Mathieson, A.C., Fei, X.G., Lu, S., 1999. Domestication of Nori for Northeast America: The Asian Experience. Bulletin Aquaculture Association of Canada 99 (1), 11–17.

Yarish, C., Neefus, C.D., Kraemer, G.P., Chopin, T., Nardi, G., Curtis, J., 2001. Development of an integrated recirculating aquaculture system for nutrient bioremediation in urban aquaculture. The Connecticut Sea Grant College Grant R/A-34, NOAA OAR's National Marine Aquaculture Initiative Project Number NA16RG1635.

Yoshida, T., Notoya, M., Kikuchi, N., Miyata, M., 1997. Catalogue of species of *Porphyra* in the world, with special reference to the type locality and bibliography. Natural History Research. Special Issue 3, 5–18.