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Enhanced production of Pacific dulse (*Palmaria mollis*) for co-culture with abalone in a land-based system: nitrogen, phosphorus, and trace metal nutrition

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Abstract

Pacific dulse (*Palmaria mollis*) is a high-quality algal feed for both red abalone (*Haliotis rufescens*) and Japanese abalone (*Haliotis discus hannai*); however, culture techniques capable of producing commercial quantities of *P. mollis* are still in development. This study was undertaken to improve our understanding of nutrient requirements and management strategies for *P. mollis* production in intensive, land-based systems.

All experiments were conducted under moderate to high artificial light of 24–52 mol photons m^{-2} day^{-1} and low seawater exchange (1 vol day^{-1}), in order to minimize seawater pumping costs. Application of nutrients every 5–7 days resulted in no significant difference in *P. mollis* growth compared with daily applications. Nutrient additions during the dark cycle compared with the light cycle were found to be effective in controlling epiphytes. The addition of *f* medium trace metals (Gran. Can. J. Microbiol. 8 (1962) 229) significantly increased growth rates compared with those of cultures supplied with just nitrate and phosphate alone. The concentration of Zn was increased to equal that of Mn (1.37 μM day^{-1}), with resulting improvement in yields. $NaNO_3$ as a source of nitrogen was found to be superior for long-term growth (9 weeks) compared with NH_4NO_3, although NH_4NO_3 was superior on a short-term basis (first 2–5 weeks). This was likely due to NH_4^+–N toxicity resulting from the experimental design. While *P. mollis* growth was not significantly different between additions of 1176–2942 μM day^{-1} NO_3^-–N (average daily N load), an increasing trend in growth was observed with increasing N loads up to 2942 μM day^{-1} NO_3^-–N. *P. mollis* showed a sharp decline in growth with P additions above 83.3 μM day^{-1} (average daily P load).

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Tissue nutrient concentrations and molar ratios (N, P, trace metals, N/P and C/N) can be used in the development of optimal nutrient management strategies to ensure maximum dulse growth rates.

Keywords: *Palmaria mollis*; Pacific dulse; Seaweed; Nutrition; *Haliotis*; Recirculating systems

1. Introduction

Over the past two decades, advances in mass culture of seaweeds (Hanisak and Ryther, 1984; Bidwell et al., 1985; Neori et al., 1996) have set the stage for the aquaculture industry to make the transition from open to closed or semi-closed polyculture systems with macroalgae acting as the primary biofilter. Interest in these living feeds, capable of both sustained vegetative growth and functioning as in situ biofilters, has resulted in a number of valuable studies. Several good examples that clearly demonstrate the key role of seaweeds in commercial, land-based marine polyculture systems were reported by the National Center for Mariculture in Elat, Israel, (Cohen and Neori, 1991; Shpigel and Neori, 1996; Neori et al., 1996, 2000).

Palmaria mollis (=*Rhodymenia palmata* var. *mollis*, Setchell and Gardner, 1903) (Rhodophyceae, Bangiales) is found in the low intertidal and subtidal rocky shore from Southeastern Alaska to San Luis Obispo County, California. At the Hatfield Marine Science Center (HMSC), Oregon State University, research has shown the effectiveness of *P. mollis* or Pacific dulse in removing NH_4^+–N, NO_3^-–N, and PO_4^- from aquaculture effluents generated by land-based salmon and abalone culture (Levin, 1991; Evans and Langdon, 2000).

The purpose of this study was to determine optimal nutrient conditions for enhancing yields of *P. mollis* over a range of naturally occurring photon flux densities (PFDs) under low seawater exchange conditions. The proper application rate and concentration of nutrients needed to be determined for mass culture so that adequate nutrition was provided without the adverse affects of over-supply, leading to either depressed growth or the proliferation of epiphytes and weed species (Schramm, 1991).

Some important culture methods have been previously described in the literature. Pulse fertilization was shown to produce good growth rates and be effective in controlling epiphytes with other cultured Rhodophyte species (Neish and Fox, 1971; Lapointe, 1985; Pickering et al., 1993). Compared to higher plants, seaweeds require a greater range of inorganic nutrients (Dring, 1982), which can quickly become limiting in land-based, low exchange culture systems. Indeed, it was known that as many as 25 elements might be required for proper seaweed nutrition (Harrison and Druehl, 1982). Nutrients that have been considered essential supplements under low flow conditions include: N, P, Fe, Zn, Mn, and Cu (Sunda, personal communication).

Since pumping seawater represents one of the most expensive operating costs of land-based aquaculture systems (Huguenin, 1976), it was also important to evaluate nutrient requirements under low seawater exchange rates. Previous experiments with *P. mollis* under low exchange rates achieved a specific growth rate (SGR) of 6.1% day^{-1} at uptake-saturating concentrations of N and P (Evans and Langdon, 2000). This was in

contrast to an SGR of 7.9% day^{-1}, when Guillard and Ryther's (1962) *f* medium was evaluated as a source of nutrients in preliminary experiments (Demetropoulos, unpublished data). These results prompted the need for a more in depth study of the nutritional requirements of *P. mollis*.

2. Materials and methods

2.1. General methods

All experiments used healthy, whole *P. mollis* rosettes provided by HMSC (OSU, C-3 strain; Demetropoulos et al., 2001). These had been previously cultured under an exchange rate of 6 vol day^{-1} and a moderate PFD of 30 mol photons m^{-2} day^{-1} under a 12:12 h photoperiod (Evans and Langdon, 2000). Triplicate 3.8-l opaque-sided vessels were placed in a seawater bath and aerated vigorously at 1.8 l min^{-1}. Vessels were stocked with *P. mollis* at a density of 0.5 kg m^{-2} (damp wt) or 2.6 g l^{-1} (damp wt) and supplied with sand-filtered seawater at a flow rate of 1 vol day^{-1}, maintained at 16 ± 1 °C and a salinity of 31 ± 1 ‰. A hexagonal "box" covered with reflective Mylar™ was placed around the vessels to block out ambient light and evenly distribute artificial light. Either 1000- or 1500-W metal halide lamps were used to illuminate the treatments. These were adjusted to produce a PFD of 300 to 1400 μmol photons m^{-2} s^{-1} (PAR) or 22.5 to 51.64 mol photons m^{-2} day^{-1} at the surface of the culture vessels. One advantage to these lamps was moderately high UV output (UV spectral power = 0.03 W nm^{-1} 1000 $lumens^{-1}$; Osram Sylvania, personal communication) that better simulated outdoor conditions than other available lamps. Cultures were acclimated for 1 week prior to measurements.

Cultures were not maintained using sterile techniques in order to approach the "commercial reality" of air/water-born contamination. All enrichments were added during the dark cycle so as to offset chlorophyte and epiphyte growth (Hanisak, 1987; J. McLachlan, personal communication). Typically, the contents of the culture vessels were damp-weighed weekly using either the spin cycle of a washing machine or a salad spinner and thinned to their original stocking density at the beginning of each week. These methods of measuring growth were found to be highly repeatable (S.D. ± 0.02). Specific growth rates were calculated according to the equation: $SGR = 100 \ln(W_t/W_0)/t$ (DeBoer et al., 1978), where SGR is expressed as % [dw] day^{-1}, W_f and W_i are the final and initial algal dry weights, and t is time (days). The equation assumes steady state exponential growth (DeBoer et al., 1978).

At the end of each experiment, whole rosettes were dipped in 0.5 M ammonium formate to remove external salts, rinsed with deionized water to remove ammonium, and either freeze-dried at −80 °C or oven dried (60 °C, 48 h) and kept at −80 °C for composition analysis.

2.2. Nutrient application rate

This experiment determined how *P. mollis* growth was affected by nutrient application rates under constant nutrient load. It was designed as a 6 × 2 factorial experiment, with six

application rates (once a day or once every 3, 5, 7, 9 or 11 days) at two light levels (22.5 or 42.34 mol photons m^{-2} day^{-1}). The nutrient load [(concentration of added nutrients in culture) × culture volume/frequency of addition (Lobban and Harrison, 1997)] for each treatment was held constant by adjusting the concentration of nutrient additions according to the different application rates that were tested. Thus, treatment 1×7 day^{-1} received seven times the concentration on day 7 vs. treatment $1 \times day^{-1}$, which received nutrients every day. Nutrients ($NaNO_3$, NaH_2PO_4, and trace metals) were supplied at 1*f* molar concentrations day^{-1} (Guillard and Ryther, 1962).

Sand-filtered seawater for the six enrichments was pretreated by passage through a nutrient-filter consisting of a 120-l tank with 1000 g of *P. mollis* in tumble culture, followed by filtration through 120- and 30-μm cartridge filters. Illumination was provided by 1000-W metal halide lamps on a 16:8 h photoperiod. Window screen was placed over experimental treatments to obtain the lowest light level. The experiment ran for 28 days.

2.3. Trace metal additions

Two experiments were conducted to determine if modifications to the *f* medium trace metal solution resulted in increased growth of *P. mollis*. Previous work had shown that addition of the *f* medium trace metals could produce better growth in *P. mollis* than additions of N and P alone (Demetropoulos, unpublished data). In both experiments, $NaNO_3$ and NaH_2PO_4 were supplied at the equivalent of 1*f* molar concentrations day^{-1} (Guillard and Ryther, 1962). N and P were added to the culture vessels every 3 days with the total nutrient load being equal to what the vessels would receive if they were fertilized daily with 1*f* medium (cf. Nutrient application rate experiment).

The two experiments were designed as follows:

2.3.1. Zinc additions

Zinc was the only individual trace metal in the *f* medium that was evaluated separately from other trace metals in this study for several reasons. Literature values indicated that typical molar concentrations of Zn in wild *P. palmata* could match or exceed those of Mn (Morgan et al., 1980a; Sirota and Uthe, 1979). Further, Munda (1978) found Zn exceeded that of Mn by 50% in the tissues of *P. palmata* and Sunda (personal communication) suggested that the concentration of Zn may need to be as high as Mn in *Palmaria* spp. Finally, cultures of *P. mollis* under high natural light, removed Zn (as supplied by the 1*f* nutrient medium) to below detectable concentrations more quickly than other trace nutrients (Demetropoulos, unpublished data).

Zinc (as $ZnSO_4$) was added to 1*f* medium at a concentration equal to that of Mn (1.82 μM day^{-1}). The chelating agent, ethylenediaminetetraacetic acid (EDTA), already present in the *f* medium, was assumed to be sufficient for chelating additional $ZnSO_4$ (Sunda, personal communication). The standard 1*f* nutrient medium (without extra Zn) was used as a control. NaH_2PO_4 was used as the source of P (average P load of 83.3 μM day^{-1} provided as 10 mg l^{-1} day^{-1} NaH_2PO_4) and $NaNO_3$ was used as the source of N (average nutrient load of 1765 μM day^{-1} provided as 150 mg l^{-1} day^{-1} $NaNO_3$). Nutrients were added every 3 days as described in the nutrient application rate experiment, tripling the concentrations added at one time. Illumination was provided by 1000-W metal halide

lamps on a 16:8 h photoperiod, producing a PFD of 22.5 or 42.34 mol photons m^{-2} day^{-1} for the low and high light levels, respectively. SGRs were measured after 35 days.

2.3.2. Concentration of Zn modified f medium trace metals

This experiment was conducted to determine the best concentration of Zn modified 1*f* trace metals to apply to cultures. Based on the results of the Zn experiment (see Results), Zn was added at a concentration equal to that of Mn (1.82 μM day^{-1}, as 1*f*). In combination with 1*f* N and P, six modified *f* medium trace metal concentrations were tested: N and P only (control), 0.25*f* day^{-1}, 0.5*f* day^{-1}, 0.75*f* day^{-1}, 1*f* day^{-1}, and 1.25*f* day^{-1}. Nutrients were added every 3 days (cf. Nutrient application rate experiment), tripling the concentrations added at one time. The experiment ran for 42 days with SGRs being measured and compared in the last 14 days. Space constraints would not allow the experiment to be run at high and low light intensities so an intermediate PFD of 37.44 mol photons m^{-2} day^{-1} (@650 μmol photons m^{-2} s^{-1}) was provided through use of 1000 W metal halide lamps on a 16:8 h photoperiod.

2.4. Ammonium nitrate vs. sodium nitrate

Experiments were conducted to test the effects of NO_3^-–N vs. NH_4^+–N on yields of *P. mollis*. Illumination was provided by 1000-W metal halide lamps, supplying approximately a PFD of 37.44 mol photons m^{-2} day^{-1} on a 16:8 h photoperiod.

The following two experiments were carried out:

2.4.1. Pulse fertilization with ammonium

Two nitrogen treatments, each providing average nutrient loads of 2353 μM N day^{-1} as either $NaNO_3$ or NH_4NO_3 were evaluated. NaH_2PO_4 was used to provide 83.3 μM P day^{-1}. Trace metals were added as 1*f* Zn-modified trace metal mix per day. Nutrients were added every 3 days (cf. Nutrient application rate experiment), tripling the concentrations added at one time. The experiment ran for 28 days.

2.4.2. Continuous fertilization with ammonium

Since pulse fertilizing resulted in cultures receiving a relatively high NH_4^+–N concentration (7059 μM N every 3 days), the effects of continuous additions of NH_4^+–N (supplied as NH_4NO_3) at a lower concentration but with the same average daily N load were determined. Three different ratios of NH_4^+–N/NO_3^-–N were tested, each ratio providing an average load of 2353 μM N day^{-1}: (A) NH_4^+–N/NO_3^-–N [0:1 M]; (B) NH_4^+–N/NO_3^-–N [1:1 M]; (C) NH_4^+–N/NO_3^-–N [2:1 M]. $NaNO_3$ and $(NH_4)_2SO_4$ were used as sources of N. NaH_2PO_4 was used to provide 83.3 μM P day^{-1} and trace metals were added at 1*f* concentration. All enrichments were pumped into the culture vessels once a day over a period of 12 h during the dark via peristaltic pumps. The experiment ran for 56 days.

2.5. Phosphorus and nitrogen additions

In the following two experiments, SGRs of *P. mollis* were determined as a function of N and P additions at two light levels. First, N additions were held constant and the

effects of different P additions were determined, then P additions were held constant and the effects of different N additions were determined. Illumination was provided by 1500-W metal halide lamps on a 16:8 h photoperiod producing a PFD of either 23.68 or 51.64 mol photons m^{-2} day^{-1} for low and high light levels, respectively. N, P and trace metals were added to the cultures every 3 days (cf. Nutrient application rate experiment), tripling the daily concentrations added at one time. Modified *f* medium (0.75*f*+Zn [1.37 μM day^{-1}]) provided trace metals (cf. Trace metal additions experiment).

The two experiments were designed as follows:

2.5.1. Phosphate additions

This experiment was designed as a 7 × 2 factorial in which eight daily PO_4^- concentrations [1.1 (seawater control), 42, 83, 125, 167, 250, 375, and 500 μM day^{-1} PO_4^- as NaH_2PO_4] were tested at two light levels (23.68 or 51.64 mol photons m^{-2} day^{-1}). $NaNO_3$ was used as the source of N (average daily N load of 2942 μM day^{-1} provided as 250 mg l^{-1} day^{-1} $NaNO_3$). SGRs were measured after 35 days.

2.5.2. Nitrate additions

This experiment was designed as a 7 × 2 factorial with seven NO_3^-–N concentrations [15.5 (seawater control), 588, 1176, 1765, 2353, 2942, and 3530 μM day^{-1} NO_3^-–N as $NaNO_3$] at two light levels (23.68 or 51.64 mol photons m^{-2} day^{-1}). These NO_3^-–N values were chosen because they represented an upper range considered potentially applicable to commercial production (Evans and Langdon, 2000; Waaland personal communication). NaH_2PO_4 was used as the source of P (average P load of 83.3 μM day^{-1} provided as 10 mg l^{-1} day^{-1} NaH_2PO_4). SGRs were measured after 49 days.

2.6. Biochemical analysis

All tissue nutrient analyses were performed by Fruit Growers Lab, Santa Paula, CA. Frozen samples were ground to a fine powder. Tissue C and N were measured using a carbon–hydrogen–nitrogen analyzer (LECO, CNS 2000). Tissue P and trace metals were determined with an inductively coupled argon–plasma spectrophotometer. Nitrate was determined spectrophotometrically by the cadmium reduction method (Dorich and Nelson, 1984).

Crude protein was determined by multiplying tissue N by 6.25. Since high NO_3^-–N additions and subsequent storage in intracellular NO_3^-–N pools were likely to interfere with protein determination, plants were starved of nutrients for 3 days prior to analysis. This reduced intracellular NO_3^-–N by approximately 96% in *P. mollis* (Demetropoulos, unpublished data; cf. Lobban and Harrison, 1997).

2.7. Statistics

Unless otherwise stated, SGRs (% day^{-1} [dw]) under the various nutrient regimes were measured during the last week of culture. The effects of nutrient application, trace metals,

ammonium, and N/P enrichment on SGR and biochemical analysis were assessed by two-factor ANOVA and differences among individual treatments were compared by the Student–Newman–Keuls (SNK) multiple comparison test ($\alpha = 0.05$). *P* values are reported for the overall ANOVA as SNK has no comparison-wise error rate. Linear regression was used to test for significant relationships between experimental variables ($\alpha = 0.05$). All error terms are presented as S.E.

3. Results

3.1. General

Initially, a light brown precipitate was noticed the day after nutrient applications in many of the treatments. This was thought to be due to interactions between phosphate and trace metals. Subsequently, N and P and trace metals were added separately and the precipitate was eliminated. Additionally, pH values for the faster growing treatments were approximately 8.60–8.75 during the last 2 or 3 days of each weekly period (before thinning the cultures to reduce stocking densities), thus, some DIC limitation was likely (Demetropoulos and Langdon, 2004a).

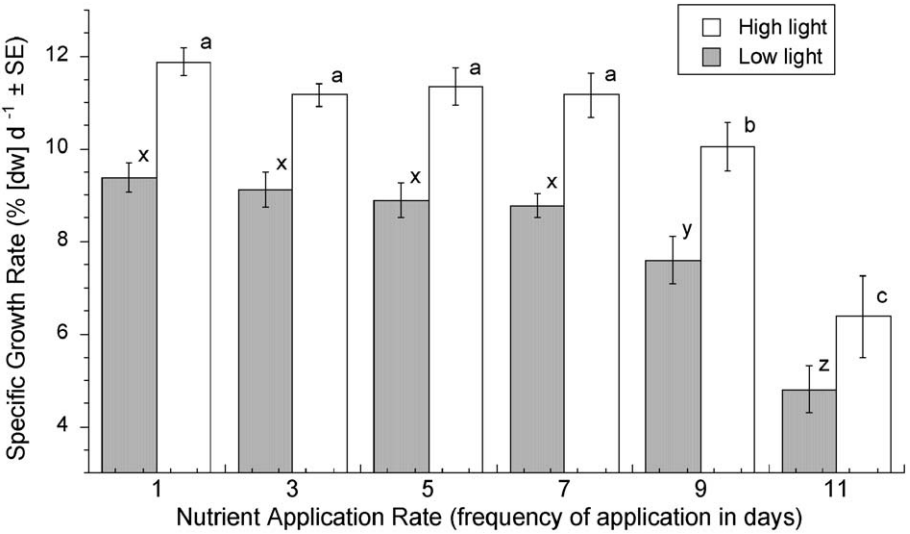

Fig. 1. Specific growth rate of *P. mollis* (± S.E.) as a function of nutrient application rate (with equal nutrient loads among all treatments) under artificial low light (PFD = 22.5 mol photons m^{-2} day^{-1}) or artificial high light (PFD = 42.34 mol photons m^{-2} day^{-1}). Cultures were supplied with $NaNO_3$, NaH_2PO_4, and trace metals at the 1*f* molar concentrations (Guillard and Ryther, 1962). Letters (a, b, c) and (x, y and z) indicate significant differences (ANOVA, SNK; $P < 0.0001$) among high and low light intensity treatments, respectively. The experiment ran for 35 days.

3.2. Nutrient application rate

This experiment showed no difference in growth for cultures provided nutrients daily vs. those provided the same daily average nutrient load at intervals of up to 7 days (Fig. 1). However, there was a significant decline (ANOVA, SNK; $P < 0.0001$) in growth when nutrient loads were provided at intervals of 9 and 11 days (Fig. 1). Mean SGR of *P. Mollis* with nutrient applications from once per day (1×1) to once every 7 days (1×7) under the low light level was equal to 9.04 ± 0.27 and under the high light level was equal to 11.4 ± 0.34 (Table 1). Daily addition of nutrients caused epiphytes (primarily *Ectocarpus siliculosis* and *Enteromorpha intestinalis*) to become a substantial problem in the last 2 weeks of the trial, accounting for as much as 15% of dry weight yields. In contrast, cultures with nutrient additions every 5 and 7 days were very healthy and free of epiphytes.

Protein content was not significantly different (ANOVA, $P = 0.11$) across application rates (Table 1). Tissue NO_3^-–N was not statistically different from zero for all treatments after 3 days without nutrient additions, indicating NO_3^-–N pools did not significantly affect tissue N content and subsequent protein measurements.

3.3. Trace metal additions

Increasing the molar concentration of Zn added daily to cultures to equal that of Mn (1.82 μM) resulted in significant (ANOVA, $P = 0.0026$) increases in SGRs at the high light level but not at the low light level (ANOVA, $P = 0.449$) (Fig. 2a). No significant differences (ANOVA, $P = 0.416$) were found between SGRs of *P. mollis* fertilized with

Table 1
Specific growth rates of *P. mollis* (% day^{-1} [dw] ± S.E.), nitrogen (% [dw] ± S.E.) and protein contents (% [dw] ± S.E.), as a function of nutrient application rate under low light (PFD = 22.5 mol photons m^{-2} day^{-1}) or high light (PFD = 42.34 mol photons m^{-2} day^{-1}) after 28 days

Application rate (in days)	Light	SGR (% day^{-1} ± S.E.)	N (% [dw] ± S.E.)	Protein (% [dw] ± S.E.)
1 (Daily)	low	9.38 ± 0.32	5.83 ± 0.52	36.4 ± 3.3
3	low	9.12 ± 0.39	5.38 ± 0.43	33.6 ± 2.7
5	low	8.89 ± 0.38	4.99 ± 0.45	31.2 ± 2.8
7	low	8.77 ± 0.27	4.42 ± 0.53	27.6 ± 3.3
9	low	7.59 ± 0.51	4.39 ± 0.44	27.4 ± 2.8
11	low	4.81 ± 0.51	4.62 ± 0.61	28.9 ± 3.8
1 (Daily)	high	11.88 ± 0.30	4.69 ± 0.33	29.3 ± 2.1
3	high	11.17 ± 0.25	4.52 ± 0.50	28.3 ± 3.1
5	high	11.36 ± 0.40	3.44 ± 0.65	21.5 ± 4.1
7	high	11.17 ± 0.48	3.81 ± 0.46	23.8 ± 2.9
9	high	10.10 ± 0.52	3.40 ± 0.71	21.3 ± 4.4
11	high	6.39 ± 0.88	3.80 ± 0.42	23.8 ± 2.6

Cultures were supplied with $NaNO_3$, NaH_2PO_4, and trace metals at 1*f* molar concentrations (Guillard and Ryther, 1962).

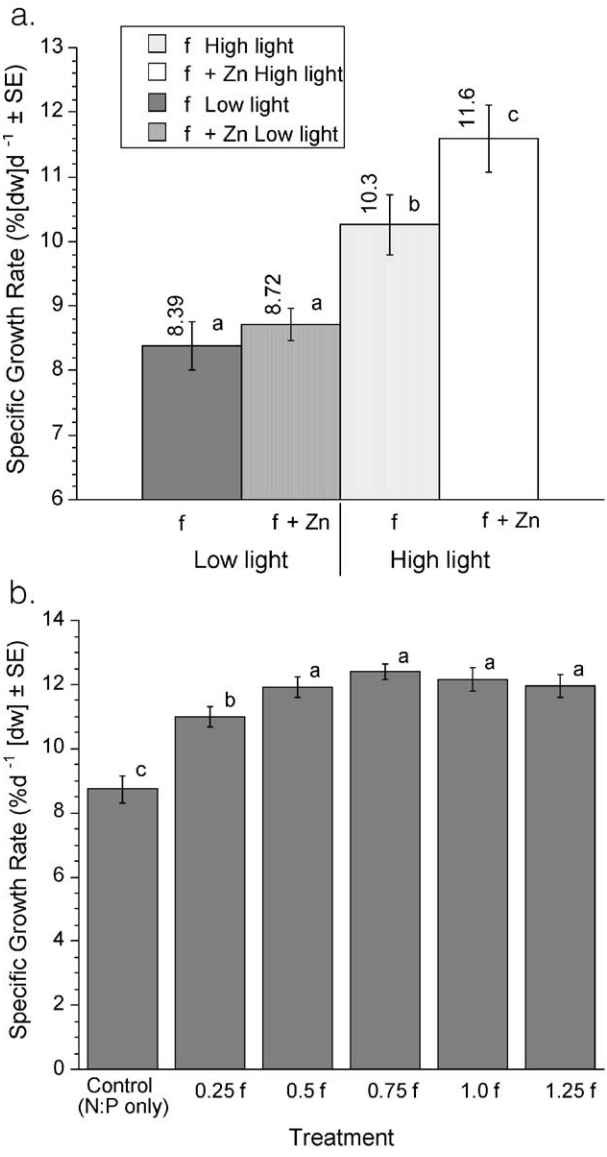

Fig. 2. (a) Specific growth rate of *P. mollis* (± S.E.) as a function of increased zinc additions (total Zn = 1.82 μM day^{-1}) under artificial low light (PFD = 22.5 mol photons m^{-2} day^{-1}) or artificial high light (PFD = 42.34 mol photons m^{-2} day^{-1}). Cultures were supplied with $NaNO_3$ and NaH_2PO_4 at 1*f* molar concentrations (Guillard and Ryther, 1962). Letters a, b, and c indicate significant differences (ANOVA, SNK; $P = 0.0026$) among treatments. The experiment ran for 35 days. (b) Specific growth rate of *P. mollis* (± S.E.) as a function of different *f* metal concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.82 μM day^{-1} for 1*f*) under a moderate PFD of 37.44 mol photons m^{-2} day^{-1}. Cultures were supplied with $NaNO_3$ and NaH_2PO_4 at 1*f* molar concentrations (Guillard and Ryther, 1962). Letters a, b, and c indicate significant differences (ANOVA, SNK; $P < 0.0001$) among treatments. The experiment ran for 42 days.

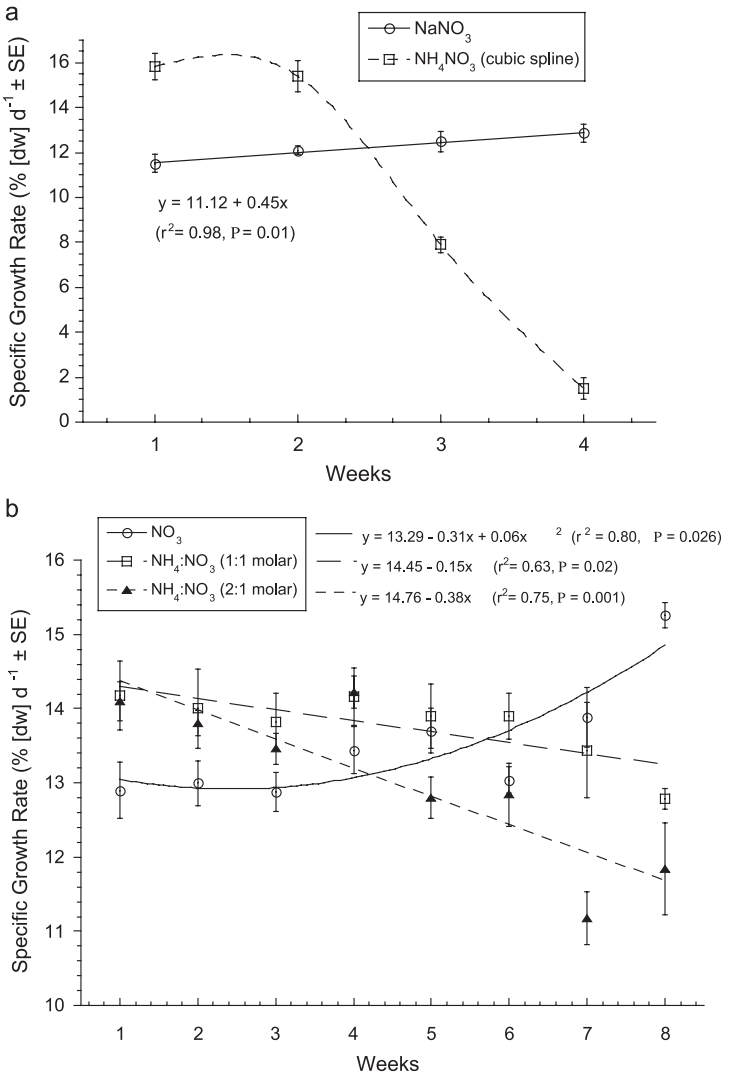

Fig. 3. (a). Specific growth rate of *P. mollis* (± S.E.) as a function of pulse fertilization with either $NaNO_3$ (linear) or NH_4NO_3 (cubic spline) as the N source. The cubic spline curve represents a series of cubic polynomials connected together and has no single equation. Every 3 days, each treatment was supplied with 7059 μM N as either $NaNO_3$ or NH_4NO_3. Cultures were supplied with NaH_2PO_4 and trace metals at 1*f* molar concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.82 μM day^{-1}). A moderate PFD of 37.44 mol photons m^{-2} day^{-1} was employed. The experiment ran for 28 days. (b) Specific growth rate of *P. mollis* (± S.E.) as a function of continuous fertilization with either $NaNO_3$ or NH_4NO_3 as the N source. Cultures were fertilized daily with 2353 μM N as either $NaNO_3$ or NH_4NO_3. Cultures were supplied with $NaNO_3$, NaH_2PO_4, and trace metals at 1*f* molar concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.82 μM day^{-1}). A moderate PFD of 37.44 mol photons m^{-2} day^{-1} was employed. The experiment ran for 56 days.

Table 2
Specific growth rates of *P. mollis* (% day^{-1} [dw] ± S.E.), tissue phosphorus (% [dw]), and protein content as a function of phosphate load day^{-1} under low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light (PFD = 51.64 mol photons m^{-2} day^{-1}) after 49 days

NaH_2PO_4 (mg l^{-1} day^{-1})	PO_4^- (μM day^{-1})	Light	SGR (% day^{-1} ± S.E.)	N (% [dw])	P (% [dw])	N/P (M)	Protein (% [dw])
0.1 (SW)*	1.1	low	2.4 ± 0.69	ND	ND	ND	ND
5	42	low	13.4 ± 0.12	4.64	0.52	8.92	29.0
10	83	low	12.7 ± 0.30	4.49	0.58	7.74	28.1
15	125	low	11.2 ± 0.68	4.70	0.75	6.27	29.4
20	167	low	9.7 ± 0.17	4.84	1.04	4.65	30.3
30	250	low	5.7 ± 0.18	4.98	1.20	4.15	31.1
45	375	low	3.8 ± 0.15	ND	ND	ND	ND
60	500	low	2.8 ± 0.12	ND	ND	ND	ND
0.1 (SW)*	1.1	high	1.5 ± 0.57	ND	ND	ND	ND
5	42	high	16.2 ± 0.20	4.00	0.49	8.16	25.0
10	83	high	17.3 ± 0.26	4.16	0.60	6.93	26.0
15	125	high	13.5 ± 0.83	4.30	0.78	5.51	26.9
20	167	high	12.5 ± 0.76	4.42	0.97	4.56	27.6
30	250	high	9.6 ± 0.17	4.62	1.16	3.98	28.9
45	375	high	7.0 ± 0.17	ND	ND	ND	ND
60	500	high	4.7 ± 0.07	ND	ND	ND	ND

Concentrations are daily averages for nutrients added once every 3 days. $NaNO_3$ was used as the source of N (mean concentration of 2942 μM day^{-1} provided as 250 mg l^{-1} day^{-1} $NaNO_3$) and trace metals were supplied as 0.75*f* molar concentrations (Guillard and Ryther, 1962) with additional Zn (total Zn = 1.37). Tissue protein was calculated from 6.25 × total nitrogen content, with no residual nitrate detected in tissue samples. SW = seawater. ND = not determined.

either 0.5*f*, 0.75*f*, 1*f*, and 1.25*f*, which were all significantly higher (ANOVA, SNK; $P < 0.0001$) than growth of *P. mollis* with additions of either 0.25*f* or the control with (N and P) alone (Fig. 2b).

3.4. Ammonium nitrate vs. sodium nitrate as a nitrogen source

3.4.1. Pulse fertilization with ammonium

During the first 14 days of the experiment, cultures supplied with 2353 μM N day^{-1} as NH_4NO_3 had significantly higher SGRs than those supplied with $NaNO_3$ (Fig. 3a). However, by the fourth week, SGRs of NH_4NO_3-supplied cultures declined to approximately 2% day^{-1} while $NaNO_3$-supplied cultures maintained a consistent SGR of approximately 12% day^{-1} (Fig. 3a). Evidence for toxicity of NH_4^+–N could be seen in the pale coloration and deterioration of apical cells of the thalli.

3.4.2. Continuous fertilization with ammonium

As in the first ammonium experiment, cultures supplied with 2353 μM N day^{-1} as NH_4NO_3 initially had significantly higher SGRs than those supplied with $NaNO_3$ (Fig.

3b). However, the high SGRs lasted for 42 days under the NH_4NO_3 (1:1 M) treatment and 28 days under the NH_4NO_3 (2:1 M) treatment after which time growth of cultures supplied with NH_4^+–N declined while SGRs of $NaNO_3$-fertilized cultures remained relatively high at approximately 13.5–14.5% day^{-1} (Fig. 3b).

3.5. Phosphate additions

The effects of phosphate addition on SGRs and tissue composition of *P. mollis* are shown in Table 2. Growth was hyperbolically related to the concentration of PO_4^{-3} additions. Background N and P (control) provided by 1 vol day^{-1} seawater exchange averaged 13.42 ± 8.1 μM NO_3^-–N and 1.09 ± 0.49 μM PO_4^- day^{-1}, respectively, and supported an SGR of 2.4% day^{-1}, with the cultures degrading during the second week of the experiment. Under a low PFD (23.68 mol photons m^{-2} day^{-1}) a concentration of 42 μM P day^{-1} (5 mg l^{-1} day^{-1} as NaH_2PO_4) provided the best growth. This was not significantly different (ANOVA, $P=0.32$) from growth rates achieved with additions of 83 μM P day^{-1} (10 mg l^{-1} day^{-1} as NaH_2PO_4). Under a high PFD (51.64 mol photons m^{-2} day^{-1}), a load of 83 μM P day^{-1} (10 mg l^{-1} day^{-1} as NaH_2PO_4) provided the best growth, but was not significantly different (ANOVA; $P=0.096$) from that achieved with 42 μM P day^{-1} (5 mg l^{-1} day^{-1}). Phosphate loads higher than these not only depressed

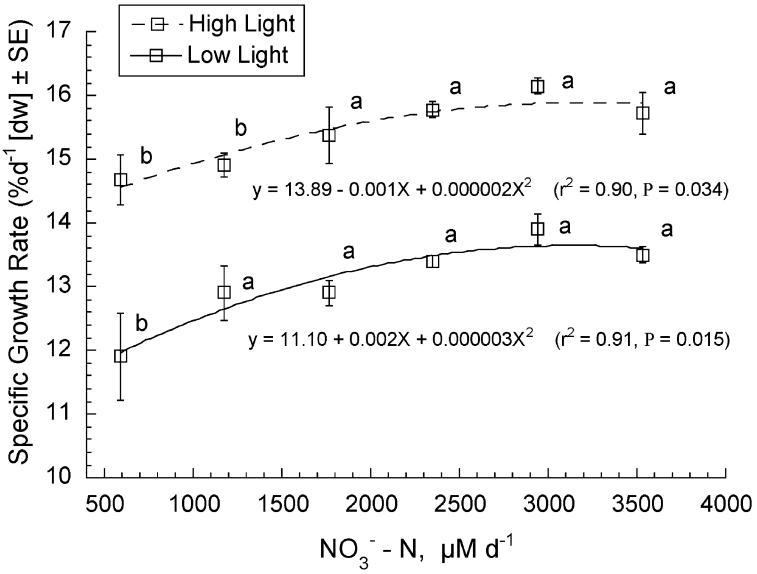

Fig. 4. Specific growth rate of *P. mollis* (± S.E.) as a function of nitrate additions (as $NaNO_3$) under low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light conditions (PFD = 51.64 mol photons m^{-2} day^{-1}). Nutrient additions were made every 3 days. Cultures were supplied with NaH_2PO_4 and trace metals at 0.75*f* molar concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.37 μM day^{-1}). Letters a and b indicate significant differences (ANOVA, SNK; $P=0.001$) among nitrate addition treatments within each tested light condition. The experiment ran for 49 days.

growth of *P. mollis* but also caused growth of macro-epiphytes, especially the chlorophytes, *Ulva* and *Enteromorpha* spp.

Under both light regimens, dry tissue P concentrations of between 0.49% and 0.60% occurred in *P. mollis* cultures showing maximum growth rates but tissue P concentrations of 0.75% or greater were correlated with lower growth rates (Table 2). Tissue P values continued to rise in spite of declining growth, indicating 'luxury consumption' of P. Under both light levels, there was a positive linear relationship between increasing ambient P and elevated tissue N (low light $r^2 = 0.78$; high light $r^2 = 0.99$).

3.6. Nitrate additions

Background N and P supplied by seawater alone (control) exchanged at 1 vol day^{-1} averaged 15.5 ± 6.37 μM NO_3^-–N and 1.63 ± 0.36 μM PO_4^- day^{-1}, respectively, supporting an SGR of 2.28% day^{-1} under low light (23.68 mol photons m^{-2} day^{-1}) and 2.07% day^{-1} under high light (51.64 mol photons m^{-2} day^{-1}). SGRs of *P. mollis* significantly increased with additions of NO_3^-–N up to additions of 2942 μM N day^{-1} provided at a concentration of 250 mg l^{-1} day^{-1} $NaNO_3$ (Fig. 4). Under low light, there was a significant difference in SGRs of *P. mollis* between NO_3^-–N additions of

Table 3
Specific growth rates of *P. mollis* (%day^{-1} [dw] ± S.E.) and tissue composition for carbon (C), nitrogen (N), phosphorus (P), molar ratios, and protein as a function of nitrate load day^{-1} (as $NaNO_3$) under low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light (PFD = 51.64 mol photons m^{-2} day^{-1}) after 49 days

$NaNO_3$ (mg l^{-1} day^{-1})	NO_3^-–N (μM day^{-1})	NO_3^-–N (g m^{-2} day^{-1})	Light	SGR (%day^{-1} ± S.E.)	C (% [dw])	N (% [dw])	P (% [dw])	C/N (M)	C/P (M)	N/P (M)	Protein (% [dw])
0 (SW)*	15.5	0.04	low	2.3 ± 0.18	40.8	2.64	0.82	15.85	49.8	3.22	16.5
50	588	1.56	low	11.9 ± 0.68	42.9	4.81	0.69	8.92	62.2	6.97	30.1
100	1176	3.11	low	12.9 ± 0.43	42.6	4.92	0.64	8.66	66.6	7.69	30.8
150	1765	4.67	low	12.9 ± 0.19	44.4	4.88	0.61	9.10	72.8	8.00	30.5
200	2353	6.23	low	13.4 ± 0.10	43.5	5.27	0.63	8.25	69.1	8.37	33.1
250	2942	7.79	low	13.9 ± 0.24	44.2	4.87	0.64	9.08	69.1	7.61	30.4
300	3530	9.34	low	13.5 ± 0.12	43.0	4.75	0.61	9.05	70.1	7.79	29.7
0 (SW)*	15.5	0.04	high	2.1 ± 0.15	40.6	2.97	0.84	13.70	48.3	3.54	18.6
50	588	1.56	high	14.6 ± 0.25	44.2	4.57	0.75	9.67	58.9	6.09	28.6
100	1176	3.11	high	14.9 ± 0.33	43.6	4.35	0.66	10.00	66.1	6.59	27.2
150	1765	4.67	high	15.4 ± 0.44	41.7	4.54	0.69	9.83	60.1	6.58	26.5
200	2353	6.23	high	15.8 ± 0.13	43.9	4.68	0.66	9.38	66.6	7.09	29.3
250	2942	7.79	high	16.2 ± 0.12	44.3	4.47	0.68	9.91	65.2	6.57	28.0
300	3530	9.34	high	15.7 ± 0.33	43.6	4.57	0.71	9.54	61.4	6.44	28.6

Nutrient concentrations are daily averages for additions every 3 days. NaH_2PO_4 was used as the source of P (mean load of 83.3 μM day^{-1} provided at a mean daily concentration of 10 mg l^{-1} day^{-1} NaH_2PO_4) and trace metals were supplied at 0.75*f* molar concentrations (Guillard and Ryther, 1962) with additional Zn (total Zn = 1.37). SGR values represent means ± S.E. Protein tissue content was calculated from 6.25 × total nitrogen content, with no residual nitrate detected in tissue samples. SW = seawater.

588 and 2942 μM N day^{-1} (ANOVA, SNK; $P<0.05$). Under high light, there was a significant difference in SGRs between NO_3^-–N additions of 1176 and 2942 μM N day^{-1} (ANOVA, SNK; $P<0.05$). Additions higher than 2942 μM N day^{-1} did not cause growth to decrease significantly, and caused no more epiphytism than lower N additions. Slightly reduced water temperatures may have caused SGRs in this experiment to be lower than those observed in the phosphate experiment.

Under both low and high light, external NO_3^-–N had a significant positive linear affect on tissue concentrations of Fe (low light: $y=167.5+0.025x$, $r^2=0.68$, $P=0.04$; and high light: $y=575.6+0.16x$, $r^2=0.90$, $P=0.04$), Zn (low light: $y=12.5+0.01x$, $r^2=0.86$, $P=0.009$; and high light: $y=27.7+0.005x$, $r^2=0.61$, $P=0.067$), and Cu (low light: $y=10.9+0.001x$, $r^2=0.70$, $P=0.04$; and high light: $y=12.27+0.0004x$, $r^2=0.41$, $P=0.18$), but not Mn (low light: $y=9.5+0.0008x$, $r^2=0.28$, $P=0.28$; and high light: $y=7.6+0.0002x$, $r^2=0.38$, $P=0.71$). The average dry tissue concentrations of trace metals ranged from 296 to 1220 μg Fe g^{-1}, from 18 to 49 μg Zn g^{-1}, from 6 to 11 μg Mn g^{-1}, and from 12 to 15 μg Cu g^{-1}. Dry tissue concentrations of metals in controls (no nutrients added) were typically higher than those of the other treatments and ranged from 1820 to 2400 μg Fe g^{-1}, from 37 to 44 μg Zn g^{-1}, from 45 to 53 μg Mn g^{-1}, and from 19 to 23 μg Cu g^{-1}. The effects of NO_3^-–N concentration on growth, tissue

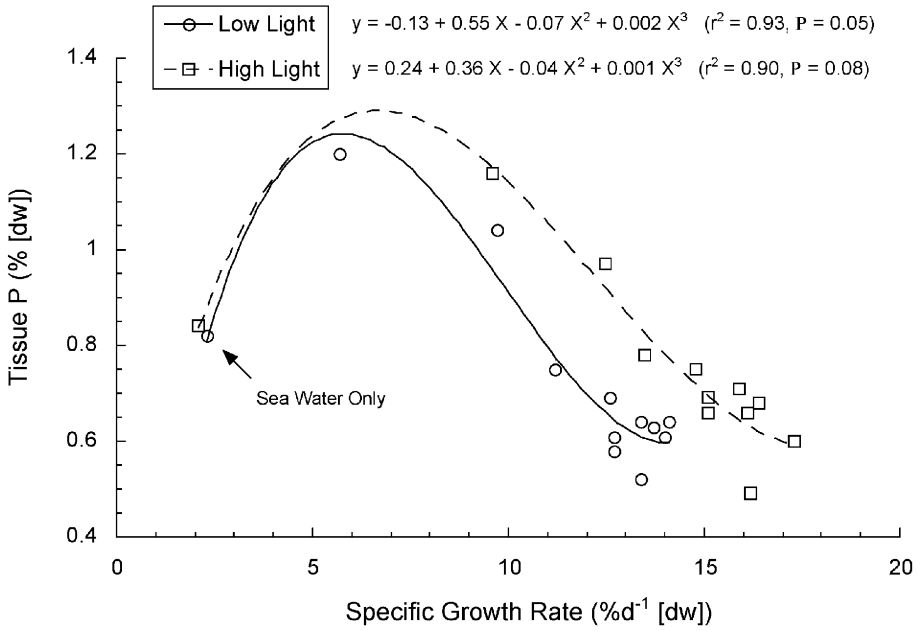

Fig. 5. Tissue phosphorus concentration of *P. mollis* as a function of specific growth rate under low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light (PFD = 51.64 mol photons m^{-2} day^{-1}). Data were pooled from the phosphate and nitrate experiments. Nutrient additions were made every 3 days. Cultures were supplied trace metals at 0.75*f* molar concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.37 μM day^{-1}).

C, N and P, and protein content under the different light and nutrient regimes are shown in Table 3. Tissue N concentrations for *P. mollis* from all seven treatments were nearly identical but tended to decline with increasing light. Dry tissue N values ranged from 4.35 ± 0.52% to 5.27 ± 0.61% with a mean of 4.72 ± 0.25% for all treatments except the controls. Increasing NO_3^-–N had no effect on tissue P in this experiment (Table 3). *P. mollis* grown under low light had a significantly (ANOVA; $P = 0.0017$) higher mean protein content (30.77 ± 1.2) than under high light (28.03 ± 1.03). The low-light cultures were also darker in color. There was no significant correlation between external N and protein content ($r^2 = 0.06$) within either light level.

3.7. Tissue C, N, and P ratios

When tissue P levels for *P. mollis* from the phosphate and nitrate experiments were plotted together, the highest growth rates were observed when dry tissue P content was approximately 0.60% under low light conditions and approximately 0.65% under high light conditions (Fig. 5). By plotting data for tissue N/P ratios from both the nitrate and phosphate experiments, tissue N/P ratios corresponding with high growth rates were approximately eight for the low light and seven for the high light conditions (Fig. 6). While there was a significant positive linear relationship ($r^2 = 0.58$) between SGR and C/N for low and high

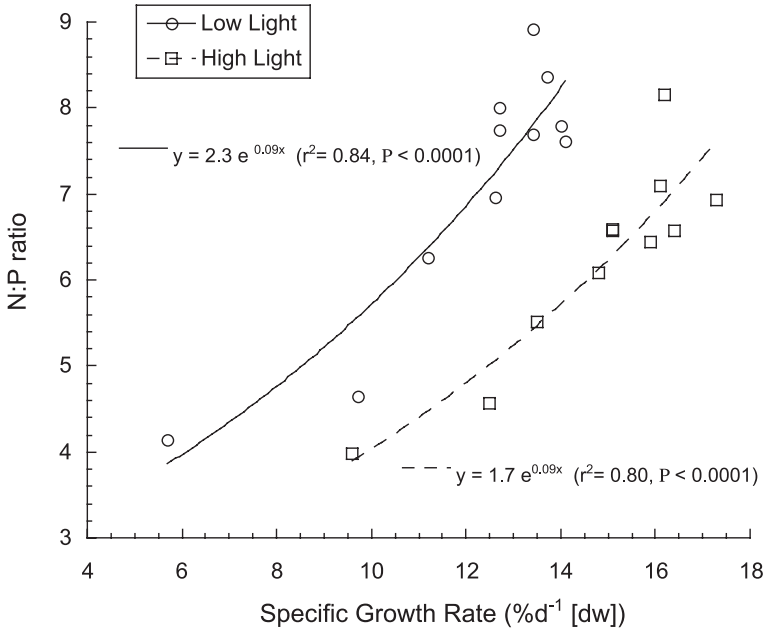

Fig. 6. N/P molar ratio of *P. mollis* as a function of specific growth rate under low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light (PFD = 51.64 mol photons m^{-2} day^{-1}). Data were pooled from the phosphate and nitrate experiments. Nutrient additions were made every 3 days. Cultures were supplied trace metals at 0.75*f* molar concentrations (Guillard and Ryther, 1962) + Zn (total Zn = 1.37 μM day^{-1}).

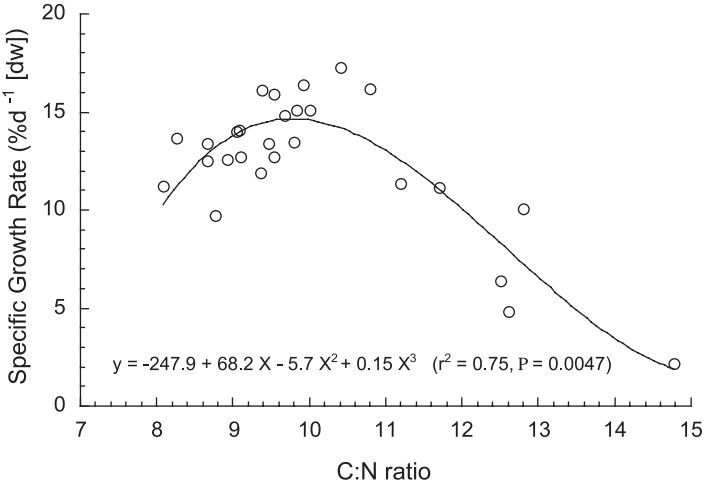

Fig. 7. Specific growth rate of *P. mollis* as a function of tissue C/N molar ratio under a PFD ranging from 22.5 to 51.64 mol photons m^{-2} day^{-1}. Data were pooled from the nutrient application rate and nitrate experiments. Nutrient additions were made every 1–7 days. Cultures were supplied with trace metals at either 0.75*f* or 1*f* molar concentrations (Guillard and Ryther, 1962) both with and without Zn (total Zn = 1.37 or 1.82 μM day^{-1}).

light treatments (except controls) in the nitrate experiment (Table 3), when these data were combined with the phosphate and nutrient application experiments the relationship between SGR and tissue C/N became parabolic ($r^2 = 0.75$; $P = 0.0047$) (Fig. 7).

4. Discussion

4.1. Pulse fertilization

Due to the large amount of apparent inter-cellular free space, macrophytes can store excess nutrients for later use when supplies are low (Thomas and Harrison, 1985). It is only when internal nutrient supplies are depleted that growth rates decline. This experiment showed *P. mollis* stored enough nutrients for a maximum period of 7 days under both light levels (Fig. 1) to maintain high growth rates. This finding agrees with results of experiments with *P. palmata* (Morgan and Simpson, 1981a) and other Rhodophytes (Lapointe and Duke, 1984; Hanisak, 1990; Pickering et al., 1993) that showed pulse-fertilizing every 7–14 days was as effective as daily additions in maintaining high growth rates. Further, it is possible that the time interval between pulses could be lengthened if the nutrient exposure period was increased beyond the 12 h used in the present study. An important advantage of periodic additions of nutrients pulsed every 5–11 days was a dramatic reduction of epiphytes and phytoplankton blooms (cf. Friedlander et al., 1991). In general, and provided other conditions described herein are met, large-scale cultures treated in this way can remain clean for months (Demetropoulos, personal observation).

4.2. Ammonium

Generally, NH_4^+–N is considered superior to NO_3^-–N for growth of most macrophytes, because NH_4^+–N can be directly incorporated into amino acids while NO_3^-–N must be reduced prior use (Lobban and Harrison, 1997). However, the growth advantage of NH_4^+–N vs. NO_3^-–N for cultured Rhodophytes vary, depending on species and especially the NH_4^+–N concentration being delivered. Under low NH_4^+–N concentrations (50 μM N day^{-1}), NH_4^+–N is typically either equal to or superior to NO_3^-–N as a source of nitrogen for most Rhodophyte species (Iwasaki, 1967; DeBoer, 1978; Levin, 1991). Under high NH_4^+–N concentrations (500–2000 μM N day^{-1}), and typically high light, NO_3^-–N is preferred by several Rhodophyte species, including *P. palmata* and *P. mollis* (Iwasaki, 1967; Lapointe and Ryther, 1979; Morgan and Simpson, 1981a; and this study).

In this study, long-term additions of NH_4NO_3 to *P. mollis* cultures were toxic (Fig. 3a and b). However, at lower NH_4NO_3 additions (2353 μM N day^{-1}), toxicity of NH_4^+–N took 49 days to become apparent (Fig. 3b). Thus, the use of NH_4^+ may result in higher growth rates for *P. mollis* compared with those obtained with NO_3^-–N additions alone, provided NH_4^+ is supplied at relatively low levels that do not become toxic. A future experiment should use an ammonium solution that is dripped into the culture vessels in amounts lower than the experimental design described here (i.e., < 50 μM N day^{-1}). Such work should examine in more detail *P. mollis* growth using combinations of NH_4^+–N and NO_3^-–N in order to benefit from the low relative costs of the former. However, use of NH_4^+–N and NO_3^-–N should be done with caution since there is evidence that even in the presence of a couple of μM of ammonium, nitrate uptake can be inhibited in some algae (Maestrini et al., 1982). In addition, uptake and utilization by *P. mollis* of low concentrations of excreted NH_4^+–N from abalone or fish in co-culture systems should be examined further (cf. Evans and Langdon, 2000).

4.3. Trace metals

While little work has been carried out on trace metal requirements and metabolism in seaweeds, the importance of their role in phytoplankton productivity is well established (McLachlan, 1982; DeBoer and Whoriskey, 1983; Martin et al., 1990; Lobban and Harrison, 1997). Since Fe is required for cell growth, respiration, and photosynthesis (Lobban and Harrison, 1997; Bidwell, 1979), additions of Fe are necessary under conditions of limited seawater exchange and high photosynthetic rates. Indeed, Fe is considered by some as a macronutrient and is reported as the fourth essential element in Irish moss aquaculture (Craigie and Shacklock, 1989). Studies indicate that Zn activates protein synthesis and Mn plays a principle role as an enzyme cofactor in photosynthesis (Lobban and Harrison, 1997). Like Fe, some researchers suggest that Mn is a macronutrient which, along with Zn, is important in mitigating photodamage under high light conditions (Craigie, personal communication); however, not all Mn accumulated by a healthy plant is physiologically necessary for growth. In this study, *P. mollis* grew more slowly without additions of nutrients (seawater controls without additions of N, P, and trace metals) but controls bio-accumulated Fe, Mn, Zn, and Cu resulting in tissue

concentrations that were well above levels in treatments with added nutrients. This may be due to EDTA used in the experiments strongly chelating the trace metals and reducing their availability *P. mollis* (Huntsman and Sunda, 1980; Sunda, 1991) or excess EDTA removing trace metals from *P. mollis* tissue (Higgins and Mackey, 1987).

This study demonstrated enhancement of *P. mollis* growth by addition of Guillard and Ryther's *f* trace metal medium. Further improvement in growth was achieved by increasing Zn concentration to equal that of Mn (Fig. 2a). Based on the relationship shown in Fig. 2b, 0.75f + Zn (total Zn = 1.37 μM day^{-1}) is likely the best trace metal concentration to support maximum growth rates at PFDs tested in this study. For polyculture systems using fish effluent, these micronutrients may already be available in sufficient quantities, even under conditions of low seawater exchange.

Like other researchers (Hanisak, 1979; DeBoer, 1981), we found that tissue nutrient status (i.e., N/P, C/N, and trace metals) was correlated with *P. mollis* growth rate. Based on the results of this study and practical experience, tissue nutrient concentrations should be kept within the ranges set forth in Table 4. Optimal, predicted trace metal values compare favorably with those reported for wild-collected *P. palmata* (Munda, 1978; Morgan et al., 1980b) and other macrophytes (DeBoer, 1981). For example, Morgan et al. (1980a,b) reported ranges of tissue Fe from 153 to 4400 μg g^{-1} [dw], Mn from 11 to 110 μg g^{-1} [dw], Zn from 41 to 200 μg g^{-1} [dw], and Cu from 22 to 48 μg g^{-1} [dw] in wild-collected *P. palmata*. Based on their work, Cu is the only tissue metal in our analysis that might be considered low. Cu supplementation is likely to be important for enhanced growth of *P. mollis* because as much as 99.7% of Cu in natural seawater of the northeastern Pacific may not be biologically available (Coale and Bruland, 1988).

For all treatments (except controls), tissue concentrations of Fe, Zn, and Cu were significantly correlated with concentrations of NO_3^-–N additions. Under high light, more Fe was accumulated in *P. mollis* tissue than under low light, a trend that also occurred to a lesser extent with Zn and Cu. It is unclear whether or not there is a greater requirement for Fe, Zn, and Cu with higher NO_3^-–N fertilization rates or whether accumulation was simply enhanced. Rice and Lapointe (1981) suggested that elevated trace metal concentrations in tissues may be due to trace metal complexation with organic nitrogen ligands.

4.4. Phosphate

Since tissue P concentrations were inversely correlated with *P. mollis* growth at PO_4^- seawater concentrations above 10 mg l^{-1} day^{-1}, there appears to have been 'luxury

Table 4
Predicted optimal tissue [dw] N, P, N/P, C/N, and trace metal concentrations (μg g^{-1} [dw] ± S.E.) for maximum growth of *P. mollis* as a function of low light (PFD = 23.68 mol photons m^{-2} day^{-1}) or high light (PFD = 51.64 mol photons m^{-2} day^{-1})

Light	N (% [dw])	P (% [dw])	N/P (M)	C/N (M)	Fe (μg g^{-1})	Zn (μg g^{-1})	Mn (μg g^{-1})	Cu (μg g^{-1})
23.68	4.9 ± 0.2	0.63 ± 0.02	7.8 ± 0.3	8.8 ± 0.4	753 ± 305	36 ± 10	8 ± 1	13 ± 2
51.64	4.6 ± 0.1	0.69 ± 0.02	6.7 ± 0.3	9.7 ± 0.3	990 ± 151	40 ± 5	7 ± 1	14 ± 1

Values were derived from mean values for dulse cultures from the nitrate and phosphate experiments that showed no significant differences in growth rate (ANOVA, $P = 0.378$).

consumption' of P. The amount of P in *P. mollis* tissue appeared to be a good indicator of both growth and P availability, with high tissue P (>0.70% [dw]) indicating excess P fertilization and potentially poor growth (cf. Tables 2, 3, and 4). In addition to lower SGRs, increasing external P beyond that required for maximum growth resulted in higher tissue N concentrations and hence a lower C/N ratio under both light levels.

Lapointe (1987) found a similar interaction between P and N enrichment for tissue N and P of *Gracilaria tikvahiae*. This result suggests that providing optimal levels of P is critical to producing high yields of *P. mollis*. Ambient seawater supplies of PO_4^- may be high enough (≥ 42 μM P day^{-1}) to support high growth rates at seawater exchange rates of 7 vol day^{-1} or higher (Mencher et al., 1983; Demetropoulos, personal observation).

4.5. Nitrate

Maximum growth rates of *P. mollis* occurred at NO_3^-–N additions of 2942 μM N day^{-1}. Additions above this level caused *P. mollis* growth to decline slightly but not significantly. Tissue N concentrations were the same for all treatments (except controls) under a given light level and it was not possible to identify tissue N concentrations that would indicate growth-limiting N conditions in the culture medium apart from those associated with *P. mollis* from the seawater control (no NO_3^-–N additions). We would expect the critical tissue N concentration lies somewhere between 2.97% [dw] (seawater control; no growth after 28 days) and 4.35% [dw] (lowest tissue N sustaining high growth for 49 days) under light conditions of 23.68 to 51.64 mol photons m^{-2} day^{-1} [dw] (Table 3).

Although Björnsäter and Wheeler (1990) suggested that tissue N contents greater than 4% [dw] can indicate N surplus, *P. mollis* may have greater N requirements than those suggested in the literature (cf. Morgan and Simpson, 1981a,b; Levin, 1991). In support of this argument, total %N has been reported as "high" for *P. palmata* in comparison to other seaweeds (Morgan et al., 1980a,b), with the highest recorded values occurring in wild *P. palmata* at 5.7% [dw] (Chaumont, 1978, reviewed in Morgan et al., 1980a,b). A tissue N value of 5.7% [dw] would place tissue protein content at approximately 35.6% [dw], comparing favorably with our highest estimated protein concentration of 36.4% [dw] (Table 1). Based on SGRs in the nitrate and phosphate experiments, optimal tissue N concentration for *P. mollis* (after 3 days of tissue NO_3^-–N purging) for maximal growth appeared to be 4.9% [dw] for the low light and 4.6% [dw] for the high light conditions (Table 4), resulting in a mean protein content of approximately 30.6% [dw] and 28.75% [dw], respectively. Providing *P. mollis* with high levels of N (as NO_3^-–N) appeared to result in high SGRs rather than increasing protein content because tissue N values were not statistically different over the range of nitrate additions tested within each light level.

Results from this study also suggest an inverse relationship between tissue N and light at this culture temperature (cf. Morgan and Simpson, 1981b,c; Levin, 1991). This relationship may be due to lower concentrations of proteins associated with protein-based photosynthetic pigments under high PFDs (Lobban and Harrison, 1997). If *P. mollis* protein content plays a role in the nutrition of herbivores, such as abalone, it is possible that *P. mollis* cultured under high light conditions may not be as nutritious as plants

cultured under low light. Davison (1991) also suggests there may be a requirement for increased N to support the need for increased photosynthetic pigment content under higher temperatures, which are generally coupled with high PFDs (cf. Demetropoulos and Langdon, 2004b). Thus, maintaining high nitrogen concentrations (2353–2942 μM day^{-1} NO_3^-–N) in *P. mollis* cultures is probably the best strategy to maintain high growth rates.

4.6. N/P

The intent of the N and P fertilization experiments was to determine nutrient requirements for growth in the context of two different PFDs and low seawater exchange conditions. Based on the two experiments, the results indicated the best load N/P molar ratio for optimal *P. mollis* growth was between 14.2 and 35.5 under low PFDs (23.68 mol photons m^{-2} day^{-1}) and between 21.3 and 35.5 under high PFDs (51.64 mol photons m^{-2} day^{-1}) (Tables 2 and 3). The optimal N/P ratio for both of these light levels occurred at average daily N and P nutrient loads of 2942 μM N day^{-1} and 83 μM P day^{-1}, resulting in an optimal tissue N/P molar ratio of between 6.57 and 8.37 for the fastest growing cultures under both light regimens of the nitrate experiment (Table 3). When data from the phosphate and nitrate experiments were combined, optimal tissue N/P molar ratios ranged from a mean of 6.7 ± 0.3 (4.6% N, 0.69% P) under high light conditions to a mean of 7.8 ± 0.3 (4.9% N, 0.63% P) under low light conditions (Fig. 6 and Table 4).

4.7. C/N

Based on the parabolic function generated in Fig. 7, the highest growth rates under both low and high light conditions occurred with C/N molar tissue ratios ranging from 9.4 to 10.4, with the optimal ratio being approximately 10 (cf. Lapointe and Duke, 1984; Lobban and Harrison, 1997). C/N ratios less than 10 are generally an indication of surplus nitrogen and a lack of light saturation (Lobban and Harrison, 1997). For example, Levin (1991) found a C/N ratio between 4.7 and 5.25 for *P. mollis* cultured exclusively on salmon effluent under low light intensities (< 18 mol photons m^{-2} day^{-1}). Above a C/N of 10, nitrate limitation under saturating light plays a more important role in decreasing SGRs of *P. mollis*. For example, Rosen et al. (2000) reported C/N ratios of 13 and 14 for *P. mollis* cultured under moderate to high light intensities (30–52 mol photons m^{-2} day^{-1}) and low seawater exchange rates (1 vol day^{-1}), which resulted in relatively low SGRs of between 2.6% and 4.9% day^{-1}.

4.8. Conclusions

Reduced exchange rates coupled with addition of nutrients are likely to be a more favorable economic strategy than pumping large volumes of seawater through a facility to provide sufficient nutrients for high seaweed productivity.

One of the most important goals of this study was to develop a nutrient regime and management protocol that would optimize *P. mollis* growth under limited seawater exchange conditions while simultaneously limiting epiphytism and the occurrence of weed species. In this regard, the strategy of adding nutrients during the dark cycle every

5–7 days has several advantages: (1) at night, water can more easily be shut off for many hours, such that nutrients are not lost to flushing yet culture temperatures can remain relatively constant, (2) the cost associated with nutrient additions is reduced because nutrient uptake is more efficient, (3) epiphyte growth is better controlled, and (4) chlorophyte species are not provided with light necessary for their nutrient uptake (cf. Hanisak, 1987; Lobban and Harrison, 1997).

The tendency of the seaweed culturist is generally to supply nutrients such that they slightly exceed demand; however, this can easily produce both depressed growth (e.g., phosphate depressed growth as shown here) and blooms of weed species such as *Ulva*, *Enteromorpha*, and filamentous diatoms (Schramm, 1991). Thus, it is best to supply nutrients on a schedule that takes advantage of the physiology of red macroalgae and the demand for nutrients based on growth. Tissue nutrient assays, similar to those used by terrestrial farmers, provide the most reliable indicator of macroalgal health and by extension, yields as a function of incident light (Hanisak, 1979, 1982; DeBoer, 1981). Tissue analysis should be adopted as a standard method for determining the nutritional status of *P. mollis*. However, tissue nutrient status may need to be tracked closely since nutrient demand will vary as a function of culture conditions, such as temperature and incident light, which can often vary over short time periods.

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