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INORGANIC NITROGEN AND PHOSPHORUS UPTAKE KINETICS IN *PALMARIA PALMATA* (RHODOPHYTA)[1]

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The N and P uptake responses were studied in a northern Spanish population of the edible red seaweed *Palmaria palmata* (Linnaeus) Kuntze. The fronds were incubated at different concentrations, and the nutrient depletion in the medium was measured at successive times to calculate uptake rates. *Palmaria palmata* uptake response was biphasic and nonsaturable for inorganic P. This would allow the species to exploit transient pulses of high P concentration in natural and fertilized conditions. Such a response is a common feature of algae avoiding nutrient deficiency. At average concentrations measured in the ocean, the response was nonsaturable for inorganic N sources, except for ammonium in autumn and winter when it is not the major N source. In contrast to the general rule of ammonium being taken at a higher rate than nitrate, we found similar affinity for both nutrients corresponding to the minor role of ammonium as N source for field populations over the year.

Key index words: **biphasic uptake; Michaelis-Menten; Monod, nitrogen uptake; nutrient kinetics; *Palmaria palmata*; phosphorus uptake; surge uptake**

Abbreviations: **ANCOVA, analysis of covariance; DW, dry weight; LOWESS, locally weighted regression scatterplot smoothing**

Inorganic nitrogen, primarily in the form of nitrate, and phosphate are the two most important nutrients limiting macroalgal growth in temperate coastal environments (Hanisak 1979, Conolly and Drew 1985). Macroalgae can take up these nutrients at extremely low concentrations, suggesting an active transport mechanism that is usually described by a Michaelis-Menten hyperbola (Lobban and Harrison 1997). Additionally, at high nutrient concentration a second transport mechanism usually operates, resulting in biphasic inorganic nutrient uptake (Ullrich 1992, Buchanan et al. 2000). This response is a common feature of higher plants to avoid nutrient deficiency and exploit all ranges of nutrient concentrations (Buchanan et al. 2000) but has received little attention in seaweeds (Lobban and Harrison 1997). Traditionally, this secondary transport has been explained by the simple diffusion of the nutrient across the membrane resulting in a *hybrid* kinetics pattern between the hyperbolic and linear functions (Lobban and Harrison 1997). This has been mathematically formulated for higher plants by the *sum function* of a Michaelis-Menten plus a straight line (Borstlap 1981).

There is a second type of kinetic relationship in unicellular algae resulting from the existence of a second active carrier rather than a diffusion phenomenon (Collos et al. 1992). *Dual uptake* models (sum function of two Michaelis-Menten hyperbolas) have been proposed to describe this response in plants (Borstlap 1981). Alternatively, a dual response may be caused by a single multiphasic carrier (Probyn and Chapman 1982). Such response has been modeled in many organisms by multimodal kinetics where each phase follows a Michaelis-Menten function (Nissen and Martin-Nieto 1998). In this study, available models of multiphasic uptake kinetics of higher plants has been applied to study these responses in seaweeds, specifically in the edible red seaweed *Palmaria palmata* (Linnaeus) Kuntze. This is the first attempt to describe the uptake kinetics for all major nutrients involved in this species' nutrition. The knowledge of *P. palmata* uptake response is also of interest to improve fertilization procedures and mariculture of this species in subsequent research. The ecological implications of time and concentration-dependent changes in nutrient transport and the higher affinity for any N source are also discussed.

MATERIALS AND METHODS

Sample collection. On 17, 23, and 30 June and 7 July 1999, 31 or 32 vegetative fronds of 0.5–1.0 g (fresh weight) from different individuals were randomly taken from a shallow subtidal population at *Playa de Estaño* on the northern Spanish coast (43°33′ N, 5°35′ W). Individuals were collected at this time of the year because contents of total N and P and major N storage compounds are very low at this time in the population (Martínez and Rico 2002). Samples were transported to the laboratory in isotherm bags (<4° C, in darkness) in 2 h.

Preincubation. Fronds were rinsed in flowing seawater and wiped with tissue to remove small epiphytes. Immediately after, they were placed in a 6-L flask filled with aged, filtered

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(GF/F filter, Whatman International Ltd., Maidstone, UK), and enriched seawater (modified half-strength Von Stosch's medium; Guiry and Cunningham 1984), with the nutrient to be used in the experiment removed from the medium. The flask was placed in a walk-in temperature-controlled room set at the same temperature as the experiment and in darkness for 18 h. Adequate mixing was ensured by an air pump.

Experimental setup. The fronds were incubated inside various vessels set at different initial concentrations. The depletion of nutrient inside the flasks was measured at different times to calculate uptake rates at different time intervals (Pedersen 1994). This mixed model incubation includes both perturbation (single vessel, measured at various time intervals) and multiple-flask methods (multiple vessels, two measures: initial and final; Pedersen 1994). Thirty-one to 32 fronds from different individuals collected in the same date were subjected to the same number of experimental concentrations for a single nutrient obtained by dilution (adding aged and filtered seawater) of a concentrated stock of orthophosphate ($H_2PO_4^-$, HPO_4^{2-}), ammonium (NH_4^{1+}), nitrite (NO_2^{1-}), or nitrate (NO_3^{1-}, Table 1). To ensure that other major nutrients were in excess, the medium was enriched as for the preincubation. Forty conical flasks filled with 250 mL of medium at different nutrient concentration were randomly placed on an orbital shaker (INNOVA 2300, Newbrunswick Scientific Co. Inc., Edison, NJ, USA) at 150 rpm inside a walk-in temperature-controlled room set at 16° C (Table 1). Saturating irradiance was provided by fluorescent lamps (cool daylight, TDL 18W/54, Philips, Chartres, France) to a final value of 125 μmol photons $\cdot m^{-2} \cdot s^{-1}$. After 30 min, an initial sample was taken from each flask to determine initial nutrient concentrations. Thirty-one to 32 fronds were then immersed in flasks, and eight to nine vessels without algae were set as controls. At regular time intervals during approximately 6 h, 10-mL samples were taken from the flasks and kept at −20° C until analysis (which was always less than 3 months after the experiment). Nitrate, nitrite, and orthophosphate were measured as described in Koroleff (1983) and ammonium as outlined in Álvarez (1993) using a Technicon II Autoanalyzer (Industrial Method no. 158-71 W/A, Dublin, Ireland). The nutrient concentrations measurements were taken to the nearest 0.01 μM.

Calculation of uptake rates. Uptake rates (μmol $\cdot h^{-1} \cdot g^{-1}$ dry weight [DW]) were calculated from changes in substrate concentration in the medium during each sampling interval as described in Pedersen (1994). The same was done with data from the controls. As the experiment proceeded, the nutrient was eventually exhausted, causing decreased number of uptake data at the final time intervals. The introduction of the algae inside the flasks at the beginning of the experiment caused ammonium levels to increase. For this reason, uptake rates measured in the first incubation period in the ammonium experiment were not used. Very high ammonium concentration media were diluted with an automatic pipette before analysis. This predilution method caused some replicates to diverge, and thus ammonium data at very high concentration were not included. Negative uptake rates were observed in several flasks (including controls) at various time intervals during the experiments. These may be explained by random variance associated to the measurement of the nutrient concentrations.

Statistical analysis. Observed uptake rates from each time interval were plotted against the substrate concentration at the beginning of the interval (Marin et al. 1986). The data were first analyzed using locally weighted regression scatterplot smoothing (LOWESS; Trexler 1993) to investigate the response with no prior assumption of its shape. This method fits curves without assuming a function to be fit. When the LOWESS graph looked like a straight line, data were fitted by least-squares linear regression (model I; Ricker 1984) and tested against the second-order polynomial fit to reject quadratic responses (Sokal and Rohlf 1995). When the response appeared hyperbolic in the LOWESS graph, the data were fitted to a second-order polynomial and its significance tested to confirm the quadratic response (Sokal and Rohlf 1995). Afterward, data were adjusted to the Michaelis-Menten function by nonlinear least-squares regression with the program GraphPad Prism (v. 4 for windows, GraphPad Software Inc., San Diego, CA, USA). The signs of the coefficients of the Taylor polynomial approximations to the Michaelis-Menten regressions and those from the "best" polynomials were then compared. The agreement of the signs showed the significance of the Michaelis-Menten model (Apostol 1990). When LOWESS fit suggested the existence of biphasic uptake patterns, these were investigated by *nonlinear breakpoint regression* (adding successive functions at different ranges of nutrient concentrations) and by nonlinear regression of the *summed function* (constructing a single function that is the sum of the more simple ones) using Graph-Pad Prism (Motulsky 1999).

Analysis of covariance (ANCOVA) (Huitema 1980) was done to compare the uptake response at different time intervals. For curve responses, polynomial ANCOVA models were used (Huitema 1980). Because the same fronds were used in all incubation periods, the independence among treatments was violated. To solve this problem, data were assigned to the different time intervals being compared. The data from a single frond were not used twice, and data from all ranges of initial nutrient concentrations were assigned to each time interval.

Linear regression was done to test the significance of the uptake response in the controls for each incubation period. The significance level for each regression coefficient was calculated by Bonferroni test departing from an overall significance level of 0.05 (Rice 1989).

RESULTS

Orthophosphate uptake kinetics. LOWESS regression suggested biphasic patterns (hyperbola followed by a line at the highest concentrations) at intervals of 0–16, 16–46, 46–76, and 76–106 min (Fig. 1A). Among all the multiphasic models tested (see the Introduction), only the Michaelis line breakpoint regression gave confident parameter estimates with logical values and a small error. This model explained more than

TABLE 1. Experimental design.

Date (1999)	Nutrient	Nutrient source	Initial range of concentrations (μM)	Temperature (° C)[a]
17 June	Orthophosphate	KH_2PO_4	0.23–36.02	16.4 ± 0.17 (7)
23 June	Ammonium	$(NH_4)_2SO_4$	0.94–21.50	16.8 ± 0.23 (7)
30 June	Nitrite	$NaNO_2$	0.22–38.21	17.0 ± 0.00 (7)
7 July	Nitrate	$NaNO_3$	5.22–65.23	16.9 ± 0.18 (9)

[a]Values are means ± SE, with *n* in parentheses.

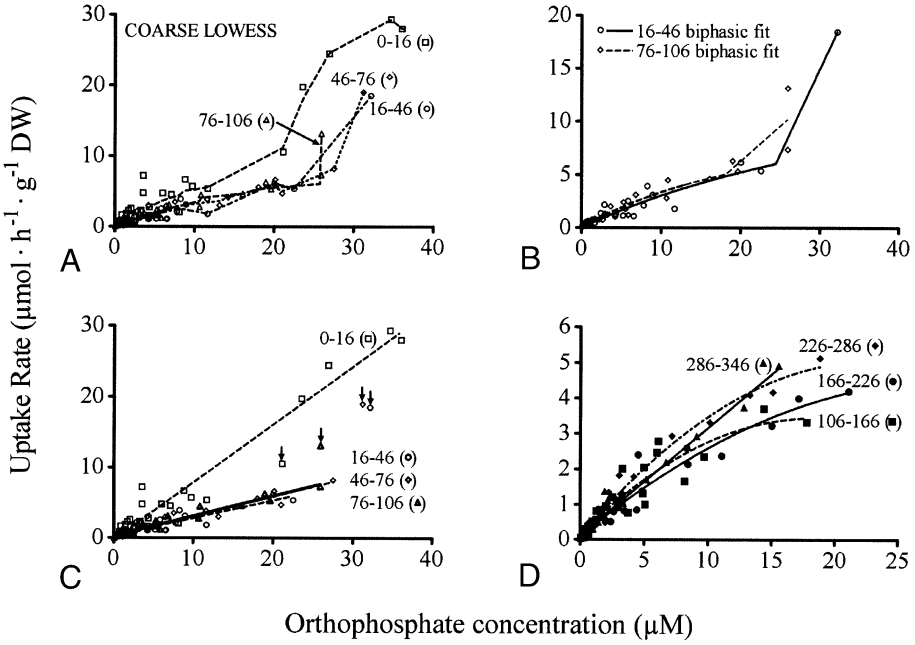

FIG. 1. (A) LOWESS regressions of the orthophosphate uptake responses at the first four time intervals (min). See text for details. (B) Biphasic regression for intervals of 16–46 and 76–166 min.(C) Final uptake responses showing the best fit for each time interval. The arrows point to the data excluded. (D) Best fits at low orthophosphate concentrations.

90% of the variance of the data at 16–46 and 76–106 min (Fig. 1B). However, both fits depended on a single point and response reverted to a line if these data were eliminated (Fig. 1C). The data at 0–16 and 46–76 min fit to a second-order concave polynomial (not shown). Again the model reverted to a line if a single point for each regression was eliminated (Fig. 1C). Lineal responses after the deletion of a single point in each of the four intervals were significant and confirmed by the nonsignificance of the quadratic polynomial function (Table 2). The lines clearly overlap, describing a common linear response except for 0 to 16-min interval (Fig. 1C). Data, apart from the values at 0–16 min, were pooled, giving a single slope and intercept estimates (Table 2). The 0–16 linear function had a higher slope as shown by the test of parallelism against the 16–46 linear regression ($F_{1,24} = 77.77$, $P < 0.001$).

In the three following intervals (106–166, 166-226, and 226–286 min), the kinetics followed a Michaelis-Menten model (Fig. 1D, Table 2). To test the significance of this model, the significance of the second-order polynomials were confirmed and the signs of the regression coefficients agreed with the coefficients of the Taylor approximation to the Michaelis-Menten function (Table 2). This is indicative of the significance of the Michaelis-Menten fits (Apostol 1990). Furthermore, the curve described by both models overlap in the plot (not shown). Nonlinear ANCOVA of the second-order polynomials revealed a common saturable response for intervals of 106–166, 166–226, and 226–286 min ($F_{1,22} = 2.80$, $P = 0.08$). Thus, data were

TABLE 2. Orthophosphate uptake responses at different time intervals for the same algae.

Sampling interval (min)	Duration (min)	95% Confidence interval		r^2	n
		Linear Model			
		Slope	Intercept (μmol · h^{-1} · d^{-1} DW)		
0–16[a]	16	0.75 to 0.89	−1.15 to 0.59	0.85	26[b]
16–46, 46–76, 76–106[a]	30	0.26 to 0.29	0.12 to 0.44	0.92	84[b,c]
		Michaelis-Menten Model			
		V_{max}(μmol · h^{-1} · d^{-1} DW)	K_s (μM)		
106–166, 166–226, 226–286[d]	30, 60	6.29 to 10.21	11.64 to 25.40	0.91	81[c]
		Linear Model			
		Slope	Intercept (μmol · h^{-1} · d^{-1} DW)		
286–346[a]	60	0.28 to 0.32	0.03 to 0.23	0.98	23

[a]Linear regressions were the only significant fit ($P < 0.0001$; 0–16: $F_{1,27} = 554.4$; 16–46: $F_{1,25} = 138.0$; 46–76: $F_{1,27} = 317.4$; 76–106: $F_{1,26} = 528.3$; 286–346: $F_{1,21} = 1031$).

[b]A single point for each interval was excluded.

[c]Pooled data.

[d]Quadratic polynomials were significant ($P < 0.01$; 106–166: $F_{1,24} = 9.16$; 166–226: $F_{1,26} = 8.96$; 226–286: $F_{1,22} = 25.61$).

K_s, half-saturation constant from the Michaelis-Menten model; V_{max}, maximal uptake rate from the Michaelis-Menten model.

pooled to give single-parameter estimates (Table 2). The response for the last incubation interval (286–346 min) was linear, and the second-order polynomial was not significant (Table 2).

The regression of the uptake rate versus orthophosphate concentration for the controls was not significant in most time intervals after Bonferroni correction. Those that showed a significant trend had very low slopes (0–16: 0.09 ± 0.018 [± SE], $n = 8$; 286–346: 0.01 ± 0.002 [± SE], $n = 7$).

Ammonium uptake kinetics. LOWESS regression suggested hyperbolic patterns in the intervals of 15–45, 45–77, 77–107, and 107–165 min (Fig. 2A). None of the multiphasic models provided confident estimations of the parameters for any interval. In contrast, Michaelis-Menten regressions at intervals of 15–45, 45–77, 77–107, and 107–165 min fitto data if a correction for the presence of minimum substrate concentration was done (Fig. 2B, Table 3). The origin of the curve was not the origin of coordinates due to the inability to turn out medium with undetectable ammonium concentration; thus this modified model was used (Rothhaupt 1996). This model explained a larger variance than the quadratic polynomial in three of four intervals and was significant against the Michaelis-Menten model from the origin in all cases (Table 3). The significance of the Monod fits were confirmed using the Taylor approximation as explained above (Table 3). The curves at different time intervals overlapped, describing a single saturable kinetics; thus data were pooled for further analysis (Fig. 2B, Table 3).

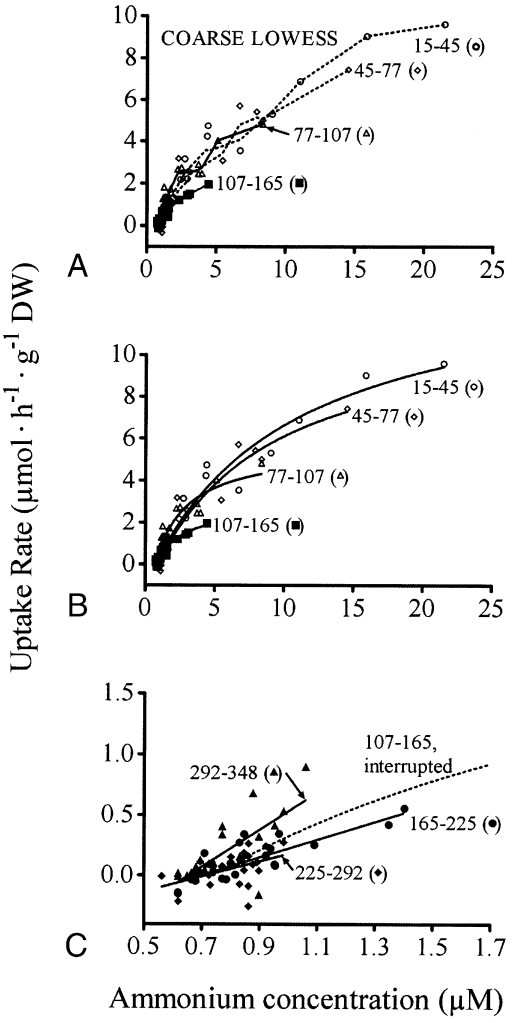

FIG. 2. (A) LOWESS regressions of the ammonium uptake responses at different time intervals (min) for the same algae. (B) Best fits at different time intervals for the same algae. (C) Best fits at low ammonium concentrations; data from the interval of 107–165 min has not been plotted.

As the experiment proceeded, the response at lower ammonium concentration was linear and the quadratic polynomials were not significant (Fig. 2C, Table 3). ANCOVA revealed a common slope for the last three incubation periods but not the same intercept, being different for the last time interval (test of parallelism: $F_{2,18} = 2.09$, $P = 0.15$; ANCOVA: $F_{2,20} = 6.81$, $P < 0.01$).

The linear regression of the uptake rate versus ammonium concentration for the controls was significant in a single interval, but the slope was very low (165–225: 0.01 ± 0.001 [± SE], $n = 6$). Initial background nitrate and nitrite levels were low in comparison with ammonium concentration (nitrate: 4.80 ± 0.072 μM [± SE], $n = 10$; nitrite: 0.20 ± 0.007 μM [± SE], $n = 10$). At the end of the experiment, the nitrate was depleted due to the uptake by the algae (0.03 ± 0.008 μM [± SE], $n = 8$) and mean nitrate uptake rate was low (1.5 ± 0.21 μmol · $h^{-1} \cdot g^{-1}$ DW [± SE], $n = 8$).

Nitrate uptake kinetics. LOWESS regression suggested a linear or biphasic pattern in the interval of 0–15 min and saturable kinetics in incubation periods of 15–45, 45–76, and 76–107 min (Fig. 3A). The Michaelis-Menten model was confirmed at intervals of 15–45, 45–76, and 76–107 min (Table 4). The hyperbolas overlapped in the plot, and thus data were pooled together to describe the common response (Fig. 3B, Table 4). In contrast, a linear response was observed in the first and last incubation periods, and the polynomials were not significant (Fig. 3, B and C, Table 4). All samples from 166–226 min and most from 107–166 and 226–291 min were lost, preventing the discussion of nitrate uptake at these incubation periods.

The regression of the uptake rate versus nitrate concentration for the controls was not significant in two intervals. In addition, when significant the slopes of the regressions were low (45–76, 0.19 ± 0.020 [± SE]; 76–107, -0.20 ± 0.038 [± SE]; 291–346, 0.01 ± 0.002 [± SE]; $n = 7$–8). Background initial concentration of nitrite and ammonium were low compared with nitrate levels (nitrite: 0.20 ± 0.007 μM [± SE], $n = 40$; ammonium: 1.36 ± 0.284 μM [± SE], $n = 23$). Final ammonium was significant (0.75 ± 0.142 μM [± SE], $n = 28$), but mean uptake rate was very low (0.1 ± 0.02 μmol · $h^{-1} \cdot g^{-1}$ DW [± SE], $n = 14$).

Nitrite uptake kinetics. The LOWESS regression clearly suggested a linear pattern at all time intervals

TABLE 3. Ammonium uptake responses at different time intervals for the same algae.

Sampling interval (min)	95% confidence interval			r^2	n
	Monod Model				
	V_{max} ($\mu mol \cdot h^{-1} \cdot d^{-1}$ DW)	X_0 (μM)	Ks (μM)		
15–45, 45–77, 77–107, 107–165[a]	12.49 to 19.4	0.46 to 0.79	9.28 to 19.81	0.93	93[b]
	Linear Model				
	Slope				
165–225, 225–292, 292–348[c]	0.55 to 1.06			0.37	70[b]

[a]Monod model regressions were significant against the Michaelis-Menten model from the origin ($P < 0.05$; 15–45: $F_{1,21} = 9.21$; 45–77: $F_{1,20} = 16.25$; 77–107: $F_{1,20} = 7.92$; 107–165: Michaelis-Menten from the origin did not converge). Quadratic polynomials were significant ($P < 0.01$; 15–45: $F_{1,21} = 21.90$; 45–77: $F_{1,20} = 16.15$; 77–107: $F_{1,20} = 8.67$; 107–165: $F_{1,20} = 8.36$).

[b]Pooled data.

[c]Linear regressions were the only significant fit ($P < 0.05$; 165–225: $F_{1,20} = 43.10$; 225–292: $F_{1,21} = 5.59$; 292–348: $F_{1,23} = 28.09$).

K_s, half-saturation constant from the Michaelis-Menten model; V_{max}, maximal uptake rate from the Michaelis-Menten model; X_0, minimum nutrient concentration from the modified Monod model.

(not shown) and all linear regressions, and none of the quadratic polynomials was significant (Fig. 4, Table 5). The response was similar at all time intervals apart from 75–105 min (Fig. 4A). The ANCOVA revealed significant differences in slope between this time interval and 0–15 (test of parallelism, $F_{1,24} = 6.84$, $P > 0.01$). In contrast, there were no differences between the slope and the intercept of 0–15 and 15–45 min (ANCOVA, $F_{1,25} = 0.90$, $P = 0.35$). The regression of the uptake rate versus nitrite concentration for the controls was not significant in three intervals. Moreover, when significant the slopes of the regressions were very low (0–15, 0.15 ± 0.006 [$\pm$ SE]; 45–75, 0.04 ± 0.003 [$\pm$ SE]; 105–166, 0.04 ± 0.003 [$\pm$ SE]; 166–225, 0.01 ± 0.001 [$\pm$ SE]; 288–345, 0.03 ± 0.001 [$\pm$ SE]; $n = 7$–9). Background ammonium and nitrate concentration were low (initial ammonium: $1.47 \pm 0.338\ \mu M$ [$\pm$ SE], $n = 19$, final: $0.89 \pm 0.171\ \mu M$ [$\pm$ SE], $n = 27$; initial nitrate: $4.95 \pm 0.051\ \mu M$ [$\pm$ SE], $n = 27$, final: $1.28 \pm 0.360\ \mu M$ [$\pm$ SE], $n = 37$), and mean uptake rate was of minor importance (ammonium: $0.2 \pm 0.02\ \mu mol \cdot h^{-1} \cdot g^{-1}$ DW [$\pm$ SE], $n = 14$; nitrate: $1.0 \pm 0.08\ \mu mol \cdot h^{-1} \cdot g^{-1}$ DW [$\pm$ SE], $n = 19$).

Higher affinity N source. When a single N source was provided, *P. palmata* took up ammonium and nitrate with similar affinity at all the initial concentrations. This was clear when both kinetic responses were plotted together (Fig. 5).

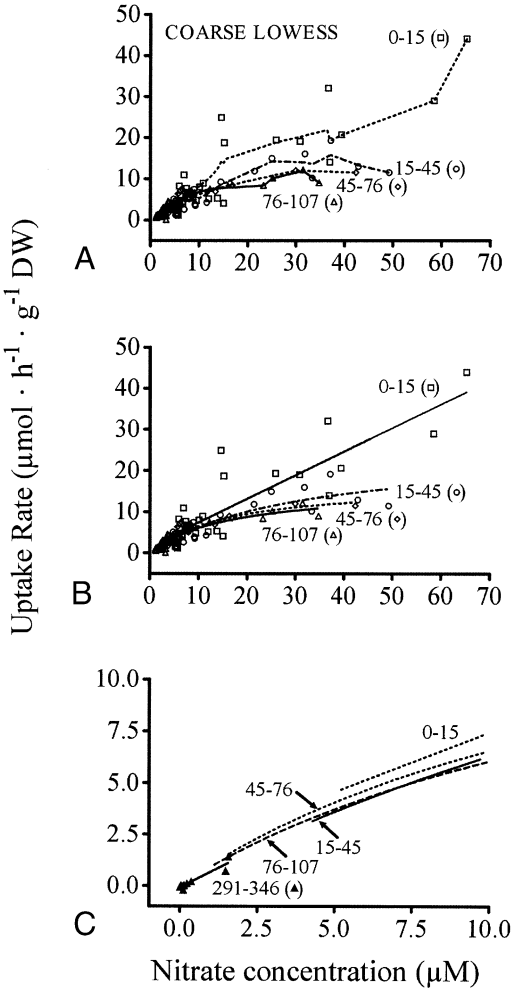

FIG. 3. (A) LOWESS regressions of the nitrate uptake responses at different time intervals (min) for the same algae. (B) Best fits for the fist four time intervals. (C) Best fits at low nitrate concentrations.

DISCUSSION

The orthophosphate transport system of *P. palmata* showed signs of biphasic kinetics, with a Michaelis-Menten response at low concentrations and a linear uptake at high phosphate concentrations. The Michaelis line breakpoint regression properly fit phosphate data in two time intervals, explaining as much as 90% of the variance. We believe that concave polynomial fits were the results of reduced number of points set at high concentrations and linear regressions at the first intervals masked biphasic uptake patterns as shown for other macroalgae (Lavery and McComb 1991). This explained why data fit lines at the first time intervals and then hyperbolas as the experiment proceeded, and finally a linear response was observed at the last time interval corresponding to the lower part of the

TABLE 4. Nitrate uptake responses at different time intervals for the same algae.

Sampling interval (min)	Duration (min)	95% confidence interval		r^2	n
		Linear Model			
		Slope	Intercept ($\mu mol \cdot h^{-1} \cdot d^{-1}$ DW)		
0–15[a]	15	0.46 to 0.70	−0.90 to 4.28	0.78	31
		Michaelis-Menten Model			
		V_{max}($\mu mol \cdot h^{-1} \cdot d^{-1}$ DW)	K_s (μM)		
15–45, 45–76, 76–107[b]	30, 31, 31	16.96 to 24.67	15.28 to 30.53	0.84	79[c]
		Linear Model			
		Slope	Intercept ($\mu mol \cdot h^{-1} \cdot d^{-1}$ DW)		
291–346[a]	55	0.59 to 0.92	−0.18 to 0.02	0.89	14

[a]Linear regressions were the only significant fits ($P < 0.0001$; 0–15: $F_{1,29} = 103.2$; 291–346: $F_{1,12} = 96.29$).
[b]Quadratic polynomials were significant ($P < 0.01$; 15–45: $F_{1,26} = 20.74$; 45–76: $F_{1,21} = 19.48$; 76–107: $F_{1,23} = 18.75$).
[c]Pooled data.

biphasic response. In contrast, when the response was saturable, data from the first time intervals fit a Michaelis-Menten function (as with ammonium data).

This biphasic response allowed *P. palmata* to accommodate a wide range of external P concentrations (from undetectable values up to 36.02 μM P). Nonsaturable response was observed at concentrations as high as 121-fold maximum mean P in the sites of origin of the plants used (orthophosphate: 0.32 ± 0.014 μM [± SE], $n = 6$, monthly sampling from June 1998 to July 1999; Martínez and Rico 2002). Transient pulses reaching such high concentrations are more frequent in nature than suggested by measured mean values because ambient concentrations represent the balance of rates of supply and consumption, so such high concentrations are being masked by a rapid flux of nutrients (Blackburn and Sørensen 1988). Microscale nutrient patches that might reach nutrient concentration as high as 860-fold mean ambient values are common in the ocean, playing a significant role in phytoplankton production (Shanks and Trent 1979). Higher seawater P content than in control areas due to urban waste was detected in the studied locality (Martínez and Rico 2002). The nonsaturable nature of the uptake response may be viewed as an adaptation to exploit such P sources by primary producers. Higher P supply caused individuals from the same population to develop larger storage P pools than individuals from a control locality 50 km away (Martínez and Rico 2002). Both physiological capabilities, the biphasic nature of the P uptake and the ability to store P, are complementary, ensuring proper P nutrition. In a laboratory growth experiment done with *P. palmata* from the same population at the same time of the year, the enrichment with N did not increase growth if P was not added to the cultivation medium. Proper enrichment with both N and P was the only way to enhance growth rate, showing the importance of P in this species' nutrition (unpublished data).

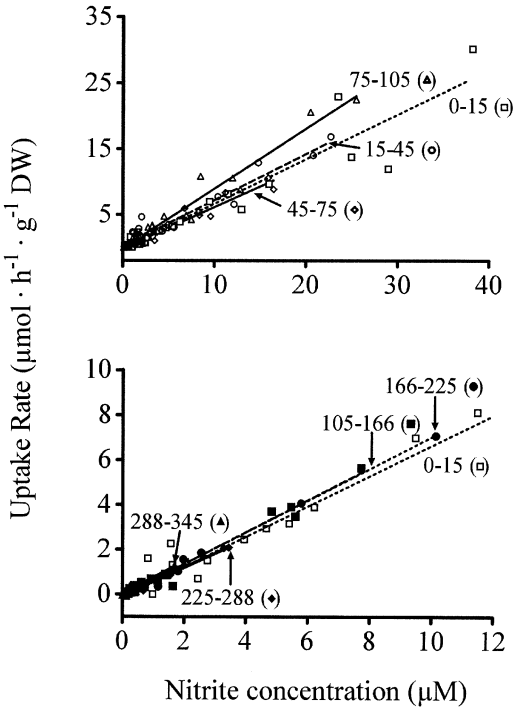

FIG. 4. (A) Nitrite uptake linear regressions at different time intervals (min) for the same algae. (B) Best fits at low nitrite concentrations.

Phosphate biphasic kinetics are well known in microalgae (Borchardt et al. 1994), but there is a single case of multiphasic phosphate uptake reported in macroalgae by Friedlander and Dawes (1985). These authors found a triphasic response: two saturable phases, plus a straight line at much higher external concentrations than those used in our study (>2 mM). This result was question by other authors because of the methodology used (Reed 1990). Most studies on P uptake in macroalgae deal with lower concentrations than that used in this study (Conolly and Drew 1985, Hurd and Dring 1990, Lotze and Schramm 2000). Higher experimental concentrations are probably needed to observe P multiphasic patterns. The transport system responsible for such nonsaturable response (>15–30 μM orthophosphate) remains unknown in algae (Borchardt et al. 1994). But based on the low membrane permeability to orthophosphate anions, this

TABLE 5. Nitrite linear uptake responses at different time intervals for the same algae.

Sampling interval (min)	Duration (min aprox.)	95% confidence interval		r^2	n
		Slope	Intercept ($\mu mol \cdot h^{-1} \cdot d^{-1} DW$)		
All intervals, except 75–105[a]	15, 30, 60	0.65 to 0.70	−0.21 to 0.11	0.92	198[b]
75–105[a]	30	0.84 to 0.97	−0.65 to 0.31	0.97	30

All linear regressions and none of the polynomial functions was significant ($P < 0.0001$).

[a]Linear regressions were the only significant fits ($P < 0.0001$; 0–15: $F_{1,26} = 220.5$; 15–45: $F_{1,25} = 402.2$; 45–75: $F_{1,24} = 476.4$; 75–105: $F_{1,28} = 803.1$; 105–166: $F_{1,24} = 1228$; 166–225: $F_{1,27} = 5688$; 225–288: $F_{1,29} = 2686$; 288–345: $F_{1,29} = 824.6$).

[b]Pooled data.

response cannot be explained by passive diffusion that has been associated with linear responses (Reed 1990, Lobban and Harrison 1997).

The linear fit in the first time interval of the nitrate experiment was consistent and did not change when one or two points were removal. Only if the three points showing the highest uptake rates were removed did a hyperbolic response at lower nitrate concentrations become evident. Following the argument used for P, this linear fit may be masking a biphasic nitrate uptake. Moreover, such a nonsaturable response would explain why growth was not nitrate saturated at concentrations as high as 2 mM in greenhouse cultures of *P. palmata* (Morgan and Simpson 1981). Nitrate biphasic kinetics are well known in macroalgae (Conolly and Drew 1985, Lavery and McComb 1991, Collos et al. 1992), and the responsible membrane transport system of such responses is well known in plants (Buchanan et al. 2000). However, evidence for a biphasic nitrate uptake response in this study is limited to three points because few points were set at high nitrate concentrations. Further research is needed to clearly prove biphasic N kinetics in seaweed.

At low nitrate and orthophosphate concentrations, the uptake response followed a Michaelis-Menten model. High-affinity Na^+-symport systems, such as those described for aquatic plants, are probably the underlying transport mechanisms for both nutrients (Reed 1990, Gracía-Sánchez et al. 2000). Enhanced uptake rates of nitrate and orthophosphate were observed in the first incubation period. Such response is well known in N-deficient macroalgae over the first 10–60 min until the internal N storage pools fill and uptake is then reduced by feedback inhibition (Lobban and Harrison 1997). Similarly, P-deficient algae possess the ability to transport orthophosphate to the cytoplasm extremely rapidly, exceeding cell requirements (Lobban and Harrison 1997). The fronds used in our experiment had low N and P content corresponding to low values of these components in wild individuals from the same population (June 1999: N, 1.8 ± 0.11% [± SE]; P, 0.3 ± 0.02% [± SE]; $n = 8$) (Martínez and Rico 2002). The content of main N storage compounds, phycoerythrin and proteins, were also low in field plants, suggesting nutrient deprivation (June 1999: phycoerythrin, 0.5 ± 0.34 $mg \cdot g^{-1}$ [± SE]; protein, 8.1 ± 0.54% [± SE]; $n = 8$) (Martínez and Rico 2002). However, rapid uptake was only three times higher than subsequent rates for orthophosphate and less for nitrate. The response is reduced when compared with measures at similar time intervals in other macroalgal species (Pedersen 1994, Lobban and Harrison 1997, Lotze and Schramm 2000). Moreover, a fraction of the nutrient entered the fronds by passive diffusion of both anions into the apparent free space, and they are not directly assimilated inside the cell (Lobban and Harrison 1997). This mechanism caused surge uptake of P in some brown algae (Hurd and Dring 1990). Diffusion into the apparent free space usually lasted less than a minute, but this space can be a significant fraction of the cell volume (up to 20%–30% in *Porphyra perforata* J. Agardh; Lobban and Harrison 1997). Using an initial time interval of 15 min, it is impossible to know whether uptake was mostly due to entrance into the apparent free space (i.e. first minute) or due to enhancement of uptake into the cell (i.e. 10–60 min). Many studies with macroalgae suffer from the same criticism because short incubation times are difficult to attain (Hurd and Dring 1990, Lotze and Schramm 2000). Harrison et al. (1989) suggested that 2 min or even shorter incubations should be conducted to avoid underestimation of uptake rates in macroalgae.

Nitrite uptake was linear at concentrations as high as 76 times greater than the maximum mean concentration in the studied area (0.52 ± 0.019 μM [± SE] , $n = 6$; Martínez and Rico 2002). Ecologically, this nonsaturable response would favor *P. palmata* in patchy environments in the same way as described for nitrate and orthophosphate. Nitrite uptake exceeded nitrate uptake in several marine phytoplankton species in

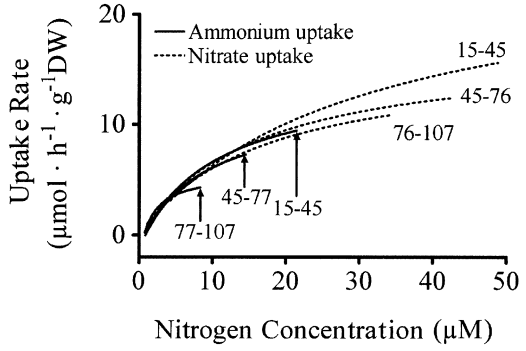

FIG. 5. Nitrate and ammonium uptake at different time intervals (min) from the nitrate and ammonium experiments.

culture and natural populations (Collos 1998) and may have been underestimated in seaweeds (Brinkhuis et al. 1989). High nitrite supply is caused by processes such as summer upwelling events (Valiela 1995), phytoplankton excretion (Collos 1998), and sewage inputs to coastal areas (Brinkhuis et al. 1989). However, the detailed description of nitrite uptake kinetics has been largely ignored in macroalgae and restricted to two studies (Hanisak and Harlin 1978, Topinka 1978). Topinka (1978) suggested saturable nitrite kinetics, but his data showed that the relationship becomes linear after excluding a single outlayer that showed an abnormal low uptake rate at high nutrient concentration. Moreover, nitrite uptake during dark conditions was clearly a linear relationship. In addition, the half-saturation constant from the Michaelis-Menten model estimates using the Michaelis-Menten function showed large standard errors. Topinka (1978) suggested that such imprecision for half-saturation constant values is common, but we believe that this was caused by using an inappropriate model. Hanisak and Harlin (1978) also used a Michaelis-Menten model; however, the data and graphs were not shown.

In higher plants, nitrite is transported into the cytoplasm by a biphasic system composed of a high affinity symport system operating at low nitrite concentrations and a low affinity symport system at high nitrite supply (Buchanan et al. 2000). This mechanism is similar to that described for nitrate transport, and in some cases the high affinity system has been shown to be the same for both nutrients (Ullrich 1992). An similar mechanism would explain the similar uptake rates for nitrite and nitrate at low concentrations in *P. palmata*. The linear uptake might be masking the biphasic transport as previously seen. The low affinity transport system plays a greater role than for nitrate in higher plants (Ullrich 1992), and this might explain the dominance of the linear phase and the absence of signs of biphasic kinetics in *P. palmata*. At an intermediate time interval, the uptake rates of nitrite were enhanced. This pattern does not correspond to the surge uptake described for nitrate and phosphate because it was observed 75 min after the start of the experiment.

Ammonium uptake showed a saturable response at concentrations five times higher than the maximum mean concentration in the field (4.32 ± 0.184 μM [± SE], $n = 6$; Martínez and Rico 2002). A saturable response was observed at all incubation periods except at last time intervals corresponding to the linear phase of the Michaelis-Menten. At concentrations typically found in autumn and early winter in the studied sites, there were signs of a saturation response. Uptake rates measured with winter fronds will be probably lower than those observed in our experiments with June fronds because of higher thallus nutrient content (Lobban and Harrison 1997, Martínez and Rico 2002). This saturated ammonium uptake did not cause the plants to be N limited in autumn and early winter because wild seaweeds were well nourished because of high nitrate supply, and ammonium played a minor role (Martínez and Rico 2002). In summer, ammonium mean concentration remained relatively high when other N sources were exhausted (Martínez and Rico 2002). At the nutrient concentrations found in summer, ammonium uptake was not saturated and partially supported summer growth together with stored N (Martínez and Rico 2002).

The *P. palmata* ammonium uptake response may be explained by a membrane transport system similar to that observed in higher plants: Cations are taken up by a saturable carrier system with high substrate affinity, which may carry out ammonium uniport at the expenses of the negative electrochemical potential (Ullrich 1992). At high ammonium concentration, the uncharged form NH_3 diffuses freely across the lipid phase of the plasmalemma (Ullrich 1992). The biphasic response associated with this system was not evident in *P. palmata*, preventing the discussion of the potential of *P. palmata* to exploit pulses of high ammonium.

Nitrate rather than ammonium was found to play a major role in *P. palmata* nutrition over the seasonal scale; ammonium was only important in summer (Martínez and Rico 2002). This is consistent with similar affinities for ammonium and nitrate but contradicts evidence of higher affinity for ammonium in *P. palmata* in tank culture when a single nutrient was added (Morgan and Simpson 1981). The saturable ammonium uptake response makes this N source less adequate at the ammonium concentration tested. Morgan and Simpson (1981) found that despite faster ammonium uptake, nitrate supported higher growth rates. Similarly, most higher plants grow better when supplied with nitrate (Mengel 1992). Nitrate is expected to be a better N source for the mariculture of this species.

Palmaria palmata nutrient uptake responses allowed the species to exploit P transient pulses of high concentration. Signs of similar mechanisms operating for N were found, but more evidence is needed for confirmation. At average concentrations measured in the ocean, uptake was not saturated, except for ammonium when was a minor N source. The biphasic mechanism should be interpreted as a way to attain linear uptake responses that maximizes uptake efficiency at all concentration ranges. In addition, P storage in *P. palmata* (Morgan and Simpson 1981, Martínez and Rico 2002) makes this species capable of uncoupling its growth cycle from P supply (Martínez and Rico 2002). More complex models such as multiphasic kinetics (Probyn and Chapman 1982) or dual functions (Borstlap 1981) were not found in this study. Our uptake patterns remarkably coincide with those observed in higher plants and in some aquatic angiosperms (Ullrich 1992, García-Sanchez et al. 2000). For this reason, we believe that similar membranes transport systems appear to operate in both algae and higher plants.

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