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EFFECTS OF LIGHT INTENSITY AND TEMPERATURE ON *CRYPTOMONAS OVATA* (CRYPTOPHYCEAE) GROWTH AND NUTRIENT UPTAKE RATES[1]

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ABSTRACT

Specific growth rate of Cryptomonas ovata *var.* palustris *Pringsheim was measured in batch culture at 14 light-temperature combinations. Both the maximum growth rate (μ_m) and optimum light intensity (I_{opt}) fit an empirical function that increases exponentially with temperature up to an optimum (T_{opt}), then declines rapidly as temperature exceeds T_{opt}. Incorporation of these functions into Steele's growth equation gives a good estimate of specific growth rate over a wide range of temperature and light intensity. Rates of phosphate, ammonium and nitrate uptake were measured separately at 16 combinations of irradiance and temperature and following a spike addition of all starved cells initially took up nutrient at a rapid rate. This transitory surge was followed by a period of steady, substrate-saturated uptake that persisted until external nutrient concentration fell. Substrate-saturated NO_3^--uptake proceeded at very slow rates in the dark and was stimulated by both increased temperature and irradiance; NH_4^+-uptake apparently proceeded at a basal rate at 8 and 14 C and was also stimulated by increased temperature and irradiance. Rates of NH_4^--uptake were much higher than NO_3^--uptake at all light-temperature combinations. Below 20 C, PO_4^{-3}-uptake was more rapid in dark than in light, but was light enhanced at 26 C.*

Key index words: ammonium; Cryptomonas*; growth rate; light intensity; nitrate; nutrient uptake kinetics; phosphate; temperature.*

The cryptomonads are an important group of microcellular algae whose physiology has largely been ignored. This study was initiated to provide basic information about the response of one member, *Cryptomonas ovata* var. *palustris* Pringsheim, to changes in light intensity and temperature. Of particular interest were the effects of irradiance and temperature on growth rate and nutrient uptake rates since these kinetics were required as input into a simulation model of *C. ovata* population dynamics in Kootenay Lake, British Columbia (7).

The growth response of nutrient-satiated algal cells is a complex function of temperature, light intensity and an interaction between the two. In general, growth rate increases exponentially with temperature up to an optimum temperature (T_{opt}), then declines rapidly as temperature exceeds this optimum (11,29,34,38). Growth rate also increases with increasing irradiance up to an optimum or saturating light intensity (I_{opt}). As irradiance exceeds this optimum, growth rate either plateaus or drops off (21, 27), depending upon the proximity of temperature to T_{opt}. At low temperatures, algal growth is inhibited by high light intensities (34,36,38), but as temperature increases, higher light intensity is required for optimum growth rate (34,36).

Steele (40) proposed an empirical relation between growth rate and irradiance:

$$\mu = \mu_m \cdot I/I_{opt} \cdot \exp(1 - I/I_{opt}), \qquad (1)$$

where μ is observed growth rate at light intensity I; μ_m is maximum growth rate; I_{opt} is the light intensity at which $\mu = \mu_m$. Assuming that the two parameters μ_m and I_{opt} both vary with temperature, Steele's equation describes the complex response to irradiance and temperature outlined. Since this approach acknowledges the ability of algal cells to change their light requirements in response to temperature, it will give a more accurate prediction of growth rate than the multiplication of independent light and temperature functions often used in phytoplankton population/productivity models (e.g. 5,9, 29). The temperature-dependence of μ_m and I_{opt} were defined for *C. ovata* in laboratory experiments described here.

Although the mechanisms of nutrient uptake by algal cells are not clearly understood, observed kinetics of nutrient uptake are generally consistent with the Michaelis-Menten function (15,32):

$$\rho = (\rho_m \cdot S)/(K_s + S), \qquad (2)$$

where ρ is the uptake velocity seen at external nutrient concentration S; ρ_m is maximum uptake velocity; K_s is the nutrient concentration where $\rho = \frac{1}{2} \rho_m$. The kinetic parameters ρ_m and K_s were originally regarded as species-specific constants. However, recent evidence (4,8,13,32) suggests that one or both parameters may vary with nutritional state and growth rate. Nutrient-uptake velocity also varies with temperature and light intensity. The rate of

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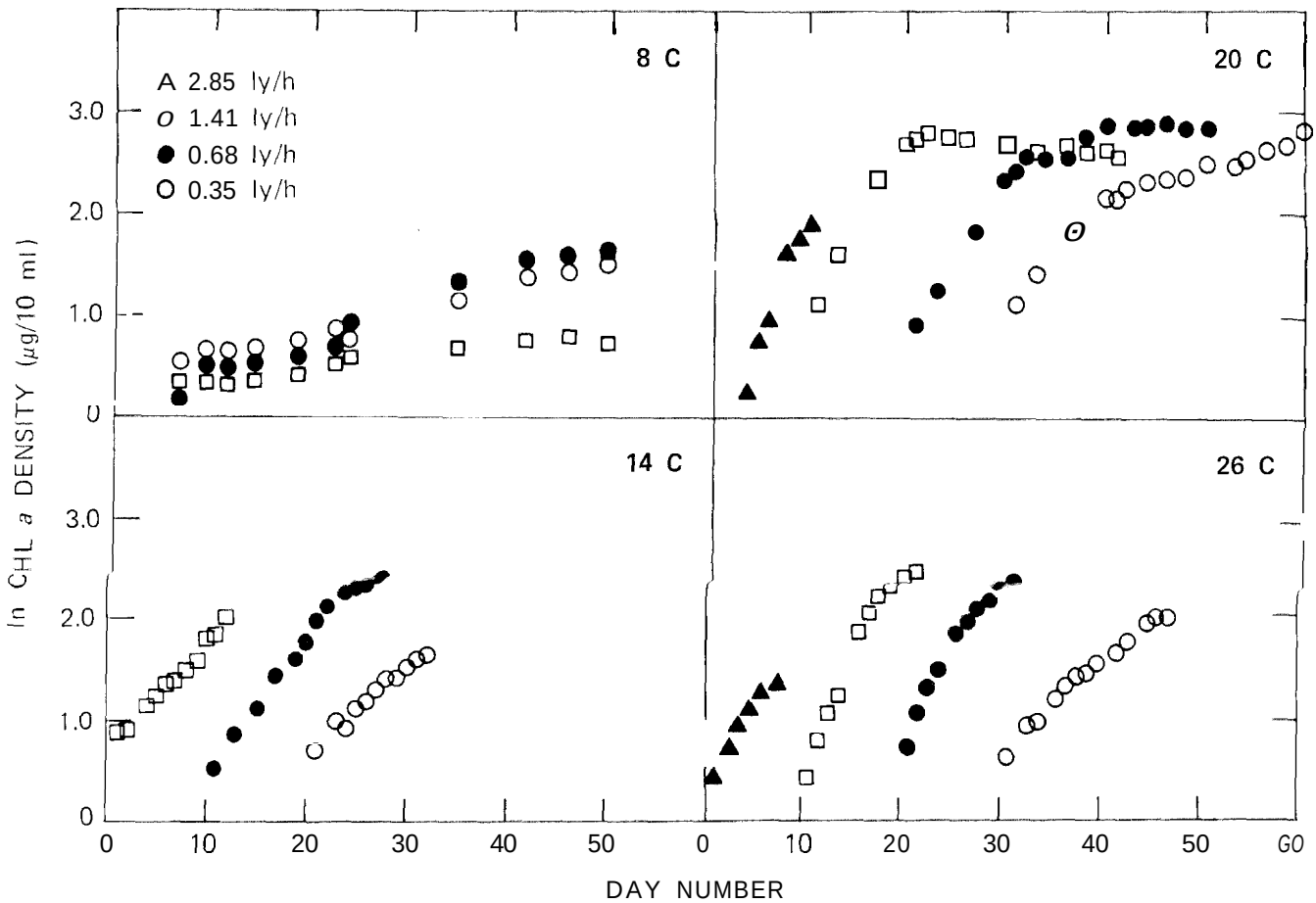

FIG. 1. Population growth curves of *Cryptomonas ovata* (successive plots were shifted 10 days for clarity).

nitrate uptake generally increases with increasing irradiance (1,12,28); the same is generally true for ammonium (10,14). Phosphate-uptake rate has been seen to increase with increasing temperature (2,20). Since uptake velocity responds to environmental changes, a question of immediate concern is whether the half-saturation constant (K,) or the maximum uptake velocity (ρ_m) or both parameters are functional variables of light and temperature. Laboratory experiments based upon the designs of Caperon and Meyer (4) and Conway et al. (8) were used to answer this question for ammonium, nitrate and phosphate uptake by *Cryptomonas ovata.*

MATERIALS AND METHODS

Growth kinetics. The experimental organism was *Cryptomonas ovata* var. *palustris,* obtained from the University of Texas Culture Collection of Algae (UTEX 58). Axenic cultures were grown on a 15:9 LD cycle, in 1 1 flasks containing 500 ml M3 medium (31) and were bubbled with air that passed through saturated sodium bicarbonate solution. Each of 14 cultures was grown in a distinct combination of light intensity and temperature. Those grown at 8 and 14 C received irradiance of either 0.39, 0.68 or 1.41 ly/h. A higher light intensity (2.85 ly/h) was included to allow for optimum growth at 20 and 26 C. Light was provided with 8 W cool-white fluorescent bulbs and irradiance was measured with a Kip radiometer.

Population growth was measured by periodically taking 10 ml samples to estimate both cell density and chl *a* concentration. Cell density was estimated by reading absorbance in a spectrophotorneter at 678 nm (37), and computing density from a regression equation previously determined by cell count (hemacytometer) vs. absorbance at 678 nm:

$$\text{cell no/ml} = (5.69 + 168 \cdot A) \times 10^4. \quad (3)$$

Chl a was extracted in 90% acetone and estimated with the trichromatic method of Strickland and Parsons (42).

Chl a was the more reliable estimate of population size, so specific growth rate (day^{-1}) between any two successive measures of population density was computed as:

$$\mu = (\ln C_2 - \ln C_1)/(t_2 - t_1), \quad (4)$$

where C_1 is chl *a* concentration on day t_1. The maximum specific growth rate for each culture was taken as the optimum growth rate associated with its light-temperature combination. Simultaneous estimates of cell number and chl a provided continuous measures of cellular chl a quota as growth progressed in each culture.

Nutrient uptake kinetics. Uptake kinetics were measured with batch culture perturbation experiments (4,8) in which the time course of nutrient uptake was followed after a spike addition of nutrient to deficient cultures. Uptake of phos-

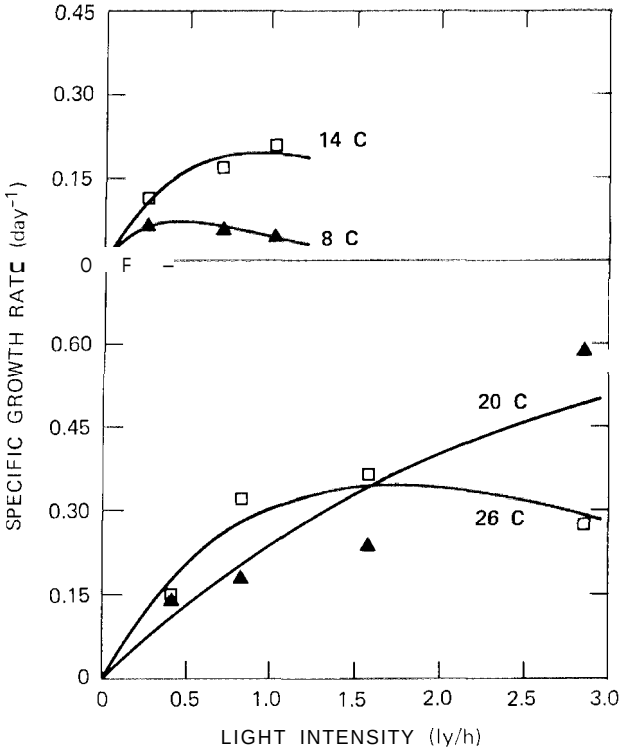

FIG. 2. Maximum growth rates of C. *ovata* observed at each experimental light-temperature combination: growth rates at each temperature fit to Steele's furrction of light intensitv.

phate, ammonium and nitrate was followed separately at 16 light-temperature combinations (see Fig. 6, 7).

Phosphate uptake. Forty ml aliquots taken from log phase stock cultures (20 C; 0.68 ly/h) were ccntrifugetl, washed in sterile phosphate-free medium (M3-P), resuspended in G0 ml M3-P, and placed in distinct light and temperature combinations (dark treatments were given irradiance of 0.35 ly/h). After 2 days, chl a and cell density were estimated from a 10 ml aliquot, then each flask received 0.50 or 0.75 ml 100 μM NaH_2PO_4 solution containing 4 μCi/ml carrier-free $H_3{}^{32}PO_4$, giving initial phosphate concentrations of 1.0 or 1.5 μM. A 2 ml sample was collected immediately and filtered onto a 25 mm HA Millipore filter; similar samples were collected over a 12 11 period. Filters were placed in vials with 10 ml liquid scintillation mix (3) and counted in a Packard Tri-Carb 2002. To correct for P-adsorption onto cells and filters, total cpm from the initial sample was subtracted from all successive samples. Correction was also made for quenching and decay.

Nitrate and *ammonium uptake.* N-deficient cultures were prepared as before, preconditioned at the same 16 light-temperature combinations for 48 h, and they then received either NH_4Cl or KNO_3 such that initial nitrogen concentrations were 6 μM. Twenty-five ml aliquots were taken imtnediately from each culture and filtered through Whatman GF/C filters. Sampling continued for 90 min during the ammonium uptake experiments and for 8–12 h during the nitrate uptake experiments. Filtrates were analyzed for nitrate concentration with a Technicon Auto-Analyzer II antl for ammonium with the method of Solórzano (35).

RESULTS

Growth kinetics. Population growth curves lor the 14 light-temperature treatments are presented in Fig.

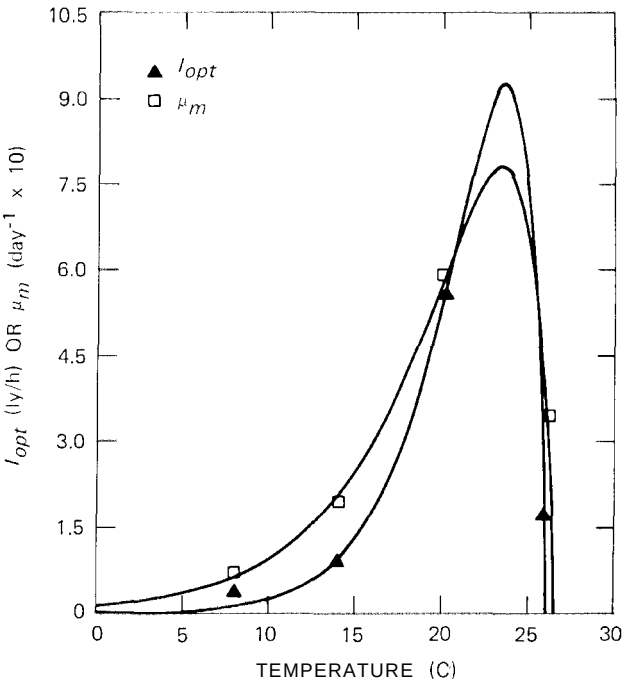

FIG. 3. Estimated maximum growth rates and optimum light intensities at each experimental temperature: also shown are fitted functions $\mu_m(T)$ antl $I_{opt}(T)$ given by Equations 6, 7.

1. Associatecl with each curve was a maximum growth rate, and within each temperature treatment these rates were fit to Equation 1 by least squares, giving the curves shown in Fig. 2. Each curve is defined by a specific μ_m and I,,,, both of which varied with temperature: μ_m antl I_{opt} increased exponentially with temperature between 8 and 20 C, and both were depressed at 26 C (Fig. 3). This is consistent with the general temperature response of biological antl biochemical rates, antl is described by a number of functions, including that derived by Logan et al. (26):

$$r(T) = \alpha \cdot \left\{\exp(\beta \cdot T) - \exp\left[\beta \cdot T_m - \left(\frac{T_m - T}{\Delta T}\right)\right]\right\}, \quad (5)$$

where α defines the rate ı at some basal temperature; β describes the rate of exponential increase up to some optimum temperature; T_m is an upper lethal temperature; ΔT defines the rate of decline as temperature (T) exceeds T_{opt}. Ihe four measured values of I_{opt} and μ_m were fit to Equation 5 with a non-lineai least squares routine (30), giving:

$$\mu_m(T) = 0.013 \cdot \left\{\exp(0.20 \cdot T) - \exp\left[5.31 - \left(\frac{26.60 - T}{2.22}\right)\right]\right\}; \quad (6)$$

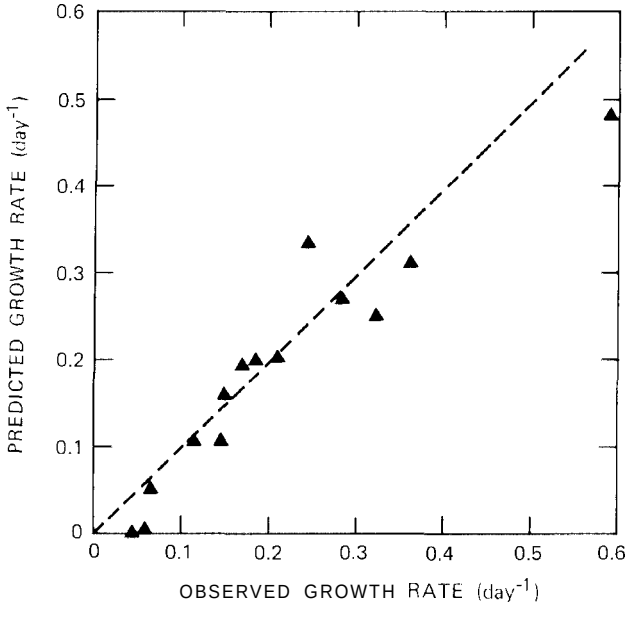

FIG. 4. Predicted specific growth rates vs. observed growth rates at experimental light-temperature combinations: --- represents an ideal one-to one correspondence between the two.

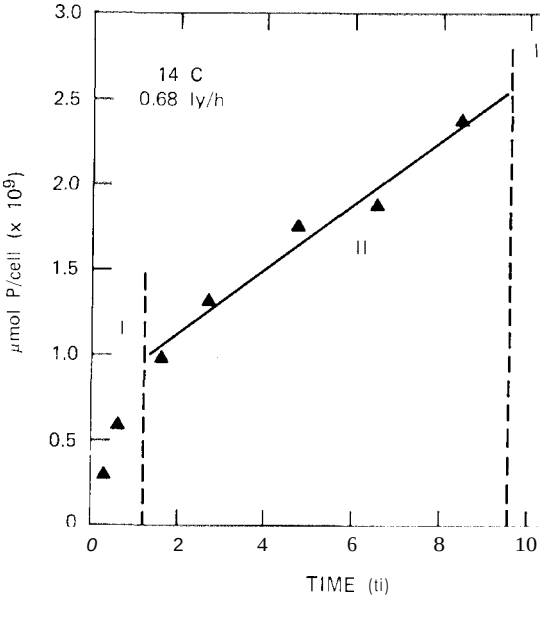

FIG. 5. Representative time course of phosphat I = initial, rapid surge; II = apparent substrate-satu take; III = substrate-limited uptake.

$$I_{opt}(T) = 0.0177 \cdot \left\{\exp(0.389 \cdot T) - \exp\left[10.184 - \left(\frac{26.173 - T}{2.43}\right)\right]\right\}. \quad (7)$$

Expected growth rates lor each experimental combination of light and temperature were computed from Equations I, 6, 7, and predicted values were plotted against their corresponding observed growth rates to demonstrate goodness-of-fit (Fig. 4).

Mean thl *a* quotas of cells grown in each light-temperature treatment are summarized in Table 1

Nutrient uptake kinetics. I he general patterns of nitrate, ammonium and phosphate uptake were similar antl are represented by a time course of phosphate uptake (Fig. 5) An initial, transient surge of rapid uptake (Phase I) was followed by a more stable, constant uptake rate (Phase 11) that persisted for several hours and then slowed (Phase 111) as external nutrient concentration fell. Conway et al. (8) saw the same uptake patterns with *Skeletonema costatum* Cleve, and they interpreted the stable uptake phase as an apparent substrate-saturated rate that slows only alter external contentrations appioach K_s. Substrate-saturated rates of nitrate, ammonium and phosphate uptake, estimated by fitting a linear equation to points comprising Phase 11, demonstrate effects of light intensity and temperature on uptake (Figs. 6–8). Only several time courses included more than two points that clearly Lell within the third (substrate-limited) uptake phase, antl this precluded meaningful estimation of half-saturation constants.

DISCUSSION

Growth kinetics. 'I he response of *Crypt ovata* growth to irradiance is described v Steele's function at 8 and 26 C where light tion was observed However, *C. ovata* is m erant of (antl may require) higher light intens the 14–20 C range, and resulting fits to Equ are not as good at the two intermediate tempe In order to estimate better μ_m and I_m at 14 an it would have been necessary to utilize high intensities However, the proposed model dc reasonably accurate estimates of growth rate wide range of temperature and irradiance (F

Note that both μ_m antl I_m were depressed a suggesting a temperature inhibition. The op growth temperature is between 20 and 26 C is consistent with findings lor other fres algae (19). The range of optimum light int

TABLE 1. *Mean chl a quota (µg/10^6 cells) of* Cryp ovata *grown nt different light-temperature combinatic confidence intervals; sample sizes included in parent*

Temperature (C)	Light Intensity (ly/h)			
	0.35	0.68	1.41	
8	1.75 ± 0.21 (9)	1.62 ± 0.16 (9)	1.34 ± 0.07 (9)	
14	2.54 ± 0.24 (9)	3.24 ± 0.36 (11)	2.61 ± 0.26 (11)	
20	3.83 ± 0.13 (12)	3.82 ± 0.44 (8)	3.21 ± 0.50 (6)	1.
26	2.65 ± 0.22 (11)	3.03 ± 0.50 (9)	2.76 ± 0.50 (10)	1.

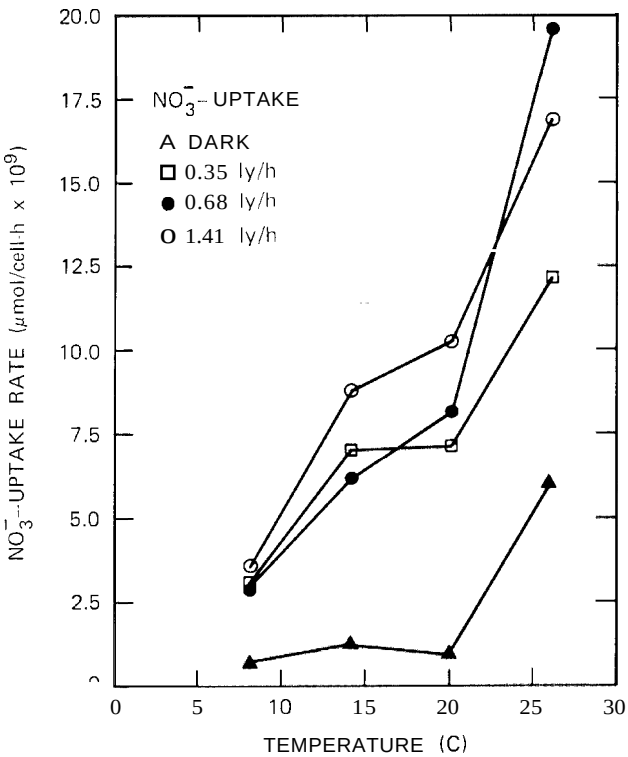

FIG. 6. Phase II nitrate-uptake velocities.

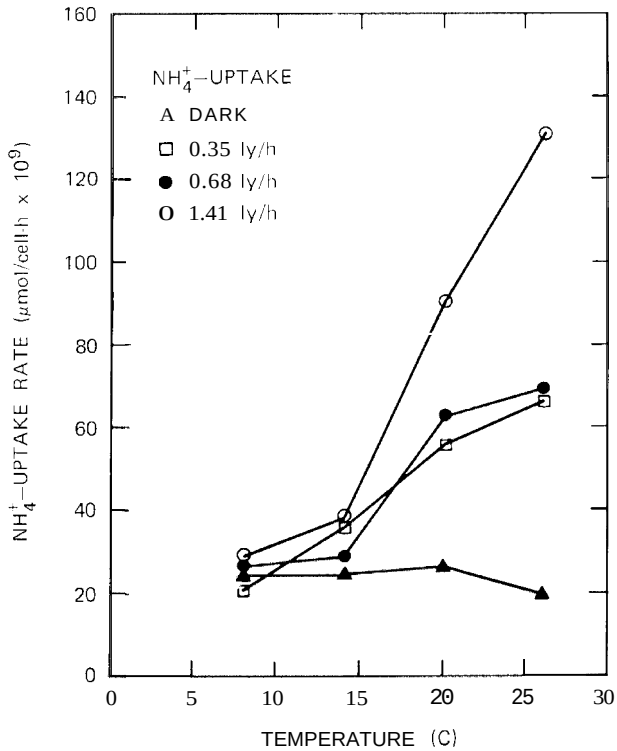

FIG. 7. Phase II ammonium-uplahe velocities.

(0.4–5.6 ly/h) is lower than reported for most phytoplankton species (e.g. 24,33), but is generally consistent with the expected range of 1.8–6.0 ly/h reported by Eppley and Strickland (17). The observed variation of $I_{s,s,s}$ with temperature has also been seen in natural tundra ponds (39) whose phytoplankton was dominated by cryptomonads and chrysophytes. The maximum observed specific growth rate of *Cryptomonas ovata* (0.5 day^{-1}) is low compared to rates for most unicellular algae (e.g. 11,22), and this may partly explain the relatively small population densities of C. *ovata* observed in Kootenay Lake, British Columbia (6).

Although chl *a* quotas were fairly constant during exponential growth in a given treatment, significant differences were found in mean chl *a* content among light-temperature treatments (Table 1). Chl *a* quota increased with temperature (note exception at 26 C) and generally decreased with increasing irradiance. Both trends have been observed in other species (16,41).

Nutrient uptake kinetics. Time courses of nitrate, ammonium and phosphate uptake by starved cells were consistent with time courses of ammonium and silicate uptake by marine diatoms (8). Detailed kinetic studies have begun to elucidate the very complicated nature of nutrient uptake by algal cells, and results presented here are consistent with the three uptake mechanisms proposed by Conway et al. (8). Since each uptake experiment was terminated before a sufficient number of substrate-limited uptake rates were measured, the question of light-temperature effects on K_s remains unanswered. However, analysis of covariance demonstrated existence of significant ($P < 0.05$) light-temperature effects on substrate-saturated (ρ_m) rates of uptake. It is clear that nitrate uptake is light-stimulated, but there is no obvious correlation between velocity and light intensity (Fig. 6). Nitrate uptake proceeds slowly in the dark (except at 26 C). Presumably the energy requirements for uptake (18) and reduction (12) are closely linked with photosynthesis, so this response to light is expected and consistent with that of other algae (10,28). The rate of nitrate uptake also varies with temperature--in the three light treatments, uptake velocities measured at 26 C were at least three times greater than those at 8 C. Measured rates of ammonium uptake also varied with light and temperature. It appears that uptake proceeds at a constant basal rate at lower temperatures when light has no effect. However, as temperature increases above 14 C, uptake velocity exceeds this basal rate in response to both increased temperature and light intensity. At the two lowest temperatures, phosphate uptake was faster in dark than in light, but at 26 C uptake was stimulated by increased light intensity. These results suggest that perhaps separate light and dark mechanisms are responsible for phosphate uptake.

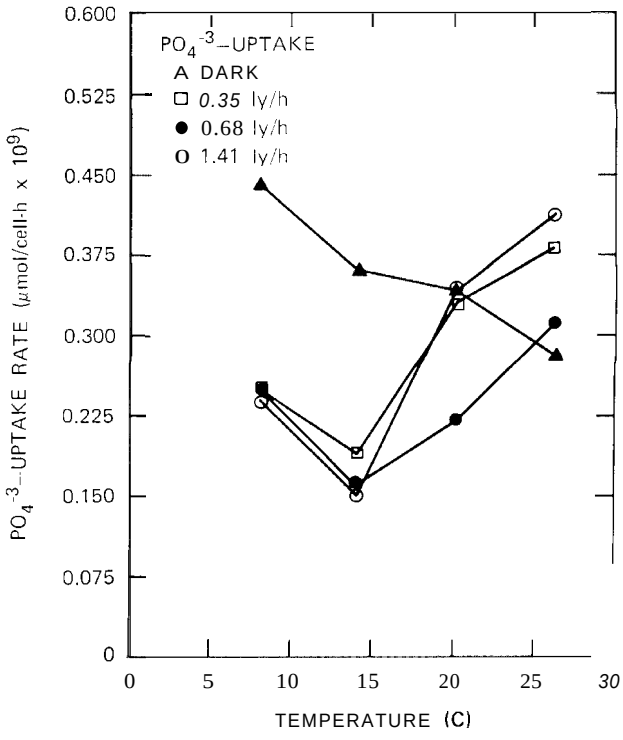

FIG. 8. Phase II phosphate-uptake velocities.

Interpretation of results presented in Figs. 6–8 must be clone with care since no single experiment was replicated. Also, since nutrient-uptake velocity is strongly dependent upon physiological condition and nutritional status (4,8,32), it is conceivable that observed differences in uptake rates resulted from a different nutritional state at the start of each experiment.

Extrapolation to lake conditions. Figures 6–8 demonstrate the range of nutrient-saturated uptake rates *C. ouatu* might exhibit in a natural system like Kootenay Lake. Ammonium uptake was faster than nitrate uptake at all experimental combinations of light and temperature, and particularly in the dark treatments. This suggests that *C. ovata* can utilize ammonium to satisfy its inorganic nitrogen needs at depths below the photic zone and perhaps at night, whereas nitrate uptake may be light-limited at depths and proceeds slowly at night. It has also been observed that when 6 μM ammonium is present, K-starved cells do not begin assimilating nitrate until ammonium is clepletecl. These observations strongly suggest that ammonium is the primary source of inorganic nitrogen for *Cryptomonas ouata* in Kootenay Lake arid other systems where both nitrate and ammonium are present. C. *ovata* takes up phosphate over a wide range of light-temperature conditions, and its ability to obtain inorganic phosphorus is probably not limited by light or temperature in Kootenay and other temperate lakes. Its rate of phosphate uptake is lower than reported rates for other algae (23,25,32), which suggests that *Cryptomonas ouatu* may be a poor competitor in natural systems where phosphorus is scarce.

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