

## SHORT NOTE

**Dark Uptake of Nitrate and Nitrate Reductase Activity of a Red-Tide Population Off Peru**Quay Dortch<sup>1\*</sup> and H. Maske<sup>2\*\*</sup><sup>1</sup> Department of Oceanography, University of Washington, Seattle, Washington 98195, USA<sup>2</sup> Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, Maine 04575, USA

**ABSTRACT:** Nitrate uptake rates and nitrate reductase activities were measured in the light and dark during a dinoflagellate bloom at one station off the coast of Peru. Although uptake rates in the light were higher than those in the dark, dark uptake rates were quite high. NR activities were generally much lower than uptake rates, but higher in the light than in the dark. The high rates of nitrate uptake in the dark support the hypothesis that dinoflagellate blooms can be maintained, when the surface layer is depleted of nitrogen, by migration to deeper, nitrate-containing layers at night. However, since nitrate uptake and reduction are not necessarily coupled, nitrate reduction in the dark is not required.

Dinoflagellate blooms are global phenomena sometimes associated with the following hydrographic conditions: stable water column, strong, shallow thermocline, and low concentration of dissolved nitrogen in the surface layer (e. g. Holmes et al., 1967; Eppley et al., 1968). These conditions may initiate and/or sustain dinoflagellate blooms because motile dinoflagellates, unlike most other phytoplankton, may be able to migrate at night down to a layer with high nitrate concentrations, take up and reduce nitrate in the dark, and return to the surface during the day (Holmes et al., 1967; Eppley et al., 1968; Packard and Blasco, 1974; Eppley and Harrison, 1975; Harrison, 1976). The importance of these abilities is the subject of continuing debate (e. g. Blasco, 1978; MacIsaac, 1978; Cullen and Horrigan, 1981; Heaney and Eppley, 1981); other mechanisms – such as encystment and horizontal transport – have also been implicated (Provasoli, 1979).

An unusually extensive dinoflagellate bloom, dominated by *Gymnodinium splendens* – a motile, non-thecate dinoflagellate – developed off the coast of Peru in the spring of 1976 along with a nitrogen depleted, warm surface layer (Dugdale et al., 1977; Blasco, 1979; Huntsman et al., 1981). These dinoflagellates migrated into the surface layer during the day, frequently forming large red patches, and out of it at night (Blasco, 1979). Despite nitrogen depletion in the surface layer, the bloom persisted throughout April, and moderately high carbon fixation rates were observed (Barber et al., 1978). We tested the hypothesis that nitrogen necessary for maintenance of the bloom could be taken up and reduced at night by making measurements of nitrate uptake rates and nitrate reductase (NR) activities in the light and the dark after nitrate additions to samples of the red tide population.

The work reported here was part of the Coastal Upwelling Ecosystem Analysis program, carried out onboard the R. V. 'Alpha Helix' during the cruise Joint II. Data for profiles of nutrient and chlorophyll *a* concentrations, 24-h simulated *in situ* <sup>14</sup>C productivity, temperature, and light measurements are available in data reports (Barber et al., 1978; MacIsaac et al., 1979).

A high-chlorophyll red patch, similar in location and chlorophyll concentration to patches observed earlier in the same cruise (Blasco, 1979) was located on April 28, 1976 beyond the shelf break at 15° 24.1'S and 76° 9.6'W by measuring *in vivo* underway fluorescence. After collecting samples at discrete depths for the routine analyses mentioned above, seawater was pumped up from 1 m depth through plastic tubing by a peristaltic pump at 16:00 h local time and stored, unenriched with nutrients, in glass carboys in the dark. The carboys, as well as all subsamples taken from them, were cooled to 19.6°C by running seawater. After

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thorough mixing, subsamples for the following experiments were taken from the carboys.

(1) Nitrate uptake during the day in natural light and in the dark. As soon as the bloom population was pumped into carboys on deck, 10  $\mu\text{M}$  nitrate was added to two 2-l subsamples. One was incubated in natural light and one was incubated in darkness. Nitrate uptake was measured at 30-min intervals until sunset (from 16:30 to 18:00). At 10:00 h the next morning the procedure was repeated with new subsamples (from 10:00 to 12:30) in order to compare the rates of light and dark uptake during the midday period of high light intensity with the rates of light and dark uptake during the late afternoon period of low light intensity.

(2) Nitrate uptake at night in artificial light and in the dark. At sunset two subsamples of the bloom population were placed in 20-l carboys. Fifteen  $\mu\text{M}$  nitrate was added to the one which was kept in the dark and 12.5  $\mu\text{M}$  to the one exposed to artificial light. At such high, saturating nitrate concentrations, the difference between the 2 carboys should not affect the uptake rates. Illumination was provided by 4 fluorescent daylight lamps. Nitrate uptake rates and NR activities were measured periodically in both carboys until sunrise.

Dissolved nitrate, nitrite, and ammonium concentrations were determined by a Technicon AutoAnalyzer<sup>®</sup> as described in Friederich and Whitledge (1972). Nitrate was taken up immediately after it was added, although there was no rapid initial uptake, and the uptake rates were constant throughout the period described, even in the large carboys. Thus, nitrate uptake rates were determined from the slopes of linear regressions of the nitrate concentrations as a function of time in experimental bottles. In all cases, the slopes were significantly different from zero (t-test;  $p \leq 0.025$ ). NR activities were measured by the method of Eppley et al. (1969).

The depth profiles of temperature and nitrate concentration show a stable water column with a steep thermocline and nitrate depletion above 20 m (Fig. 1). Ammonium and nitrite concentrations in the upper 20 m were also low,  $0.35 \pm 0.017 \mu\text{M}$  (mean and standard error,  $n = 9$ ) and  $0.09 \pm 0.003 \mu\text{M}$  ( $n = 10$ ) respectively. Microscopic examination of a sample from a depth of 1 m showed a phytoplankton population dominated by dinoflagellates (T. Cowles, pers. comm.). Light at 16:00 h (Fig. 1) showed strong attenuation close to the surface which can be attributed to the surface accumulation of dinoflagellates. However, the location of the maximum chlorophyll concentration at 2.5 m (Fig. 1) indicated that the downward migration had already begun. A half hour earlier the maximum concentration had been observed by *in vivo* fluorescence measurements at the surface. Both the 24-h car-

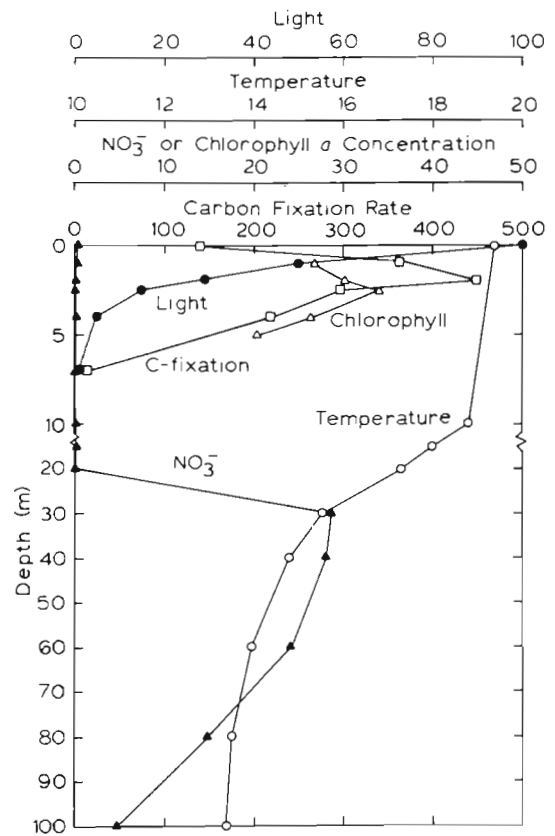


Fig. 1. Light level ( $\%I_0$ ,  $I_0 = 240 \mu\text{Einsteins m}^{-2} \text{s}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ), nitrate ( $\mu\text{M}$ ) and chlorophyll *a* ( $\mu\text{g l}^{-1}$ , SCOR:UNESCO) concentrations and carbon fixation rates ( $\mu\text{gC l}^{-1} \text{d}^{-1}$ ) at Station 66

bon fixation rates and chlorophyll concentrations were reasonably high and well within the range observed earlier in the bloom (Barber et al., 1978), suggesting that the population was not senescent.

Although nitrate uptake rates in the light were always higher than those in the dark (Table 1), dark uptake rates were quite high, averaging 69 % of light uptake rates. There was no difference between nitrate uptake rates as a result of the level or kind of light (natural vs. artificial), except that uptake rates were highest in the late afternoon of the first day (Table 1, Exp. 1a). Nitrate uptake in the dark by organisms passing through a Gelman GFA filter was negligible. Such a test was necessary since denitrifying bacteria were proposed to be active in the near-surface waters (Dugdale et al., 1977) and could have caused significant dark uptake of nitrate.

The low ratios of rates of nitrate uptake in the light over those in the dark were similar to those observed by Eppley and Harrison (1975) and Harrison (1976) with cultures of *Gonyaulax polyedra* or a red tide population dominated by *G. polyedra*. In contrast, Mac Isaac (1978) obtained very high ratios for a red tide population also dominated by *G. polyedra* off Baja

Table 1. Rates of nitrate uptake and NR activity ( $\mu\text{mole l}^{-1} \text{h}^{-1}$ ) and their standard errors in the light and the dark

Description of experiment	Rate or activity		
	Light	Dark	L/D
(1) Uptake during day in natural light and darkness			
Late afternoon (16:30–18:00)	1.17 $\pm$ 0.10 (n = 4)	1.01 $\pm$ 0.06 (n = 4)	1.16
Mid-day (10:00–12:30)	0.89 $\pm$ 0.16 (n = 4)	0.53 $\pm$ 0.06 (n = 4)	1.68*
(2) a Uptake during night in artificial light and darkness (18:00–06:00)	0.83 $\pm$ 0.03 (n = 10)	0.50 $\pm$ 0.03 (n = 10)	1.66*
b NR activity during night in artificial light and darkness (18:00–06:00)	0.0277 $\pm$ 0.00367 (n = 6)	0.0160 $\pm$ 0.00376 (n = 6)	1.73*

\* Rate in the light was significantly higher than in the dark (one-tailed Student's t-test;  $p \geq 0.05$ )

California. She argues that low ratios of uptake in the light and dark result when uptake rates in the light are low since rates in the dark are uniformly low (Mac Isaac, 1978). However, when the light uptake rates in Table 1 are converted to specific rates based on chlorophyll *a* and compared with those in MacIsaac (1978) using the appropriate chlorophyll *a* values (Whitledge and Bishop, 1972) the rates reported here are much higher. Instead the differences in the light to dark ratios may be a function of differences in the nutrient regimes since the high nitrate layer was much nearer the surface off Baja California than it was off Peru.

NR activities, measured in Experiment 2 (Table 1), were quite low both in the light and the dark and represented between 2 and 3 % of the corresponding nitrate uptake rate. The activities were significantly greater in the light than in the dark and the ratio of 1.73 was quite similar to the light/dark ratios for nitrate uptake in the same experiment. There was little variation in activity during the course of the experiment. In fact, instead of the usual post-sunset decrease, nighttime minimum, and pre-sunrise increase in activity (Packard and Blasco, 1974), the maximum in both light and dark was observed between 20:00 and 24:00 h.

The absence of a large diel periodicity of the NR in some dinoflagellates has been used as supporting evidence for the hypothesis that dinoflagellates take up nitrate when they migrate to depth at night under certain conditions (Packard and Blasco, 1974; Eppley and Harrison, 1975; Harrison, 1976). However, it has been pointed out that since dinoflagellates are capable of storing large quantities of unassimilated nitrate, there is no necessity for high NR activities in the dark (Hersey and Swift, 1976; Bhovichitra and Swift, 1977). Furthermore, the NR activities were so low that it is difficult to assess the significance of differences in

activities. Since ammonium concentrations were quite low, it is unlikely that the low NR activities were due to ammonium inhibition. Nitrate reduction may be accomplished by a second, unidentified nitrate reducing enzyme which has been postulated to function under conditions of high ambient nitrate concentrations (Collos and Slawyk, 1976; Dortch et al., 1979). If so, it is further evidence that the dinoflagellates are obtaining nitrate in the high nitrate layer at depth.

We have shown that the phytoplankton encountered in large, red, surface patches off Peru in the spring of 1976 were capable of high nitrate uptake rates in the dark. NR activities were low, but this does not argue against uptake at night, since uptake and reduction are not necessarily coupled. However, in order to prove that the dinoflagellate bloom was sustained by dark uptake of nitrate, nightly migration down to the nitricline must be demonstrated. Otherwise, other nitrogen sources – such as ammonium and organic nitrogen supplied by excretion or internally stored nitrogen – may have also supported the bloom. Blasco (1979) observed earlier in the cruise that surface patches of dinoflagellates disappeared before sunset, and that maximum dinoflagellate concentrations were found at times as deep as 21 m. We also saw the disappearance of the surface patches, but did not measure chlorophyll profiles in the water column during the experiment. In order to reach the nitricline, found below 20 m, nightly, speeds of migration considerably in excess of  $2 \text{ m h}^{-1}$ , the maximum speed observed for most dinoflagellates (e. g. Eppley et al., 1968; Blasco, 1978), would be required. Alternatively, perhaps only part of the population migrates the full 20 m, possibly in response to depletion of internal stores of nitrogen. Understanding the process of migration off Peru is complicated by recent studies showing that the ability to migrate is found in N-deficient dinoflagellates, but that under

those conditions, some species, including *Gymnodinium splendens*, do not migrate to the surface during the day (Cullen and Horrigan, 1981; Heaney and Eppley, 1981).

During the same dinoflagellate bloom off Peru in 1976, Huntsman et al. (1981) measured the ratio of diatoms to dinoflagellates, nitrogen concentrations, and stability of the water column for 6 wk at an inshore station where dinoflagellates predominated, but did not reach the high concentrations observed elsewhere (Blasco, 1979). They proposed that the dinoflagellates were maintained in the area because of an interaction between horizontal water movements at different levels in the water column and the vertical migration of the dinoflagellates. They also suggest that the sizeable diatom population which coexisted with the dinoflagellates persisted because of slight mixing of the surface layer and because the nitrogen concentrations, which were too low in the surface layer for the dinoflagellates to utilize, were adequate to maintain the diatoms. However, there must also have been an adequate nitrogen source for the dinoflagellates to have persisted for so long at this inshore station. Since we have shown that these organisms are capable of high nitrate uptake rates in the dark, it seems likely that they obtained their nitrogen when they migrated to depth at night.

Although it seems apparent that the ability of dinoflagellates to take up nitrate in the dark may be a factor during some dinoflagellate blooms, its precise role is not yet clear. What is needed is a systematic study which measures nitrate uptake, storage, and reduction, documents vertical migration, and determines water movements during the course of a bloom.

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