93820433

STATUS: PENDING 20120816 OCLC #: 5736913

REQUEST DATE: 20120816 NEED BEFORE: 20120820 SOURCE: FSILLSTF

BORROWER: SRR RECEIVE DATE: DUE DATE:

RENEWAL REQ: NEW DUE DATE: SPCL MES:

LENDERS: *EKC

LHR SUMMARY: 2-5(1981-1983)
TITLE: Marine biology letters.
ISSN: 0165-859X
IMPRINT: Amsterdam, Elsevier/North-Holland Biomedical Press. 1979 1984
ARTICLE TITLE: Simultaneous uptake of ammonium and nitrate ..
ARTICLE AUTHOR: Maestrini, et al.
VOLUME: 3
ISSUE DATE: 1982
PAGES: 143-153
VERIFIED: WorldCat CODEN: MBLED7 Desc: v.Type: Serial
SHIP TO: Frederick Lont/Cardno ENTRIX/108 W. Jefferson St./Ste. 300/Syracuse NY US 13202

BILL TO: Frederick Lont/Cardno ENTRIX
SHIP VIA: Email/best
COPYRIGHT COMPLIANCE: CCL
EMAIL: Frederick.Lont@cardno.com
BORROWING NOTES: We need this by tomorrow morning. Thanks Sonya!

PATRON: June Schneider/I

Marine Biology Letters, 3 (1982) 143–153
Elsevier Biomedical Press

SIMULTANEOUS UPTAKE OF AMMONIUM AND NITRATE BY OYSTER-POND ALGAE*

SERGE Y. MAESTRINI[1], JEAN-MICHEL ROBERT[2] and ISABELLE TRUQUET[3]

[1] Station marine d'Endoume, 13007 Marseille, [2] Laboratoire de Biologie marine, Université de Nantes, 44072 Nantes Cedex, and [3] Institut scientifique et technique des Pêches maritimes, B.P. 1049, 44037 Nantes Cedex, France

(Received 20 January 1982; revised version received and accepted 23 February 1982)

SUMMARY

Natural micro-algal populations of oyster ponds have been grown in situ, in the presence of ammonium and nitrate as nitrogen sources. Both ions were added at varied concentrations, up to 50 μg-at.$\cdot l^{-1}$; other nutrients were in excess.

The uptake of nitrate was prevented by the presence of ammonium above a threshold concentration which was an order of magnitude higher than the highest values reported for offshore phytoplankton species. When nitrate uptake resumed, it occurred at a reduced rate until the ammonium had decreased to $\approx$ 7 μg-at.$\cdot l^{-1}$; the uptake mechanism then operated at a rate which was similar or equal to the ammonium uptake rate. Cultures with initial ammonium concentration lower than the threshold values lacked the reduced-rate phase and/or the uptake lag phase.

Data reported and those appearing in the literature demonstrate that the frequently accepted limit of 1 μg-at.$\cdot l^{-1}$ NH_4-N for a concomitant uptake of ammonium and nitrate does not apply to some micro-algae at least; the algae of salt-marsh, estuarine and pond communities are suspected to react at quite higher nutrient concentrations.

Key words: nitrogen uptake, ammonium, nitrate, micro-alga, oyster pond.

INTRODUCTION

That ammonium is a suitable nitrogen source for algae is a concept which goes back to Krüger (1894) for freshwater species, and to Brandt (1899) for marine species. Moreover, as early as 1903, Chick demonstrated that the green alga *Chlorella pyrenoidosa*, growing in axenic conditions, preferred ammonium to nitrate; when supplied with both ions this alga exhausted first ammonium and left nitrate and nitrite free from uptake. Further contributions reviewed by Pringsheim (1949), Myers (1951), Fogg (1953), Syrett (1962, 1981) and Morris (1974) have

*This research was supported by the 'Centre national d'Exploitation des Océans'; grant numbers 80/2248 and 80/2278.

demonstrated that nitrate uptake resumes when ammonium concentration decreases to near zero. The mechanism originates from the repression by ammonium of the nitrate reductase formation (Hattori, 1962a, b; Morris and Syrett, 1963, Syrett and Morris, 1963). On the basis of measurements which involved marine phytoplankton, Eppley et al. (1969) established that the specific ammonium threshold values at which nitrate reductase production stops range within 0.5 and 1.0 μg-at.NH_4-N$\cdot l^{-1}$. Otherwise, the nitrate reductase content of algal cells is critically maximum when nitrate is available to the cells (Packard, 1979); yet some species are able to produce slight amounts of this enzyme when nitrate is lacking in the growth medium (Syrett and Hipkin, 1973).

Thus, a recorded nitrate reductase activity will mean the algae harvested have taken up the nitrate in growing; while a lack of nitrate reductase activity will mean either the algae were taking up ammonium or that they had totally exhausted the nitrogen supply of sea water. According to this concept, nitrate reductase measurements provide a useful tool for studying the time course of phytoplankton blooms (Eppley et al., 1969) and for separating new from regenerated production (Dugdale and Goering, 1967).

Some algae have nevertheless appeared not to respect this 'rule' of 1 μg-at.$NH_4\cdot l^{-1}$ upper limit, and have shown that they are capable of taking up nitrate when ammonium is present at a higher concentration. These increased specific limits are quite high for the freshwater species studied by Prochazkova et al. (1970) and Toetz (1981), but remain low for the marine species which have been studied so far (e.g. Conover, 1975; Bates, 1976; Garside, 1981). Here we report some data which demonstrate that natural populations of micro-algae in oyster ponds can take up nitrate, even when ammonium is also present in the water at concentrations which range over an order of magnitude higher than the previously reported limit values.

MATERIAL AND METHODS

Water was collected in March 1979, 1980 and 1981, from several oyster ponds adjacent to the bay of Bourgneuf (Vendée, France; for more details concerning the area of sampling, see Robert, 1975), and filtered through a 50 μm mesh. After mixing, aliquot samples were distributed into 25-l glass vessels. Natural micro-algal populations were harvested separately from the same ponds; after filtration through a 200 μm plankton-net mesh to remove most of the grazers, the water was refiltered through a 50 μm mesh which retained most of the oyster-pond algae. The concentrated algal suspension was inoculated into the 25-l jars, in order to obtain an initial cell density of $4–5 \times 10^5$ cells$\cdot l^{-1}$, which gave a biomass of near 5 μg chlorophyll-$a\cdot l^{-1}$. The ammonium sulfate and potassium nitrate solutions were added, while the water was gently but continuously stirred. Phosphorus and silica salts were added, in order to prevent any growth limitation prior to exhaustion of

total nitrogen supply. The cultures were incubated in situ in an oyster pond in 1979 and 1980 and, in 1981, in a large swimming-pool which mimicked the natural conditions quite well.

Samples for analysis were taken once a day in the early experiments, and 4 times a day later on. NO_3-N was immediately analysed by using an automatic Technicon analyser (Strickland and Parsons, 1972); NH_4-N was manually analysed by the method of Koroleff (1970). A biomass estimation was also made (i.e. chlorophyll-*a*; SCOR-UNESCO, 1966), to confirm that the yield index did not vary more than within tolerable limits.

The threshold values below which the concomitant assimilation of both ions begins was obtained both from the plot of concentration versus growth duration (see Fig. 1) and the use of a preferential index. McCarthy et al. (1977) have used a 'relative preference index' (RPI) to establish the degree to which a particular form is selected:

$$\mathrm{RPI}_{NO_3} = \frac{U_{NO_3}}{U_{NO_3} + U_{NO_2} + U_{NH_4} + U_{\mathrm{Urea}}} \Big/ \frac{[NO_3]}{[NO_3] + [NO_2] + [NH_4] + [\mathrm{Urea}]}, \qquad (1)$$

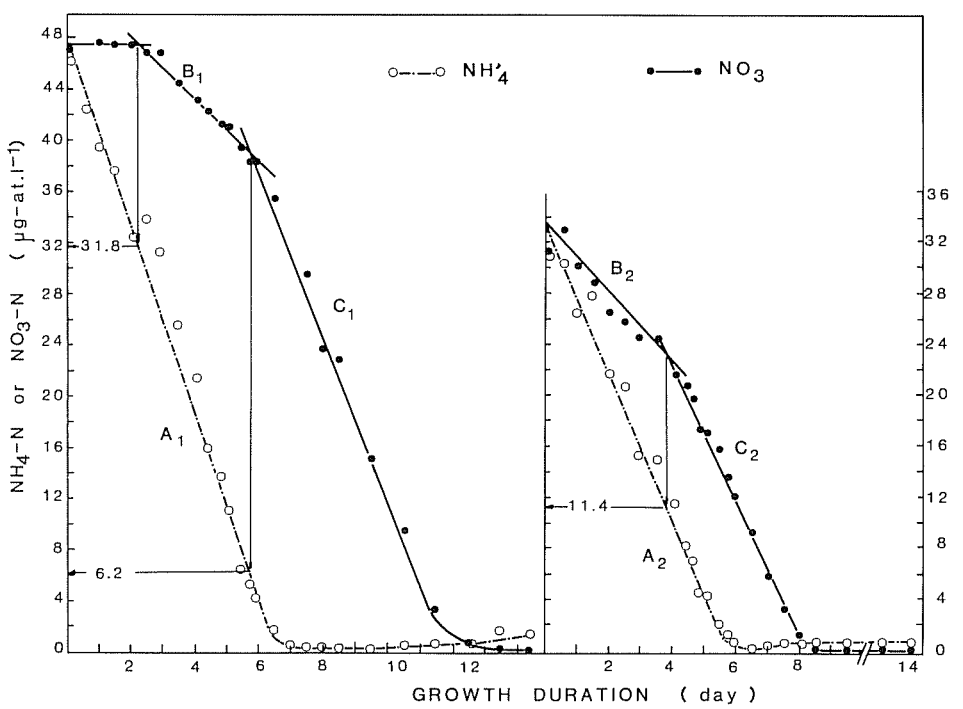

Fig. 1. NH_4 and NO_3 concentration decreases due to algal growth, during experiment number 5 (left section of the figure) and experiment number 12 (right section of the figure).

where U_{NO_3}, U_{NO_2}, U_{NH_4}, U_{urea} = rate of uptake of respective nitrogen source, as measured with ^{15}N. $[NO_3]$, $[NO_2]$, $[NH_4]$, [Urea] = respective actual nitrogen concentration. This index is relevant when both nitrogen uptake and inorganic nitrogen contents of sea water refer to temporally scattered measurements (i.e. only one measurement per station), but it may be improved when more complete data on nutrient consumption are available, as was the case in our experiments. Thus we modified the index of McCarthy et al. and plotted its values versus the actual ammonium concentration:

$$\frac{\sum U_{NO_3}}{\sum U_{NO_3} + \sum U_{NH_4}} \times \frac{[NO_3]t_0 + [NH_4]t_0}{[NO_3]t_0} \times 100 = f([NH_4]t_a), \quad (2)$$

where $[NO_3]t_0$ and $[NH_4]t_0$ = respective initial concentrations, $[NH_4]t_a = NH_4$ concentration, at any time of the growth duration or subsequent time course of nitrogen uptake; ΣU_{NO_3} and ΣU_{NH_4} = respective numbers of nitrogen atoms taken up, from the beginning of growth (t_0) to actual time (t_a); the values of our NO_3 RPI are expressed in percent; they are computed from respective Day 0 to the last Day (i.e. NO_3 exhaustion), by steps of 0.5 or 1 μg-at-NH_4; each value is plotted against the mean NH_4 concentration value between the step limits.

The common algal species present in the oyster-pond waters during the experiments in 1979, 1980 and 1981 are listed in Table I.

RESULTS

Altogether 14 experiments have been carried out. They involved different ammonium and nitrate concentrations (Table I), up to the maximum of inorganic nitrogen levels occurring in this type of coastal water. In all cases, ammonium nitrogen was immediately taken up by algae, while, on the contrary, the uptake of nitrate nitrogen was prevented by high ammonium concentrations. Thus, the behaviour of nitrate uptake was dependent on the initial ammonium concentration.

Experiment number 5 provides a typical case with both high ammonium and nitrate initial concentrations, i.e. 48.2 μg-at.$\cdot l^{-1}$ and 50.0 μg-at.$\cdot l^{-1}$, respectively. Ammonium uptake began without any lag phase (Fig. 1; left side), whereas nitrate was not taken up for 2 days. When the ammonium concentration had decreased to $\approx$ 32 μg-at.$\cdot l^{-1}$, nitrate uptake began, but at a reduced rate, i.e. 0.10 μg-at.$\cdot l^{-1} \cdot h^{-1}$. This reduced rate occurred for approximately 4 days, until the ammonium reservoir had decreased to $\approx$ 6.2 μg-at.$\cdot l^{-1}$. At that point the nitrate uptake increased markedly and became roughly equal to the ammonium uptake rate: 0.27 μg-at.$\cdot l^{-1} \cdot h^{-1}$ and 0.30 μg-at.$\cdot l^{-1} \cdot h^{-1}$, respectively.

Figure 1 (right side) depicts a quite different result (experiment number 12). The first data points are confusing, as far as the nutrient concentrations were concerned, because those belonging to the 12-h sample were higher than initial values. This

TABLE I

Respective list of main algal species present in the oyster-pond waters used for the 1979, 1980 and 1981 experiments, with relative cell density (as percent of total cell density); total cell densities were 548,000 cells $\cdot l^{-1}$ in 1979, 243,000 cells $\cdot l^{-1}$ in 1980 and 307,000 cells $\cdot l^{-1}$ in 1981.

1979		1980		1981	
Species	%	Species	%	Species	%
Skeletonema costatum	47.1	*Navicula ostrearia*	44.1	*Amphora hyalina*	24.5
Nitzschia closterium	33.1	*Nitzschia closterium*	13.4	*Gyrosigma formosum*	23.4
Dinoflagellates	9.9	*Amphora hyalina*	12.4	*Navicula ostrearia*	21.8
Nitzschia rigida	2.7	*Pleurosigma sp.*	6.4	*Navicula ramosissima*	17.0
Navicula ostrearia	2.0	*Amphora ostrearia*	5.7	*Nitzschia closterium*	6.1
Nitzschia longissima	2.0	*Nitzschia longissima*	4.1	*Gyrosigma balticum*	1.3
Nitzschia communata	1.0	Unknown pennata	3.8	*Nitzschia acicularis*	0.9
Other diatoms (6 species)	1.2	*Amphora sp.*	2.4	Other diatoms (15 species)	4.2
		Nitzschia acuminata	1.5	Dinoflagellates	0.5
		Surirella sp.	1.3		
		Navicula sp.	1.1		
		Skeletonema costatum	0.8		
		Other diatoms (9 species)	3.2		

probably resulted from an imperfect mixing following the addition of enrichment mixtures, because the sampling was made without delay in order to get the initial values prior to any algal uptake. This assumption is supported by the fact that the correlation factors are quite high, i.e. 0.99 for the NH_4 regression (A) and 0.98 for the slow NO_3 regression (B). Hence it is more realistic to use the values given by the intercept between the regression lines and the yy′ axis than to use the data given by the first analysis. Similar features were observed with some other experiments, irrespective of the great care we took in order to obtain a good mixing. In any case, it is clear that the initial inhibition of nitrate uptake did not occur here; the slow rate of uptake (0.12 μg-at.$\cdot l^{-1}\cdot h^{-1}$) was immediately apparent. Here again, a full nitrate uptake rate (0.22 μg-at.$\cdot l^{-1}\cdot h^{-1}$) equal to that of ammonium uptake (0.23 μg-at.$\cdot l^{-1}\cdot h^{-1}$) was restored when ammonium concentration had reached the value of 11.4 μg-at.$\cdot l^{-1}$.

When the initial ammonium concentration was close to a value of 12 μg-at.$\cdot l^{-1}$ nitrate uptake was never prevented or reduced. Figure 2 depicts such a situation (experiment number 14) which refers to an initial ammonium concentration of 12.5 μg-at.$\cdot l^{-1}$ and 13.5 μg-at.$\cdot l^{-1}$ nitrate. There, both ions were immediately taken up at the same rate: 0.11 μg-at.$\cdot l^{-1}\cdot h^{-1}$.

The RPI values are consistent with these estimations. Figure 3 depicts some typical curves we obtained. The threshold limits appear very clearly, but an artefact was apparent in a few experiments where initial ammonium concentration was lower than the threshold value. In such conditions, ammonium and nitrate were both immediately taken up at the same rate (Fig. 2); therefore the NO_3 RPI should have had the same value from the beginning to the end of the uptake period. This does

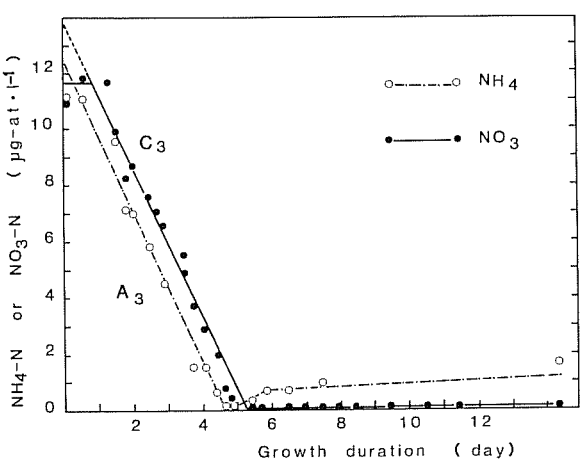

Fig. 2. NH_4 and NO_3 concentration decreases due to algal growth, during experiment number 14.

TABLE II

Initial NH_4 and NO_3 concentrations, maximum chlorophyll-*a* concentrations (chl-*a*), rate of ammonium uptake, slow and fast NO_3 uptake rates and ammonium concentrations at which they began, of natural oyster-pond micro-algal populations.

Reference of the experiment	$[NH_4]t_0$ (μg at.$\cdot l^{-1}$)	$[NO_3]t_0$ (μg at.$\cdot l^{-1}$)	Chl-*a* ($\mu g \cdot l^{-1}$)	NH_4 uptake rate (μgat.$\cdot l^{-1} \cdot h^{-1}$)	Slow NO_3 uptake		Fast NO_3 uptake	
					$[NH_4]$ when began (μg at.$\cdot l^{-1}$)	Rate (μgat.$\cdot l^{-1} \cdot h^{-1}$)	$[NH_4]$ when began (μg at.$\cdot l^{-1}$)	Rate (μgat.$\cdot l^{-1} \cdot h^{-1}$)
Experiment of March 1979								
1	12.0	6.3	–	0.07	–	–	8.6	0.06
Experiments of March 1980								
2	6.6	34.2	84	0.13	–	–	6.6	0.37
3	11.3	18.6	–	0.05	11.3	0.02	4.1	0.12
4	17.2	36.8	81	0.21	17.2	0.03	4.4	0.62
Experiments of March 1981								
5	48.2	50.0	109	0.30	31.8	0.10	6.2	0.27
6	47.5	51.3	143	0.29	29.4	0.12	8.8	0.27
7	37.5	51.7	123	0.27	35.1	0.10	7.8	0.27
8	35.0	50.1	134	0.32	31.3	0.09	7.7	0.34
9	29.6	49.9	108	0.26	28.4	0.17	10.9	0.32
10	27.3	50.0	121	0.21	20.2	0.19	4.1	0.40
11	23.2	49.9	113	0.18	18.5	0.19	3.8	0.37
12	31.3	30.2	84	0.23	27.4	0.12	11.4	0.22
13	18.7	20.0	78	0.18	16.9	0.19	5.1	0.28
14	11.1	11.0	56	0.11	–	–	10.2	0.11

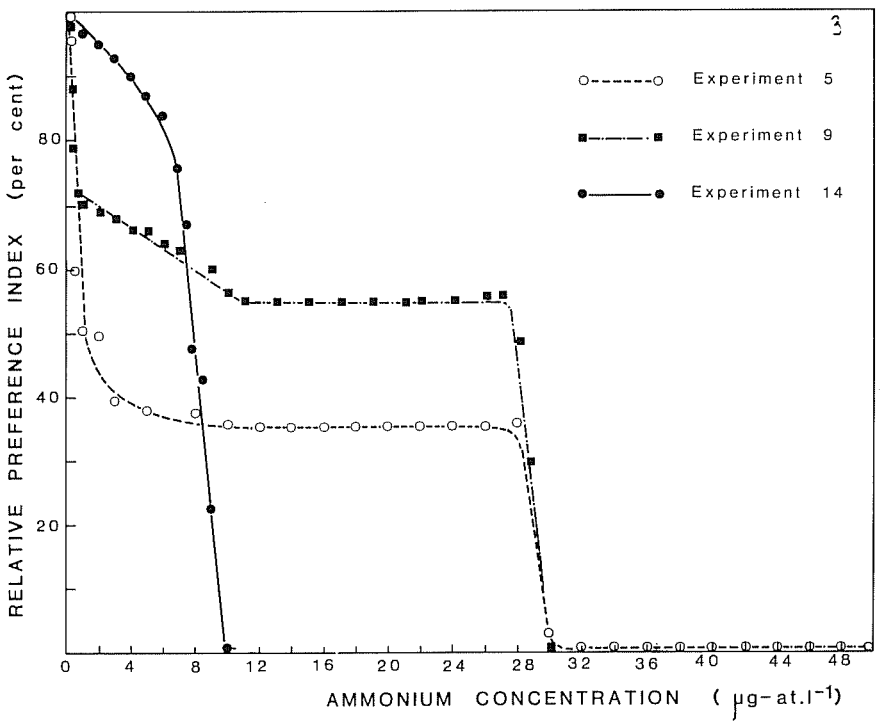

Fig. 3. Preference index values of NO_3 uptake versus ammonium concentrations, during three 1981 experiments.

not result when computations are made from analytical data, because, as we suggested above, the initial mixing was never good enough to allow the respective concentrations to be immediately at maximum. However, in the worst case depicted in Fig. 3 (experiment number 14) the slope of the adjustment part of the curve is so steep that no mistake is possible.

The possibility that ammonia oxidation by bacteria may have affected these results was excluded by repeating the experiments with axenic strains of local algal isolates. Figure 4 shows the results of one such experiment with a starting ammonium concentration of 2.5 μg-at. $\cdot l^{-1}$.

DISCUSSION

Altogether, the threshold values we have recorded clearly demonstrate that nitrate ions were taken up when ammonium ions were present at concentrations far exceeding 1 μg-at. $\cdot l^{-1}$ (Table I).

The prevention of nitrate uptake could nevertheless be observed; the threshold concentration was simply very high. An average value involving all ammonium

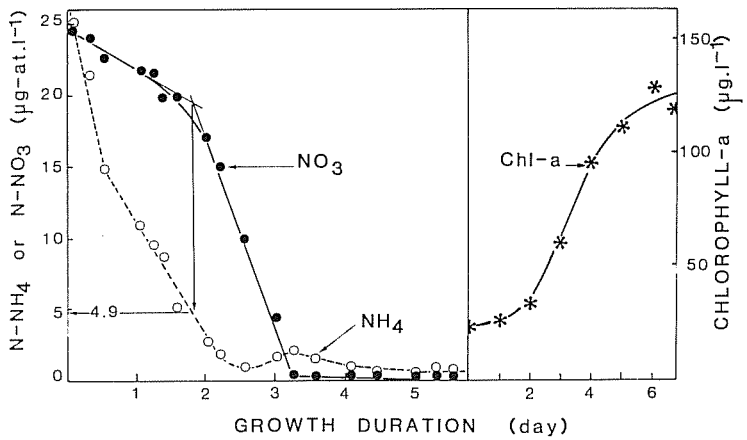

Fig. 4. NH_4 and NO_3 concentration decreases and chlorophyll-*a* content increase, during the time course of growth of the oyster-pond diatom *Navicula ostrearia* Bory, grown in axenic conditions.

concentrations from which nitrate uptake began is not really pertinent here, because several of the initial ammonium concentrations were lower than most of the threshold values we recorded. Therefore, we deleted all experiments whose threshold values did not correspond to a diminution of at least 1 μg-at.$\cdot l^{-1}$ in relation to the initial ammonium concentration (i.e. experiments 1, 2, 3, 4 and 14); the others give a mean value of 26.6 μg-at.$\cdot l^{-1}$ ($s = 6.46$). Such a high value is surprising and has not been reported previously. Yet, several previous papers provide support for our results, when reconsidered. Thus, ZoBell (1935) claimed that his data was evidence for the preferential assimilation of ammonium in the presence of nitrate, which was true in regard to the ammonium which was depleted first, but it is apparent from his results that uptake of both ions began simultaneously, despite the fact that ammonium was originally present at 50 μg-at.-N$\cdot l^{-1}$. Proctor (1957) also stated that the alga he studied assimilated ammonium nitrogen almost exclusively as long as it was available, but his data demonstrated that the uptake of nitrate nitrogen had undoubtedly begun while ammonium was still present in the culture medium at roughly 79 μg-at.$\cdot l^{-1}$. Moreover, the paper of Grant et al. (1967) which is frequently cited to support the concept of repression of nitrate uptake by the presence of ammonium contains this sentence: 'wherever ammonium and nitrate are supplied together, nitrate is not assimilated until ammonia is reduced to approximately 1 mg N per litre' (p. 132). Since 1 mg N$\cdot l^{-1} = 71.4$ μg-at.$\cdot l^{-1}$, their conclusion is somewhat confusing. Natural freshwater phytoplankton populations have also been reported not to respect the 1 μg-at.N$\cdot l^{-1}$ threshold limit and to show a nitrate uptake concomitant with ammonium concentration up to 11.4 μg-at.$\cdot l^{-1}$ (Prochazkova et al., 1970) or 15.0 μg-at.$\cdot l^{-1}$ (Toetz, 1981).

In our experiments, when the prevention of nitrate uptake ceased, as a result of decrease of ammonium concentration, the rate of nitrate uptake was never

immediately maximum, but acted at a reduced rate until the ammonium concentration had reached a second threshold concentration. The full nitrate uptake which was then restored usually paralleled that of ammonium, thus indicating that the cell machinery was working at full rate with nitrate. The different values of this threshold are rather scattered (Table I); however the mean, viz. 6.9 μg-at.$\cdot l^{-1}$ NH_4-N ($s = 2.60$), is significantly higher than 1 μg-at.$\cdot l^{-1}$, the limit hitherto considered to apply to all algae.

A reduced rate of nitrate uptake in the presence of ammonium has been observed before. Caperon and Myers (1972) have described a similar behaviour (see their Fig. 2) to that which we have presented here. Bates (1976), Conway (1977), McCarthy et al. (1977) and Garside (1981) have all observed depressed rates of nitrate uptake in the presence of ammonium concentrations higher than 1 μg-at.$\cdot l^{-1}$, by marine phytoplankters. However, all these contributions refer to a range of ammonium concentration which only slightly exceeds 3 μg-at.$\cdot l^{-1}$.

Thus, the main feature which results from our data is that the processes involving the respective uptake of ammonium and nitrate occur at approximately an order of magnitude higher levels with oyster-pond micro algae than with other phytoplankton. Yet, the reduced rate and the subsequent existence of two thresholds are not explained by any clearly known biochemical mechanism; they therefore call for further research.

REFERENCES

Bates, S.S., 1976. Effects of light and ammonium on nitrate uptake by two species of estuarine phytoplankton. Limnol. Oceanogr. 21, 212–218.

Brandt, K., 1899. Über den Stoffwechsel im Meere. Wiss. Meeresunt. Abt. Kiel, Neue Folge 4, 213–230.

Caperon, J. and Meyer, J., 1972. Nitrogen-limited growth of marine phytoplankton. II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep-Sea Res. 19, 619–632.

Chick, H., 1903. A study of a unicellular green alga, occurring in polluted water, with special reference to its nitrogenous metabolism. Proc. R. Soc. London 71, 458–476.

Conover, S.A.M., 1975. Nitrogen utilization during spring blooms of marine phytoplankton in Bedford Basin, Nova Scotia, Canada. Mar. Biol. 32, 247–261.

Conway, H.L., 1977. Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Mar. Biol. 39, 221–232.

Dugdale, R.C. and Goering, J.J., 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12, 196–206.

Eppley, R.W., Coatsworth, J.L. and Solórzano, L., 1969. Studies on nitrate reductase in marine phytoplankton. Limnol. Oceanogr. 14, 194–205.

Fogg, G.E., 1953. The metabolism of algae. Wiley and Sons Publishers, New York, 149 pp.

Garside, C., 1981. Nitrate and ammonia uptake in the apex of the New York Bight. Limnol. Oceanogr. 26, 731–739.

Grant, B.R., Madgwick, J. and Dal Pont, G., 1967. Growth of *Cylindrotheca closterium* var. *californica* (Mereschk.) Reimamm and Lewin on nitrate, ammonia, and urea. Aust. J. Mar. Freshwater Res. 18, 129–136.

Hattori, A., 1962a. Light-induced reduction of nitrate, nitrite and hydroxy-lamine in a blue-green alga, *Anabaena cylindrica*. Plant Cell Physiol. (Tokyo) 3, 355–369.

Hattori, A., 1962b. Adaptative formation of nitrate reducing system in *Anabaena cylindrica*. Plant Cell Physiol. (Tokyo) 3, 371–377.

Koroleff, F., 1970. Direct determination of ammonia in natural waters as indophenol blue (revised). Cons. intern. Explor. Mer, Information on techniques and methods for sea water analysis, Interlab. Rep. 3, 19–22.

Krüger, W., 1894. Beiträge zur Kenntniss der Organismen des Saftlusses (sog. Schleimflusses) der Laubbäume. Beiträge Physiol. Morphol. nied. Organismen, Leipzig 4, 69–116.

McCarthy, J.J., Taylor, W.R. and Taft, J.L., 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preference. Limnol. Oceanogr. 22, 996–1011.

Morris, I., 1974. Nitrogen assimilation and protein synthesis. In: Algal Physiology and Biochemistry, pp. 583–609. Editor: W.D.P. Stewart. Bot. monographs, Vol. 10, Blackwell Scientific Publications, Oxford, 989 pp.

Morris, I. and Syrett, P.J., 1963. The development of nitrate reductase in *Chlorella* and its repression by ammonium. Archiv. für Mikrobiol. 47, 32–41.

Myers, J., 1951. Physiology of the algae. Ann. Rev. Microbiol. 5, 157–180.

Packard, T.T., 1979. Half-saturation constants for nitrate reductase and nitrate translocation in marine phytoplankton. Deep-Sea Res. 26, 321–326.

Pringsheim, E.G., 1949. Pure cultures of algae. Cambridge.

Procházková, L., Blažka, P. and Králová, M., 1970. Chemical changes involving nitrogen metabolism in water and particulate matter during primary production experiments. Limnol. Oceanogr. 15, 797–807.

Proctor, V.W., 1957. Preferential assimilation of nitrate ion by *Haematococcus pluvialis*. Am. J. Bot. 44, 141–143.

Robert, J.-M., 1975. Le verdissement des huîtres dans les claires de la baie de Bourgneuf. Bull. Soc. Sci. Natl. Ouest, France 73, 123–129.

SCOR-UNESCO, 1966. Determination of photosynthetic pigments in sea water. Monographs Oceanogr. Meth. UNESCO, Paris, 1, 69 pp.

Strickland, J.D.H. and Parsons, T.R., 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Canada 167, 2nd ed., 310 pp.

Syrett, P.J., 1962. Nitrogen assimilation. In: Physiology and Biochemistry of Algae, pp. 171–188. Editor: R.A. Lewin. Academic Press, New York, 929 pp.

Syrett, P.J., 1981. Nitrogen metabolism in microalgae. Can. Bull. Fish. Aquat. Sci., in press.

Syrett, P.J. and Hipkin, C.R., 1973. The appearance of nitrate reductase activity in nitrogen-starved cells of *Ankistrodesmus braunii*. Planta (Berlin) 111, 57–64.

Syrett, P.J. and Morris, I., 1963. The inhibition of nitrate assimilation by ammonium in *Chlorella*. Biochim. Biophys. Acta 67, 566–575.

Toetz, D.W., 1981. Effects of pH, phosphate and ammonia on the rate of uptake of nitrate and ammonia by freshwater phytoplankton. Hydrobiologia 76, 23–26.

ZoBell, C.E., 1935. The assimilation of ammonium nitrogen by *Nitzschia closterium* and other marine phytoplankton. Proc. Natl. Acad. Sci., Washington 21, 517–522.