# Amino acid uptake by marine phytoplankters[1,2]

*Patricia A. Wheeler, Barbara B. North, and Grover C. Stephens*
Department of Developmental and Cell Biology, University of California, Irvine 92664

*Abstract*

Axenic cultures of 25 species of unicellular marine algae were tested for their ability to utilize nine common amino acids, supplied at high concentrations in batch culture, as a nitrogen source; most species were able to use several amino acids, although growth was often slower than on nitrate nitrogen. The algae were also tested for their ability to take up $^{14}$C-labeled amino acids supplied at low, natural concentrations. In most cases, species that could grow on an amino acid at high concentration in culture could also take up amino acids at low concentrations. Uptake rates were higher in cells that had been deprived of nitrogen during growth. In some cases, uptake rates at low concentrations—if sustained—would be sufficient to support moderate growth rates. The ability to accumulate amino acids from dilute solution occurs in many phytoplankters, particularly in species that normally occur in inshore and littoral habitats.

The growth of phytoplankton in the sea often appears to be controlled by the availability of nitrogen (Dugdale and Goering 1967). Ambient concentrations, uptake rates, assimilation and metabolism of the inorganic forms of nitrogen—nitrate and ammonia—have been studied to explore the relation between nitrogen nutrition and phytoplankton ecology, but the role of organic nitrogen has received much less attention. We wish to focus attention on the potential significance of amino acids as a nitrogen source for phytoplankters under natural conditions.

Concentrations of dissolved free amino acids are usually in the range of 0.2 to 2.0 $\mu$g-atoms N liter$^{-1}$ for total amino acids (Bohling 1970; Clark et al. 1972; Riley and Segar 1970). A number of phytoplankters have been grown axenically with various organic sources of nitrogen, including amino acids (Guillard 1963). However, in such growth experiments nitrogenous compounds are usually 1,000 times more concentrated than in natural waters. The ability of phytoplankton to use amino acids as nitrogen sources at high concentrations does not necessarily provide any insight into their potential significance at low concentrations.

To study growth directly in laboratory culture at natural concentrations it would be necessary to maintain axenic conditions in a large volume, to supply nutrients continuously to mimic a steady state, and to devise methods of assessing slow growth in a sparse suspension of cells. We have chosen to approach the issue by studying the short-term rate of amino acid uptake by phytoplankters from dilute solution in conjunction with growth studies at higher concentrations. Analysis of the kinetics of uptake allows estimation of entry rates under natural conditions, while growth experiments establish the substrate as a metabolically adequate nutrient.

We have demonstrated uptake of amino acids from low concentrations and their oxidation and assimilation into synthetic pathways by the unicellular algae *Platymonas subcordiformis* and *Nitzschia ovalis* (e.g. North and Stephens 1967, 1969, and later). Amino acid transport systems in phytoplankters are extremely labile. Restriction of nitrogen availability in the culture decreases the nitrogen content of the cells and slows their growth rate; it also greatly accelerates the rate of amino acid uptake. In some cases, no uptake is observed until nitrogen in the growth medium is restricted. The possible contribution of amino acids to the nitrogen required for growth appears to depend on the nutritional history of the cells. Amino acids supplied at low levels

[1] This work was supported by NSF Grant GA 33904X.

[2] N. Kaji and V. Cooke provided technical assistance and help in manuscript preparation. D. Staley assisted in the uptake measurements.

*Table 1. Experimental organisms.*

| | Obtained from* | Code or clone | Isolator | Location | Culture medium† |
|---|---|---|---|---|---|
| | | | Chlorophyta | | |
| Chlamydomonas sp. | FCRG | Chlamy. | R.W. Eppley | Estuary near San Luis Obispo, Calif. | GPM |
| Chlorella sp. | ICC | 636 | Scott | --- | PAHNVSi |
| Dunaliella tertiolecta | Strickland | Dun-1 | I.J. Pintner | --- | PAHNVSi |
| Platymonas sp. | Lowenstam | A | --- | --- | PAHNVSi |
| P. subcordiformis | ICC | 171 | R.A. Lewin | --- | PAHNVSi |
| | | | Chrysophyta | | |
| Chaetoceros affinis | FCRG | 44-a | J. Jordan | Off sewage outfall Pt. Loma, Calif. | SD |
| Coscinodiscus granii | FCRG | 19-3 | J. Jordan | 0.8-3.2 km W UCSD | SD |
| Cyclotella nana | FCRG | 3H | R.L. Guillard | Forge River, Moriches Bay, Long Island, N.Y. | GPM |
| Leptocylindrus danicus | FCRG | 43-F | J. Jordan | Off sewage outfall Pt. Loma, Calif. | SD |
| Melosira sp. | Guillard | Mel-3 | R.L. Guillard | --- | GPM |
| Navicula incerta | ICC | 1262 | R.A. Lewin (69-M) | On shore of Salton Sea (1956) | PAHNVSi |
| Navicula salinarum | ICC | 682 | R.A. Lewin (41-M) | Sea of Galilee (1954) | PAHNVSi |
| Nitzschia sp. | ICC | 684 | R.A. Lewin (35-M) | On surface of Chondrus Nova Scotia | PAHNVSi |
| N. frustulum | Lewin | Strain 205 M | R.A. Lewin | Muroran, Japan | PAHNVSi |
| N. ovalis | ICC | 13-M | R.A. Lewin | From tide pool Nova Scotia | PAHNVSi |
| Phaeodactylum tricornutum | ICC | 642 | Pringsheim | --- | PAHNVSi |
| P. tricornutum | ICC | 646 | M.R. Droop | --- | PAHNVSi |
| Skeletonema costatum | FCRG | Skele | Haskins Lab | --- | SD |
| Stephanopyxis turris | FCRG | S. tur. | L. Provasoli | --- | SD |
| Thalassiosira fluviatilis | Guillard | Actin-G | R.L. Guillard | Long Island Sound (1956) | PAHNVSi |
| Coccolithus huxleyi | FCRG | F-5 | E. Paasche | Oslo Fjord, Norway | GPM |
| Cricosphaera carteri | FCRG | Cr.cart. | I.J. Pintner | --- | GPM |
| Isochrysis galbana | FCRG | Iso | R.L. Guillard M. Parke | Rock pool | GPM |
| | | | Pyrrophyta | | |
| Amphidinium carterae | FCRG | A. cart. | L. Provasoli | --- | GPM |
| Cachonina niei | FCRG | 87-1 | W.F. Blankley | Salton Sea, Calif. | GPM |
| | | | Rhodophyta | | |
| Porphyridium sp. | ICC | 637 | R.A. Lewin | --- | PAHNVSi |

*FCRG - Food Chain Research Group, University of California, San Diego; ICC - Indiana Culture Collection.

†Culture media: GPM - Gonyaulax polyedra medium. Natural seawater medium for dinoflagellates, A. R. Loeblich and V. E. Smith 1968. With PII metals, Provasoli 1964. PAHNVSi - Artificial seawater medium (North and Stephens 1972). SD - Natural seawater medium for diatoms developed by the Food Chain Research Group, UCSD.

(0.5 $\mu$g-atom N liter$^{-1}$) can make only a minor contribution to cells that were grown in the presence of abundant nitrogen. However, the same concentration can supply ample nitrogen for cells that have been grown on nitrogen-restricted medium, because their nitrogen content is reduced and uptake rate increased. Since the latter conditions are closer to natural ones we conclude that amino acids may be a significant nitrogen source for these phytoplankters.

Both *P. subcordiformis* and *N. ovalis* are most abundant in eutrophic or benthic habitats; neither is an important component of the phytoplankton in oligotrophic waters. Here we report on the ability of 25 species of marine phytoplankters to take up and to grow on the nine amino acids most abundant in the sea. We did two kinds of experiment: axenic growth of each species in batch culture using amino acids at high concentrations as a N source and short-term uptake experiments using $^{14}$C-labeled amino acids to examine each species' ability to remove amino acids from dilute solution. Uptake rates for each species were examined before and after N deprivation.

## *Materials and methods*

Marine phytoplankters from 21 genera were included in the survey. All cultures were axenic. Algae were cultured on artificial seawater medium whenever possible, but about half the species required a natural seawater medium (Table 1). Nine amino acids were selected, eight because they are the most common ones found in the sea. These can be placed in two main groups according to their relative abundances. Glycine and serine are always the most abundant amino acids. Six others (alanine, aspartate, threonine, valine, glutamate, ornithine) constitute a second group, each about 1/10 to 1/3 as concentrated as glycine or serine. Other amino acids are often present, but in smaller amounts (Andrews and Williams 1971; Chau and Riley 1966; Degens et al. 1964; Webb and Wood 1967). Lysine is in this category and was included in the survey to provide information about transport of polybasic amino acids.

### Growth experiments

Each alga was inoculated into 13 culture flasks, with nitrogen sources as shown in Table 2. The mixture of amino acids approximated that normally found in the sea: five parts each of glycine and serine, two parts each of alanine, aspartate, threonine, valine, glutamate, and ornithine. A flask containing the amino acid mixture was incubated in the dark to check for heterotrophic utilization of the amino acid carbon. Nitrogen, when present, was always supplied at $2 \times 10^{-3}$ g-atom N liter$^{-1}$.

Air saturated with water vapor was bubbled through the culture flask through cotton plugs. Cell samples were withdrawn periodically with a sterile syringe through a sidearm closed with a serum stopper. Cultures were maintained on a 16-hr light–8-hr dark regime at 20°C, under "cool-white" fluorescent light. Growth in culture was followed by measuring the absorbance of the cell samples with a spectrophotometer; growth rates are presented as doubling times for absorbance.

After each experiment, samples from each flask were tested for contaminating bacteria and fungi by incubation in a glucose medium (Peterson and Torrey 1968) and a peptone medium ZoBell 1946).

### Uptake experiments

Cells used in uptake experiments were grown in batch culture with nitrate as a N source. Kinetics of amino acid uptake in *Platymonas* and *Nitzschia* respond to the total amount of N in the medium, but are independent of its chemical form (North and Stephens 1971, 1972). Uptake rates were measured at three times during growth on batch culture: during or at the end of exponential growth, 24–48 hr after transfer to N-free culture medium, and 72–120 hr after transfer.

For each measurement cells were harvested, washed, and resuspended in Millipore-filtered artificial seawater (Cavanaugh 1956). Uniformly labeled $^{14}$C amino acids of the L-configuration (except ornithine which was DL) were then added to give about 1 $\mu$Ci per 25 ml of cell suspension,

*Table 2. Growth of marine phytoplankters in batch culture. Nitrogen was supplied as nitrate, individual amino acids, an amino acid mixture (in light and in dark), or was withheld from the culture medium. Growth rates are presented as generation time (days) estimated from optical density measurements.*

| N. source | $NO_3$ | Ala | Asp | Glu | Gly | Lys | Orn | Ser | Thr | Val | Mix | None | Mix Dark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chlamydomonas | 3.4 | 3.4* | † | ǂ | ǂ | ǂ | † | ǂ | † | † | 2.1* | ǂ | § |
| Chlorella | 1.5 | ǂ | ǂ | ǂ | ǂ | ǂ | 5.5 | ǂ | 11.0 | ǂ | 23.5 | ǂ | ǂ |
| Dunaliella | 2.2 | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ |
| Platymonas sp. | 1.6 | 1.2 | 3.6 | 3.5 | 1.7 | ǂ | 6.9 | 1.5 | 27.5 | 23.5 | 1.5 | ǂ | ǂ |
| P. subcordiformis | 2.2 | 2.2 | 2.5 | 2.6 | 1.4 | ǂ | 9.1 | 1.7 | 3.2* | 6.5* | 3.1 | ǂ | ǂ |
| Chaetoceros | 2.5 | 3.6* | ǂ | ǂ | 8.2* | ‖ | 3.8* | ǂ | ‖ | § | 4.2* | ǂ | § |
| Cyclotella | 2.1 | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ |
| Melosira | ‖ | ‖ | ǂ | ǂ | ‖ | ǂ | ǂ | ‖ | ‖ | ǂ | ǂ | ǂ | ǂ |
| Navicula incerta | 3.8 | 4.0 | 3.0 | 44.0 | 9.4 | 7.6 | 13.8 | 4.7 | 3.8 | 4.0 | 4.0 | ǂ | ǂ |
| N. salinarum | 11.3 | 7.5 | ǂ | ǂ | ǂ | ǂ | ** | ǂ | ǂ | ǂ | 8.0 | ǂ | ǂ |
| Nitzschia sp. | 1.8 | 4.2 | 3.0 | 1.5 | 2.0 | 2.9 | 2.7 | 2.0 | 3.0 | 3.7 | 3.0 | ǂ | ǂ |
| N. frustulum | 3.4 | 4.3 | 5.8 | 6.5 | 7.3 | 4.7 | 5.2 | 6.5 | 6.0 | 5.7 | 6.5 | ǂ | ǂ |
| N. ovalis | 3.5 | 3.3 | 1.7 | 3.8 | 5.4 | 4.4 | 4.0 | 4.8 | 9.5 | 4.4 | 2.0 | ǂ | ǂ |
| Phaeodactylum tricornutum (642) | 3.5 | 4.1* | 2.5 | 5.6 | 5.6 | 3.6 | 5.7 | 4.4 | ǂ | 1.7* | 5.9 | ǂ | ǂ |
| P. tricornutum (646) | 1.5 | 1.0 | 2.2 | 1.7 | 2.1 | 2.3 | 1.7 | 1.8 | ǂ | 3.0 | 2.0 | ǂ | ǂ |
| Skeletonema | 5.5 | ǂ | ǂ | ǂ | 6.0 | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ |
| Stephanopyxis | 1.7* | ǂ | ǂ | ǂ | † | ǂ | † | ǂ | ǂ | ǂ | ǂ | ǂ | § |
| Thalassiosira | 1.6 | ǂ | ǂ | ǂ | ǂ | ǂ | 2.5 | 5.3 | ǂ | ǂ | 4.1 | ǂ | ǂ |
| Coccolithus | 2.0 | ǂ | ǂ | ǂ | 3.5 | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ |
| Cricosphaera | 2.2 | 3.3* | ǂ | ǂ | ǂ | 3.0 | 2.6 | ǂ | ǂ | 3.5 | 2.4 | ǂ | § |
| Isochrysis | 1.5 | 3.7 | ǂ | ǂ | 3.6 | 5.1 | 5.0 | 6.6 | ǂ | 5.5 | 6.5 | ǂ | § |
| Amphidinium | 3.0 | 8.2 | ǂ | ǂ | ǂ | 4.5 | 3.3 | ǂ | ǂ | 9.2 | ǂ | ǂ | ǂ |
| Cachonina | 2.6* | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | ǂ | 8.0* | ǂ | ǂ |
| Porphyridium | 2.5 | ** | ** | † | † | † | ** | † | ** | ** | ** | § | ** |

*contaminated, good algal growth; †contaminated, possible algal growth; ǂno growth; §contaminated, no algal growth; ‖very slow algal growth; **not done.

corresponding to an initial concentration of about 1 $\mu$g-atom N liter$^{-1}$ as amino acid. Suspensions contained $7 \times 10^3$ large cells ml$^{-1}$ or to $2 \times 10^7$ small cells ml$^{-1}$. Samples were removed from the cell suspension every few minutes for 90 min, or until 30% of the initial radioactivity had been taken up, whichever came first. The cells were centrifuged out, and the supernatant acidified to remove any respired $^{14}C$-$CO_2$.

Uptake rates were computed from the decrease in counts per minute in the supernatant samples. A straight line was fitted to the data by an unweighted least squares analysis. The slope was taken as the influx rate.

Cultures were checked for contaminating microorganisms as above before each uptake incubation. Cell nitrogen was measured with a nitrogen analyzer.

## *Results*

### Growth experiments

About 75% of the phytoplankters tested could utilize amino acids as a nitrogen source for growth in batch culture, although growth was often somewhat slower than on an equal concentration of nitrate nitrogen. Among the Chlorophyta, two strains of *Platymonas* grew well on all amino acids except lysine and *Chlorella* could utilize several amino acids.

In the Chrysophyta *Phaeodactylum*, *Nitzschia*, and *Navicula* utilized amino acids very well while *Stephanopyxis* and *Cyclotella* were completely unable to use any amino acid. (Results for *Melosira* and a second species of *Navicula* are extremely questionable because growth on all nitrogen sources, including nitrate, was slow.) *Cricosphaera*, *Isochrysis*, and the dinoflagellate *Amphidinium* utilized several amino acids for growth.

Species that could use individual amino acids could also grow on the amino acid mixture, usually at comparable rates. Apparently, amino acids supplied together or separately were utilized equally well; there were no obvious synergistic effects. In all, 9 of the 25 species tested could use at least half the amino acids as a nitrogen source for growth (*see Table 2*).

None of the algae was able to use amino acids for heterotrophic growth in the dark. Hellebust (1970) found this also and suggested that amino acids cannot enter metabolic pathways leading to gluconeogenesis.

### Uptake experiments

Short-term uptake rates are presented as $V$ (nitrogen taken up hr$^{-1}$ cell N$^{-1}$). The units reduce to hr$^{-1}$; $V$ is simply a growth constant in terms of cell nitrogen.

This rate expression, $V$, is a function of changes in both uptake rate and cell nitrogen. Its use bypasses difficulties associated with expressing uptake in terms of cell number. Many species grow in clumps, sheets, or mats, making routine cell counts difficult and inaccurate. Even so, some of our cell counts are reliable and show that changes in $V$ result from stimulation of uptake rate by N deprivation, as well as from decrease in cell N; in no case did N/cell decrease by more than 50% after transfer to nitrogen-free medium. Rates for different amino acids were calculated for an external amino acid concentration of 1 $\mu$g-atom N liter$^{-1}$.

The ability of species to accumulate amino acids from low concentrations shows a general correlation with their ability to grow on high concentrations in culture. *Platymonas*, several species of *Nitzschia*, *Phaeodactylum*, *Melosira*, and *Navicula* showed rapid uptake of nearly all the amino acids, and rates increased substantially after the cells were transferred to nitrogen-free growth medium. The remaining phytoplankters had restricted uptake capacities under all conditions (Table 3).

In spite of our attempts to maintain asepsis, several cultures became contaminated by fungi, bacteria, or both during transfer to nitrogen-free medium. Uptake measurements were however in no way correlated with the presence of contaminating microorganisms. North and Stephens (1967) established that contaminating microorganisms had no measurable effect on short-term uptake by *Platymonas*.

*Table 3. Amino acid uptake rates in marine phytoplankters subjected to nitrogen starvation for different periods. Uptake rates were determined for $^{14}$C-labeled amino acids at 1 μg-atom N liter$^{-1}$.*

| | Period of N deprivation (hr)* | | Amino acid uptake rate† = $V \times 10^3$ $V$ = [(mass nitrogen taken up)(hr)$^{-1}$(mass cell nitrogen)$^{-1}$] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | Ala | Asp | Glu | Gly | Lys | Orn | Ser | Thr | Val |
| Chlamydomonas | 0 | | 0.1 | 0 | 0.2 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| Chlorella | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 |
| | 48 | 24 | 0 | 0 | 0 | 0 | 7.0 | 0 | 0 | 0 | 0 |
| | 96 | 96 | 0 | 0 | 0 | 0 | 21 | 0.1 | 0 | 0 | 0 |
| Dunaliella | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.3 | 0 |
| Platymonas sp. | 0 | 0 | 1.1 | 0 | 0.1 | 0.3 | 2.3 | 0.6 | 0.2 | 0.1 | 0.4 |
| | 48 | 24 | 5.5 | 0 | 0.1 | 3.1 | 5.8 | 1.4 | 1.2 | 0.6 | 1.3 |
| | 96 | 72 | 19 | 0 | 0.2 | 17 | 20 | 15 | 7.3 | 3.9 | 8.7 |
| P. subcordiformis | 0 | 0 | 2.4 | 0 | 0 | 0.3 | 0.7 | 0.5 | 0.2 | 0.4 | 1.1 |
| | 48 | 48 | 9.0 | 0 | 0.1 | 4.6 | 5.9 | 1.8 | 1.9 | 1.9 | 4.4 |
| | 96 | 120 | 16 | 0 | 0.2 | 12 | 20 | 12 | 5.8 | 4.0 | 11 |
| Chaetoceros | 0 | | 0.8 | 0 | 0.1 | 0.3 | 0 | 0.1 | 0.3 | 14 | 2.0 |
| | 48 | | 2.9 | 0.1 | 0 | 0.5 | 0 | 0 | 0.3 | 0.4 | 4.4 |
| | 96 | | 1.7 | 0 | 0 | 0.3 | 0 | 0 | 0.3 | 0.2 | 2.4 |
| Coscinodiscus | 0 | | 0.1 | 0 | 0 | 0 | 1.8 | 0.1 | 0 | 0 | 0 |
| | 48 | | 0 | 0.1 | 0.1 | 0 | 0.2 | 0.3 | 0 | 0 | 0.1 |
| | 96 | | 1.0 | 0.4 | 0.5 | 0.4 | 3.1 | 1.4 | 0.6 | 0.1 | 0.6 |
| Cyclotella | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48 | | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptocylindrus | 0 | | 0 | 0.1 | 0 | 0.1 | 0 | 0.4 | 2.2 | 0 | 0 |
| | 48 | | 0.2 | 0 | 0.4 | 0 | 1.9 | 0 | 2.4 | 0 | 0 |
| | 96 | | 0 | 0.1 | 0 | 0.1 | 0.1 | 0.3 | 0 | 0.3 | 0.2 |
| Melosira | 0 | 0 | 11 | 3.3 | 0.4 | 2.2 | 5.0 | 1.6 | 1.0 | 3.1 | 17 |
| | 48 | 48 | 12 | 0 | 1.9 | 4.0 | 34 | 3.7 | 10 | 4.4 | 16 |
| | 96 | 120 | 33 | 7.7 | 5.2 | 10 | 39 | 9.2 | 13 | 0.8 | 31 |
| Navicula incerta | 0 | 0 | 0.6 | 1.3 | 1.5 | 0.1 | 9.9 | 0.1 | 0.1 | 0.1 | 0.4 |
| | 24 | 24 | 1.0 | 9.0 | 2.2 | 0.1 | 5.8 | 0 | 0.1 | 0.4 | 2.4 |
| | 96 | 120 | 0.2 | 8.7 | 4.6 | 0.4 | 23 | 0 | 1.3 | 0.2 | 0.6 |
| N. salinarum | 0 | | 0.1 | 0.4 | 0.2 | 0 | 2.5 | 0.2 | 0.1 | 0 | 0.2 |
| | 48 | | 1.1 | 1.4 | 1.1 | 0.5 | 5.1 | 0.6 | 0.9 | 2.8 | 1.1 |
| | 96 | | 1.3 | 1.4 | 0.8 | 0.3 | 7.7 | 0.5 | 0.9 | 0.6 | 0.6 |
| Nitzschia sp. | 0 | | 0.1 | 3.4 | 0.8 | 0 | 0 | - | 0 | 0 | 0 |
| | 48 | | 0.8 | 9.3 | 1.8 | 0.1 | 0.1 | - | 0.1 | 0.7 | 0.3 |
| | 96 | | 0.9 | 6.6 | 1.8 | 0.2 | 0.9 | - | 0.3 | 0.5 | 0.5 |
| N. frustulum | 0 | | 0 | 0.4 | 0.2 | 0 | 2.7 | 0.7 | 0.1 | 0 | 0 |
| | 48 | | 0 | 0.2 | 0.3 | 0 | 3.5 | 0.3 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 6.5 | 0.3 | 0 | 0 | 0 |
| N. ovalis | 0 | 0 | 0 | 0.2 | 0.1 | 0 | 0.2 | 0.1 | 0 | 0 | 0 |
| | 48 | 48 | 0.7 | 1.2 | 0.4 | 0.3 | 1.1 | 0.2 | 0.3 | 0.4 | 0.2 |
| | 120 | 96 | 5.0 | 2.4 | 0.6 | 0.5 | 1.7 | 0.2 | 2.6 | 2.0 | 1.3 |
| Phaeodactylum tricornutum (642) | 0 | 0 | 0.1 | 0.1 | 0.1 | 0 | 0.5 | 0.2 | 0 | 0 | 0 |
| | 48 | 24 | 0.4 | 10 | 0 | 0 | 0.3 | 0 | 0 | 0 | 0.3 |
| | 96 | 72 | 1.0 | 29 | 0.5 | 0.4 | 0.5 | 0 | 0.5 | 0 | 0.6 |
| P. tricornutum (646) | 0 | 0 | 5.1 | 0 | 0.1 | 1.7 | 4.9 | 0.2 | 0.3 | 0 | 0.7 |
| | 48 | 48 | 11 | 0 | 0 | 1.3 | 18 | 0.3 | 0.3 | 0 | 0.8 |
| | 96 | 120 | 20 | 0 | 0.1 | 2.7 | 20 | 0.5 | 0.5 | 0 | 1.7 |
| Skeletonema | 0 | | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 |
| | 48 | | 0.3 | 0 | 0 | 0 | 2.2 | 0.1 | 1.0 | 0 | 0.2 |
| | 96 | | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.3 |
| Stephanopyxis | 0 | | 0.3 | 0 | 0 | 1.1 | 0 | 0 | 0.1 | 0.2 | 1.9 |
| | 48 | | 0.8 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 1.2 |
| | 96 | | 0.1 | 0.1 | 0 | 0 | 0 | 0.1 | 0.2 | 0 | 0.3 |

*Table 3. (Continued.)*

| | Period of N deprivation (hr)* | | Amino acid uptake rate† = $\gamma \times 10^3$ $V = [(\text{mass nitrogen taken up})(\text{hr})^{-1}(\text{mass cell nitrogen})^{-1}]$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | Ala | Asp | Glu | Gly | Lys | Orn | Ser | Thr | Val |
| Thalassiosira | 0 | 0 | 0 | 0 | 0.1 | 0 | 11 | 0.2 | 0 | 0.1 | 0.1 |
| | 48 | 48 | 0 | 0 | 0 | 0 | 3.1 | 0.6 | 0 | 0 | 0 |
| | 96 | 120 | 0 | 0 | 0 | 0 | 7.6 | 1.5 | 0 | 0 | 0 |
| Coccolithus | 0 | | 0 | 0 | 0 | 0 | 0 | 0.6 | 0 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| Cricosphaera | 0 | | 0 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0.2 | 0.1 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0 |
| Isochrysis | 0 | | 0 | 0 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0 |
| | 48 | | ' | 0.3 | 0 | 0 | 0.3 | 0.3 | 0 | 0 | 0 |
| | 96 | | 0.4 | 0.8 | 0.1 | 0 | 0.2 | 0.2 | 0 | 0 | 0 |
| Amphidinium | 0 | | 0 | 0 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 |
| | 96 | | 0 | 0.1 | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0 |
| Cachonina | 0 | | 0 | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 0 | 0 |
| | 48 | | 0 | 0 | 0 | 0 | 0 | 0.3 | 0 | 0.1 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 |
| Porphyridium | 0 | | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 0.2 | 0 | 0.1 |
| | 48 | | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 96 | | 0 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0 | 0 |

*Where two sets of deprivation periods are given for one species, two experiments were done. Deprivation periods under column *a* refer to those used for uptake measurements of ala, asp, val, thr, and orn. Deprivation periods under column *b* refer to these used for uptake measurements of gly, lys, glu, and ser.

†Rates were calculated for 1 µg-atom N/liter by assuming that rates were proportional to external concentration in the range tested.

*Discussion*

Our results show that many phytoplankters are able to take up amino acids from low concentrations. Lysine is taken up most readily. Patterns of uptake for the other amino acids are extremely variable, both with respect to rates and the type of amino acid that is taken up. The absence of clearer patterns is not surprising, because amino acid uptake systems are very labile. However, uptake rates of several species are clearly stimulated by nitrogen deprivation during growth (Table 3: *Platymonas*, *Melosira*, *Phaeodactylum*, etc.).

Growth on high concentrations of amino acids (Table 2) shows a general correlation with uptake rates at low concentrations (Table 3), but there are several exceptions. Three species of Chlorophyta and the diatom *Thalassiosira* failed to grow on lysine even though they were able to take it up rapidly. This is consistent with the results of Hellebust (1970) who found that lysine strongly inhibited growth of cultures of *Melosira nummuloides*. *Chaetoceros* was the only other alga we found unable to grow on amino acids that it had taken up—threonine and valine. The growth experiments show that, with the exception of lysine, any common amino acid that can be taken up by phytoplankters can also serve as a nitrogen source for growth.

The question, therefore, is whether uptake rates from low concentrations are sufficiently rapid to support a reasonable rate of growth. If amino acid nitrogen is present at 1 $\mu$g-atom N liter$^{-1}$, 14 of the phytoplankters we tested accumulated one or more amino acids rapidly enough to support a cell doubling at least every 10 days (Table

*Table 4. Potential contribution of amino acids to the nutrition of marine phytoplankters. Generation times were calculated on the basis of nitrogen required for cell doubling, assuming that all the nitrogen is supplied by ambient amino acids at 1 μg-atom N liter$^{-1}$. Amino acid nitrogen uptake rates were determined for cells subjected to various periods of nitrogen deprivation. Dashes represent generation times > 10 days.*

| | Period of N deprivation (hr)* | | Calculated generation time = $G_e$† (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | Ala | Asp | Glu | Gly | Lys | Orn | Ser | Thr | Val |
| Chlorella | 0 | | – | – | – | – | 2.3 | – | – | – | – |
| | 24 | | – | – | – | – | 4.2 | – | – | – | – |
| | 96 | | – | – | – | – | 1.3 | – | – | – | – |
| Platymonas sp. | 0 | 0 | – | – | – | – | – | – | – | – | – |
| | 48 | 24 | 5.2 | – | – | 9.2 | 4.9 | – | – | – | – |
| | 96 | 72 | 1.5 | – | – | 1.7 | 1.5 | 3.4 | 4.2 | 7.4 | 3.3 |
| P. subcordiformis | 0 | | – | – | – | – | – | – | – | – | – |
| | 48 | | 3.1 | – | – | 6.2 | 4.9 | – | – | – | 6.5 |
| | 120 | | 1.8 | – | – | 2.3 | 1.4 | 2.4 | 5.0 | 7.2 | 2.7 |
| Chaetoceros | 0 | | – | – | – | – | – | – | – | 2.1 | – |
| | 48 | | 10 | – | – | – | – | – | – | – | 6.5 |
| | 96 | | – | – | – | – | – | – | – | – | – |
| Coscinodiscus | 0 | | – | – | – | – | – | – | – | – | – |
| | 48 | | – | – | – | – | – | – | – | – | – |
| | 96 | | – | – | – | – | 9.3 | – | – | – | – |
| Melosira | 0 | 0 | 2.5 | 8.7 | – | – | 5.7 | – | – | 9.3 | 1.7 |
| | 48 | 48 | 2.8 | – | – | 7.2 | 0.8 | 7.8 | 2.9 | 6.5 | 1.3 |
| | 96 | 120 | 0.9 | 3.7 | 5.5 | 2.9 | 0.7 | 3.1 | 2.3 | – | 0.9 |
| Navicula incerta | 0 | 0 | – | – | – | – | 2.9 | – | – | – | – |
| | 24 | 24 | – | 3.2 | – | – | 5.0 | – | – | – | – |
| | 96 | 120 | – | 3.3 | 6.1 | – | 1.2 | – | – | – | – |
| N. salinarum | 0 | | – | – | – | – | – | – | – | – | – |
| | 48 | | – | – | – | – | 5.6 | – | – | 10 | – |
| | 96 | | – | – | – | – | 3.7 | – | – | – | – |
| Nitzschia sp. | 0 | | – | 8.5 | – | – | – | – | – | – | – |
| | 48 | | – | 3.1 | – | – | – | – | – | – | – |
| | 96 | | – | 4.4 | – | – | – | – | – | – | – |
| N. frustulum | 0 | | – | – | – | – | – | – | – | – | – |
| | 48 | | – | – | – | – | 8.2 | – | – | – | – |
| | 96 | | – | – | – | – | 4.4 | – | – | – | – |
| N. ovalis | 0 | 0 | – | – | – | – | – | – | – | – | – |
| | 48 | 48 | – | – | – | – | 2.6 | – | – | – | – |
| | 120 | 96 | 5.7 | – | – | 5.6 | 1.7 | – | – | – | – |
| Phaeodactylum | 0 | 0 | – | – | – | – | 5.8 | – | – | – | – |
| tricornutum (642) | 48 | 24 | – | 2.8 | – | – | – | – | – | – | – |
| | 96 | 72 | – | 1.0 | – | – | 6.2 | – | – | – | – |
| P. tricornutum (646) | 0 | 0 | 5.6 | – | – | – | 5.9 | – | – | – | – |
| | 48 | 48 | 2.6 | – | – | – | 1.6 | – | – | – | – |
| | 96 | 120 | 1.4 | – | – | – | 1.4 | – | – | – | – |
| Thalassiosira | 0 | | – | – | – | – | 2.7 | – | – | – | – |
| | 48 | | – | – | – | – | 9.3 | – | – | – | – |
| | 120 | | – | – | – | – | 3.8 | – | – | – | – |

*Same as Table 3.

†$G_e = \frac{\ln 2}{V \times 24}$ values for $V$ are given in Table 3.

4), probably sufficient to contribute to the growth of natural populations; it is estimated that populations in the sea double every 4 to 5 days on the average (Eppley and Strickland 1968). Species with rapid amino acid uptake rates tend to occur in nearshore areas or in tide pools where amino acid concentrations may be higher than farther offshore (Clark et al. 1972).

Thus, amino acids may have some nu-

tritional significance for some natural populations. Our extrapolation of laboratory results to the field is speculative, however, and subject to several sources of error. One difficulty is that $^{14}C$ is not necessarily an accurate tracer for uptake and utilization of the nitrogen from the amino acid. The amino acid molecules accumulated by phytoplankton cells may be metabolized immediately, and carbon dioxide or organic compounds derived from it may be quickly excreted back into the medium. In our experiments, rapid uptake of amino acids was often accompanied by metabolic production of carbon dioxide; in some cases, 10% or more of the total $^{14}C$ supplied to the cells was converted to a volatile form—presumably carbon dioxide—that could be driven out of the medium by acidification. We have reported this in some detail (Stephens and North 1971); both *Platymonas* and *Nitzschia* take up glycine, alanine, and arginine, metabolize them so that the amino nitrogen is retained, and then return a considerable fraction of the $^{14}C$ in the carbon skeleton to the medium as carbon dioxide and other, nonvolatile carbon compounds (Stephens and North 1971).

Schell (1971, 1974) obtained a similar result when he measured assimilation of $^{15}N$- and $^{14}C$-labeled amino acids by natural populations of microorganisms in marine waters. He reported that $^{15}N$ is assimilated more rapidly than $^{14}C$ when glycine is supplied to the population. Alternatively, he found that more $^{14}C$ than $^{15}N$ is assimilated when glutamic acid is supplied. Since he used incubation times of several hours, and did not distinguish between the metabolic activities of different microorganisms, it is difficult to evaluate the role of the phytoplankton in the total assimilation pattern. In similar experiments, Williams (1970) has shown that most of the $^{14}C$ from amino acids becomes associated with microorganisms in the bacterial size range after several hours, rather than with larger phytoplankton cells. One can speculate that, in mixed populations, some of the carbon excreted by phytoplankton becomes assimilated by other microorganisms.

Extrapolating from our earlier results (Stephens and North 1971), it seems probable that the $^{14}C$ uptake measurements we report here underestimate the amount of nitrogen taken up because of excretion of carbon as carbon dioxide and organic molecules.

Another experimental difficulty was that many of the cultures tested in this survey may have been unhealthy. Many of the species could not be grown in a completely defined, artificial growth medium (*see Table 1*), yet the cells had to be transferred to artificial seawater for uptake measurements to ensure that no extraneous amino acids were present.

A more general problem involves using nitrogen deficient cells for uptake measurements, since several uptake systems in algae are stimulated when cultures are deprived of nitrogen, including those for nitrate, ammonia, and possibly urea (Eppley et al. 1969; Fitzgerald 1968; McCarthy 1972*a*). Nitrogen deficient laboratory cultures also show a decrease in cell nitrogen, in assimilation ratio ($CO_2$ assimilated g Chl $a^{-1}$), and a rise in the C:N ratio of the cell (Thomas 1970; Strickland et al. 1969; Hobson and Pariser 1971). But if natural populations are not growing under conditions of nitrogen stress, then uptake rates based on nitrogen starved cultures would be overestimated. Thomas (1970), using assimilation ratios to estimate the degree of nitrogen stress, concluded that tropical Pacific populations in oligotrophic regions are only somewhat nitrogen deficient, but populations in eutrophic, upwelling regions seem well supplied with nitrogen. Another evaluation of the nutritional condition of phytoplankton in the ocean comes from measurements of C:N uptake ratios: natural populations are incubated with $^{14}C$-labeled $CO_2$, and $^{15}N$-labeled nitrogen as some combination of ammonia, nitrate, and urea. Goering et al. (1970) found low values for C:N uptake in nutrient deficient waters, possibly produced by high uptake rates associated with a nitrogen deficient population. Such evidence suggests that nitrogen deficiency does occur in natural populations, but its extent is uncertain.

Uptake measurements based on nitrogen deficient laboratory populations may be reasonable.

We chose to restrict the nitrogen supply in these experiments by simply transferring the cells to a nitrogen-free medium. However, such drastic restriction is not required to stimulate amino acid uptake. Amino acid uptake in *Platymonas* and *Nitzschia* is stimulated when cells are grown in media containing nitrogen well in excess of natural concentrations (North and Stephens 1971, 1972; W. North et al. 1972). Such media still support log phase growth in fixed volume culture, even though cell nitrogen is somewhat reduced.

There is some evidence that amino acid nitrogen is actually used by natural populations. Dugdale and Goering (1967) reported that $^{15}N$-labeled glycine was accumulated by a natural population. McCarthy (1972*b*) suggested that an unusually high C:N uptake value in waters near the Whites Point sewage outfall in Los Angeles may be due to a failure to consider the contribution of amino acid uptake, along with those of urea, nitrate, and ammonia.

There is now considerable evidence that organic nitrogen, in the form of urea, may be important in the sea. Urea concentrations in surface waters, ranging from 0.1 to 5.0 $\mu$g-atoms N liter$^{-1}$, have been reported for oceanic waters (Remsen 1971). Experiments from both field and laboratory suggest that urea uptake by phytoplankton may, in fact, account for a considerable fraction of the total nitrogen used by some natural populations (Carpenter et al. 1972*b*; McCarthy 1972*a*).

A study on urea utilization by Carpenter et al. (1972*a*) is particularly interesting because it provides a direct comparison of the uptake behavior of phytoplankton in the laboratory and in the field. Natural populations of *Skeletonema costatum* accumulated urea 30 times more rapidly than a laboratory culture of the same species. It seems possible that nutritional stress in the field population, as well as the genetic and temperature factors cited by the investigators, may have contributed to this difference in rate.

Obviously, the application of laboratory measurements of the utilization of organic nitrogen to natural populations is a complex problem. However, we can conclude from our study that many phytoplankters have transport systems that allow them to accumulate and assimilate amino acids commonly found in the sea. At least some of the uptake systems are sensitive to nitrogen stress. Uptake is fastest, or at least easiest to demonstrate, in genera like *Nitzschia*, *Platymonas*, *Phaeodactylum*, and *Navicula* that are often found in nearshore or littoral areas.

## *References*


ANDREWS, P., AND P. J. LEB. WILLIAMS. 1971. Heterotrophic utilization of dissolved organic compounds in the sea. 3. J. Mar. Biol. Assoc. U. K. **51**: 111–125.

BOHLING, H. 1970. Untersuchungen über freie gelöste Aminosäuren in Meerwasser. Mar. Biol. **6**: 213–225.

CARPENTER, E. J., C. C. REMSEN, AND B. W. SCHROEDER. 1972*a*. Comparison of laboratory and in situ measurements of urea decomposition by a marine diatom. J. Exp. Mar. Biol. Ecol. **8**: 259–264.

———, ———, AND S. W. WATSON. 1972*b*. Utilization of urea by some marine phytoplankters. Limnol. Oceanogr. **17**: 265–269.

CAVANAUGH, G. M. [ED.]. 1956. MBL Formula (4). *In* Formulae and methods IV of the Marine Biological Laboratory chemical room. Mar. Biol. Lab., Woods Hole, Mass.

CHAU, Y. K., AND J. P. RILEY. 1966. The determination of amino-acids in sea water. Deep-Sea Res. **13**: 1115–1124.

CLARK, M. E., G. A. JACKSON, AND W. J. NORTH. 1972. Dissolved free amino acids in southern California coastal waters. Limnol. Oceanogr. **17**: 749–758.

DEGENS, E. T., J. H. REUTER, AND K. N. F. SHAW. 1964. Biochemical compounds in offshore California sediments and sea waters. Geochim. Cosmochim. Acta **28**: 45–66.

DUGDALE, R. C., AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. **12**: 196–206.

EPPLEY, R. W., J. N. ROGERS, AND J. J. MCCARTHY. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. **14**: 912–920.

———, AND J. D. H. STRICKLAND. 1968. Kinetics of marine phytoplankton growth, p. 23–62. *In* M. R. Droop and E. J. F. Woods [eds.], Advances in microbiology of the sea, v. 1.

FITZGERALD, G. P. 1968. Detection of limiting or surplus nitrogen in algae and aquatic weeds. J. Phycol. **4**: 121–126.

GOERING, J. J., D. D. WALLEN, AND R. M. NAUMAN. 1970. Nitrogen uptake by phytoplankton in the discontinuity layer of the eastern subtropical Pacific Ocean. Limnol. Oceanogr. **15**: 789–796.

GUILLARD, R. R. L. 1963. Organic sources of nitrogen for marine centric diatoms, p. 93–104. *In* C. H. Oppenheimer [ed.], Marine microbiology. Thomas.

HELLEBUST, J. A. 1970. The uptake and utilization of organic substances by marine phytoplankters, p. 225–256. *In* D. W. Hood [ed.], Organic matter in natural waters. Inst. Mar. Sci. (Alaska) Occas. Publ. 1.

HOBSON, L. A., AND R. J. PARISER. 1971. The effect of inorganic nitrogen on macromolecular synthesis by *Thalassiosira fluviatilis* Hustedt and *Cyclotella nana* Hustedt grown in batch culture. J. Exp. Mar. Biol. Ecol. **6**: 71–78.

MCCARTHY, J. J. 1972a. The uptake of urea by marine phytoplankton. J. Phycol. **8**: 216–222.

———. 1972*b*. The uptake of urea by natural populations of marine phytoplankton. Limnol. Oceanogr. **17**: 738–748.

NORTH, B. B., AND G. C. STEPHENS. 1967. Uptake and assimilation of amino acids by *Platymonas*. Biol. Bull. **133**: 391–400.

———, AND ———. 1969. Dissolved amino acids and *Platymonas* nutrition, p. 263–273. *In* R. Margalef [ed.], Proc. 6th Int. Seaweed Symp., Madrid.

———, AND ———. 1971. Uptake and assimilation of amino acids by *Platymonas*. 2. Increased uptake in nitrogen-deficient cells. Biol. Bull. **140**: 242–254.

———, AND ———. 1972. Amino acid transport in *Nitzschia ovalis* Arnott. J. Phycol. **8**: 64–68.

NORTH, W. J., G. C. STEPHENS, AND B. B. NORTH. 1972. Marine algae and their relations to pollution problems, p. 330–340. *In* M. Ruivo [ed.], Marine pollution and sea life. FAO, Fish. News.

PETERSON, D. M., AND J. G. TORREY. 1968. Amino acid incorporation in developing *Fucus* embryos. Plant Physiol. **43**: 941–947.

REMSEN, C. C. 1971. The distribution of urea in coastal and oceanic waters. Limnol. Oceanogr. **16**: 732–740.

RILEY, J. P., AND D. A. SEGAR. 1970. The seasonal variation of the free and combined dissolved amino acids in the Irish Sea. J. Mar. Biol. Assoc. U. K. **50**: 713–720.

SCHELL, D. M. 1971. Uptake and regeneration of dissolved organic nitrogen in southeastern Alaskan marine waters. Ph.D. thesis, Univ. Alaska. 142 p.

———. 1974. Uptake and regeneration of free amino acids in marine waters of Southeast Alaska. Limnol. Oceanogr. **19**: 260–270.

STEPHENS, G. C., AND B. B. NORTH. 1971. Extrusion of carbon accompanying uptake of amino acids by marine phytoplankters. Limnol. Oceanogr. **16**: 752–757.

STRICKLAND, J. D. H., O. HOLM-HANSEN, R. W. EPPLEY, AND R. J. LINN. 1969. The use of a deep tank in plankton ecology. 1. Limnol. Oceanogr. **14**: 23–34.

THOMAS, W. H. 1970. On nitrogen deficiency in tropical Pacific oceanic phytoplankton: photosynthetic parameters in poor and rich water. Limnol. Oceanogr. **15**: 380–385.

WEBB, K. L., AND L. WOOD. 1967. Improved techniques for analysis of free amino acids in sea water. Automat. Anal. Chem., Technicon Symp., 1966, p. 440–444.

WILLIAMS, P. J. LEB. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. J. Mar. Biol. Assoc. U. K. **50**: 859–870.

ZOBELL, C. E. 1946. Marine microbiology. Chronica Botanica.

*Submitted: 26 July 1973*
*Accepted: 16 November 1973*