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Effect of nitrogen source and growth rate on phytoplankton-mediated changes in alkalinity[1]

Abstract—Continuous cultures of the marine chrysophyte *Dunaliella tertiolecta* were grown on four nitrogen sources: NO_3^-, NO_2^-, NH_4^+, and urea. Alkalinity changes were consistent with a simple stoichiometric model in which OH^- production is balanced by NO_3^- and NO_2^- uptake, H^+ production is balanced by NH_4^+ uptake and no change occurs when the uncharged species urea is assimilated. Neither the influent N concentration nor the growth rate had any effect on the 1:1 stoichiometry between N uptake and alkalinity change. These results preclude the possibility of excretion of an organic acid (e.g. glycolic acid) stronger than carbonic acid. However, excretion of a weak organic acid or a salt of a strong organic acid cannot be ruled out. In general, the results are consistent with the notion that excretion of glycolic acid by healthy marine phytoplankton cells is minimal.

In our earlier work with three phytoplankton species we observed alkalinity shifts that were consistent with a simple model of charge balance in which NO_3^- uptake is balanced by OH^- production and NH_4^+ uptake leads to H^+ generation (Brewer and Goldman 1976). These results demonstrated in effect that NO_3^- ion was taken up unaccompanied by a cation, and NH_4^+ ion unaccompanied by an anion. However, we were unable to demonstrate the exact stoichiometry of this relationship due to an apparent acid offset in the data; nor did we examine either the effect of uptake of other N species, or the validity of this relationship over a wide range of substrate concentrations and specific growth rates. We report here our measurements of alkalinity changes associated with growth of phytoplankton on NO_3^-, NO_2^-, NH_4^+, and urea as nitrogen sources, over a concentration range from 50 to 500 μg-atoms·liter^{-1} and a specific growth rate of from 0.1 to 1.1·d^{-1}.

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The marine chlorophyte *Dunaliella tertiolecta* (Dun) was grown in continuous culture with the four nitrogen sources. The experimental protocols were similar to those used previously (Brewer and Goldman 1976), except that in this study a bank of eight 0.5-liter continuous cultures was used (*see* Goldman and McCarthy 1978). Temperature and continuous light were kept at 19.2°C and 0.06 ly·min^{-1}. Bubbled air plus stirring served to prevent inorganic carbon limitation and kept the pH between ≈8.0 (medium) and <8.4 (culture). The cultures were nonaxenic, for the reasons cited by Goldman (1977).

Media were enrichments of 1-μm-filtered natural seawater (32‰ salinity) from Vineyard Sound, Cape Cod, Massachusetts. The enrichments included trace metals and vitamins in the amount used by Brewer and Goldman (1976), phosphate at 10 μg-atoms·liter^{-1} when the N source was 50, 100, 150, and 500 μg-atoms·liter^{-1}, and 30 when 450 μg-atoms N·liter^{-1} was used. Each of the four media was fed to two replicate cultures concurrently.

Twenty-three steady state dilution rates (=specific growth rates) for each N source were established in the range of 0.18–1.11·d^{-1}. Culture washout was estimated to be 1.34·d^{-1}. Measurements were made at each steady state of culture particulate nitrogen (PN), and of alkalinity (ALK), NO_3^-, NO_2^-, NH_4^+, urea, and PO_4^{3-} in both influent medium and the culture filtrates according to the techniques described by Brewer and Gold-

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Table 1. Effects of pretreatment of glass-fiber filters on alkalinity changes in seawater.

	ALK	Difference from control
	μeq	
Experiment 1		
Unfiltered medium (control)	2,087±8(3)*	—
Filtered medium filter unrinsed	2,122±2(2)	+35
filter rinsed-medium	2,099±3(2)	+12
filter rinsed-distilled water	2,055±1(2)	−32
Experiment 2		
Unfiltered medium (control)	2,107(1)	
Filtered medium filter rinsed-distilled water	2,055±1(2)	−38

* No. replicates.

man (1976). Care was taken to confine alkalinity titration points to the pH region above pH 3.2 to avoid protonating NO_2^- in the nitrite uptake experiments. Samples were taken directly from the cultures. Urea was measured according to the technique of Newell et al. (1967). Culture samples (100 ml) for alkalinity measurements were first filtered through 47-mm Gelman A-E glass-fiber filters that were precombusted at 500°C and prerinsed with 100 ml of distilled water. Medium samples were not filtered. All samples were preserved with a few drops of saturated mercuric chloride and stored at 10°C until analyzed, usually within 24 h. In one experiment, alkalinity was measured in filtered and unfiltered culture medium. Both unrinsed and prerinsed filters were compared. The rinsed filters were treated with either distilled water or medium. In addition, titrations were made on seawater with and without reagent-grade glycolic acid added at concentrations of 1.64 mM and 0.164 mM.

The consistent acid offset that we observed earlier (Brewer and Goldman 1976) was again found in these experiments. We later determined that the source of this offset was associated with ion-exchange processes on the glass-fiber filters used to remove algal cells from the culture medium before titrations. For filters that were prerinsed with 100 ml of distilled water, there was a 35-μeq decrease in alkalinity, whereas with no prerinse, there was a 35-μeq increase in alkalinity (Table 1). These effects could be minimized by rinsing the filters with culture medium before filtration of the sample (Table 1). Hence, a correction of 35 μeq was applied to the alkalinity data collected by using prerinsed filters.

Clearly, the filters act as ion exchangers. The results in Table 1 are consistent with the hypothesis that silanol groups are formed by hydration of the oxide surface of the glass-fiber filters during the distilled water prerinse, leading to an alkalinity increase in the rinse water. The subsequent filtration of culture medium is accompanied by exchange with Mg^{2+} ions (Table 2), resulting in release of H^+ and an acidic offset in the data. These

Table 2. Changes in alkalinity due to prerinsing procedures with glass-fiber filters.

Solvent	Sequence	Effect on culture ALK
None	Unused filter—$Si\langle^{O^-}_{O^-}$	
Distilled water	$Si\langle^{O^-}_{O^-} + 2H_2O \rightarrow Si\langle^{OH}_{OH} + 2\,OH^-$	Increase
Culture medium (filtration)	$Si\langle^{OH}_{OH} + Mg^{2+} \rightarrow Si\langle^{O}_{O}\rangle Mg + 2\,H^+$	Decrease

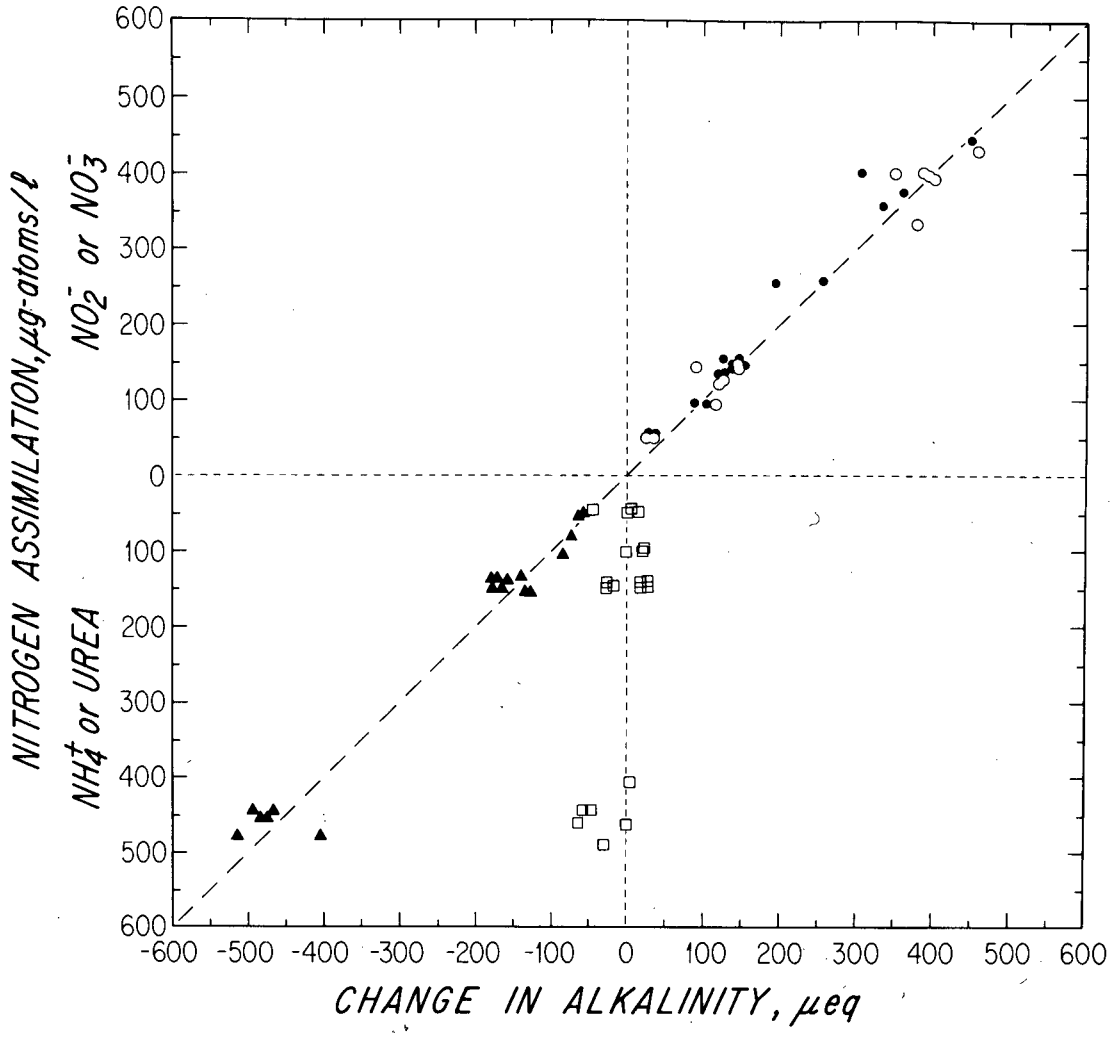

Fig. 1. Alkalinity change as a function of source of medium nitrogen assimilated by *Dunaliella tertiolecta* at steady state in N-limited continuous cultures. Broken line represents theoretical 1:1 stoichiometry between alkalinity change and ionic N assimilation (●—NO_2^-; ○—NO_3^-; ▲—NH_4^+; □—urea).

effects should be considered by other workers attempting high-precision alkalinity measurements on filtered samples.

With the above adjustments in the alkalinity, we found that the simple 1:1 stoichiometric relationship between alkalinity change and N species uptake held for NO_3^-, NO_2^-, and NH_4^+ uptake over a range of N concentrations spanning an order of magnitude; in contrast, urea assimilation had no effect on alkalinity (Fig. 1). It was also evident that the 1:1 stoichiometry between alkalinity change (ΔALK) and ionic N assimilation (ΔN) was unaffected by growth rate (Fig. 2). Although the ratio ΔALK:ΔN varied within a range of 0.75–1.25 over the entire growth rate spectrum, this variability appeared random and most likely was due to experimental error.

By titrating seawater containing added glycolic acid, we demonstrated that this acid did indeed titrate the seawater CO_2 system, leading to alkalinity reductions.

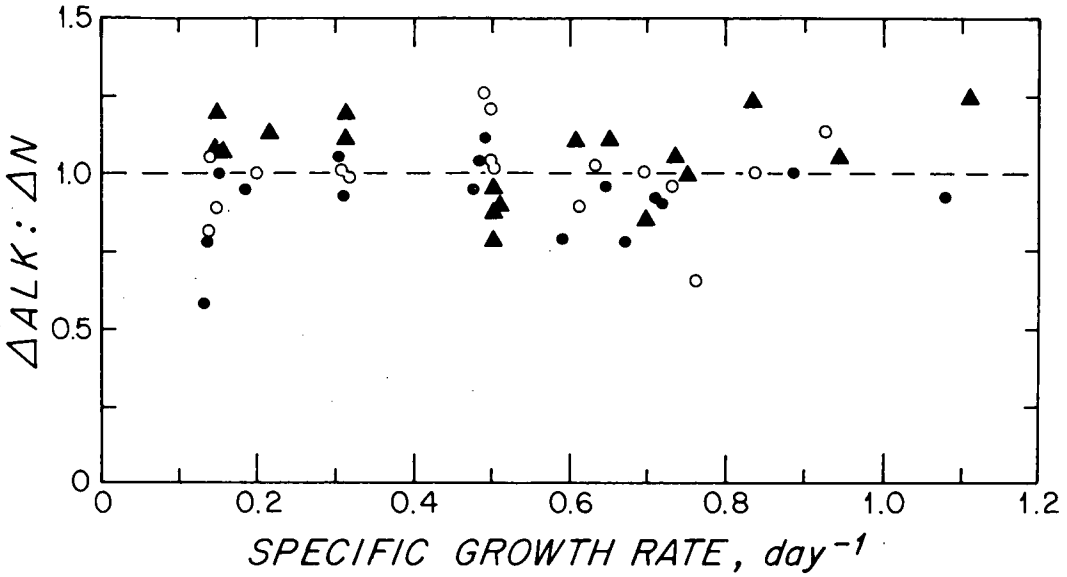

Fig. 2. Relationship between ratio of alkalinity change to ionic nitrogen assimilated (ΔALK:ΔN) and specific growth rate of *Dunaliella tertiolecta* at steady state in N-limited continuous cultures (●—NO_2^-; ○—NO_3^-; ▲—NH_4^+).

From these results (Table 3), we calculated an effect equivalent to 85% of the stoichiometric ratio.

The 1:1 stoichiometry we observed between alkalinity shift and ionic N uptake places some limits on the various hypotheses of acidic excretory products of phytoplankton. Glycolic acid ($HOCH_2COOH$) is a ubiquitous product of photosynthesis, and frequently it has been suggested as a major excretory compound of both freshwater and marine phytoplankton (Fogg 1966: Merrett and Lord 1973). Both Hellebust (1974) and Fogg (1977) claim that it is the major organic acid excreted by algae.

Glycolic acid ($pK = 3.6$) is a stronger acid than carbonic acid ($pK_1' = 6.0$) (Table 3). Hence, if it was excreted as a true acid in our earlier and current experiments, we would have observed an acidic offset in the alkalinity data directly correlated with biomass. Yet, no such effect was found. In most previous studies of this topic, reference is made to measurements of the anion; the product invariably is referred to as "glycolic acid" or "glycolate," with no apparent distinction made between the two forms. Clearly, it is the acidic moiety that is germane to our present study. In our previous and current studies, which include data with three species (*D. tertiolecta, Phaeodactylum tricornutum, Monochrysis lutheri*)—each representing a different taxonomic group grown over a wide range of conditions (e.g. varying exponential growth rates and biomass levels and different nitrogen sources)—the 1:1 stoichiometry between ionic N uptake and alkalinity change held. Thus true

Table 3. Alkalinity titrations of seawater with and without glycolic acid added.

	ALK	Difference from control
	μeq	
Experiment 1		
Seawater (control)	2,419	
1,640 μM glycolic acid in seawater	1,028	−1,391
Experiment 2		
Seawater (control)	2,439	
164 μM glycolic acid in seawater	2,295	−144

glycolic acid excretion could not have been more than about 20 μeq even at the highest cell densities produced.

However, the above conclusion does not exclude the possibility that a salt of glycolic acid (e.g. sodium glycolate) was being excreted in our experiments, nor does it address the problem of excretion by stressed cells. Moreover, because we did not measure dissolved organic carbon, we do not know if excretion of other organic compounds was significant. Hellebust (1965) found only minor glycolic acid excretion in 18 of 22 marine species, representing in most cases <0.1% of the photosynthetically assimilated CO_2. Yet, the general consensus of opinion is that under stressed conditions of cell stagnation—high light intensity, low inorganic carbon supply, or both—glycolic acid production is most pronounced, and in certain species can represent a sizable fraction of the assimilated CO_2 (Hellebust 1974; Fogg 1977). However, it appears that healthy marine phytoplankton excrete very little of their assimilated carbon (Berman and Holm-Hansen 1974; Sharp 1977). Our results are consistent with these findings, at least in demonstrating almost undetectable glycolic acid production by exponentially growing marine phytoplankton. The 1:1 stoichiometry between ionic N assimilation and alkalinity change, exclusive of any release of organic acid with a pK less than that of carbonic acid, should be a common feature of natural waters and lead to alkalinity and pH changes that are a function of the N source available.

In addition to the effect of alkalinity changes caused by ionic N-species uptake, it should also be possible to observe any alkalinity change caused by phosphate uptake. Brewer and Goldman (1976) suggested several possible relationships, including uptake of phosphate without an accompanying cation as in

$$HPO_4^{2-} + 2\ H_2O \rightarrow H_3PO_4 + 2\ OH^- \quad (1)$$

thus resulting in no alkalinity increase, similar to the noneffect of CO_2 uptake or production by microbes, and uptake accompanied by a cation as in

$$HPO_4^{2-} + Mg^{2+} \rightarrow MgHPO_4^0 \quad (2)$$

resulting in a decrease. With urea as an N source, any alkalinity change observed should then be due solely to phosphate uptake according to Eq. 2. No change was indicated (Fig. 1), tending to support uptake as depicted in Eq. 1; however, the experimental error and the narrow range of phosphate concentrations (1–30 μg-atoms·liter^{-1}) covered preclude a completely satisfactory observation of this effect.

In our earlier study, we suggested that estimates of primary productivity and $CaCO_3$ dissolution and precipitation could be in error if consideration was not given to changes in alkalinity due to N transformations carried out by microbes. Kinsey (1978) has pointed out that these effects are of marginal significance in estimates of coral reef calcification where carbonate removal is much greater than in open ocean waters. However, the effects are definitely important in the reverse case, the study of deep-sea dissolution of carbonates (Brewer et al. 1975). Furthermore, a detailed knowledge of these processes is important in estimating the penetration of fossil fuel CO_2 into the ocean (Brewer 1978; Chen and Millero 1979), where corrections for changes in alkalinity induced by N-species uptake is critical.

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