ing the detrital material must also be determined. A significant deviation from the δ ^{13}C value of the whole plant tissue could result, for example, if a heterotroph selectively assimilated specific carbon compounds, e.g. lipids, amino acids, or sugars. These fractions can have δ ^{13}C values very different from that of the whole organism (Park and Epstein 1961; Degens et al. 1968). The marsh grasshopper, however, did not appear to fractionate fresh *S. alterniflora* carbon.

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Morphometric changes in *Asterionella formosa* colonies under phosphate and silicate limitation[1]

Abstract—Steady state populations of *Asterionella formosa* show a marked dependence of the number of cells per colony on growth rate under both phosphate and silicate limitation. At high steady state growth rates the cells per colony average 8. Under phosphate limitation, the number of cells per colony decreases with decreasing steady state growth rate in a linear manner to less than 2 cells colony^{-1} at very low growth rates. Under silicate limitation, the number of cells per colony increases with decreasing steady state growth rate in an approximately exponential manner to over 20 cells colony^{-1} at very low growth rates.

When terrestrial plants are deficient in a particular nutrient, there are specific symptoms that allow easy detection of the nutrient involved (Sprague 1964). Holmes (1966) has reported cytological changes induced by a specific nutrient limitation for several species of marine planktonic diatoms. Such changes allow an investigator to rapidly determine which nutrient may be limiting the growth of these species. Morphological changes, with an apparent relation to seasonal population cycles, have been reported for *Asterionella formosa* Hass., a major planktonic diatom in midlatitude mesotrophic lakes and impoundments (Lund et al. 1963; Lund 1949;

Gardiner 1940–1941; Hutchinson 1967). For instance, Lund et al. (1963) reported that natural populations of *Asterionella* averaged 8 cells colony⁻¹ during the period of maximal growth in the spring bloom of Windermere, England. Just after the bloom peaked, at which time the growth rate of *Asterionella* was considered to be limited by silicate, colony size increased to 10 or more cells colony⁻¹. By midsummer, after the *Asterionella* population had fallen precipitously, the number of cells per colony decreased to 2–4. This was followed by an increase to over 10 cells colony⁻¹ during the relatively minor autumnal diatom bloom, also characterized as silicate limited. Phosphate and silicate are the two nutrients most often found to limit the growth rate of phytoplankton in lakes, although other nutrients may be important in some cases.

During short term growth experiments on *Asterionella* in the laboratory (Kilham 1975; Titman 1976*a*; Tilman and Kilham 1976) we observed changes in the number of cells per colony which seemed dependent on two factors: the rate of growth of the population and the particular nutrient limiting that growth. Specifically, under conditions in which phosphate was limiting the growth of *Asterionella*, the number of cells per colony decreased from the usual 8 to as low as 2. The more slowly growing populations (i.e. more phosphate limited) had fewer cells per colony. Under silicate limiting conditions, we never observed cultures that averaged <7 or 8 cells colony⁻¹. The more silicate limited cultures were, the more cells per colony there were. Under extreme conditions we observed 20 cells colony⁻¹ as a population average.

To explore this observation further, we decided to look at colony size in populations of *Asterionella* grown at various steady state growth rates under silicate or phosphate limitation. We assumed that the tendencies we observed in short term cultures would approach a limit as the population reached specific steady state growth rates under nutrient limiting conditions.

We used a flow-through, semicontinuous method. The *A. formosa* used is an axenic clone (FraAf) isolated from Frains Lake, Michigan. Cultures were grown at 20°C, on a 14–10-h light–dark cycle, with illumination of ca. 100 μEin m⁻² s⁻¹, in medium "WC" (Guillard and Lorenzen 1972), modified so that either phosphate or silicate would be the limiting nutrient. The *Asterionella* population was allowed to grow to steady state in semicontinuous cultures. For the phosphate experiments, the influent concentrations were 0.25 μM and 1.00 μM PO_4-P (with 100 μM SiO_2-Si). For the silicate experiment, the influent concentration was 8.5 μM SiO_2-Si (with 50 μM PO_4-P). For each influent concentration, cultures were run at several (5–6) flow rates from 0.05 to 0.6 d⁻¹. Cultures were manually diluted each day by removing a portion of the culture suspension and replacing it with an equal volume of sterile medium. Flow rates (f) are expressed as the volume removed per day to the total culture volume. For semicontinuous cultures, the steady state growth rate (D; equal to the dilution rate at steady state) depends on f as: $D = \ln[1/(1-f)]$. All cultures were sampled periodically at which time extracellular and internal levels of phosphate (Menzel and Corwin 1965; Strickland and Parsons 1972) and silicate (Strickland and Parsons 1972) were measured, and population size and cells per colony were determined microscopically. Experiments were ended when nutrient concentrations and cell numbers had been at steady state for a week, usually after about 25 days.

As cultures approach steady state (i.e. extracellular and internal concentrations and population size become constant), the number of cells per colony also approaches a constant value. The steady state values for colony size vs. growth rate are shown in Fig. 1. Under phosphate limitation, cultures at the lowest growth (flow) rate studied average <2 cells colony⁻¹. As growth rate increases to the maximal rate of growth (0.80 d⁻¹), the number of cells per colony increases up to 8 in a linear
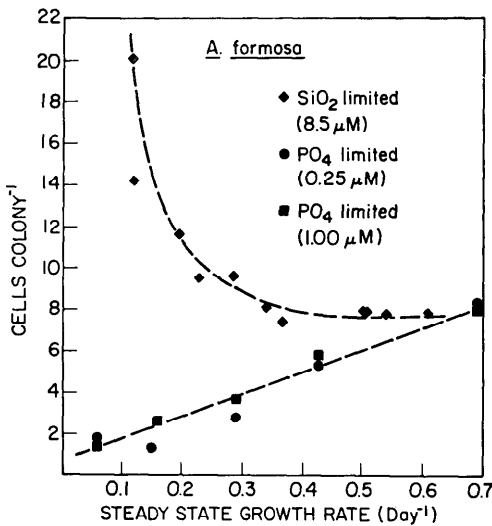
Fig. 1. Nutrient dependent morphometric changes in colony size of steady state cultures of *Asterionella formosa* grown under phosphate or silicate limitation in flow-through culture. Steady state growth rates shown are equal to dilution rate of each culture and thus are expressed in terms of natural logarithm (ln).

manner (correlation coefficient, $r = 0.98$). Under silicate limitation there are over 20 cells colony^{-1} at a steady state growth rate of 0.13 d^{-1}, decreasing in an approximately negative exponential manner ($r = 0.78$) with increasing growth rate, to a lower limit of ca. 8 cells colony^{-1} at higher steady state growth rates. At rates near the maximal rate of growth, *Asterionella* is barely limited by any nutrient.

Because it is unlikely that steady state conditions prevail in lakes, colony morphology should not be used to estimate the absolute rate of growth of natural *Asterionella* populations. If silicate and phosphate are the nutrients most likely limiting *Asterionella* populations in nature, increases in colony size above 8 cells colony^{-1} would be indicative of silicate limitation, and decreases below 6–8 cells colony^{-1} would be indicative of phosphate limitation. The more the colony size deviates from the value of 8 cells colony^{-1}, the greater should be the extent or duration of limitation by the growth rate limiting nutrient.

These morphological differences indicate that colony size of *Asterionella* may be used to assess whether a natural population is silicate or phosphate limited. If *Asterionella* in Windermere responds to nutrient limitation as it does in Frains Lake, the suggestion of Lund et al. (1963) that the decline of *Asterionella* after the spring bloom may have been due to silicate limitation would be supported by our results, because colonies averaged >10 cells colony^{-1} in that period. Our results would also indicate that after the population decline in Windermere, the cells that were left (at very low population levels) probably became phosphate limited (or at least not silicate limited) because the colony size decreased to about 4–2 cells colony^{-1}. Gardiner (1940–1941) reported a decrease in colony size of *Asterionella* during the spring bloom in three London reservoirs from a high of 6.9 cells colony^{-1} to 2.1 cells colony^{-1} 40 days later; our results would indicate that the *Asterionella* was probably phosphate limited at this time.

Similar variations in colony size or shape, or in cytology of cells, have been observed in other species of freshwater algae (Hutchinson 1967; Trainor 1969; Lind and Croasdale 1966; Shubert and Trainor 1974). These species may also prove useful in understanding the nutrient status of phytoplankton and deserve careful laboratory and field investigation.

Morphological indications of the nutrient status of one species must be used with caution in application to other species in a phytoplankton community. For instance, if another diatom uses silicate more efficiently than *Asterionella*, its growth rate may be limited by a different nutrient (e.g. phosphate) at the same time that *Asterionella* is silicate limited (Titman 1976a). Many careful experiments in nutrient physiology must be done for the important species. Then, by knowing the ratio of silicate to phosphate at which each species is equally limited by both nutrients (*see* Titman 1976a,b; Droop 1974; Rhee 1974), we may be able to use nutrient-related morphological changes of one or a few species in a

community to assess the nutrient status of each species for which physiological information is available.

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