

# Effects of Ammonium Concentration and Dilution on the Competition between the Cyanobacterium *Microcystis novacekii* and the Green Alga *Scenedesmus quadricauda*.

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**Abstract:** The chemostat theory on two species competition has shown that the dilution rate where transition of dominance occurs is independent of limiting-nutrient concentration. However, we obtained the experimental data indicating that the transition-dilution rate changed with variations in limiting-ammonium concentrations, using the chemostat mixed-culture of the cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda*. The transition-dilution rate was dependent on the concentration of limiting ammonium in the influx culture medium. We tried to simulate the experimental results. We introduced the effective dilution rate that depended on nutrient concentration (ammonium concentration in this study). A Monod-type hyperbolic function was used to represent the effective dilution rate for each species. The maximum dilution rate of the function was set to be the mechanical dilution rate (nominal dilution rate) of the chemostat culture. The calculation showed that the nominal transition-dilution rate where transition of dominance occurs decreased with increased concentration. This simulation was well consistent with our experimental data.

**Keywords:** Algae; Chemostat; Competition; Dilution rate; Limiting nutrient

## 1. INTRODUCTION

Dominant species in algal blooms is often determined by the competition between algae for resources such as nutrients. Mixed algal chemostat theory and cultures have been used as a model to study the competition (Grover, 1997). In the chemostat, the culture medium containing a limiting nutrient continuously flows into a culture vessel and the same amount of cultured algal suspension as that of influx medium flows out. Tilman (1982) applied successfully this system to elucidate phytoplankton dominance in nature.

In the chemostat system, only one species often survives and others become extinct. However, the dominating species varies, depending on the dilution rate and concentration of limiting nutrient. According to the chemostat theory, two species cultures in this system are expected to show that changes in dominant species occurs at constant dilution rate, irrespective of concentrations of limiting nutrients (Kuwata and Miyazaki, 2000). However, it has not been examined well whether this is the case in the wide range of nutrient concentrations. There is a possibility that the dominance change depends on nutrient concentrations. If the change depends on the

concentration, there would be a need to reconsider the formulation of the chemostat theory

In this study, we examined the competition between freshwater algae; the cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda*, using the chemostat systems. *M. novacekii* blooms often in summer in temperate lakes and deteriorates water quality (Collins, 1978; Skulberg *et al.*, 1984; Turner *et al.*, 1990). The blooms usually occur in still waters. Though *S. quadricauda* is present in most lakes, it seldom makes large blooms. Using these two algae as model species may be useful to consider algal competition in the field. We measured the dominance of the two species in competition experiment, and obtained the relationship between the dilution rate and the concentration of limiting nutrient. Based on the experimental results, we assumed that the dilution rate (the volumetric flow rate/the volume of culture vessel) was a function of the nutrient concentration and deviated from constant especially under low nutrient concentration. Calculation based on this assumption explained the dominance change of the algae in the culture system. The function of dilution rates could be species-specific. We try to discuss the possibility that the species-specificity

can cause coexistence of phytoplankton under low nutrient conditions.

## 2. MATERIALS AND METHODS

### 2.1. Competition experiment in chemostat culture

The cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda* were precultured in batch at room temperature at an irradiance of 20  $\mu\text{E m}^{-2} \text{s}^{-1}$  in 14:10 LD cycles in modified WC medium (Guillard and Lorenzen, 1972).

Chemostat cultures were carried out at 25 °C and illuminated continuously at an irradiance of 150  $\mu\text{E m}^{-2} \text{s}^{-1}$  (Watanabe and Miyazaki, 1996; Nagao and Miyazaki, 2002). Ammonium chloride was used as the limiting nitrogen source. The cultures were continuously stirred with magnetic stirrers. Samples of 8 ml were removed from the culture vessels at predetermined time and fixed with a few drops of Lugol's solution. The number of cells was counted with an inverted microscope (ZEISS Axiometer 135). The time courses of changes of cell number were monitored for 20-35 days. Then, dominance of algae was estimated after duplicate experiments in each condition.

### 2.2. Simulation

We used the model equations based on chemostat theory (Tilman 1982; Smith and Waltman, 1995; Kuwata and Miyazaki, 2000). Calculation was made using Mathematica 3.0.

$$\frac{dQ_m}{dt} = V_m - \mu_m Q_m \quad (1)$$

$$\frac{dQ_s}{dt} = V_s - \mu_s Q_s \quad (2)$$

$$\frac{dS}{dt} = D(S_0 - S) - V_m C_m - V_s C_s \quad (3)$$

$$\frac{dC_m}{dt} = (\mu_m - D)C_m \quad (4)$$

$$\frac{dC_s}{dt} = (\mu_s - D)C_s \quad (5)$$

where  $S$  is ambient ammonium concentration,  $D$  is dilution rate,  $V$  is ammonium uptake rate per cellular carbon,  $\mu$  is specific growth rate,  $Q$  is cell quota of nitrogen expressed as cellular N:C,  $C$  is biomass. Subscripts m and s refer to *M. novacekii* and *S. quadricauda*, respectively. Eqs (1) and (2) express the mass balance of intracellular nitrogen content of *M. novacekii* and *S. quadricauda*, respectively. Eq. (3) formulates the nitrogen mass balance within the culture

system. Eqs. (4) and (5) show the carbon balance of the *M. novacekii* and of *S. quadricauda* in the culture system, respectively.

Ammonium uptake rates are expressed by the Michaelis-Menten type equation (Dugdale, 1967; Legovic and Cruzado, 1997).

$$V = V_{\max} \frac{S}{K_u + S} \quad (6)$$

where  $V_{\max}$  is the maximum ammonium uptake rate per cellular carbon and  $K_u$  is the half saturation constant.

The relationship between growth rate ( $\mu$ ) versus cellular N:C, which is an alternative indicator of cell quota of nitrogen,  $Q$  (Droop, 1973), is given by the linear function (Healey and Hendzel, 1988; Watanabe and Miyazaki, 1996).

$$\mu_m = a_m (Q_m - Q_0) \quad (7)$$

$$\mu_s = a_s (Q_s - Q_{0s}) \quad (8)$$

where  $a$  is the slope of  $\mu$  versus  $Q$ .

To improve fitness to the experimental data, the dilution was assumed to depend on nutrient concentration. In this study, we used a Monod-type hyperbolic function as a first approximation:

$$D(S) = \frac{D_0 S}{k + S} \quad (9)$$

where  $D(S)$  was effective dilution rate at  $S$  (concentration of limiting nutrient),  $D_0$  is the nominal dilution rate that is a dilution rate in usual chemostat and  $k$  is a constant. At a given  $D_0$ , if  $S$  becomes large enough,  $D(S)$  becomes  $D_0$  in which case the chemostat corresponds to the usual one. If  $S$  is low,  $D(S)$  is dependent on  $S$ . At higher  $D_0$ ,  $D(S)$  becomes close to  $D_0$ , because  $k/D_0$  becomes small. When  $D(S)$  is  $D_0$ , dominance change would not occur irrespective of nutrient concentrations. When  $D(S)$  differs from  $D_0$ , the dominance change will be dependent on nutrient condition. Calculation was carried out by changing  $D_0$  and  $S$ . The parameters used in this study (Table 1) were obtained from the culture experiments by Watanabe and Miyazaki (1996).

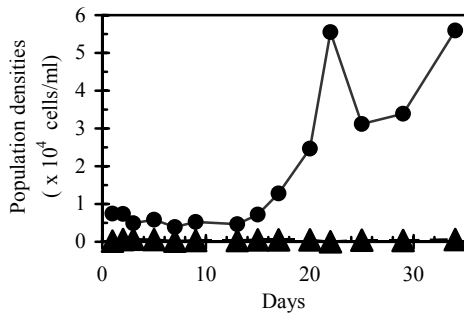
## 3. RESULTS

### 3.1. Culture experiments

Figure 1 shows the time-series changes of cell densities in mixed chemostat culture of *M. novacekii* and *Scenedesmus quadricauda* at 0.1  $\text{d}^{-1}$  of dilution rate and at 10  $\mu\text{M}$  of ammonium. In this case, *S. quadricauda* expelled *M. novacekii* from the culture medium. In this way, we determined dominant species in the mixed cultures at different

Table 1. Parameters for calculation used in the present study.

| Parameter | <i>M. novacekii</i> | <i>S. quadricauda</i> | Unit                                      |
|-----------|---------------------|-----------------------|---|
| $V_{max}$ | 0.206               | 0.696                 | mol N mol C <sup>-1</sup> d <sup>-1</sup> |
| $K_u$     | 0.5                 | 1.25                  | μM  |
| $a$       | 6.33                | 32.26                 | mol N <sup>-1</sup> mol C d <sup>-1</sup> |
| $Q_0$     | 0.0237              | 0.465                 | mol N mol C <sup>-1</sup>                 |
| $k$       | 1                   | 1                     | μM  |



**Figure 1.** Competition between *M. novacekii* and *S. quadricauda* at 0.1 d<sup>-1</sup> of dilution rate and 10 μM of ammonium

sets of dilution rates and concentrations of limiting nutrient (ammonium).

Dominance of *M. novacekii* or *S. quadricauda* at steady state was shown in Table 2. The dilution rate where transition of dominance occurred depended on limiting ammonium concentration in the supply medium. At 0.3 d<sup>-1</sup> of nominal dilution, *S. quadricauda* was dominant at all ammonium concentrations examined. At 0.1 d<sup>-1</sup>, *S. quadricauda* was dominant at 50 and 100 μM, whereas *M. novacekii* dominated at 10 μM. At 0.01 d<sup>-1</sup>, *M. novacekii* was dominant at 100 μM. According to the chemostat theory where dilution rate is not a function of nutrient concentration, dominance transition occurs at a constant dilution rate in spite of nutrient concentration changes (Kuwata and Miyazaki, 2000)

### 3.2. Simulation

We calculated the time courses of population density using the effective dilution rate (9) that

depends on nutrient concentration. Figure 2 shows that time courses at 0.3 μM of ammonium. *M. novacekii* dominates at lower dilution rates of 0.01 d<sup>-1</sup>, whereas *S. quadricauda* dominated at a higher dilution rate of 0.95 d<sup>-1</sup>. The transition-dilution rate where dominance changed was obtained from these time courses. Table 3 exhibits the nominal dilution rate at which dominance-transition occurred. At 0.1 μM of ammonium the dominance change occurred at the nominal dilution rate of 0.86 d<sup>-1</sup>. At 100 μM, it occurred at 0.08 d<sup>-1</sup>. This indicates that dominance change occurs at different dilution rates at the different limiting nutrient concentration, and corresponds to the experimental results.

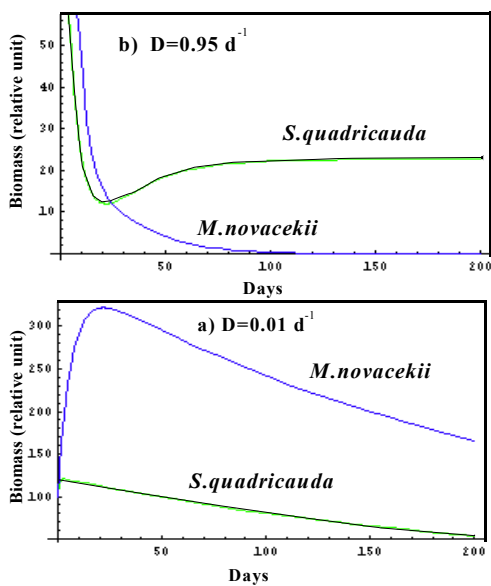
## 4. DISCUSSION

The present culture experiments suggest the dilution and the concentration of limiting nutrient were mutually dependent. The lower dilution and lower nutrient concentration favored the dominance of *M. novacekii*, compared with *S. quadricauda*. In natural waters, turnover of water was usually smaller than that in chemostat culture experiments, and the concentration of ammonium was less than several μM. Thus, it may be necessary to consider the interdependency of nutrient concentration and dilution when considering algal blooms and competition in natural waters.

We used, as a first-order approximation, the Monod-type function for the expressing the relationship between dilution rate and limiting-nutrient concentration, though other types of function might be possible. This introduction into chemostat equations explained the results of chemostat cultures in the present study. At lower concentration of limiting ammonium, dominance transition occurred at lower nominal dilution rates.

**Table 2.** Dominant species in two species mixed culture of *S. quadricauda* and *M. novacekii* under different sets of dilution rate and ammonium concentration in supply medium. Culture were carried out at 25°C. ND: no data.

| Ammonium concentration | Dilution rate (d <sup>-1</sup> ) |                       |                       |
|------------------------|----------------------------------|-----------------------|-----------------------|
|                        | 0.01                             | 0.1                   |                       |
| 10 (μM)                | ND                               | <i>M. novacekii</i>   | <i>S. quadricauda</i> |
| 50 (μM)                | ND                               | <i>S. quadricauda</i> | ND                    |
| 100 (μM)               | <i>M. novacekii</i>              | <i>S. quadricauda</i> | ND                    |



**Figure 2.** Time courses of biomass changes at 0.3 μM of ammonium in simulation. a) dilution rate = 0.01 d<sup>-1</sup>. b) dilution rate = 0.95 d<sup>-1</sup>.

**Table 3.** Calculated transition-dilution rate vs. ammonium concentration in the supply medium.

| Ammonium Concentration μM | transition-dilution rate (nominal) d <sup>-1</sup> |
|---------------------------|--|
| 0.1                       | 0.86   |
| 0.5                       | 0.24   |
| 5                         | 0.095  |
| 10                        | 0.085  |
| 50                        | 0.08   |
| 100                       | 0.08   |

The equation (9) indicates that effective dilution rates are lower than nominal ones at lower concentrations of limiting nutrient. The parameter  $k$  of the equation (9) can be species-specific. If so, effective dilution might be also species-specific. Then, turnover of each species could be different even in the same nominal dilution. In this situation, more than one species can coexist under the same dilution of water in the homogeneous environments, since the equilibrium might be different for each species.

In the chemostat system, only one species often survives and others become extinct. The maximum number of coexisting species is the number of limiting nutrients (Grover, 1997). However, many species coexist in natural waters where the number of limiting nutrients is small. Hutchinson proposed the coexistence in homogenous water environment as "the paradox of plankton" (Hutchinson, 1961). Disturbance to water environments may create such a diversity of phytoplankton. In the chemostat, fluctuation of nutrient inflow is supposed to cause the coexistence (Tilman, 1962).

In apparently homogeneous water environments, the environment around each alga would be inhomogeneous at lower nutrient concentrations, because of local deprivation of nutrients due to growth and the slow supply of nutrients. Some algae may grow well, and others may not. Algal distribution in the culture medium will be inhomogeneous. On the whole, algal growth may become lower than that expected from the nominal dilution rate. Thus, the expected growth may be attained at higher nominal dilution. At higher concentrations algae could grow enough at lower nominal dilution since nutrient deprivation did not occur due to higher nutrient concentrations, and so nominal dilution rates were almost the same as effective ones.

Is it possible for phytoplanktonic taxa to coexist, without disturbance? High diversity of phytoplankton has been observed in still waters with low nutrients. At lower concentrations of limiting ammonium, nominal dilution rates were more different from effective dilution rates. Furthermore,  $k$  in the equation (9) can be species-specific. If so, more than one algal species in homogeneous environments could coexist at lower concentrations of a limiting nutrient, because each species with different dependency of dilution on nutrient concentration (9) would reach the different equilibrium. This coexistence processes might explain the higher diversity of phytoplankton in oligotrophic waters (Leibold, 1999).

Several explanations for species diversity in water environment have been presented (Richerson et. al., 1970; Levins, 1979; Sommer, 1985; Padisak et.al.,

1993). These explanation used external perturbation such as temporal variability, inhomogeneous of space, predation.

Hulsman and Welssing (2002) showed that the diversity was explained without considering external perturbation, because resource competition models could generate oscillations and chaos. The dependency of dilution on nutrient concentration can also explain the diversity without consideration of external perturbation.

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