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Effects of Temperature and Salinity on the Growth and the Optimum Nitrogen to Phosphorus Ratio for the Culture of Diatoma tenue Isolated from a Temporary Arctic Pond in Svalbard, Norway

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ABSTRACT

We investigated the effects of temperature and salinity on the growth of *Diatoma tenue* isolated from a temporary arctic pond in a high-latitude region of Svalbard, Norway. We also examined the optimum nitrogen to phosphorus (N:P) ratio of the culture media. The highest growth rate (0.38 day^{-1}) of *D. tenue* was achieved at 15°C, while growth was completely inhibited above 20°C. *D. tenue* could not grow when cultured at above 5‰ salinity. The final cell density of *D. tenue* was increased with an increasing phosphate concentration in the culture media from 0 to 5 μmol P L^{-1}. The final cell density became stable at above 5 μmol P L^{-1}. The N:P molar ratio of the media containing 5 μmol P L^{-1} was 34.8. These results show that *D. tenue* growth is limited by phosphorus at N:P ratios greater than 34.8, with a nitrogen limitation below 34.8. This finding indicates that this alga has a higher optimum N:P ratio than the Redfield ratio of 16.

INTRODUCTION

The polar region is a unique environment characterized by an annual freeze-thaw cycle coinciding with a cycle of winter darkness and summer daylight. Organisms that have adapted to the low temperatures are responsible for most of the biological production in this region during the short summer season. High latitude biota and environments have been considered relatively stable compared with those of more temperate regions, where human civilization has made a greater impact. However, Overpeck et al. (1997) discovered that the arctic region has been affected by climate changes since the 19th century.

Freshwater algae are sensitive indicators of environmental change. Diatoms are particularly useful for long-term environmental monitoring, since they are often well preserved in lake sediments (Birks et al. 2004, Jones and Birks 2004). Such limnological data are becoming critical as the polar region is increasingly being recognized as susceptible to the impacts from atmospheric pollution and to global warming (IPCC 1997).

Diatoma tenue is an important type of pennate diatom found in cold regions (Bothwell et al. 1989, Antoniades and Douglas 2002). For example, this species was previously reported to be in the ponds of Svalbard during the summer (Ki et al. 2006). Additionally, this species was also found in the lake sediment records of Svalbard (Jones

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and Birks 2004). Thus, this species has probably played a significant role in biological production in this cold region. However, the growth characteristics of this alga have not yet been well described. For example, *D. tenue* was described as a halophilic phytoplankter by Tuchman et al. (1984). In contrast, Bothwell (1989) studied this species in a freshwater environment. The growth potential of the *D. tenue* of Svalbard in brackish water has not yet been examined. Furthermore, the optimum temperature and the nitrogen to phosphorus ratio have not yet been determined for this organism. Therefore, we studied the growth characteristics of this alga to obtain a better understanding of these unique arctic ecosystems.

METHODS AND MATERIALS

For the isolation of *D. tenue*, a water sample was collected from a lake in Svalbard on 12 August 2005 (Ki et al. 2006). A portion of the sample was inoculated into DM media (Kang et al. 2005) and incubated at 10°C for one week under a 12:12 hour light:dark cycle with a light intensity of approximately 50 μE m^{-2} s^{-1}. A portion of the culture was spread onto DM agar plates (1.2% agar) and incubated under the same conditions for three weeks. Cells from subsequent brownish colony were transferred to DM media in a 96-well plate and cultured. A unialgal culture was confirmed and was selected to establish a clonal culture, labeled as the strain HYNP006. The organism was subsequently identified as *Diatoma tenue* by morphological data using a scanning electronic microscope and 18S rDNA sequencing. This strain was cultured in DM media at 10°C.

Growth rates were examined under various temperatures (4, 10, 15, 20, 25, and 30°C) and under various salinities (0, 5, 10, 15, 20, 25, and 30‰). Salinities were adjusted using NaCl and were confirmed using a salinity refractometer (model S/Mill-E, Atago). To examine the optimum N:P ratio for *D. tenue* growth, six media mixtures were prepared, each with a different phosphorus concentration (Table 1). In these experiments, cultures were incubated at 10°C under approximately 100 μE m^{-2} s^{-1} and a 12:12 hour light:dark cycle for 23 days.

All experiments were conducted in triplicate using 30 mL test tubes. Initial cell densities for all experiments were adjusted to approximately 1 x 10^4 cells mL^{-1}. A 1 mL sample was obtained each day, and fixed with a Lugol's solution. The cell densities were enumerated with a hemocytometer.

Table 1. Nitrogen and phosphorus concentrations and the N:P molar ratio of the media used for the examination of optimum N:P ratio for *D. tenue* growth. The nitrogen and phosphorus concentrations in the DM media were 174.3 μmol N L^{-1} and 91 μmol P L^{-1}, respectively.

Nitrogen concentration (μmol N L^{-1})	Phosphorus concentration (μmol P L^{-1})	N:P ratio
174.3	0	-
174.3	2.5	69.6
174.3	5	34.8
174.3	10	17.4
174.3	25	6.96
174.3	91	1.91

RESULTS AND DISCUSSION

D. tenue grew well from 4 to 20°C, though it did not grow at 25°C and above (Fig. 1). When incubated at 25°C, cells disappeared after seven days; at 30°C, the cells disappeared after three days. The highest growth rate (0.38 ± 0.07 day^{-1}) was recorded at 15°C. The final cell densities in cultures grown at 4, 10, 15, and 20°C were almost the same. Surprisingly, *D. tenue* could not grow even in the low salinity media of 5‰. The active growth was found only in media of 0‰ salinity (Fig. 2).

Growth curves under various phosphorus concentrations are shown in Fig. 3 and appear to be similar to each other. However, final cell densities were different among the treatments. When *D. tenue* cells were inoculated into a 0 μmol P L^{-1} culture, the final cell density was 40 x 10^4 cells mL^{-1}. With an increased phosphorus concentration of 5 μmol P L^{-1}, the final cell density also increased to 52 x 10^4 cells mL^{-1}. At phosphorus concentrations greater than 5 μmol P L^{-1}, final cell densities were stable.

It is well known that some diatom species can grow rapidly even in low water temperatures (e.g., Kozhov 1963, Ventela et al. 1998). In Lake Köyliönjärvi, Finland, *Aulacoseira islandica* formed a bloom in April when the water temperature was below 4°C (Ventela et al. 1998). Richardson et al. (2000) studied the growth rate of *Aulacoseira baicalensis*, which is an endemic diatom of Lake Baikal, under various temperatures. The highest growth rate of the alga was recorded at 2-3°C. *A. baicalensis* could not grow at temperatures above 11°C. As compared with these diatoms, the optimum temperature for the growth of *D. tenue* (15°C, Fig. 1) was somewhat higher.

When the water sample for the isolation of *D. tenue* was obtained, the water temperature of the lake was about 8°C, which is probably the yearly maximum. Since

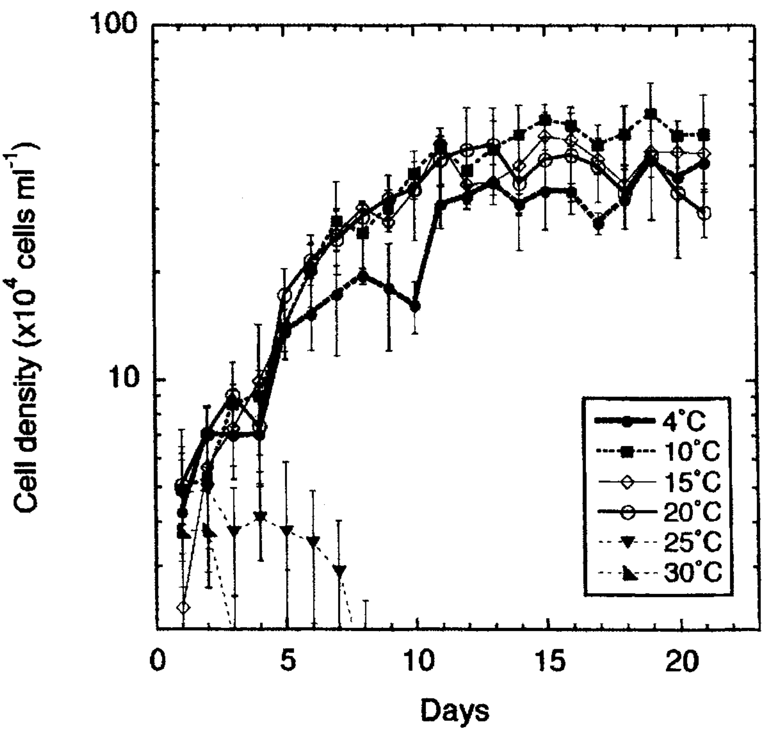

Figure 1. Growth curves of *Diatoma tenue* under various temperatures. The data are mean values for triplicates, and vertical bars indicate standard deviations.

the optimum growth rate was achieved at 15°C, *D. tenue* may grow faster as temperatures in Svalbard increase until they reach 15°C. The 8°C observed in Svalbard is higher than the temperature during the *A. islandica* blooms in Lake Köyliönjärvi (<4°C, Ventela et al. 1999) and *A. baicalensis* bloom in Lake Baikal (<4°C, Kozhov 1963). Therefore, it is reasonable to conclude that the optimum temperature is 15°C. It is likely that *D. tenue* adapted to the temperature regime of Svalbard.

This study clearly demonstrated that *D. tenue* lacks halotolerance (Fig. 2), while Tuchman et al. (1984) described *D. tenue* as a halophilic phytoplankter. *D. tenue* was found in some lake sediment records in Svalbard (Jones and Birks 2004), which indicates that this species has existed for a long time. Since Svalbard is isolated from other landmasses such as Norway and Greenland, a freshwater diatom of *D. tenue* may possibly have evolved to adapt to this unique environment. Thus, although the 18S rDNA sequence and morphological data reveal the strain as *D. tenue*, the strain possibly possesses unique characteristics.

The use of batch culture experiments is not a suitable means for examining nutrient limitation in algae. This is due to the fact that, when the nutrient concentration exceeds the saturated concentration, which is usually lower than those of the media, the growth rate will be equal for the various nutrient concentrations. In addition, the nutrient concentrations are successively decreased as the culture progresses during the incubation. Therefore, it is difficult to measure the growth rate accurately at a certain nutrient concentration. Indeed, the growth rates among the variable phosphorus treatments in this study were quite similar. Therefore, the final cell densities were used to examine the

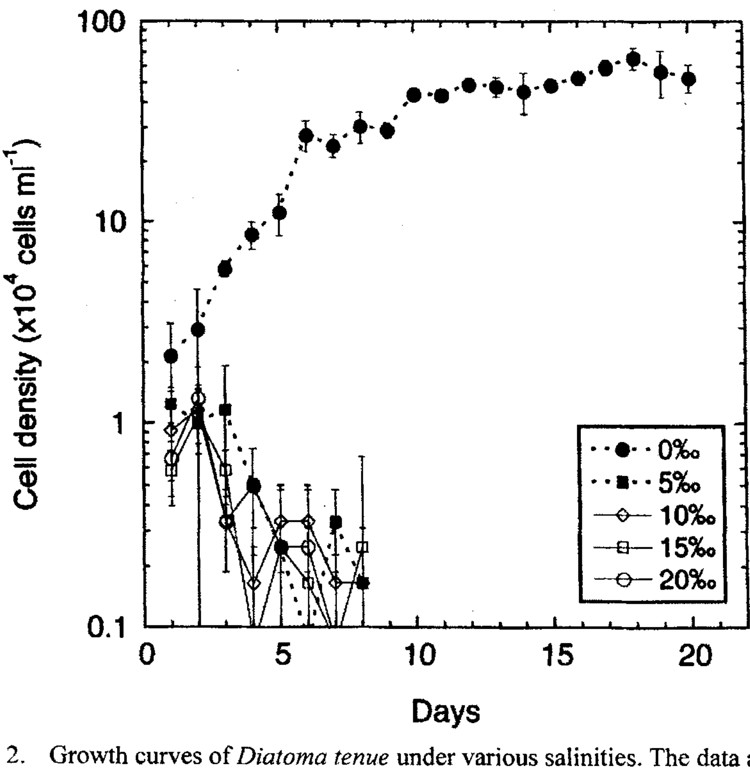

Figure 2. Growth curves of *Diatoma tenue* under various salinities. The data are mean values for triplicates, and vertical bars indicate standard deviations.

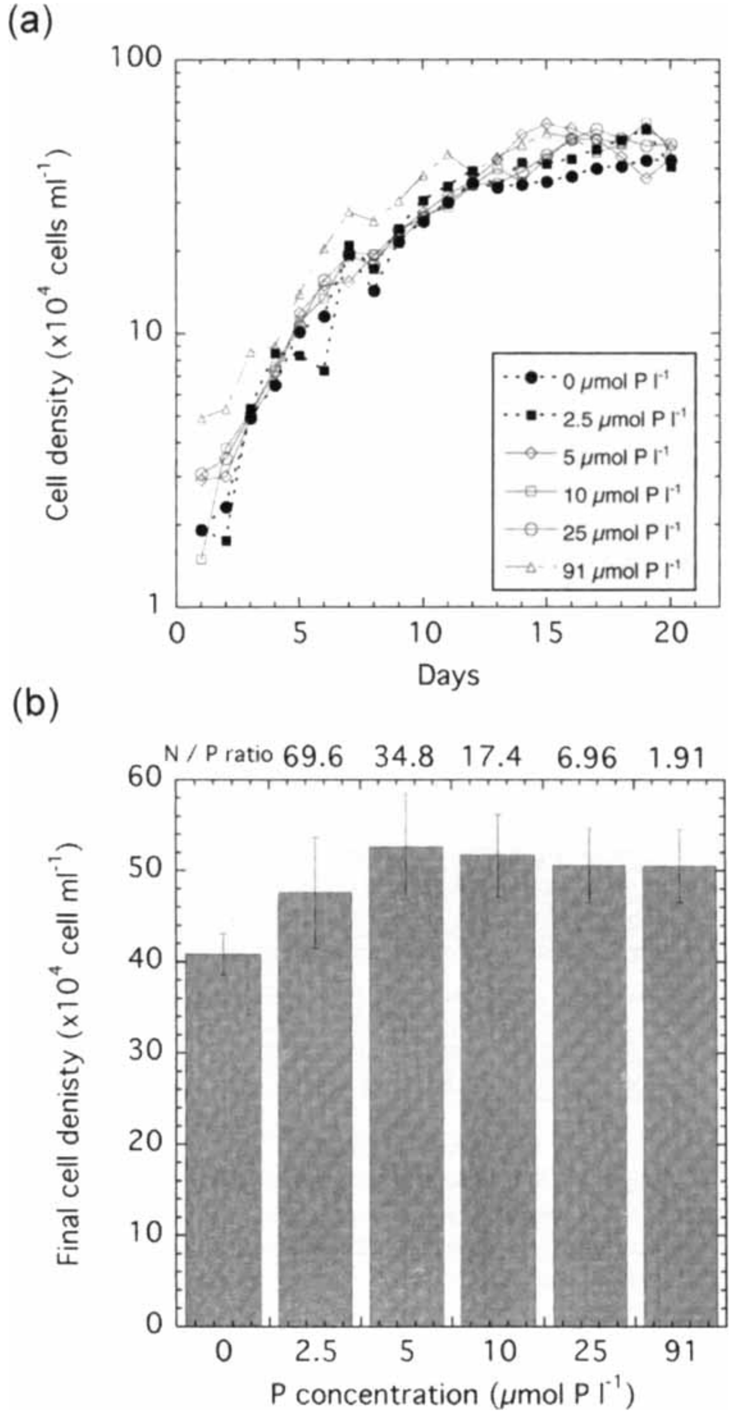

Figure 3. Growth curves of *Diatoma tenue* under various phosphorus concentrations (a) and final cell density at the stationary phase at each phosphorus concentration (b). Data are mean values for triplicates, and vertical bars indicate standard deviations.

systematic limitations (Hashimoto and Nakano 2003). In this analysis, the final cell densities were compared under various nutrient concentrations. When the algal cells are limited by the tested nutrient, with an increase in the nutrient concentration, the final cell density should also increase. When the cells are not limited by the tested nutrient, the final cell density should not change. Therefore, with the tested nutrient concentration increasing from 0 μmol L^{-1}, the final cell density should increase up to a certain level. Around this concentration, the systematically limiting nutrient would shift to other nutrients. Since the N:P ratio of the DM media was 1.96, the cells grown in DM media may be limited by nitrogen. Thus, the phosphorus concentration was changed to find the optimum N:P ratio for *D. tenue*.

The results clearly showed that from 0 to 5 μmol P L^{-1}, *D. tenue* cell densities were limited by the controlled amount of phosphorus (Fig. 3). At levels above 5 μmol P L^{-1}, the limiting nutrient for the final cell densities most likely shifted from phosphorus to nitrogen. At levels greater than 5 μmol P L^{-1}, the nitrogen concentration in these cultures at the stationary phase may be exhausted. Therefore, nitrogen may be a limiting nutrient on the cell yield of *D. tenue* at concentrations greater than 5 μmol P L^{-1}. The N:P ratio of the media at 5 μmol P L^{-1} was 34.8 (Table 1). Therefore, the preliminary result indicates that the optimum N:P ratio for *D. tenue* growth may be approximately 34.8. At 0 μmol P L^{-1}, the final cell density was 40 x 10^4 cells mL^{-1} (Fig. 3b). The grown cells probably utilized phosphorus, which was acquired in the previous culture or carried over from the previous media. Consequently, this detail should be carefully examined in future experiments that utilize continuous culture.

Generally, it is understood that the optimum N:P ratio for phytoplankton is similar to the Redfield ratio of 16. Marks and Power (2001) reported that *Diatoma* spp. dominated in habitats with higher N:P ratios in the Great Lakes Region. This finding supports the results of this study, which show that *D. tenue* favors a higher N:P ratio than Redfield ratio. Freshwater phytoplankters are frequently limited by phosphorus. This is also the case for colder lakes, such as Lake Baikal (Satoh et al. 2006) and Lake Stechlin (Padisak et al. 1997). These findings may indicate an advantage for *D. tenue* in prevailing over other algal species in the phosphorus-limited lakes of Svalbard.

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