TEMPERATURE DEPENDENCE OF GROWTH AND CARBON ASSIMILATION IN NITZSCHIA PUNGENS F. MULTISERIES, THE CAUSATIVE DIATOM OF DOMOCIC ACID POISONING

Youlian Pan\textsuperscript{1,2}, D. V. Subba Rao\textsuperscript{2}, K. H. Mann\textsuperscript{2}, W. K. W. Li\textsuperscript{2} and R. E. Warnock\textsuperscript{2}

\textsuperscript{1} Department of Biology, Dalhousie University, Halifax, N. S. Canada, B3H 4J1; \textsuperscript{2} Department of Fisheries and Oceans, Bedford Institute of Oceanography, P. O. Box 1006, Dartmouth, N. S., Canada, B2Y 4A2

ABSTRACT

Batch cultures of Nitzschia pungens f. multiseries grown for three days at 10\textdegree C and acclimated for one day at 0\textdegree, 5\textdegree, 10\textdegree, 15\textdegree, 20\textdegree and 25\textdegreeC were utilized to study photosynthetic rates (\delta^{13}C uptake) as a function of photosynthetic photon flux density (PPFD) at these temperatures. The division rate (K) increased from 0.3 d\textsuperscript{-1} at 0\textdegreeC to 1.7 d\textsuperscript{-1} at 15\textdegreeC and remained the same at higher temperatures. The initial slope of the photosynthesis-PPFD curves (\alpha\textsuperscript{0}) was low at 0\textdegreeC (0.17 x 10\textsuperscript{-3} \mu g C [\mu g Chl a]\textsuperscript{-1} h\textsuperscript{-1} [\mu mol m\textsuperscript{-2} s\textsuperscript{-1}]) and increased to 5.8 x 10\textsuperscript{-3} \mu g C [\mu g Chl a]\textsuperscript{-1} h\textsuperscript{-1} [\mu mol m\textsuperscript{-2} s\textsuperscript{-1}] at 15\textdegreeC. The corresponding assimilation numbers (P\textsuperscript{0}\textsubscript{m}) were 0.08 \mu g C [\mu g Chl a]\textsuperscript{-1} h\textsuperscript{-1} and 3.42 \mu g C [\mu g Chl a]\textsuperscript{-1} h\textsuperscript{-1}. Compared to data on other diatoms, the assimilation numbers of Nitzschia pungens f. multiseries were low. Cellular chlorophyll a increased 3-times between 0\textdegree and 25\textdegreeC, but carbon and nitrogen decreased to 44\% and 55\%, respectively. In the temperature range from 5\textdegree to 15\textdegreeC, Q\textsubscript{10} for K was 2.8, which is in agreement with data on other algae. However, the Q\textsubscript{10} for \alpha\textsuperscript{0} and P\textsuperscript{0}\textsubscript{m} were 10.3 and 16.9, respectively.

INTRODUCTION

Unlike the seasonal spring and summer phytoplankton blooms in most coastal waters [1], the toxic blooms of Nitzschia pungens f. multiseries occur in late fall and winter [2, 3, 4] when the prevailing seawater temperature is low (-1\textdegree to 3\textdegreeC). Blooms of Nitzschia pungens resulted in serious commercial losses to the shellfish industry and loss of human lives [5]. Photosynthetic rates of phytoplankton are dependent on temperature, light and overall cell metabolism, thus cell division and photosynthesis are ultimately regulated by the environmental factors affecting these processes. The existence of non-linear relationships between growth (photosynthetic) rate and temperature has been well documented [6, 7, 8, 9, 10]. Studies on the effects of temperature on growth and photosynthesis of Nitzschia pungens f. multiseries are important in understanding the development of the toxic bloom. This paper presents results on the growth and photosynthesis of Nitzschia pungens f. multiseries at different temperatures and examines their relationship to temperature, cellular carbon, nitrogen and chlorophyll a.

MATERIALS AND METHODS

Non-axenic stock cultures of N. pungens f. multiseries isolated from Cardigan Bay, PEI, Canada were grown in medium FE [4], at 10\textdegree - 12\textdegreeC under continuous cool white fluorescence light of 410 \mu mol m\textsuperscript{-2} s\textsuperscript{-1}. For the temperature experiments, 200 ml of exponentially growing (5 days) N. pungens f. multiseries were inoculated into a Fernbach flask containing 2 litres of FE medium. On the third day of growth, 250 ml of the culture were dispensed into each of six flasks and these were transferred to 0\textdegree, 5\textdegree, 10\textdegree, 15\textdegree, 20\textdegree and 25\textdegreeC, with photosynthetic photon flux density (PPFD) ranging
from 350 to 440 μmol m⁻² s⁻¹.

Photosynthesis-PPFD (P-I) relationships were determined at corresponding temperatures using the ¹⁴C method [11] for cells harvested on the 4th day. High specific activity ¹⁴C-HCO₃⁻ (~120 K Bq ml⁻¹) was added to about 65 ml culture. Same methods and calculations for P-I work were employed as our previous work on the same diatom [12].

Samples for cell concentration were taken on day 0, 1, 3 and 4. Particulate carbon, nitrogen and chlorophyll a were measured in cultures at all the temperatures at the same time as the P-I experiments. Cells were enumerated on a 1 ml aliquot settled in plankton chambers and counted using an inverted plankton microscope. Chlorophyll a determinations were based on duplicate samples employing the fluorometric method [11]. Particulate carbon and nitrogen were analyzed using Perkin-Elmer 240B CHN Elemental Analyzer [11]. Dissolved inorganic nitrate, phosphate and silicate concentrations in the medium were measured with a Technicon Autoanalyzer II [11]. Growth PPFD was measured with a LICOR model Li-185B light meter.

The division rates of N. pungens f. multiseries in the cultures based on the cell densities on day 3 and day 4 were calculated according the equation of Guillard [13]:

\[ K \text{(division d}^{-1}) = 3.322 \times \log (N_2/N_1) / (t_2-t_1). \]

RESULTS

(i) Growth and Biomass

Cell concentrations increased at all temperatures (Fig. 1A), the division rates ranging from 0.3 to 1.8 d⁻¹, reaching a plateau at 15°C and remaining constant between 15°C and 25°C (Fig. 1B). The Q₁₀ was 2.8 between 5°C and 15°C.

![FIG. 1. Nitzschia pungens f. multiseries: A) cell concentrations and B) growth rate vs. temperature.](image)

![FIG. 2. Cellular levels of A) Chlorophyll a, B) carbon and C) nitrogen vs. growth temperature.](image)
Growth rates were positively correlated to cellular chlorophyll $a$ levels ($r = 0.8$, $p < 0.05$). There were parallel increases in growth rate and cellular chlorophyll $a$ in the temperature range of 10 - 15°C (Fig. 1B, 2A). However, cellular carbon and nitrogen decreased to 44% and 55%, respectively, when the growth rate increased from 0.3 $d^{-1}$ at 0°C to 1.7 $d^{-1}$ at 15°C (Fig. 2B, C). The carbon:nitrogen ratio (C:N, by atoms) decreased slightly as growth rate and temperature increased (Fig. 3B). The ratio of carbon to chlorophyll $a$ (by weight), however, decreased drastically, from 306 to 52 (Fig. 3A).

Nutrient concentrations in the medium remained high throughout the experiment. Nitrate was in the range of 2.32 - 2.70 mM, phosphate 72 - 91 μM and silicate 106 - 154 μM. None of the major nutrients limited growth.

**FIG. 3.** Intracellular ratios of A) carbon to chlorophyll $a$ (by weight) and B) carbon to nitrogen (by atoms) vs. growth temperature.

**FIG. 4.** Relationships between photosynthetic rate and photosynthetic photon flux density (P-I curves) at A) 0°C to 25°C; B) at 15°C and C) at 0°C.

(ii) Photosynthesis

Typical P-I curves occurred at all temperatures (Fig. 4A, B, C): with increasing PPFD, photosynthesis increased to a maximum, then decreased at higher PPFD (>1500 μmol m$^{-2}$ s$^{-1}$). The maximum assimilation number ($P_{m}$) was 3.42 μg C [μg Chl $a$]$^{-1}$ h$^{-1}$ at 15°C and the minimum was 0.08 μg C [μg Chl $a$]$^{-1}$ h$^{-1}$ at 0°C. The temperature dependence of the P-I responses depended upon the biomass index chosen (Fig. 5A, B). When photosynthesis is normalized in relation to chlorophyll $a$, the maximum photosynthetic rate ($P_{m}$; μg C [μg Chl $a$]$^{-1}$ h$^{-1}$) increases exponentially, from 0.08 at 0°C to 3.42 at 15°C, with $Q_{10} = 10.8$ between 5°C and 15°C and then decreased at temperatures >15°C. When normalized to carbon, the maximum photosynthetic rate showed a plateau between 15°C and 25°C.
Similar patterns characterized the initial slope of the P-I curve (α), which increased exponentially, from $0.17 \times 10^{-2}$ to $6.50 \times 10^{-2}$ [µg Chl a]$^{-1}$ h$^{-1}$ [µmol m$^{-2}$ s$^{-1}$] at 0°C to 20°C with $Q_{10} = 16.9$ between 5°C and 15°C. The patterns were similar for biomass normalization as both carbon and chlorophyll a, but there was a temperature difference of 5°C between the two curves (Fig. 5B).

**DISCUSSION**

The acclimation time of phytoplankton varies from 0 to more than 100 hours, and from 0 to 4 divisions [14]. If *N. pungens* adapted to the new temperatures after one generation (one division), the cultures at 10°C, 15°C, 20°C and 25°C would have and the one at 5°C would almost (0.8 division) acclimated to the new temperature before the P-I experiments. In contrast, cells transferred from 10°C to 0°C grew slowly and photosynthesized at greatly reduced rates (Fig. 4C).

Division rates (Fig. 1B), photosynthetic rates (Fig. 5A) and cellular chlorophyll a (Fig. 2A) of *Nitzschia pungens* f. *multiseries* increased with temperature up to 20°C. The exponential increase of $P_{\text{Tm}}$ from 0.08 at 0°C to 3.42 at 15°C was similar to that reported for well acclimated, temperate microphytoplankton [15]. But the $Q_{10}$ of $P_{\text{Tm}}$ was much higher than some well acclimated coastal phytoplankton [16]. The $Q_{10}$ of photosynthesis at low temperatures (<10°C) may be as high as 16 [17]. Harris and Piccinin [8] reported a $Q_{10} = 16.0$, between 2.5°C and 5°C, for photosynthesis of natural phytoplankton populations.

Our data on *N. pungens* f. *multiseries* showed that $\alpha$, the indicator of photosynthetic activity in the light limited portion, varied significantly (Fig. 5B) in the growth temperature range of 0°C to 15°C. $\alpha$ increased exponentially with increasing temperature, similar to the sigmoid pattern of *Leptocylindrus danicus* [10], but the magnitude of temperature dependent variations of $\alpha$ for *N. pungens* was much higher than for *L. danicus* [18]. For *Nitzschia americana*, at salinity lower than 30%, $Q_{10}$ for $\alpha$ was variable, the maximum was 13.1 between 10 - 20°C at 8%o [19].

Cellular carbon and nitrogen in *N. pungens* were the highest at the beginning of the batch cultures [12], but decreased substantially as the cells entered the exponential phase. Cellular levels did not recover when the cells entered the stationary phase [12]. In the present study, the highest cellular carbon and nitrogen occurred at 0°C when the growth of *N. pungens* was restricted by the low temperature (see the negative correlation of growth rate with carbon: $r = -0.94$, $p < 0.01$; and with nitrogen: $r = -0.98$, $p < 0.01$).
The response of the cells, i.e. growth, chlorophyll $a$, C:Chl $a$ and P-I parameters varied with changes in temperature. Growth and photosynthesis were promoted as a result of increase in temperature from 10° to 15°C [Figs. 1B, 5A, B]; and the cells responded consistently to the increasing PPFD (Fig. 4B). When temperature decreased from 10° to 0°C, on the other hand, the cells seemed to be stressed, growth and photosynthetic rates were drastically reduced, and no clear P-I relationship was established (Fig. 4C).

Upon transfer from 10°C to higher temperatures, cellular chlorophyll $a$ increased immediately, more than doubling within a day, coincident with obvious increases in growth and photosynthetic rates. The C:Chl $a$ ratio decreased with increase in temperature, consistent with observations on Phaeodactylum tricornutum [14], Skeletonema costatum [20, 21] and Dunaliella tertiolecta [1, 22]. When transferred to lower temperatures, on the other hand, variations in cellular chlorophyll $a$ were very small, even though growth and photosynthetic rates decreased. However, our data showed that in temperature controlled batch cultures of N. pungens f. multiseriatus, growth rate was significantly correlated to cellular chlorophyll $a$ ($r = 0.81, n = 6$).

In conclusion, growth and photosynthesis of N. pungens were sensitive to temperature. Optimal growth and photosynthesis of N. pungens f. multiseriatus occurred in the temperature range of 15 - 20°C. Monospecific blooms of N. pungens f. multiseriatus occurred in late fall or winter when the prevailing water temperature was low (-1°C to 3°C). Since the temperature for optimal growth is much higher than this, it is evident that factors other than temperature must have initiated the development of blooms.

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**Reference**