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ENVIRONMENTAL FACTORS CONTROLLING *ALEXANDRIUM TAMARENSE* (DINOPHYCEAE) GROWTH RATE DURING A RED TIDE EVENT IN THE ST. LAWRENCE ESTUARY (CANADA)[1]

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The dinoflagellate *Alexandrium tamarense* (Lebour) Balech 1985 is responsible for recurrent outbreaks of paralytic shellfish poisoning in the St. Lawrence Estuary. In July 1998, an *A. tamarense* red tide developed in the estuary with maximum cell concentrations reaching 2.3×10^6 cells·L^{-1} in brackish surface waters. To estimate the growth rate of these cells, surface water samples from different locations and days during the bloom were incubated for 5 to 9 days under *in situ* temperature and light conditions. Growth rates varied both spatially and temporally between 0 and 0.55 day^{-1}, reaching the maximum growth rate reported for this species in culture. High growth rates were measured even during the peak of the red tide, suggesting that the extremely high cell concentrations observed did not solely result from aggregation or physical concentration but also involved active cellular growth. *Alexandrium tamarense* cells were found over a large range of salinity (20.8–29.5 psu), but high densities and significant growth were only measured when salinity was lower than 24.5 psu. Under these conditions, the number of divisions achieved by *A. tamarense* was proportional to the amount of nitrate available at the beginning of the incubations, whereas variations in growth rate were apparently controlled by the availability of phosphate. We hypothesize that the ability of *A. tamarense* to perform vertical migrations and acquire nitrate at night pushes this species toward phosphate limitation in the St. Lawrence Estuary.

Key index words: ***Alexandrium tamarense*; growth rate; nitrate; phosphate; salinity; St. Lawrence Estuary**

Harmful algal blooms are a recurrent problem in many areas of the world (Hallegraeff 1993). To predict these blooms and therefore their environmental and economic impacts, considerable efforts have been devoted to understanding the factors controlling their development, maintenance, and decline. Despite these efforts, we still know little of the relative importance of the key factors governing these blooms, partly due to the complex interplay between the physical, chemical, and biological factors involved (Anderson 1995). Biophysical models represent appropriate tools to study such complex systems (Franks 1997).

One parameter central to the development of harmful algal bloom models is the specific *in situ* growth rate. Growth rates are easy to determine in the laboratory, but field determinations remain a challenge. The few existing studies have shown that the growth rate of harmful algal bloom species can vary significantly both temporally and spatially during bloom events (Chang and Carpenter 1985, Garcés et al. 1999, Garcés and Maso 2001). The causes for this variability are not known, limiting our ability to distinguish between the role of biology (e.g. growth and loss of cells, vertical migrations) and physics (e.g. horizontal advection, passive accumulation at fronts) in harmful algal bloom development.

In the St. Lawrence Estuary (Canada), blooms of the dinoflagellate *Alexandrium tamarense* are responsible for recurrent paralytic shellfish poisoning outbreaks (Blasco et al. 2003). In this large-scale system, high concentrations of *A. tamarense* are confined to brackish water plumes formed by freshwater input from the

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upper estuary and local rivers (Therriault et al. 1985, Cembella and Therriault 1989, Larocque and Cembella 1990). The presence of *A. tamarense* in these brackish river plumes has been attributed to the well-known preference of dinoflagellates for vertically stable conditions, although the positive effects of river-borne growth factors (e.g. humic substances) cannot be discarded. Weise et al. (2002) reported that windy conditions could prevent the development of *A. tamarense* blooms or cause their dissipation in the St. Lawrence, probably due to an increase in turbulent mixing. The exact cause for the presence of *A. tamarense* in brackish waters and whether the cells are growing more rapidly in these water masses remain unknown.

The specific objective of this study was to determine the spatiotemporal variations in growth rate of *A. tamarense* during a bloom event and to identify environmental factors responsible for the observed variations. This information will be used in the future development of a biophysical model of this toxic dinoflagellate for the St. Lawrence Estuary.

MATERIALS AND METHODS

Study area. The lower St. Lawrence Estuary is a large-scale estuary (30–50 km wide, Fig. 1), and its major bathymetric feature is the Laurentian channel with depths exceeding 300 m (Ingram and El-Sabh 1990). The mesoscale water circulation, strongly influenced by Coriolis effects, is complex and exhibits important spatial and temporal variability (Ingram and El-Sabh 1990, Vézina et al. 1995). In April and May, the freshwater runoff peak induces a drastic decrease in the mean surface salinity of the estuary and the establishment of a strong stratification that persists until the fall (Therriault and Levasseur 1986).

Sampling. Several sampling strategies were used to maximize the number of samples collected for growth rate determination during the bloom event: a weekly or daily sampling at a coastal monitoring station, a 4-day helicopter survey during the peak of the bloom, and a 1-day cruise on the Canadian Coast Guard Ship *Martha L. Black* just after the peak of the bloom.

The development, maintenance, and dissipation of the bloom were followed at the coastal monitoring station of the Maurice Lamontagne Institute (Fisheries and Oceans Canada, Fig. 1) from May to October 1998. Surface water samples were collected with a bucket once a week from the pier of the Institute. The sampling took place at high tide and during daylight hours (for details on the monitoring program, see Blasco et al. 2003). On each sampling day, water temperature and salinity were measured and water samples were collected for nutrient concentration measurements and *A. tamarense* enumeration. When the *A. tamarense* bloom was detected at the monitoring station, the sampling frequency was increased from weekly to daily and water samples were taken for growth rate estimation (described below).

At the peak of the bloom, on 8 and 9 July, additional stations were sampled by helicopter. Surface water samples were collected using a Niskin bottle (General Oceanics, Miami, FL, USA) at stations 32 to 35 on 8 July and at stations 6 to 14, 31 to 33, 35, and 37 to 41 on 9 July (Fig. 1). A last helicopter survey was conducted on 12 July (stations 1 to 14 and 25 to 30). At each station, surface water temperature and salinity were measured and water samples were taken for nutrient concentration measurements, *A. tamarense* enumeration, and growth rate estimation. In addition, a shallow conductivity, temperature, density (CTD) cast was made from the helicopter at every station sampled on 12 July.

Finally, sampling along a south–north transect across the estuary (stations 10 to 24, Fig. 1) was conducted on 13 July on the C.C.G.S. *Martha L. Black*. During this cruise, a CTD cast was made at each station and water samples were collected for the determination of nutrient concentrations and *A. tamarense* enumeration at 2, 5, 10, 15, and 25 m using a rosette equipped with Niskin bottles. In contrast with all previous samplings, this cruise allowed us to determine the vertical distribution of *A. tamarense* during daytime. Because *A. tamarense* cells were always concentrated close to the surface during this transect (data not shown), we are confident that *A. tamarense* surface concentrations measured during this study are representative of maximum concentrations present in the water column.

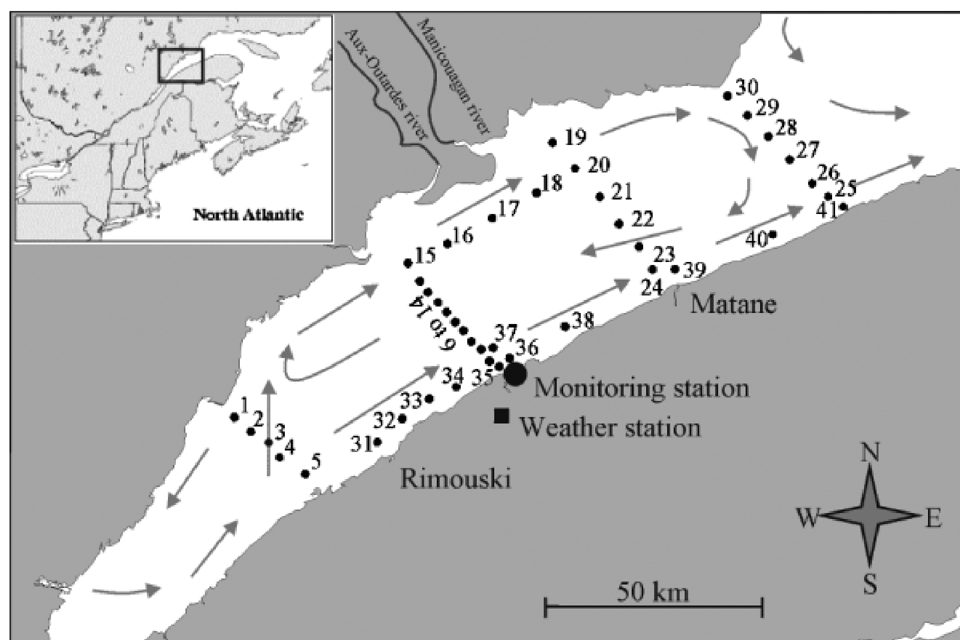

FIG. 1. Location of sampling stations in the St. Lawrence Estuary during summer 1998. The gray arrows indicate the mean summer surface circulation in the Estuary (adapted from El-Sabh 1976). The location of the Environment Canada weather station of Mont-Joli is also indicated.

Salinity and temperature measurements. For the samples collected at the coastal monitoring station, water temperature was measured with a mercury thermometer and salinity with a Guildline salinometer (model Autosal 8400 T, Smith Falls, Ontario, Canada). For stations sampled by helicopter on 8 and 9 July, water salinity and temperature were measured using a salinometer (model LF330, WTW, Weilheim, Germany). For the stations sampled from the helicopter and the ship on 12 and 13 July, water salinity and temperature were obtained from the CTD casts. All instruments used to measure salinity and temperature during this study were intercalibrated.

Nutrient analysis. Subsamples of 5 mL were filtered through 0.7-µm Acrodisc filters (Pall-Gelman, Ann Arbor, MI, USA), frozen at $-80°$ C in acid-cleaned polypropylene cryogenic vials, and analyzed for nitrate + nitrite, nitrite, and phosphate concentrations using a Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY, USA).

Wind measurements. Wind speed and direction were recorded at the weather station of Mont-Joli (Fig. 1) by Environment Canada.

Alexandrium tamarense enumeration and growth rate determination. Water samples were fixed with acid Lugol for later identification and enumeration of *A. tamarense* cells using the Utermöhl technique (Lund et al. 1958). To estimate *A. tamarense* growth rate, 28 incubation experiments were performed with water sampled from the coastal monitoring station and from other stations in the estuary on 7, 8, 9,12, 17, and 20 July. After a gentle prefiltration on a 53-µm mesh (to remove potential grazers of *A. tamarense*), a 2-L volume of water was incubated in polystyrene culture bottles (BD Falcon, Franklin Lakes, NJ, USA) that were placed in an outdoor incubator under temperature and natural light conditions similar to *in situ* conditions. Incubation experiments lasted between 5 and 9 days and were terminated after the end of the exponential growth phase, when *A. tamarense* concentrations were either decreasing or had reached a plateau, except for two experiments that had to be stopped for logistical reasons when the cells were still in the growth phase (station 8 on July 9 and monitoring station on 17 July). For each incubation experiment, the abundance of *A. tamarense* was estimated at the beginning of the experiment and then once a day. A 50-mL subsample of water was taken from each bottle, and cell counts were made in triplicates using a Multisizer™ particle sizer (Coulter, Hialeah, FL, USA) equipped with a 140 × 168-µm orifice. The data were analyzed by AccuComp™ software (Coulter). Because the particle sizer also counts all other species of similar size, this technique tends to overestimate the abundance of *A. tamarense* compared with light microscopy (LM). To estimate this bias, we took a sample at the beginning of each incubation to enumerate *A. tamarense* cells by LM and compared this result with the abundance obtained with the particle sizer. Concentrations measured using the particle sizer were linearly correlated with concentrations estimated by LM ($r^2 = 0.98$; $P < 0.001$). The abundance obtained with the particle sizer was thus corrected with the following equation, which is the regression line between the particle sizer data and the microscopy data:

$$y = 0.65x - 1935 \quad (1)$$

where y and x are *A. tamarense* abundance ($\text{cells} \cdot L^{-1}$) measured with LM and with the particle sizer, respectively. This comparison between the particle sizer and LM was done with samples from the first day of every incubation, which should be representative of the exponential growth conditions when growth rates were measured.

The growth rate of *A. tamarense* during the incubations was calculated during the period of exponential growth following the equation

$$\mu = \text{Ln}(B_t/B_{t0})/(t - t_0) \quad (2)$$

where μ is the growth rate (day^{-1}) and B_{t0} and B_t are *A. tamarense* abundance ($\text{cells} \cdot L^{-1}$) at incubation times t_0 and t (in days), respectively. Cells were enumerated once a day, and because exponential growth lasted only 1 to 3 days in the incubations, our growth rate estimates rely only on two to four consecutive data points.

Estimation of nitrogen and phosphorus required by Alexandrium tamarense *during incubations.* For each incubation, the amounts of nitrogen and phosphorus required to theoretically support the measured increase in *A. tamarense* biomass (N_{req} and P_{req}, µM) were estimated, assuming a constant cellular C:N:P ratio, using a cellular nitrogen quota of 26 $\text{pmol} \cdot \text{cell}^{-1}$ as measured by Levasseur et al. (1995) for an *A. tamarense* strain isolated from the St. Lawrence Estuary, and a cellular phosphorus quota of 2 $\text{pmol} \cdot \text{cell}^{-1}$ (Yamamoto and Tarutani 1999 and a cellular N:P ratio close to the Redfield ratio). The parameters N_{req} and P_{req} were computed following the equations

$$N_{req} = (B_{t\max} - B_{t0}) \times Q_N \quad (3)$$

$$P_{req} = (B_{t\max} - B_{t0}) \times Q_P \quad (4)$$

where B_{t0} is the initial abundance of *A. tamarense* ($\text{cells} \cdot L^{-1}$), $B_{t\max}$ the maximum *A. tamarense* abundance ($\text{cells} \cdot L^{-1}$) measured during the incubation at the end of the growth period, and Q_N and Q_P the cellular nitrogen and phosphorus quotas ($\mu\text{mol} \cdot \text{cell}^{-1}$), respectively.

The nitrogen and phosphorus regeneration rates (R_N and R_P, $\text{nmol} \cdot L^{-1} h^{-1}$) required to sustain *A. tamarense* growth during the incubations were then computed following the equations

$$R_N = (N_{req} - N_{t0})/T_G \text{ and } R_P = (P_{req} - P_{t0})/T_G \quad (5)$$

where N_{t0} and P_{t0} are external nitrate + nitrite and phosphate concentrations (µM) measured at the beginning of the incubations, respectively, and T_G is the length of the growth period, that is, the time interval during which the abundance of *A. tamarense* increased during the incubation (h).

RESULTS

Temporal variations in Alexandrium tamarense *abundance and growth rate.* Temporal variations in *A. tamarense* abundance and growth rate and salinity and wind speed measured at the coastal monitoring station in July and August 1998 are presented in Figure 2. *Alexandrium tamarense* was first detected at this station at the end of May, but its abundance remained low during June (<1000 $\text{cells} \cdot L^{-1}$, data not shown). *Alexandrium tamarense* concentrations increased abruptly to 433,942 $\text{cells} \cdot L^{-1}$ on 7 July (Fig. 2a) and reached 972,400 $\text{cells} \cdot L^{-1}$ 2 days later (with visible discoloration of the surface water). *Alexandrium tamarense* remained present in the estuary until the beginning of August but exhibited considerable day-to-day variations. Large concentrations of *A. tamarense* were always found in low salinity surface waters: 972,400 $\text{cells} \cdot L^{-1}$ and 21.4 psu on 9 July, 262,800 $\text{cells} \cdot L^{-1}$ and 25 psu on 20 July, and 39,960 $\text{cells} \cdot L^{-1}$ and 24.7 psu on 5 August (Fig. 2, a and b). The growth rate of *A. tamarense* also exhibited considerable variability between 7 and 20 July (Fig. 2a). Growth rates were very high during the peak in

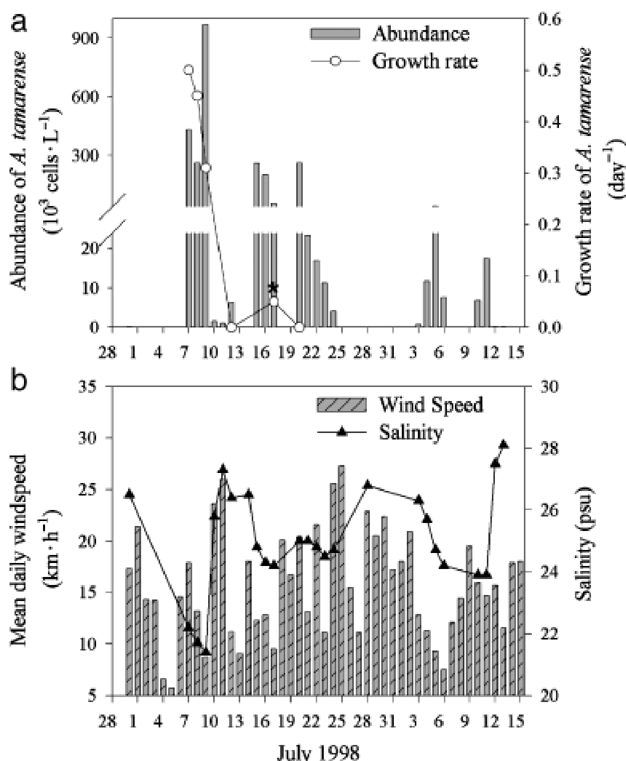

FIG. 2. Temporal variations of (a) *Alexandrium tamarense* abundance and growth rate and (b) salinity and mean daily wind speed at the coastal monitoring station during the period of maximum *A. tamarense* abundance (28 June to 15 August). The asterisk indicates that the value of the growth rate on that day is minimal (see Table 1 for more details).

cell abundance (up to 0.50 day^{-1}, Fig. 2a and Table 1) and became undetectable on 12 July. The decrease in cell abundance and growth rate measured after 10 July corresponded to an increase in surface water salinity from 21.4 to 27.3 psu and in wind speed from 8.7 to 26 km · h^{-1} (Fig. 2b). Temporal variations in surface water salinity at the coastal monitoring station closely followed the variations in wind speed, with salinity always higher than 24–25 psu when wind speed exceeded 20 km · h^{-1} (Fig. 2b). The sharp increase in salinity and decrease in cell number measured on 10 and 11 July corresponded to a strong northeast wind.

Results from the helicopter and ship surveys allowed us to increase the number of growth rate determinations during the peak in cell abundance (8 and 9 July) and after the passage of the wind event when cell abundance was lower (12 and 13 July). As observed at the monitoring station, *A. tamarense* abundance and growth rates were generally higher in the estuary on 8 and 9 July (655,000 cells · L^{-1} on average and up to 0.55 day^{-1}, respectively; Fig. 3, a and b) than after the wind event of 10 and 11 July (34,261 cells · L^{-1} on average and almost no growth; Fig. 3, d and e, and Table 2). Surface water salinities in the estuary showed the same pattern as those at the monitoring station, with values lower and higher than 24 psu before and after the wind event, respectively. Only two stations located close to the north shore exhibited salinity below 24 psu after the wind event (Table 2), and interestingly, one of these is the only station where *A. tamarense* grew on 12 July (0.49 day^{-1} at station 1; Fig. 3e and Table 2).

Alexandrium tamarense *growth rate variability: the importance of salinity.* Results from the incubation experiments are presented in Table 1 for the coastal monitoring station and Table 2 for stations sampled during the helicopter survey. As can be seen in Figure 4, which includes all measurements (monitoring and surveys), high abundances of *A. tamarense* were only found in high temperature (>10° C) low salinity (<26 psu) waters. *Alexandrium tamarense* growth rates exhibited a similar relationship with salinity (Fig. 4c); significant growth occurred only at stations where salinity was below 24.5 psu. The only exception is station 10 (9 July), where surface water salinity was 23.3 psu and *A. tamarense* did not grow (Table 2). The absence of growth at high salinity does not result from the absence of *A. tamarense* cells in the incubations or from very scarce seeding populations. Initial concentrations of *A. tamarense* in nongrowing

TABLE 1. Incubation experiments at the coastal monitoring station: initial conditions and results of *Alexandrium tamarense* growth.

Dates	Salinity (psu)	Temperature (° C)	Initial NO_3^- (μM)	Initial PO_4^{3-} (μM)	NO_3^- : PO_4^{3-}	Initial biomass (cells · L^{-1})	Growth rate (day^{-1})	Number of divisions (growth period[a])	Growth curve
7 July	22.2	14.8	0.2	0.75	0.3	433,942	0.50	1.4 (2)	a
8 July	21.7	14.5	1.4	0.28	5.1	262,200	0.45	1.7 (3)	b
9 July	21.4	13.0	n.a.	0.17	—	972,400	0.31	1.2 (3)	b
12 July	26.4	11.0	7.1	1.11	6.4	6360	0	—	—
17 July	24.2	16.2	0.1	0.30	0.2	55,000	0.05 ?[b]	—	—
20 July	25.0	15.5	0.1	0.27	0.4	262,800	0	—	—

The growth curves a and b refer to Figure 5 (see text for details).

[a]The growth period (i.e. the number of days during which an increase in *A. tamarense* abundance was observed in incubations) is indicated in parentheses for each incubation.

[b]We do not have a precise estimation of this growth rate because *A. tamarense* concentration was only measured on the first and the fourth days of incubation. The absolute value of this growth rate is not considered here; however, it is worth noting that *A. tamarense* cells sampled at the coastal monitoring station on that day were able to grow in incubation.

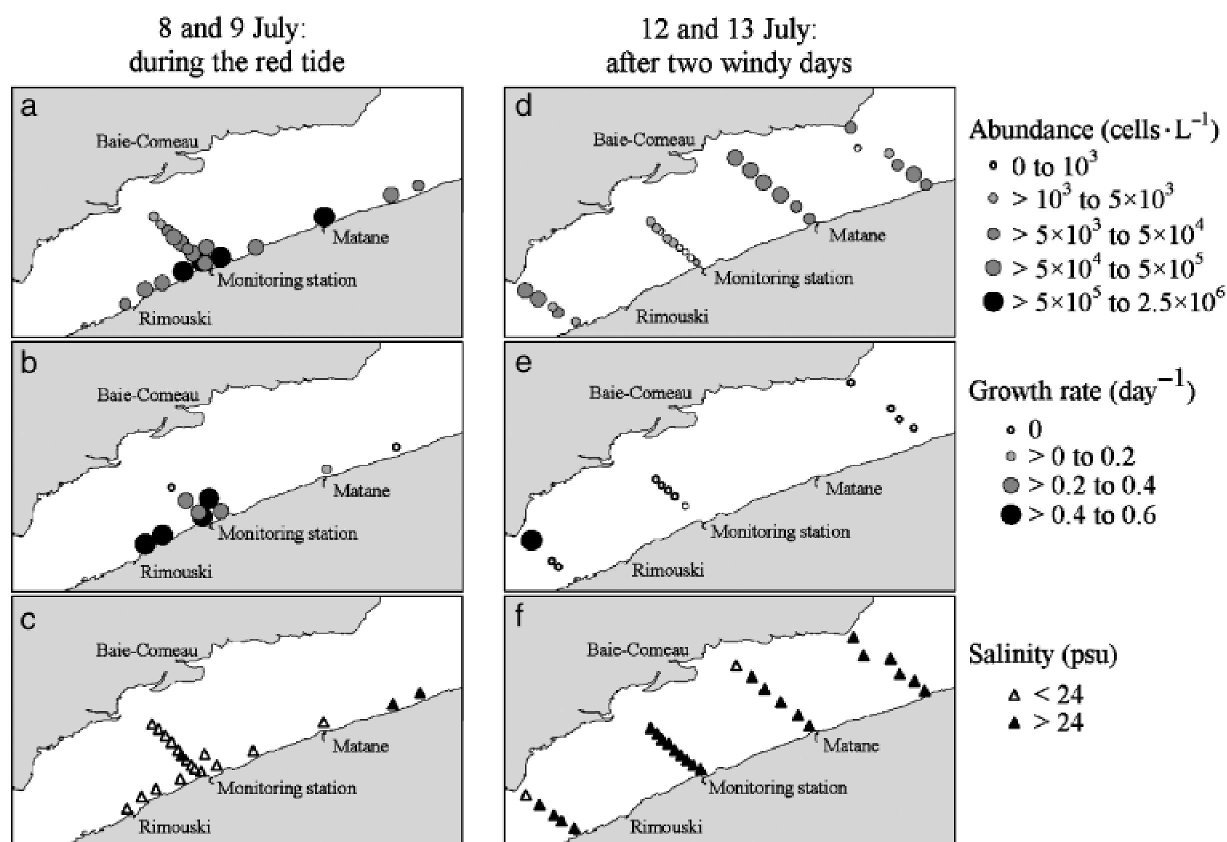

FIG. 3. Spatial distribution of (a, d) *Alexandrium tamarense* abundance, (b, e) *A. tamarense* growth rate, and (c, f) salinity in surface waters of the St. Lawrence Estuary during the red tide on 8 and 9 July and on 12 and 13 July, after two windy days. The mean wind speed during the previous 2 days (10 and 11 July) was $24.8\,km \cdot h^{-1}$.

incubations were always above $400\ cells \cdot L^{-1}$, and half of the incubations had more than $5000\ cells \cdot L^{-1}$. Furthermore, initial concentrations of nitrate and phosphate were not lower in higher salinity waters (Tables 1 and 2).

Alexandrium tamarense *growth rate variability: the influence of nutrients.* During the incubations where positive growth of *A. tamarense* was detected, the variations in cell numbers exhibited two different patterns. The growth curves presented in Figure 5, a and b, are representative of these patterns. During four incubations (Tables 1 and 2), the abundance of *A. tamarense* increased until it reached a plateau that persisted several days (e.g. station 32 on 8 July, Fig. 5a). During seven incubations (Tables 1 and 2), *A. tamarense* concentrations decreased just after the exponential growth phase (e.g. station 36 on 8 July, Fig. 5b). There was no difference in salinity, temperature, initial nitrate concentration, initial phosphate concentration, nitrate-to-phosphate ratio, or *A. tamarense* initial concentrations between the two types of growth curves (Tables 1 and 2). The difference between incubations where *A. tamarense* abundance remained high and those where it decreased cannot be explained with our present data. During the exponential growth phase of the incubations where *A. tamarense* growth was measured, variations in growth rates were not related to salinity ($r^2 = 0.13$, $P = 0.26$) or to initial nitrate concentrations ($r^2 = 0.04$, $P = 0.69$) but exhibited a nonlinear Michaelis-Menten type relationship to the initial phosphate concentration (Fig. 6, $r^2 = 0.58$, $P < 0.05$). Growth rates increased with increasing initial phosphate concentration up to 0.4 μM and then tended to level off at around $0.5\ day^{-1}$. Interestingly, the total number of divisions that the cells performed during the incubations was a linear function of the amount of nitrate present at the beginning of the incubations (Fig. 7, $r^2 = 0.96$, $P < 0.001$). These results suggest that when growth occurred (at salinity below 24.5 psu), phosphate concentration controlled the growth rate of *A. tamarense* cells whereas nitrate limited the number of division that the cells could achieve during the incubations.

Nitrogen and phosphorus budget. Nitrate and phosphate concentrations were low at the beginning of the incubations (Tables 1 and 2) and could only explain 1% to 28% of N_{req} and 5% to 44% of P_{req}. Clearly, initial nitrate and phosphate concentrations cannot explain the large increases in *A. tamarense* biomass measured. Between 4 and $64\,nmol\ P \cdot L^{-1} \cdot h^{-1}$ should have been regenerated to fulfill *A. tamarense*

TABLE 2. Incubation experiments at different stations sampled by helicopter in the estuary: initial conditions and results of *Alexandrium tamarense* growth.

Dates	Station	Salinity (psu)	Temperature (° C)	Initial NO_3^- (μM)	Initial PO_4^{3-} (μM)	NO_3^- : PO_4^{3-}	Initial biomass (cells · L^{-1})	Growth rate (day^{-1})	Number of divisions (growth period[a])	Growth curve
8 July	35	21.6	12.9	1.1	0.26	4.2	458,400	0.47	1.7 (4)	b
8 July	33	21.3	14.6	1.7	0.34	4.9	112,000	0.55	1.7 (4)	a
8 July	32	21.1	14.6	n.a.	n.a.	—	253,200	0.44	1.2 (4)	a
8 July	36	23.0	12.3	n.a.	n.a.	—	195,200	0.45	2.1 (4)	b
9 July	37	21.7	13.0	0.3	0.25	1.1	2,332,804	0.32	1.3 (3)	a
9 July	39	22.6	13.7	n.a.	0.16	—	1,812,186	0.20	0.4 (2)	b
9 July	6	20.8	12.1	2.8	0.36	7.8	223,600	0.38	1.9 (5)	b
9 July	8	24.5	12.2	n.a.	n.a.	—	145,600	0.29	—	—
9 July	41	25.1	14.8	0.3	0.20	1.5	54,600	0	—	—
9 July	10	23.3	11.1	6.9	0.64	10.7	51,480	0	—	—
12 July	1	22.0	12.8	4.7	0.54	8.6	332,000	0.49	2.4 (4)	b
12 July	12	24.4	12.2	2.0	0.41	5.0	960	0	—	—
12 July	13	24.6	12.3	2.1	0.41	5.1	1240	0	—	—
12 July	10	24.9	12.3	1.7	0.42	4.0	3920	0	—	—
12 July	11	24.6	12.1	2.1	0.42	5.0	2160	0	—	—
12 July	9	25.4	11.7	1.4	0.72	2.0	400	0	—	—
12 July	4	25.7	9.3	11.2	0.83	13.5	11,520	0	—	—
12 July	3	25.4	9.3	11.2	0.88	12.7	3560	0	—	—
12 July	26	26.3	12.3	1.9	0.41	4.6	58,120	0	—	—
12 July	27	27.9	13.7	0.1	0.18	0.6	6360	0	—	—
12 July	28	28.3	13.8	0.2	0.25	0.6	1200	0	—	—
12 July	30	27.9	14.8	0.1	0.28	0.3	17,600	0	—	—

The growth curves a and b refer to Figure 5 (see text for details).
[a]The growth period (i.e. the number of days during which an increase in *A. tamarense* abundance was observed in incubations) is indicated in parentheses for each incubation.

phosphorus requirements (Table 3), whereas N_{req} imply nitrogen regeneration rates (R_N) of 64 to 875 nmol $N \cdot L^{-1} \cdot h^{-1}$ (Table 3). These rates are minimum values because they do not take into account the phosphorus and nitrogen requirements of non-*Alexandrium* cells present in the incubations. However, N_{req} could also be overestimated because they do not take into account a possible decrease in *A. tamarense* cellular nitrogen quotas under nitrogen limitation.

DISCUSSION

Here we present the first estimates of *A. tamarense* growth rates for natural populations under *in situ* conditions for the St. Lawrence Estuary. During the incubations, *A. tamarense* growth rates varied between 0 and 0.55 day^{-1}. The highest rates are in agreement with previous growth rates determined in culture for *A. tamarense* strains from the St. Lawrence Estuary (Levasseur et al. 1995, MacIntyre et al. 1997, Parkhill and Cembella 1999) and show that *A. tamarense* can reach high growth rates *in situ*. It is interesting to note that growth occurred even during the peak of the red tide when 2.3×10^6 cells · L^{-1} divided at least once during our incubations. These results indicate that the extremely high cell concentrations did not solely result from aggregation or physical concentration but also involved active cellular growth. As previously reported during other field experiments (Chang and Carpenter 1985, Garcés et al. 1999, Garcés and Maso 2001), the growth rates of *A. tamarense* cells were highly variable during the bloom, both temporally and spatially.

The relationship between Alexandrium tamarense *and salinity in the St. Lawrence Estuary.* Most of the variability in cell abundance was related to changes in surface water salinity, with *A. tamarense* cells found mainly in waters of salinity below 26 psu. More interestingly, *A. tamarense* growth was only detected in low salinity surface waters (<24.5 psu). Surface salinities below 26 psu in summer in the studied area of the St. Lawrence Estuary typically mark the presence of freshwater plumes from the Manicouagan and Aux-Outardes Rivers (Therriault and Levasseur 1986) located on the north shore (Fig. 1). The presence of *A. tamarense* has been previously associated with river runoff in the estuary and the gulf of St. Lawrence (Therriault et al. 1985, Weise et al. 2002), but this is the first demonstration that growth of *A. tamarense* also occurs in these plumes. During a laboratory culture experiment, Parkhill and Cembella (1999) reported no significant difference in the growth rate of an *A. tamarense* strain isolated from the St. Lawrence Estuary at salinities between 20 and 30 psu. It is thus unlikely that the inhibition of growth observed at salinities above 24.5 psu would reflect a direct physiological effect of salinity on *A. tamarense* growth. The inhibition of growth at high salinity may, however, result either from the absence of a growth factor or, assuming that increases in salinity may reflect recent vertical mixing, from the negative effect of turbulence on *A. tamarense* growth.

The stimulating effect of river-borne humic substances on dinoflagellate growth is well known (Granéli and Moreira 1990, Doblin et al. 1999), and recent

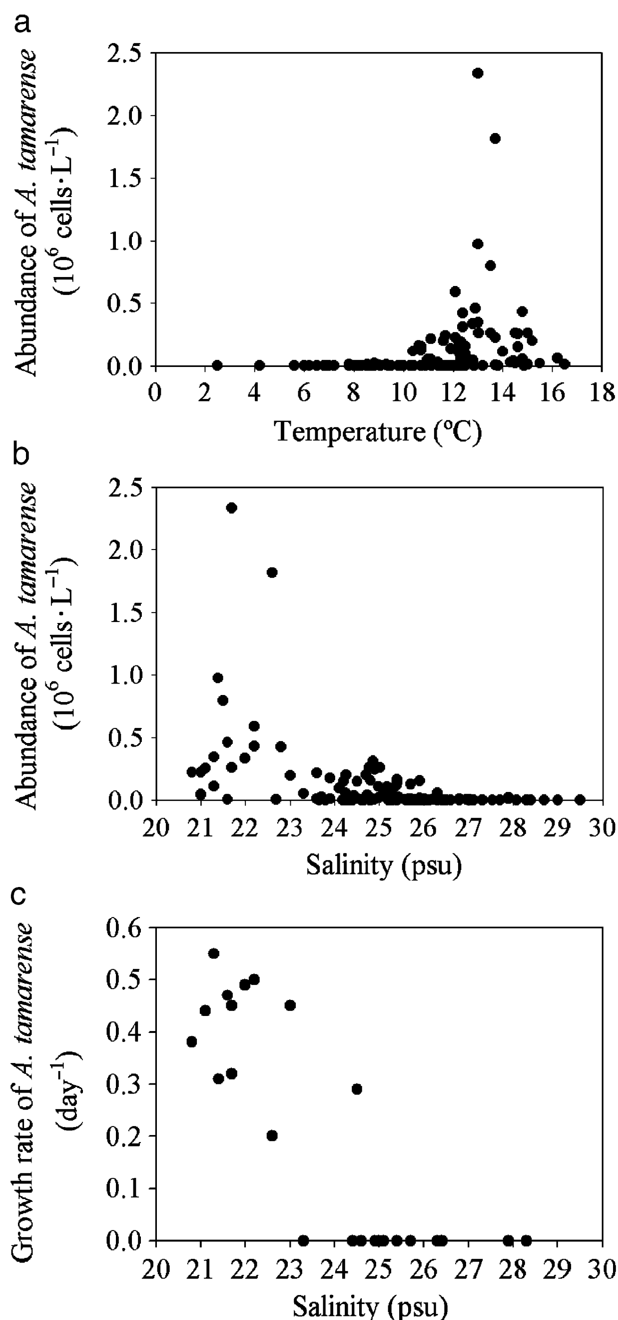

FIG. 4. Abundance of *Alexandrium tamarense* as a function of (a) temperature and (b) salinity, and (c) growth rate of *A. tamarense* as a function of salinity for the coastal monitoring station and other stations in the estuary during summer 1998.

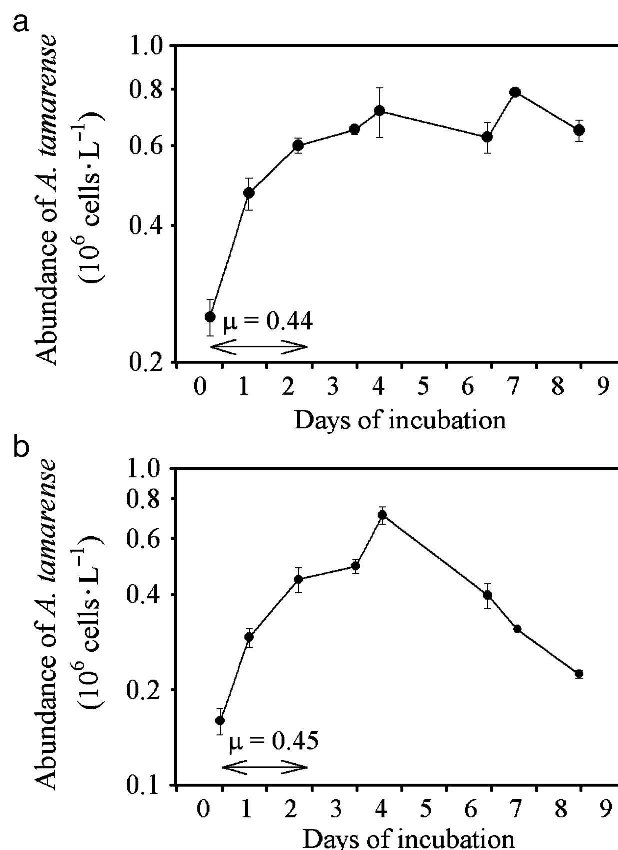

FIG. 5. Temporal evolution of the abundance of *Alexandrium tamarense* during two incubation experiments: (a) station 32 on 8 July and (b) station 36 on 8 July. The growth rate (μ, day^{-1}) is indicated for each incubation. The arrows indicate the periods over which the growth rates were calculated. The error bars represent the SD between triplicate counts determined using the particle sizer.

work from our laboratory demonstrated that humic substances extracted from the Manicouagan River on the north shore of the estuary (Fig. 1) stimulate the growth of a local *A. tamarense* strain in culture (Gagnon et al. 2005). It is thus possible that a lack of humic substances (or another river-borne growth-promoting compound) was responsible for the absence of *A. tamarense* growth at salinities higher than 24.5 psu.

During our study, the sharp increase in surface water salinity measured on 10 July coincided with a wind event, suggesting that wind mixing could be responsible for the change in salinity. The fact that the increase in surface salinity was observed almost all over the estuary on 12 July supports this interpretation rather than advection of a new water mass. Several laboratory experiments showed that the growth of many dinoflagellate species, including *A. tamarense*, could be negatively affected by turbulence (White 1976, Thomas and Gibson 1990, Berdalet 1992, Juhl et al. 2001, Juhl and Latz 2002). Field confirmation of this sensitivity to turbulence is scarce, but some studies have also reported a wind speed threshold above which dinoflagellate blooms cannot develop or are destroyed: 28 km · h^{-1} for *A. tamarense* blooms in the Gulf of St. Lawrence (Weise et al. 2002), 25 km · h^{-1} for *A. tamarense* in Spain (Blanco et al. 1985), and 18 km · h^{-1} for *Gymnodinium catenatum* in Tasmania (Hallegraeff et al. 1995). These values are close to the threshold of 20 km · h^{-1} suggested by the results of the present study. These results suggest that the turbulence

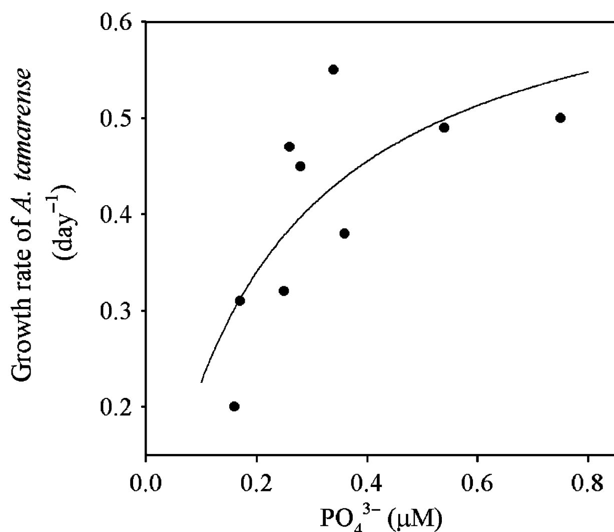

FIG. 6. Growth rate of *Alexandrium tamarense* as a function of the phosphate concentration at the beginning of the incubation experiments. The curve represents the regression between both variables, with a Michaelis-Menten function: $y = 0.69\, x/(0.21 + x)$ ($r^2 = 0.58$, $P < 0.05$).

produced by northeast winds exceeding $20\,\text{km} \cdot \text{h}^{-1}$ could be sufficient to erode and deepen the halocline and inhibit the growth of *A. tamarense* in the St. Lawrence Estuary. The proposed wind-induced turbulence hypothesis to explain the absence of growth in high salinity waters is, however, challenged by some of our observations. Inhibition of *A. tamarense* growth at high salinity persisted for several days (5–9) in the absence of turbulence during our incubations, suggesting that several calm days are required for *A. tamarense* to recover after a wind event. This seems too long a time, considering that physiological acclimation of phytoplankton to environmental conditions may occur on an hourly scale (e.g. light regime) (Cullen and Lewis 1988). Also challenging the wind-induced turbulence hypothesis are the recent laboratory results of Sullivan and Swift (2003) showing that the growth rate of two clones of *A. tamarense* was unaffected by turbulence as encountered by the cells in the field. Whether the inhibition of *A. tamarense* growth during our incubations conducted with saltier waters resulted from a limiting concentration of a river-borne growth-promoting factor or from an increase in turbulent mixing cannot be resolved with our current data set.

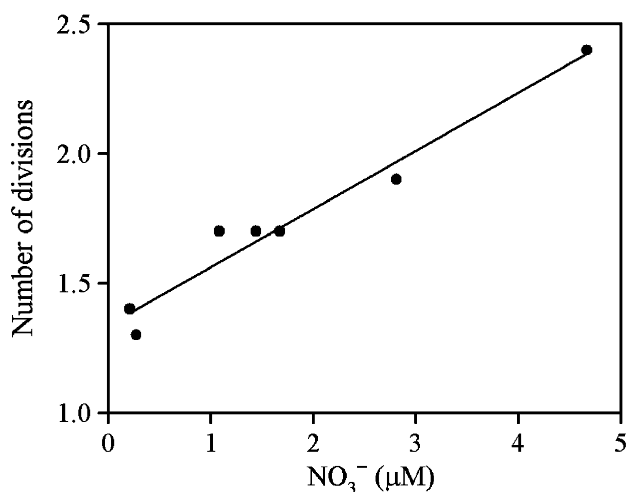

FIG. 7. Number of divisions completed by *Alexandrium tamarense* during the incubations as a function of the nitrate concentration at the beginning of the incubations. The solid line represents the linear regression between variables ($r^2 = 0.96$, $P < 0.001$).

Nutrients and Alexandrium tamarense *growth in the brackish waters of the St. Lawrence Estuary.* Our results suggest that in the brackish water plume where *A. tamarense* thrives in the St. Lawrence Estuary, the bloom dynamics are controlled by both phosphate and nitrogen. We first demonstrated that the growth rate of *A. tamarense* was limited by phosphate availability. Our calculations show that the initial amounts of phosphate present in the incubation bottles were not sufficient to explain the increase in *A. tamarense* biomass. On the other hand, our calculations show that *A. tamarense* in the incubators could have grown, albeit not at maximum rates (see below), on regenerated phosphate. Phosphate regeneration rates required to support the observed *A. tamarense* growth during the incubations are realistic: most of them are in the same order of magnitude as those reported by Harrison (1983) for the Bedford Basin (Eastern Canada, up to $5.75\,\text{nmol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$) and by Sorokin (2002) for the sea of Okhotsk (Russia, up to $3.9\,\text{nmol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$). However, the relationship between phosphate and the growth rate of *A. tamarense* evidenced during our study suggests that phosphate regeneration rates may be insufficient to fulfill the phosphorus demand of this species in the St. Lawrence Estuary.

The nitrate-to-phosphate ratio in surface waters where *A. tamarense* grew was between 0.2 and 8.6, always below the Redfield ratio ($\text{N:P}_{\text{Redfield}} = 16$), indicating that nitrate was also in short supply for phytoplankton cells in surface waters. This is clearly demonstrated by our nitrogen budget showing that the amount of nitrogen needed to support the sole production of *A. tamarense* cells in the incubations was up to 210 times higher than the initial nitrate concentrations. Ammonium concentrations were not measured during this study, but concentrations higher than 2 μM have rarely been measured in the St. Lawrence Estuary (Therriault and Levasseur 1985, Zakardjian et al. 2000). The contribution of the ammonium present at the beginning of the incubations was thus probably minimal. Urea, another potential nitrogen source, is not considered here, because local strains of *A. tamarense* seem not to be able to grow on urea (Levasseur et al. 1995). There is no information on ammonium regeneration in the surface layer of the St. Lawrence Estuary, but we calculated that ammonium regeneration rates required to support the increase in *A. tamar-*

TABLE 3. Nitrogen and phosphorus requirements for measured *Alexandrium tamarense* growth during incubations and calculated nitrogen and phosphate regeneration rates necessary to meet these requirements.

Station	Dates	Nitrogen requirement (μM)	Required nitrogen regeneration rate ($nmol \cdot L^{-1} \cdot h^{-1}$)	Phosphorus requirement (μM)	Required phosphate regeneration rate ($nmol \cdot L^{-1} \cdot h^{-1}$)
MS	7 July	22	302	1.7	13
35	8 July	22	217	1.7	15
33	8 July	10	89	0.8	5
MS	8 July	14	107	1.1	7
37	9 July	63	875	4.9	64
6	9 July	11	64	0.8	4
MS	9 July	38	—	2.9	38
1	12 July	26	222	2.0	15

MS, monitoring station.

ense biomass are 3 to 35 times higher than the greatest rates reported for marine ecosystems at similar latitudes (Maguer et al. 1998, 2000, Diaz and Raimbault 2000). Overall, our results and calculations suggest that *in situ* nitrogen concentrations and regeneration rates were probably not sufficient to explain the increase in *A. tamarense* biomass during the incubations. We thus hypothesize that the missing nitrogen comes from internal nitrate pools accumulated at night during vertical migrations.

There is increasing evidence that *A. tamarense* strains from the St. Lawrence Estuary perform diel vertical migrations. Results from microcosm experiments demonstrated the ability of an *A. tamarense* strain from this region to perform diel vertical migrations under nitrogen-limiting conditions to acquire nitrate at night (MacIntyre et al. 1997). More recently, we demonstrated that such diel vertical migrations could take place in the field when nitrate concentrations are low in surface waters (unpublished results). Dinoflagellates can transiently accumulate intracellular nitrogen pools under situations where nitrate inputs occur sporadically, allowing them to buffer the variability of nitrogen supply by utilizing internal nitrogen pools when external nitrogen concentrations are low (Dortch 1982, Dortch et al. 1985, Bode et al. 1997). Interestingly, the number of divisions completed by *A. tamarense* during our incubations (between 0.4 and 2.4) is in agreement with a previous study by Dortch et al. (1984), showing that a dinoflagellate species could store enough nitrogen to complete 1.3 divisions without an external nitrogen supply. *Alexandrium tamarense* could thus probably store transient internal nitrogen pools when migrating down to the nitracline at night during calm conditions. Therefore, the missing nitrogen in the incubation budgets might come from intracellular nitrogen pools that *A. tamarense* cells would have accumulated at depth before the sampling. In our incubations, the cells probably stopped growing when intracellular nitrogen pools and external nitrate were exhausted.

In conclusion, we demonstrated that important variations in *A. tamarense* growth rate might occur in the St. Lawrence Estuary during bloom events, variations that will need to be taken into account in the development of biophysical models for this toxic species. Significant growth was only measured in low salinity waters (<24.5 psu), providing additional support to the hypothesis that freshwater runoff is crucial for the development of this toxic dinoflagellate in the St. Lawrence Estuary. The inhibition of growth at high salinity remains unexplained but could result either from the absence of a growth factor (e.g. humic substances) or from the negative effect of turbulence. In incubations from the brackish waters, the number of divisions achieved by *A. tamarense* was proportional to the amount of nitrate available at the beginning of the incubation, whereas variations in growth rate were apparently controlled by the availability of phosphate. We hypothesize that the ability of *A. tamarense* to perform vertical migrations and acquire nitrate at night pushes this species toward phosphate limitation in the St. Lawrence Estuary. Thus, phosphate regeneration rates could dictate the growth rate of *A. tamarense* in the St. Lawrence Estuary, whereas the duration of the low wind periods and the ability of *A. tamarense* to perform vertical migrations under these conditions might determine how much biomass can be reached for this species in the estuary.

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