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On the nature of *Alexandrium fundyense* blooms in the Gulf of Maine

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Abstract

Blooms of the toxic dinoflagellate, *Alexandrium fundyense*, are a common feature during the summer months in the Gulf of Maine, potentially resulting in paralytic shellfish poisoning when human beings consume shellfish that have ingested these dinoflagellates. Factors that control the dynamics of offshore blooms, including their timing, distributions and cell densities were investigated on three research cruises in the Gulf of Maine: 25 April to 3 May and 5–14 June, 2000, and 19–28 July, 2001; additional samples were collected by our colleagues on separate cruises in May and June, 2001. Measurements included hydrographic data, concentrations of phytoplankton chlorophyll, inorganic nutrients, cell densities of *Alexandrium* at standard depths, and near-surface densities of major phytoplankton taxa. The *Alexandrium* bloom in 2000 began sometime between the April–May cruise, when we observed low *Alexandium* cell densities (<200 cells L^{-1}), and June, when broad patches of >1000 cells L^{-1} were observed. In July of 2001 we observed high cell densities of *Alexandrium* ($>10{,}000$ cells L^{-1}), which were most abundant at subsurface depths. Vertical pump samples collected at 1-m resolution in July 2001 revealed high densities of *Alexandrium* cells in thin layers at depths corresponding to the pycnocline and nutricline. We present evidence that the distributions, abundances and timing of onset of the seasonal *Alexandrium* bloom may be related to oceanographic processes that control differences in the relative concentrations of dissolved inorganic nitrogen (DIN) and silicate. While by no means conclusive, results are suggestive of an allelopathic interference by diatoms on *Alexandrium* growth, which might impede the development of high densities of *Alexandrium* cells.

Keywords: *Alexandrium*; Red tide; Nutrients; Diatoms; Thin layers; Allelopathy

1. Introduction

The Gulf of Maine is subject to periodic outbreaks of paralytic shellfish poisoning (PSP), which occur when people consume molluscan shellfish that have ingested the toxic dinoflagellate *Alexandrium* spp. (Anderson, 1997). While the immediate pubic health concern from PSP usually involves consumption of intertidal shellfish, our recent ECOHAB research results indicated that blooms of *Alexandrium* spp. in the Gulf of Maine are most prominent in offshore waters (Townsend et al., 2001; Keafer et al., 2005). In our earlier study of the 1998 summer *Alexandrium* bloom (Townsend et al., 2001), we showed that patches of high cell densities in offshore waters were related to ambient light levels and

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hydrographic conditions that promoted elevated concentrations and/or flux rates of dissolved inorganic nitrogen. By parameterizing light and nutrients as a ratio of water clarity to the depth of a critical dissolved inorganic nitrogen concentration, and correcting for advection, we were able to explain the locations of two expansive *Alexandrium* patches in the Gulf: one in the mouth of the Bay of Fundy and a second patch in waters associated with the eastern Maine Coastal Current.

Our fundamental observation in 1998 was perhaps our most significant: that highest cell densities were well offshore, away from most coastal shellfish beds, and in agreement with earlier preliminary observations reported by Martin and White (1988). With few exceptions, we observed that *Alexandrium* spp. populations appeared to be restricted to waters associated with the eastern Maine coastal current (EMCC), which throughout much of its length lies beyond the coastal boundary layer, and beyond the strongest influence of coastal freshwater discharge. Those results helped place in an oceanographic context earlier observations of coastal PSP, such as the ''sandwich'' phenomenon described by Hurst and Yentsch (1981), Yentsch et al. (1986), and Shumway et al. (1988), in reference to the usual absence of PSP along a stretch of coastline between western Penobscot Bay and the area east of Mount Desert Island (Fig. 1). We now suspect that this void in PSP is coincident with the location of the offshore–directed flow of the EMCC (Brooks and Townsend, 1989; Bisagni et al., 1995; Pettigrew et al., 1998), which carries with it the highest *Alexandrium* cell densities. Of course, cells in these offshore high-density patches must be delivered to coastal waters in order to present PSP problems; the problem of cell transport to inshore waters is addressed by Hetland et al. (2003), McGillicuddy et al. (2003), and several papers in this issue (Luerssen et al., 2005; Keafer et al., 2005; McGillicuddy et al., 2005a,b).

Results from Townsend et al. (2001) also demonstrated that subsurface (20 m) cell densities of *Alexandrium* spp. (on the order $10–100 \times 10^3$ cell L^{-1}) are often greater than near-surface cell densities, but the vertical sample resolution employed (a surface sample at 1–2 m, and a second sample at either 20 m or the depth of the subsurface

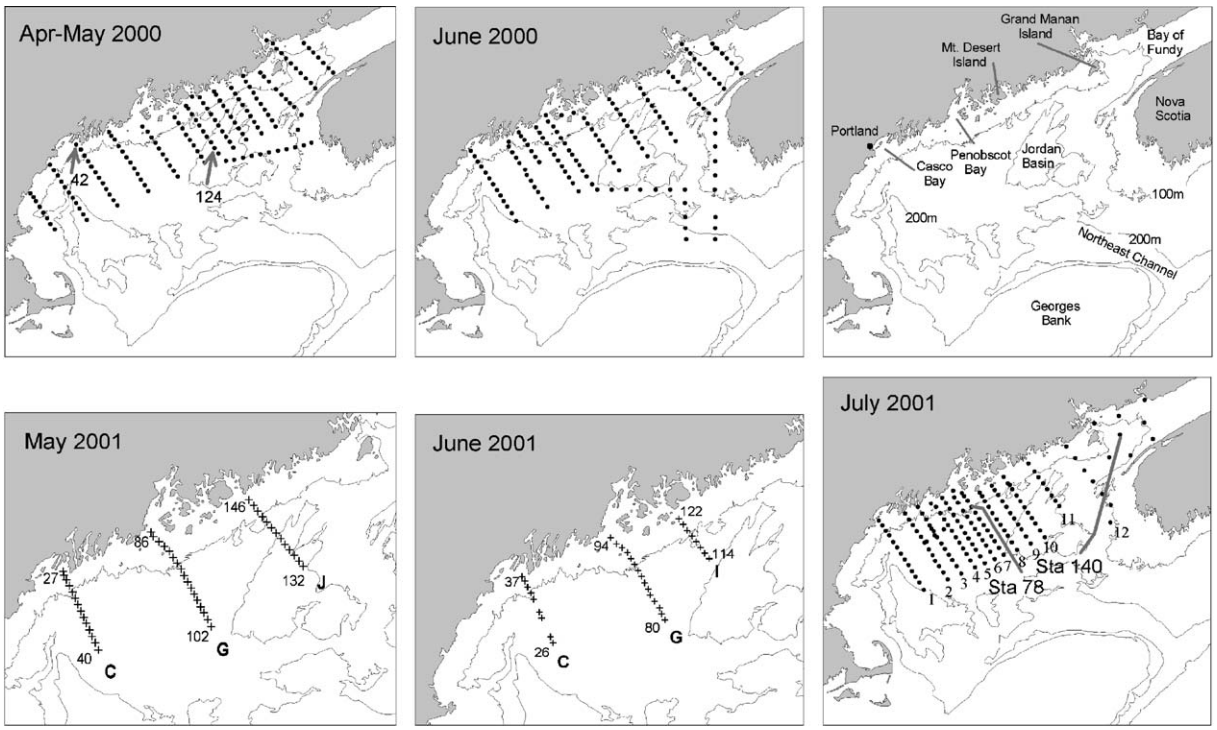

Fig. 1. Station locations for each of the cruises reported on here, as indicated, along with a map of the region and locations referred to in the text. The beginning and ending station numbers for each transect are indicated for the May and June 2001 samples. The two pump stations sampled in July 2001 are indicated (Stations 78 and 140), as are the transect numbers referred to in the text.

chlorophyll maximum layer) did not allow close examination of vertical distributions in relation to hydrographic structure or nutrient levels. For example, we were unable to determine the relationship between *Alexandrium* subsurface distributions and details of frontal features associated with the high-nutrient waters of the EMCC during the 1998 season (Townsend et al., 2001).

The purpose of this communication is to report on follow-up studies conducted in 2000 and 2001 that focused on unanswered questions and otherwise interesting observations from our 1998 surveys, such as details of *Alexandrium* vertical distributions. In addition, our 1998 results left unanswered questions about the timing of onset of the summer bloom in offshore waters. It already has been reported that inshore *Alexandrium* populations in the Casco Bay area of the Gulf of Maine begin to develop as early as April (Anderson, 1997; McGillicuddy et al., 2003), but our first cruise in 1998 was in mid-June, at which time we observed an offshore bloom that was already well developed. Thus, we could not be certain of how the seasonal bloom offshore commenced in relation to the spring–summer oceanographic transition and the annual spring phytoplankton bloom in the Gulf of Maine without repeating cruises earlier in the spring. Finally, we were interested in examining the possibility that both the timing and areal distributions of high densities of *Alexandrium* cells might be somehow correlated with the annual spring diatom bloom and diatom populations that persist in coastal waters following the spring bloom; e.g., we questioned whether *Alexandrium* blooms might be related to a species succession indirectly controlled by variable concentrations and flux rates of dissolved inorganic nitrogen and silicate, as had been shown for diatoms and dinoflagellates on the nearby Georges Bank (Townsend and Thomas, 2001, 2002). We report here the results of research cruises in 2000, designed to capture the important elements of this seasonal transition, and the following year (2001), during which we focused on discerning in greater detail than in 1998 the horizontal and vertical distributions of *Alexandrium* spp. in relation to the hydrographic and nutrient fields.

2. Methods

We conducted oceanographic surveys of the coastal and offshore waters of the northern Gulf of Maine from New Hampshire to the outer Bay of Fundy during two research cruises in the spring and summer of 2000 (25 April–3 May and 5–14 June) and a third cruise in summer of 2001 (19–28 July), each aboard the R/V *Cape Hatteras*; we refer to these three cruises as our main cruises. We also analyzed and report on nutrient and phytoplankton samples collected for us by our colleagues on two additional cruises in May and June of 2001 (discussed in Keafer et al., 2005).

Standard hydrocasts were made at the stations shown in Fig. 1 on our main cruises using a SeaBird CTD with a Wet Labs in situ fluorometer and SeaBird carousel water sampler equipped with 5 L Niskin bottles. The CTD package was lowered to within 5 m of the bottom at all survey stations and water was collected during the upcast. Water samples were collected for analyses of phytoplankton chlorophyll (fluorometric analyses of acetone extracts of particulate material collected from 100 mL on GF/F glass fiber filters; Parsons et al., 1984) and inorganic nutrients. Nutrient samples, taken at depths between the surface and near-bottom, were filtered through Millipore HA filters, placed immediately in a sea water–ice bath for 5–10 min, and frozen at $-18^{\circ}C$ to be analyzed following the cruises for $NO_3 + NO_2$, NH_4, $Si(OH)_4$ and PO_4 using an autoanalyzer and standard techniques. Water samples for the enumeration of *Alexandrium* spp. cell densities were collected from standard depths of 1, 10, 20, 30, and 50 m during the 2000 cruises (April–May, and June 2000); subsequently we moved the 50 m sampling depth to 40 m for the July 2001 cruise. Enumeration of *Alexandrium* spp. cell densities was performed by sieving 2 L of water from each sample depth through a 20-μm mesh screen; the concentrate was preserved in a 5% formaldehyde sea water solution and stored in 20-mL vials in the dark in a refrigerator. Quantitative cell counts were performed within 14 months of collection, and were based on epifluorescence microscopy and an immunological stain specific to the genus *Alexandrium*, based on the method of Adachi et al. (1993). Slides were prepared by drawing a subsample of the formalin-preserved, 20-μm-seived sample through a 5-μm Nucleopore filter. The particles retained on the filter were stained in a two–step process. First, the filters were incubated in a solution containing a primary antibody specific to *Alexandrium* spp. cell surface proteins, M8751 (MAB); the antibody was provided by D.M. Anderson, Woods Hole Oceanographic

Institution. The filters were then incubated with a secondary antibody that was bonded with FITC, a fluorescent molecule. The filters were washed free of excess antibody and placed on a microscope slide; a drop of 80% glycerin was applied to the filter that was then covered with a cover slip. This procedure makes *Alexandrium* spp. cell surface proteins fluoresce green when excited by light of 494 nm wavelength, thus easing microscopic identification and enumeration. The entire area under the cover slip was counted using a Nikon epifluorescent microscope at a magnifications of 100× or 200×. All slides were counted within four days of being prepared, as the fluorescence fades over time.

Three toxic species of the dinoflagellate genus *Alexandrium* have been identified in the Gulf of Maine: *Alexandrium tamarense, A. fundyense,* and *A. ostenfeldii* (Anderson, 1997; Gribble et al., 2005). *A. ostenfeldii* is a larger cell than either *A. tamarense* or *A. fundyense* (Tomas, 1997), and both *A. ostenfeldii* and *A. tamarense* are significantly less abundant than *A. fundyense*. We did not distinguish among these three species, and it is likely that the vast majority of the cells we counted in this study are *A. fundyense*; therefore, despite potential uncertainties, we refer to cells identified with the immunofluorescent stain as *A. fundyense*, or simply *Alexandrium* for short.

Surface (1 m depth) water samples were collected at various stations on our July 2001 cruise (100 mL preserved in Lugol's iodine solution) for determination of densities and taxonomic composition of the phytoplankton populations (e.g., not just *Alexandrium*). Samples were concentrated by settling 50 mL of the sample for 24 h in a graduated cylinder. The top 40 mL were siphoned off and the remaining 10 mL mixed; a 1 mL subsample of the five-fold concentrated sample was placed in a 1 mL counting chamber, and cells enumerated using an inverted compound microscope and identified to major taxonomic group (dinoflagellate, diatom or other flagellates). The dominant genera in those taxonomic groups were determined qualitatively to the extent possible. We also enumerated phytoplankton samples from May and June of 2000 collected for us by our colleagues on three of their transects (Fig. 1; see Keafer et al., 2005), along with nutrient samples at the same stations, but at various depths from the surface to the bottom.

On the July 2001 cruise we conducted vertical pump profiles at two stations to determine fine scale vertical distributions (1-m resolution) of *Alexandrium*. We used a commercially available, electric submersible pump and 1.5-cm inside-diameter hose. The intake end of the hose was attached to the CTD-rosette frame in order to sample water from the same depth as the CTD pressure sensor; the other end of the hose was connected to the pump, which was kept just below the surface alongside the ship. A second hose from the pump brought water on board the ship where water samples were collected. The pumping rate of $0.107\,L\,s^{-1}$ (or, $6.4\,L\,min^{-1}$) required 103 s to completely clear the sampling system. Thus, we raised the CTD-rosette and hose intake in discrete steps of 1 m every 2 min, which allowed time to flush the hose between depth intervals, collecting a 2 L sample at each 1-m step for *Alexandrium* cell counts; we collected a 20 ml nutrient sample at 5-m intervals. An estimate of error in our *Alexandrium* cell counts was performed by making seven replicate counts of three samples collected in July 2001, with which we computed standard deviations and confidence intervals.

Contour plots of *Alexandrium* cell densities and hydrographic parameters were made using a commercial software package (Surfer, produced by Golden Software, Golden, Co., USA). All contour plots were checked against actual data to ensure fidelity. Satellite sea-surface temperature images for each cruise period were made using AVHRR (Advanced Very High Resolution Radiometer) satellite data received and processed at the University of Maine's ground station.

3. Results and discussion

3.1. April–May and June 2000 Cruises

The areal distributions of *Alexandrium* on each of our three main cruises (April–May and June 2000, and July 2001) are presented as contour plots of cell densities in the form of areal slices at each of our sample depths, along with eight-day composite satellite images of sea-surface temperature corresponding with each cruise period. Composite images effectively remove areas obscured by clouds in individual satellite over passes, and they are more representative of conditions throughout the duration of our cruises.

Densities of *Alexandrium* at the surface (1 m) during the April–May cruise were low at all stations (Fig. 2), reaching a maximum density of 118 cells L^{-1} at Station 42 (see Fig. 1). However, on the last day of the cruise (3 May 2000) upon the

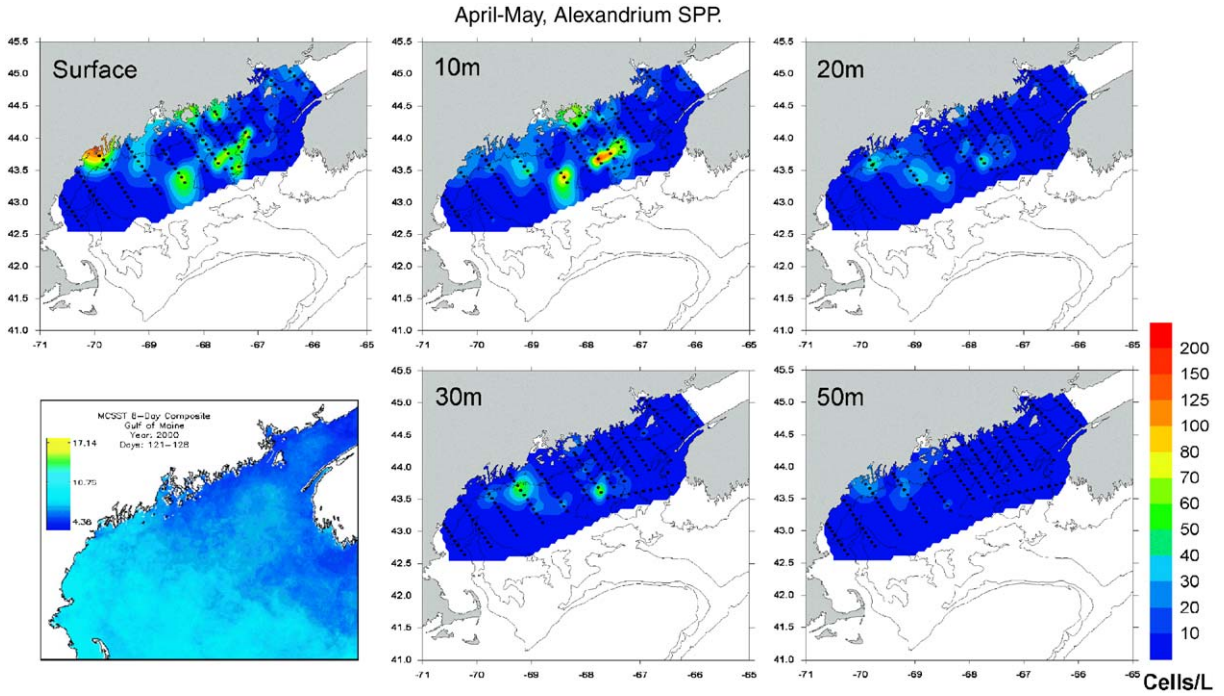

Fig. 2. Areal contour plots of densities of *Alexandrium fundyense* cells presented as slices from each of the standard depths sampled during the April–May 2000, cruise. Densities are given in cells L^{-1}. Also shown is an eight-day composite satellite image of sea-surface temperature for year days 121–128.

completion of the survey, we re-sampled the third transect (Fig. 1) on our way back into port (Portland, Maine), and at Station 42 the surface cell densities had increased to 337 cells L^{-1}. Because our cruise track began with the western-most transect and proceeded to the east, those data collected on the way into port were out of sequence by more than a week and are thus aliased. For this reason, we have not included them in Fig. 2. Aside from this caveat, the maximum density of cells at any of the depths we sampled on this spring cruise was 190 cells L^{-1} at 10 m at Station 124 (see Fig. 1). This April–May cruise preceded, or occurred at just the onset of, the annual *Alexandrium* bloom which we described earlier for the 1998 season (Townsend et al., 2001), but it followed the spring phytoplankton bloom, as indicated by the nutrient fields and phytoplankton chlorophyll concentrations (Fig. 3).

All *Alexandrium* cell densities given in this paper are based on single 2-L water samples collected at sea, and a single subsample counted in the lab (e.g., no replicates were taken). Table 1 shows that the coefficient of variation associated with these samples is in the range of about 8–16%.

Surface phytoplankton chlorophyll concentrations for the April–May 2000 period exceeded 2 μg L^{-1} in only two small patches located offshore, and a third patch in the offing of Penobscot Bay, Maine (Fig. 3), which was most likely in association with processes controlled by freshwater runoff from the Penobscot River estuary. Throughout the rest of the Gulf of Maine, surface chlorophyll concentrations were below 1.5 μg L^{-1}. Surface concentrations of dissolved silicate ($Si(OH)_4$ and dissolved inorganic nitrogen (DIN; defined here as the sum of nitrate, NO_3, and nitrite, NO_2; NH_4 was always well below 1 μM), as well as the total quantity of each integrated vertically from the surface to 25 m, show evidence of nutrient draw-down (Fig. 3), which we assume resulted from an earlier spring phytoplankton bloom. DIN concentrations were highest in association with the eastern Maine coastal current (EMCC) waters, as has been described earlier (Townsend et al., 1987; Brooks and Townsend, 1989; Pettigrew et al., 1998; Townsend et al., 2001), and were at or just below 3 μM throughout most of the remainder of the Gulf of Maine. Silicate was also drawn down over much of the offshore Gulf

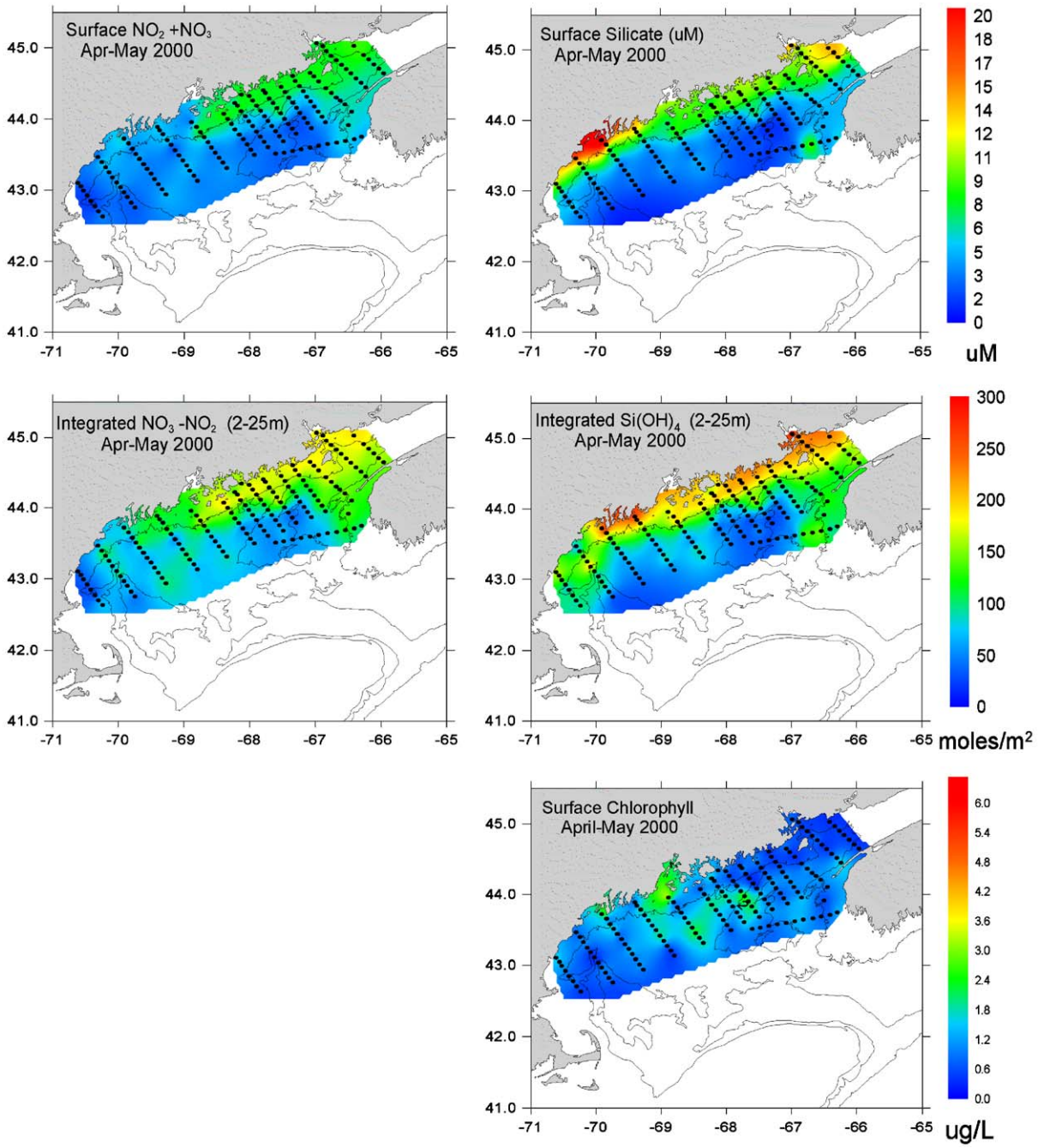

Fig. 3. Areal contour plots of concentrations of nitrate (NO_3) plus nitrite (NO_2), and silicate ($Si(OH)_4$) in surface waters (1 m depth; units are μM), as well as integrated totals of each from the surface to 25 m (units are moles L^{-1}), and surface concentrations of extracted phytoplankton chlorophyll *a* (μg L^{-1}), for the April–May 2000 cruise. Station locations are given.

waters (Fig. 3), but its distribution differed from that of DIN in that it reflected strongly a major source in coastal freshwater runoff; the highest silicate concentrations corresponded with the locations of major river systems, especially as seen in the vertically integrated silicate loads. Note the close correspondence between coastal silicate distributions in Fig. 3 and low salinities in Fig. 4. While both nutrients dropped off markedly beyond the coast and the influence of the EMCC, the silicate

Table 1
Results of replicate cell counts from samples collected at three stations in July 2001 in the eastern Gulf of Maine (see methods)

Replicate	Sample 1 (cells L^{-1})	Sample 2 (cells L^{-1})	Sample 3 (cells L^{-1})
1	952	4067	5003
2	938	4202	5069
3	1122	3229	5085
4	883	3080	4087
5	1198	3455	4933
6	889	2871	5025
Mean	997	3484	4867
Lower 95% CI	859	2917	4462
Upper 95% CI	1135	4051	5272
Standard error	54	221	158
Coefficient of variation	13.2%	15.5%	7.9%

drop was somewhat sharper than that of DIN, as seen in the vertically integrated plots. This difference reflects the generally greater concentrations of DIN than silicate in the deep source waters of the Gulf of Maine (Townsend et al., 2004).

Approximately five weeks later, during the June 2000 cruise, the cell densities of *Alexandrium* had increased (Fig. 5). The areal distribution of cells was uneven, with highest surface (1 m) cell densities of >1000 cells L^{-1} occurring in several patches between the Bay of Fundy, in the east, and the central Gulf of Maine. The general pattern of surface cells tended to encircle the northern Jordan Basin area of the Gulf of Maine in an inverted cup-like pattern, in apparent association with a surface temperature frontal zone that encompassed warmer waters offshore, as seen in the accompanying satellite image of sea surface temperature (Fig. 5). A second surface patch of *Alexandrium* cells was seen in the Bay of Fundy, just east of Grand Manan Island. Beneath the surface, high densities of cells (500 to >1000 cells L^{-1}) were associated with the warm side of the surface temperature front, and deeper layers of cells were all but absent nearer the coast.

The extension of our sampling domain in June 2000, to include waters on the southwest Nova Scotian Shelf, in the southeastern Gulf of Maine (Fig. 1), was intended to document whether *Alexandrium* cells might be imported into the Gulf of Maine by way of advection with Scotian Shelf waters, which enter the Gulf as a surface flow from the east around Nova Scotia; however, as shown in Fig. 5, those waters were virtually devoid of *Alexandrium* cells.

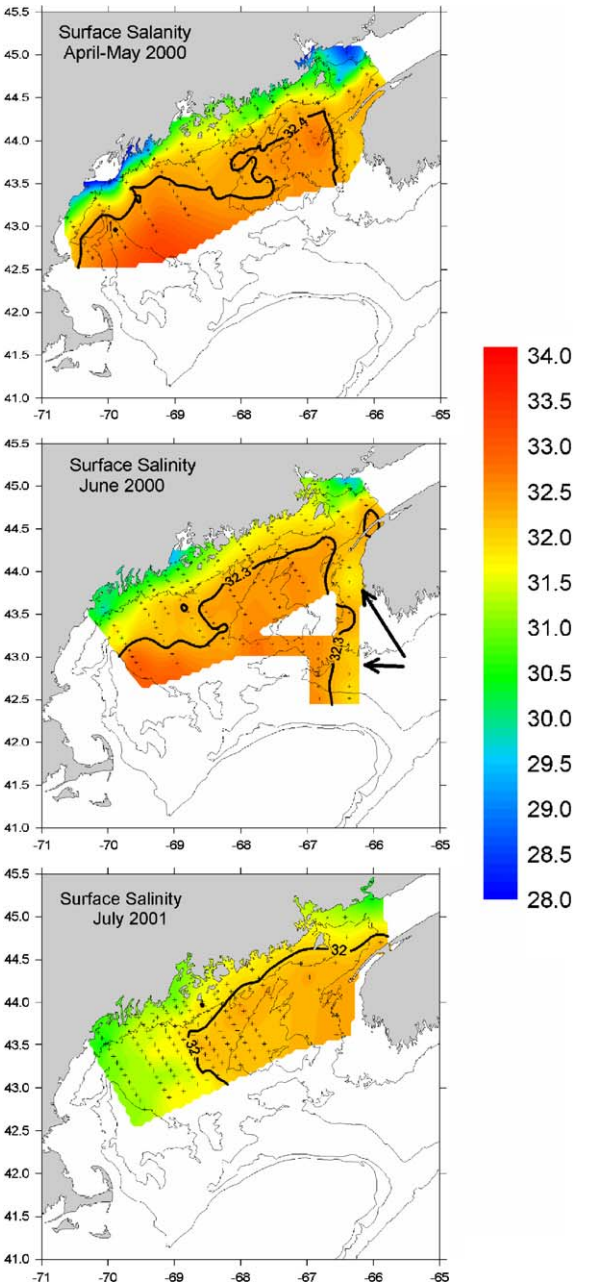

Fig. 4. Areal contour plots of surface salinity (1 m) for each of the three main cruises: April–May 2000, June 2000, and July 2001. The station locations are given. The heavy contour line corresponds to the surface at which highest densities of *Alexandrium* cells and lowest concentrations of silicate were observed (see text). The two arrow in the middle panel indicate intrusions of low salinity Scotian Shelf waters (discussed in text).

The phytoplankton and nutrient fields in June 2000, (Fig. 6) show that DIN had been further reduced from concentrations seen during the preceding April-May cruise (Fig. 3), both as viewed in

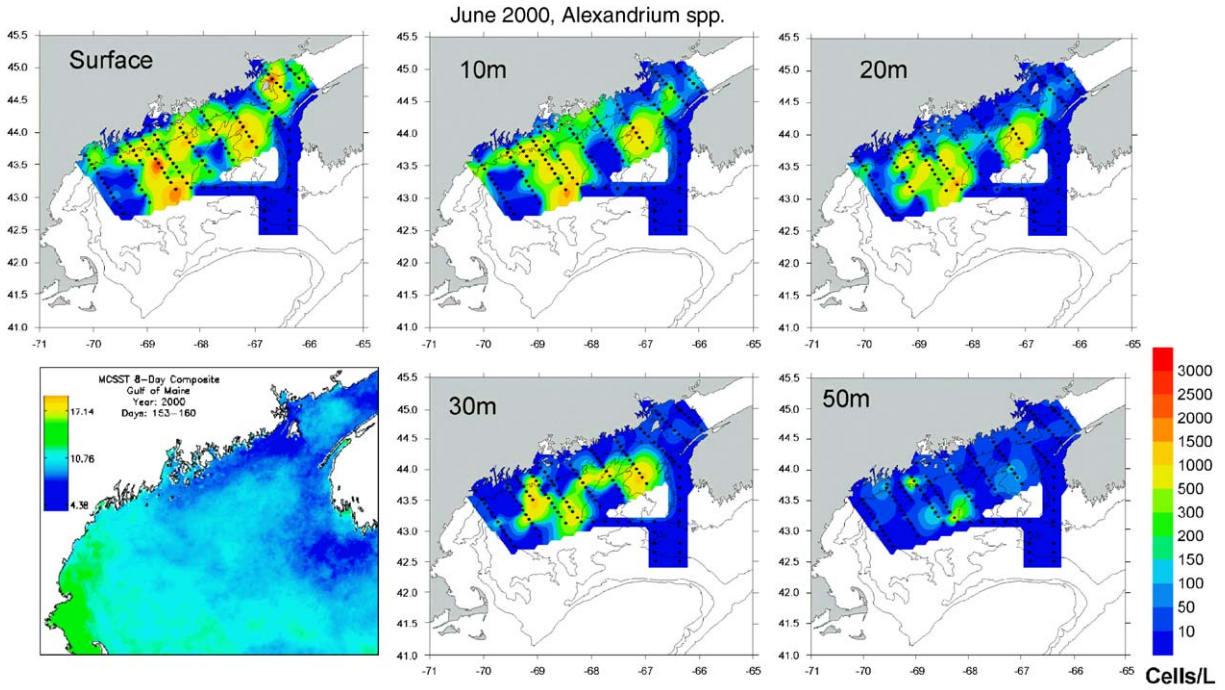

Fig. 5. Areal contour plots of densities of *Alexandrium fundyense* cells presented as slices from each of the standard depths sampled during the June 2000, cruise. Densities are given in cells L^{-1}. Also shown is an eight-day composite satellite image of sea-surface temperature for year days 153–160.

the surface concentrations (which were at or below 1.0 μM $NO_3 + NO_2$) and as vertically integrated totals. Note especially the scale change between the integrated DIN plots in Figs. 3 and 6. Highest surface concentrations of DIN were confined to the northeastern-most sector of our sampling domain, and may reflect anthropogenic nutrient loads carried into the Bay of Fundy by the St. John River in New Brunswick (Fig. 6). Elevated concentrations of DIN concentrations were, in general, again seen in the eastern Gulf of Maine in the vicinity of Grand Manan Island and the mouth of the Bay of Fundy, as discussed above. Silicate concentrations in June still reflected the influence of coastal freshwater sources, especially as vertically integrated totals, but unlike the earlier April–May cruise period, there appeared to be low concentrations of silicate, but nonetheless significant loads, in the eastern-most sectors of our sampling domain (2–3 μM $Si(OH)_4$ at the surface), including the offshore waters off southwest Nova Scotia. This eastern Gulf of Maine silicate load was associated with an influx of Scotian Shelf water (SSW), which is characterized by low temperature and low salinity, and includes an important contribution from the Gulf of St. Lawrence and the St. Lawrence River. The influence of colder and fresher SSW in the eastern Gulf of Maine is visible in the sea-surface temperature satellite image in Fig. 5 and the surface-salinity distributions in Fig. 4; note especially the fresher waters off southwest Nova Scotia, at the positions indicated by arrows in Fig. 4. Much of the Gulf of Maine surface salinity was fresher in June than the April–May period (Fig. 4), and would suggest both a spreading of Gulf of Maine coastal runoff as well as an influx and spreading of cold and fresh SSW.

The phytoplankton chlorophyll concentrations increased between the April–May and June 2000 cruises (Fig. 6), but the highest chlorophyll concentrations corresponded closely with the colder waters of the EMCC, as seen in the sea-surface temperature image in Fig. 5, and would appear to have been the result of the elevated nutrient loads carried in the EMCC (Townsend et al., 1987).

The coastal band of high silicate concentrations, seen both at the surface and as vertically integrated loads, during our April–May cruise (Fig. 3), and again during our June cruise (Fig. 6), may hold important clues to oceanographic factors that determine the timing and areal distributions of

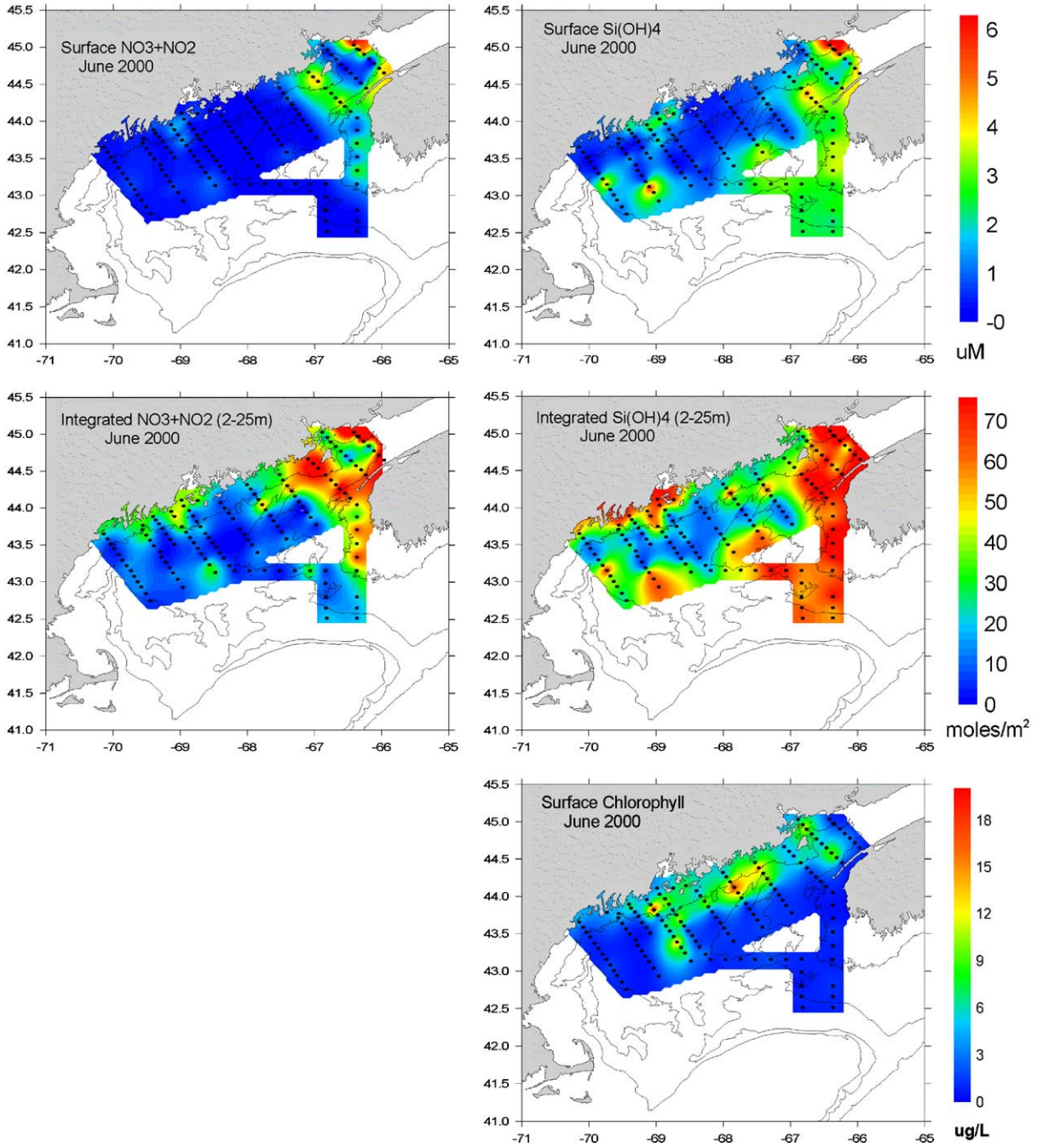

Fig. 6. Areal contour plots of concentrations of nitrate (NO_3) plus nitrite (NO_2), and silicate ($Si(OH)_4$) in surface waters (1 m sample depth; units are μM), as well as integrated totals of each from the surface to 25 m (units are moles L^{-1}), and surface concentrations of extracted phytoplankton chlorophyll *a* (μg L^{-1}), for the June 2000 cruise. Station locations are given.

seasonal *Alexandrium* blooms in the Gulf of Maine. The coastal band of high silicate concentrations is the result of spring runoff of high-silicate river waters (200–400 μM $Si(OH)_4$; Schoudel, 1996). This is plainly seen in Fig. 7, where silicate concentrations are plotted against salinity for all water samples from all depths sampled during our three main cruises. During the April–May cruise, when freshwater runoff is typically greatest and coastal salinities lowest, we see a V shaped pattern in the

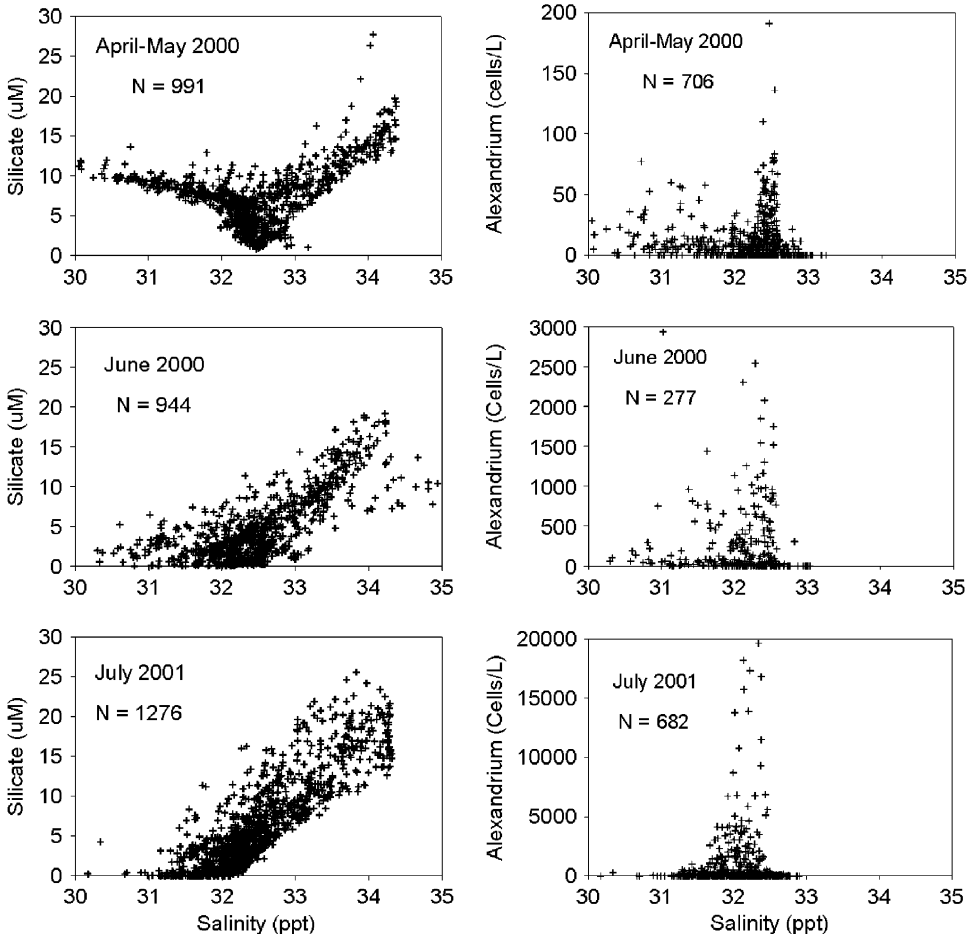

Fig. 7. Plots of silicate concentrations versus salinity, and *Alexandrium* cell densities versus salinity for all samples (total number of data pairs given) collected on the three main cruises: April–May 2000, June 2000 and July 2001.

silicate vs salinity plot (Fig. 7), with higher silicate concentrations at both low and high salinities, and minimal silicate concentrations at the intermediate salinity of about 32.4. This pattern is the result of two mixing curves, one curve with the silicate source end member being river water, and a second curve where the silicate source end member is saltier (and deeper) slope waters of the Gulf of Maine. Later, in June of 2000 (Fig. 7), the high silicate content of the fresher waters had clearly been reduced from that seen in April–May, leaving what would appear to be waters dominated more by the single deepwater mixing curve. Of course, two processes likely were involved in reducing silicate concentrations at those lower salinities and in reducing the volume of low-salinity water in June (as indicated by fewer numbers of samples at the lower salnities). They are biological uptake of silicate by diatoms, between the beginning of May and the middle of June of 2000, and a reduction in volume of freshwater being discharged into coastal waters in June.

Also shown in Fig. 7 are plots of *Alexandrium* cell densities as a function of salinity for each of our three main cruises in 2000 and 2001 (we discuss specifics of our July 2001 cruise below). In each case, the maximum densities of cells correspond to the same salinity at which we observed the lowest silicate concentrations. Those salinities were: 32.4 in April–May 2000, 32.3 in June 2000 and 32.0 in July 2001; those surface isohalines are plotted in Fig. 4 and discussed further below. Based on this observation it might be tempting to argue that *Alexandrium* populations grow well only when silicate concentrations—and, presumably, diatom populations—are low, but that argument, of course is confounded simply because we routinely see in the ocean an

inverse relationship between standing stocks of phytoplankton and nutrients. That is, phytoplankton populations typically reach highest concentrations after having exhausted their nutrient sources. In fact, plots of *Alexandrium* cell densities for our three main cruises as a function of nutrient concentrations (not shown) exhibit highest cell densities at lowest nutrient concentrations, as also discussed by Love et al. (2005).

We proposed earlier a model of *Alexandrium* growth dynamics in the offshore waters of the Gulf of Maine as being determined by light and dissolved inorganic nitrogen levels (DIN) (Townsend et al., 2001; see also McGillicuddy et al., 2005a,b) and the purpose of this discussion is to explore why, under conditions of high DIN and light, such as exists near shore in spring, we nonetheless do not routinely see high cell densities of *Alexandrium* develop. Silicate would seem to be an important clue, because unless *Alexandrium* is limited to a narrow salinity range, there does not seem to be a straightforward relationship between *Alexandrium* populations and coastal water mass properties. The presence of the coastal band of high silicate concentrations observed on these two survey cruises in 2000 might prompt one to propose the hypothesis that there is an allelopathic inteference between diatoms and the dinoflagellate *Alexandrium*, which is important in determining areal distributions and seasonal development of *Alexandrium* blooms in the Gulf of Maine. Observations of such allelopathic interactions in the plankton are common but only poorly understood (reviewed in Legrand et al., 2003). We do not purport to document such an interaction based on these field measurements in the Gulf of Maine, but our results do not exclude the possibility that allelopathy might be operating, as we discuss below.

Highest cell densities of *Alexandrium* in the Gulf of Maine, during our 1998 surveys (Townsend et al., 2001), were offshore, and removed by some distance from the adjacent coast, which has higher silicate, except in instances of shoreward advection of offshore waters, or localized near shore bloom phenomena, such as has been documented by Anderson (1997). Also, it is well known that the classical pattern of planktonic species succession proceeds from an initial spring bloom of diatoms, which require both silicon and nitrogen in approximately equal molar proportions, followed later in the spring and summer by dinoflagellates and other non-diatom species, which do not require silicon. On nearby Georges Bank in the southern part of the Gulf of Maine, e.g., the spring diatom bloom has been shown to be limited by silicate, which is drawn down before dissolved inorganic nitrogen, leaving a surplus of DIN that fuels the subsequent growth of non-diatom phytoplankton (Townsend and Thomas, 2001, 2002). In the Georges Bank case, excess nitrogen remains after the diatom bloom because the source waters (deep waters of slope water origin) have excess DIN (DIN-to-silicate ratio of about 1.4–1.5). Coastal waters of the Gulf of Maine are somewhat similar, except that silicate is in excess not because of the properties of deep source waters in the Gulf of Maine, but because of the influence of silicate-rich freshwater runoff. Following our two cruises in April–May and June of 2000 we suspected that such might be the case, that the presence of high densities or growth rates of diatoms somehow interacts with the development of high population densities of *Alexandrium*. Because our group's already-scheduled cruise for the following field season was set for July 2001, we made arrangements with our colleagues to collect samples for us along selected transects on their two cruises in May and June of 2001 (Keafer et al., 2005) with which we measured nutrients and determined the composition of major phytoplankton taxa.

3.2. May and June 2001

Fig. 8 shows vertical cross sections of DIN and silicate, and surface-water cell densities of major phytoplankton taxa in May 2001, along the three transects shown in Fig. 1. The spring diatom bloom near shore was evident, with diatom cell densities of several hundred mL^{-1} (= several hundred thousand cells L^{-1}), dropping off with distance offshore. The high-density diatom populations were dominated by a few taxa, principally *Chaetoceros* spp., *Skeletonema* spp., *Thalassiosira* spp., and *Leptocylindrus* spp. The distance offshore of high diatom densities increased from west to east, from Transect C to G to J (Fig. 1). Smaller cells ($<10\,\mu m$), given as *Other* in Fig. 8, were dominated by *Phaeocystis* spp., cryptomonads, and microflagellates. Those smaller cells were very abundant at offshore stations on Transects G and J (Fig. 8). Dinoflagellates ranged in abundance from 1 to 8 cells mL^{-1} in the west, to 60 cells mL^{-1} at a point one-third the way out on Transect G, to about 200 cells mL^{-1} at the farthest station offshore on Transect J in the east. Dominant dinoflagellate species included *Gymnodinium* spp.,

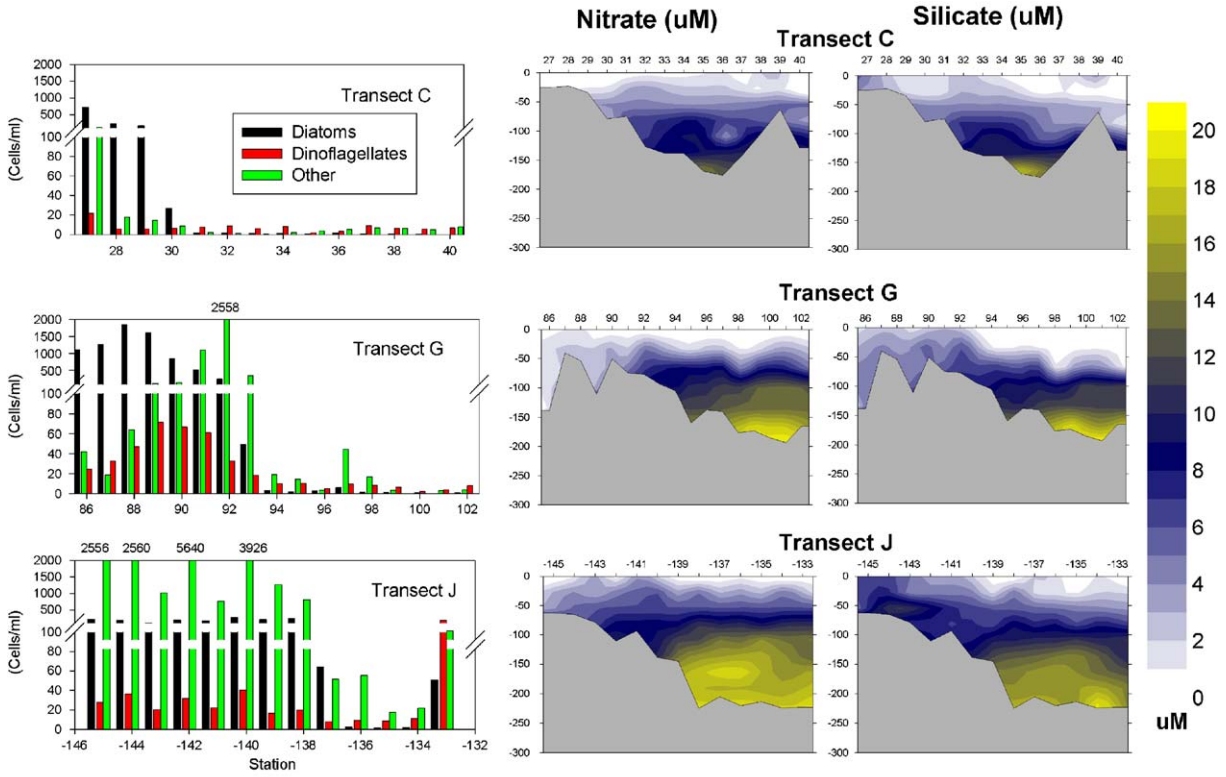

Fig. 8. Bar graphs of phytoplankton cell densities, by major taxonomic group, at the surface (1 m), and vertical cross sections of nitrate (NO_3) plus nitrite (NO_2), and silicate ($Si(OH)_4$), along the three transects shown in Fig. 1 for samples collected May 2001 (see text for explanation).

Gyrodinium spp., *Prorocentrum* spp., *Ceratium* spp., and *Heterocapsa* spp. Unlike these other dinoflagellates, *Alexandrium* spp. cells were sampled only as trace occurrences in these samples; Keafer et al. (2005) report that densities of *Alexandrium* reached 50–100 cells L^{-1} ($= 0.05$–0.10 cells mL^{-1}) at midpoints on Transects G and J, which is less than 0.1% the densities of other dinoflagellates present.

The distributions of phytoplankton along the three transects in May 2001 corresponded with what might be expected based on the nutrient distributions, also shown in Fig. 8. Concentrations of DIN were low at the surface in inshore waters in the west, while silicate levels were 1–4 μM $Si(OH)_4$. Both DIN and silicate were higher at shallower depths closer to shore on Transects G and J in the central and eastern Gulf, respectively.

Phytoplankton cell densities and nutrients along three transects in June 2001 are presented in Fig. 9. Diatom cell densities remained high at the inshore stations on all three transects in June, but they extended farther offshore into the Gulf of Maine on the middle transect (Transect G). Diatom taxa did not differ markedly from that in May except that species of *Thalassiosira* and *Rhizosolenia* became more common. Cell densities and taxonomic composition of dinoflagellates were similar to the previous month, but the densities of smaller cells, dominated by microflagellates, were lower than in May. Silicate concentrations were elevated (2–10 μM $Si(OH)_4$ at the surface at Stations 34–36 at the inshore end of Transect C, while DIN concentrations were undetectable (Fig. 9); we observed high diatom cell densities (300–800 cells mL^{-1}) associated with, and just inshore of, those high-silicate surface waters. As was the case in May 2001, surface waters of the central and eastern transects in June were elevated in both DIN and silicate.

In both these May and June 2001 data sets we can see, in general, an inshore–offshore transition in phytoplankton species composition, from diatoms to dinoflagellates to microphytoplankton, which is spatially analogous to a seasonal transition from

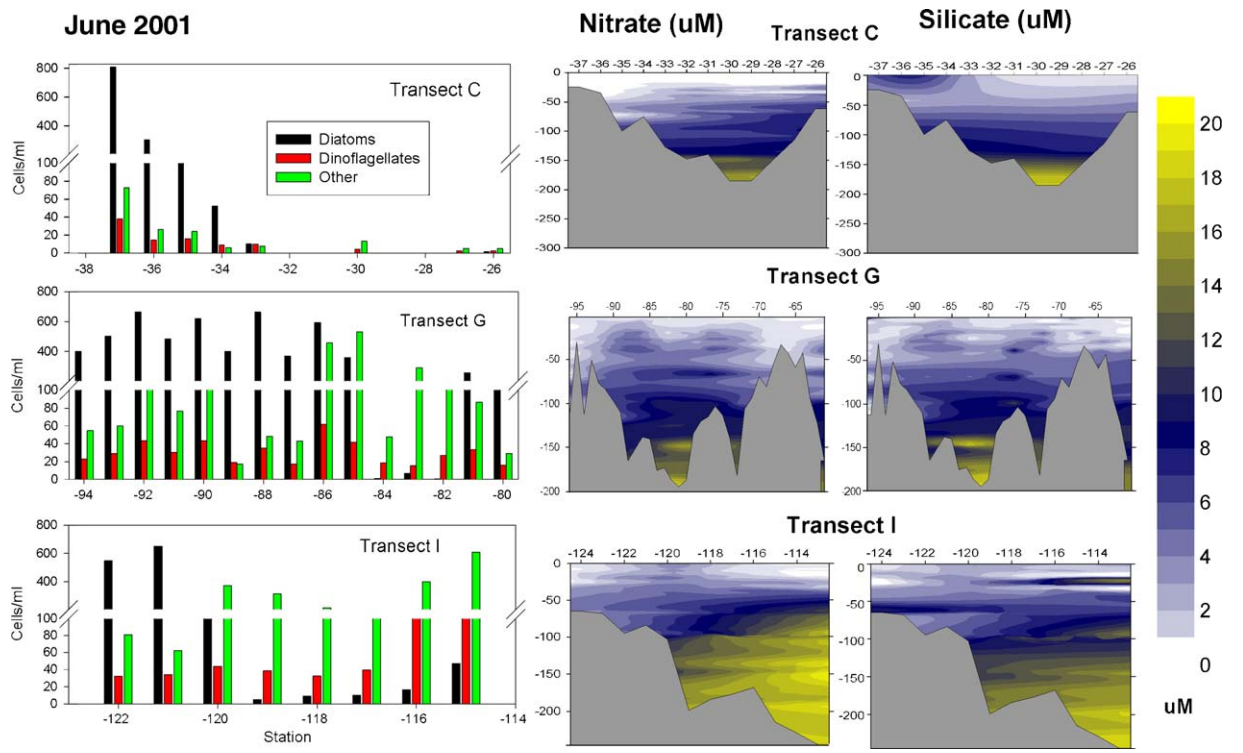

Fig. 9. Bar graphs of phytoplankton cell densities, by major taxonomic group, at the surface (1 m), and vertical cross sections of nitrate (NO_3) plus nitrite (NO_2), and silicate ($Si(OH)_4$), along the three transects shown in Fig. 1 for samples collected June 2001 (see text for explanation).

spring to summer. This general pattern is not inconsistent with the hypothesis that near shore, silicate-rich and nitrate-poor waters of persistently high densities and/or growth rates of diatoms might interfere with developing *Alexandrium* populations. On the other hand, these distributions may simply reflect the growth histories of cells growing in different water masses. Keafer et al. (2005) observed densities of 200–500 *Alexandrium* cells L^{-1} at the surface and 10 m depth at the innermost two stations on Transect J, which represented the highest densities of *Alexandrium* they encountered on their June 2001 cruise. Diatom populations were high at those same innermost stations (>500 cells mL^{-1}; [or, >500,000 cells L^{-1}]; Fig. 9). Compared with *Alexandrium* cell densities the next month (July 2001, discussed below), the June 2001 densities of 200–500 *Alexandrium* cells L^{-1} are relatively low. That is, these data do not resolve the issue of whether there is an inhibitory effect of diatoms on the development of *Alexandrium* blooms, and the question remains: were the 200–500 *Alexandrium* cells L^{-1} in June being held in abeyance, or, were they actively growing and thus representing an initiation of the bloom we observed the following month?

3.3. July 2001

Our next sampling period was in July 2001, and we remind the reader that while it is tempting to do so, we must be careful not to draw conclusions about seasonal patterns among our three main survey cruise periods, which span two different years (two cruises in April–May and June 2000, and a third cruise in July 2001). However, this July cruise does follow the May and June 2001 cruises discussed in the preceding section, the detailed results of which are presented in Keafer et al. (2005).

The densities of *Alexandrium* cells in July of 2001 were high, with large surface patches of greater than 1000 cells L^{-1} and one smaller patch with surface densities greater than 10,000 cells L^{-1} (Fig. 10). The surface pattern of cells appeared to be positioned broadly about the cooler EMCC waters off the mid-Maine coast, as seen in the satellite image of sea-surface temperature in Fig. 10. Comparing the satellite image of sea-surface temperature in Fig. 10

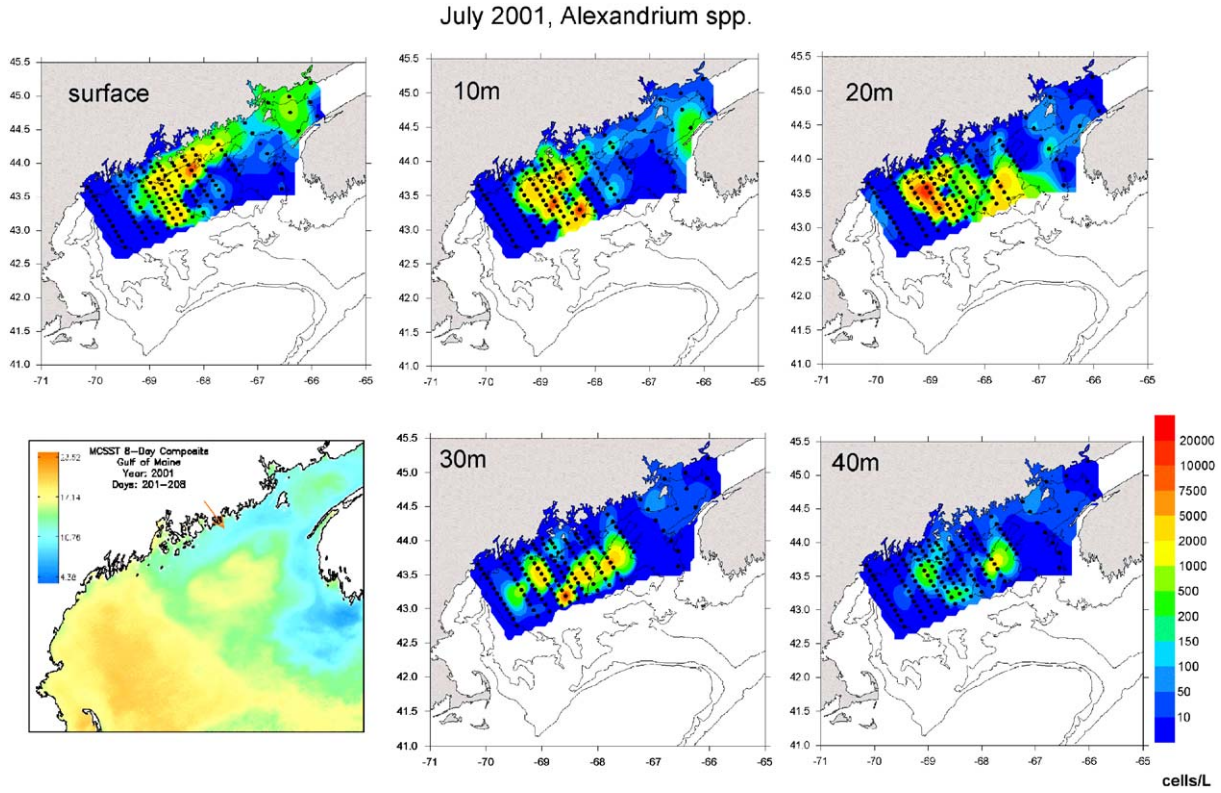

Fig. 10. Areal contour plots of densities of *Alexandrium fundyense* cells presented as slices from each of the standard depths sampled during the July 2001, cruise. Densities are given in cells L^{-1}. Also shown is an eight-day composite satellite image of sea surface temperature for year days 201–208.

with the distributions of *Alexandrium* cells shows that cell densities increase some distance "downstream" within the EMCC, beginning at the position indicated by the arrow drawn on the satellite image, in association with progressively warmer and more vertically stratified surface waters with increasing distance downstream in the EMCC. That is, surface water temperatures become warmer, and vertical stratification becomes more pronounced along the long axis of the EMCC from its origins in the northeastern Gulf near Grand Mannan Island downstream to the central Gulf of Maine.

High densities of *Alexandrium* cells ($>10{,}000\,L^{-1}$) were beneath the surface in patches that were located, in general, at progressively greater distances to the southwest from where we saw the highest cell densities at the surface. In addition, the relative positions of the high-density *Alexandrium* patches with increasing depth strata suggest a subsurface advection of cells in a counterclockwise direction out over the central Gulf. Such advection of cells is consistent with the presumed flow of the EMCC, as revealed in the satellite image of sea-surface temperature (Fig. 10), which shows the EMCC turning offshore and becoming part of a counter clockwise, gyre-like feature around the western perimeter of Jordan Basin. As current speeds diminish with distance along the axis of the EMCC, a major filament of the EMCC recirculates back toward the east in the southern limb of a cyclonic gyre over Jordan Basin. The sluggish current speeds at depths of 30 and 40 m, in conjunction with moderate *Alexandrium* growth rates in the vicinity of the nutricline, may have supported the growth and accumulation of high densities of *Alexandrium* cells we see in Fig. 5, and which we observed in the summer of 1998 (Townsend et al., 2001).

The distributions of nutrients (DIN and silicate) and phytoplankton chlorophyll during the July 2001 cruise are presented in Fig. 11. Elevated DIN and silicate concentrations at the surface were confined to the northeastern Gulf, as discussed earlier for our

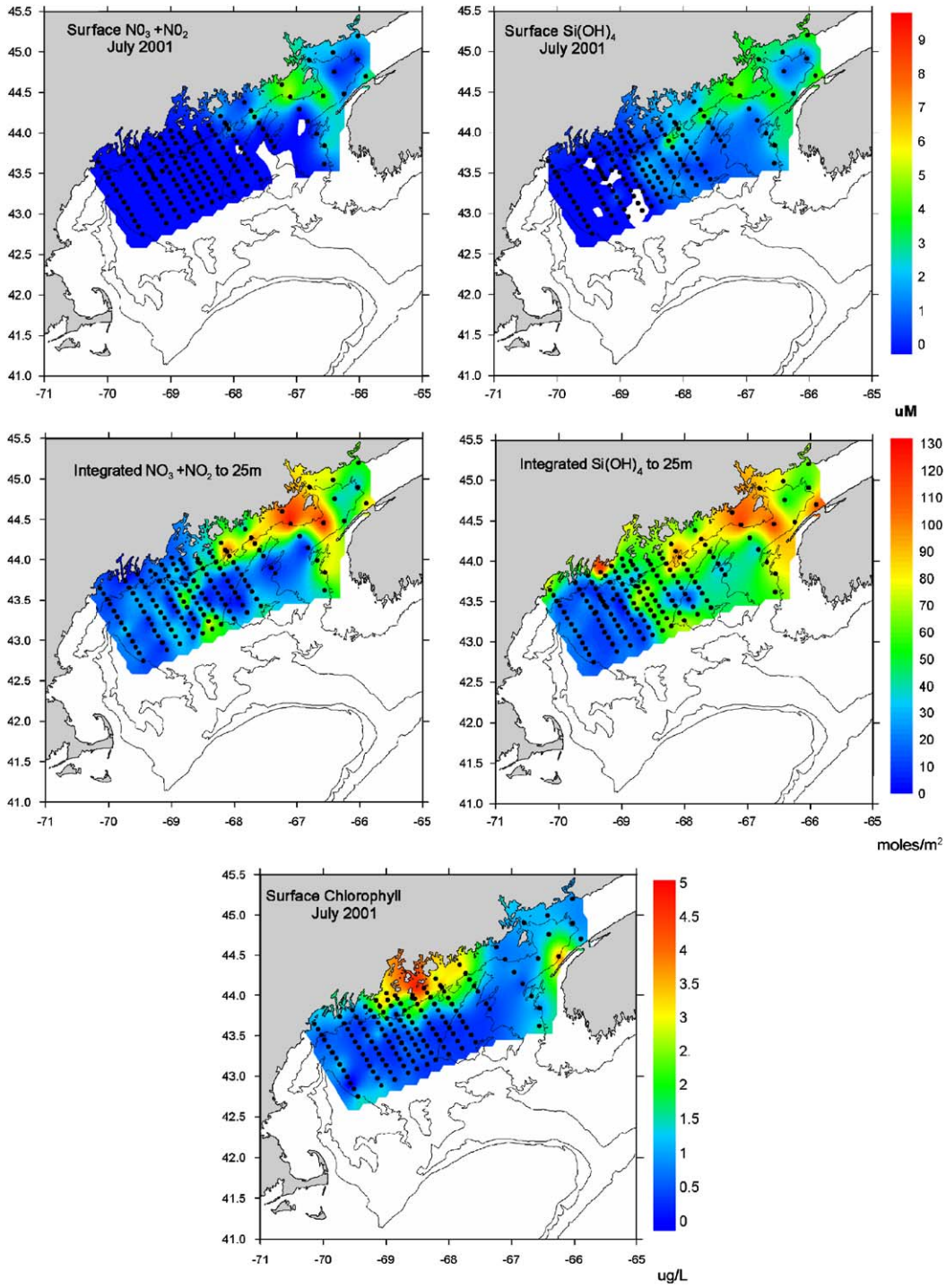

Fig. 11. Areal contour plots of concentrations of nitrate (NO_3) plus nitrite (NO_2), and silicate ($Si(OH)_4$) in surface waters (1 m sample depth; units are μM), as well as integrated totals of each from the surface to 25 m (units are moles L^{-1}), and surface concentrations of extracted phytoplankton chlorophyll *a* (μg L^{-1}), for the July 2001 cruise. Station locations are given.

June 2000 cruise, and were associated with the eastern Maine coastal current system, which injects nutrients into surface waters in the northeastern Gulf (Townsend et al., 1987; Pettigrew et al., 1998). Those elevated nutrient loads, both DIN and silicate, can be traced to the southwest within the EMCC.

The concentrations of silicate throughout most of the eastern Gulf of Maine in July 2001 were greater than what we observed for the two earlier cruises (April–May, and June) the previous year. This relative increase in silicate may have been be related to a greater volume of SSW (Scotian Shelf Water) in the eastern Gulf of Maine in July of 2001; Fig. 4 clearly shows that the Gulf of Maine is fresher in July 2001 than in either of the two cruises the previous year. Note, however, that the vertically integrated loads of DIN within the EMCC, versus the remainder of the Gulf, are slightly greater than silicate (Fig. 11). This is an important point, which may help to understand how *Alexandrium* populations are apparently initiated within the northeastern Gulf of Maine and EMCC. We revisit this discussion of *Alexandrium* cell growth in relation to DIN and silicate in Section 3.3.3.

The distributions of major phytoplankton taxa in the surface waters in July 2001 are presented in Fig. 12. Like the May and June 2001 data sets (Figs. 8 and 9), cell densities were least in the western Gulf, but unlike the earlier months, diatoms in July were confined to the central and eastern coastal waters. The overall cell densities in July were lower than either May or June, and reflected overall reduced nutrient concentrations in surface waters in July (discussed below).

3.4. Fine-scale vertical distributions

Two pump profiles of *Alexandrium* cell densities were completed during the July 2001 cruise at the two stations shown in Fig. 1 (Stations 78 and 140). Station 78 was located in a region of relatively high *Alexandrium* cell densities, as determined by shipboard qualitative examination of 20-μm mesh, 25-cm diameter plankton net samples collected in the top 10–20 m (Fig. 13). The station was thermally stratified, with a gradual thermocline between the

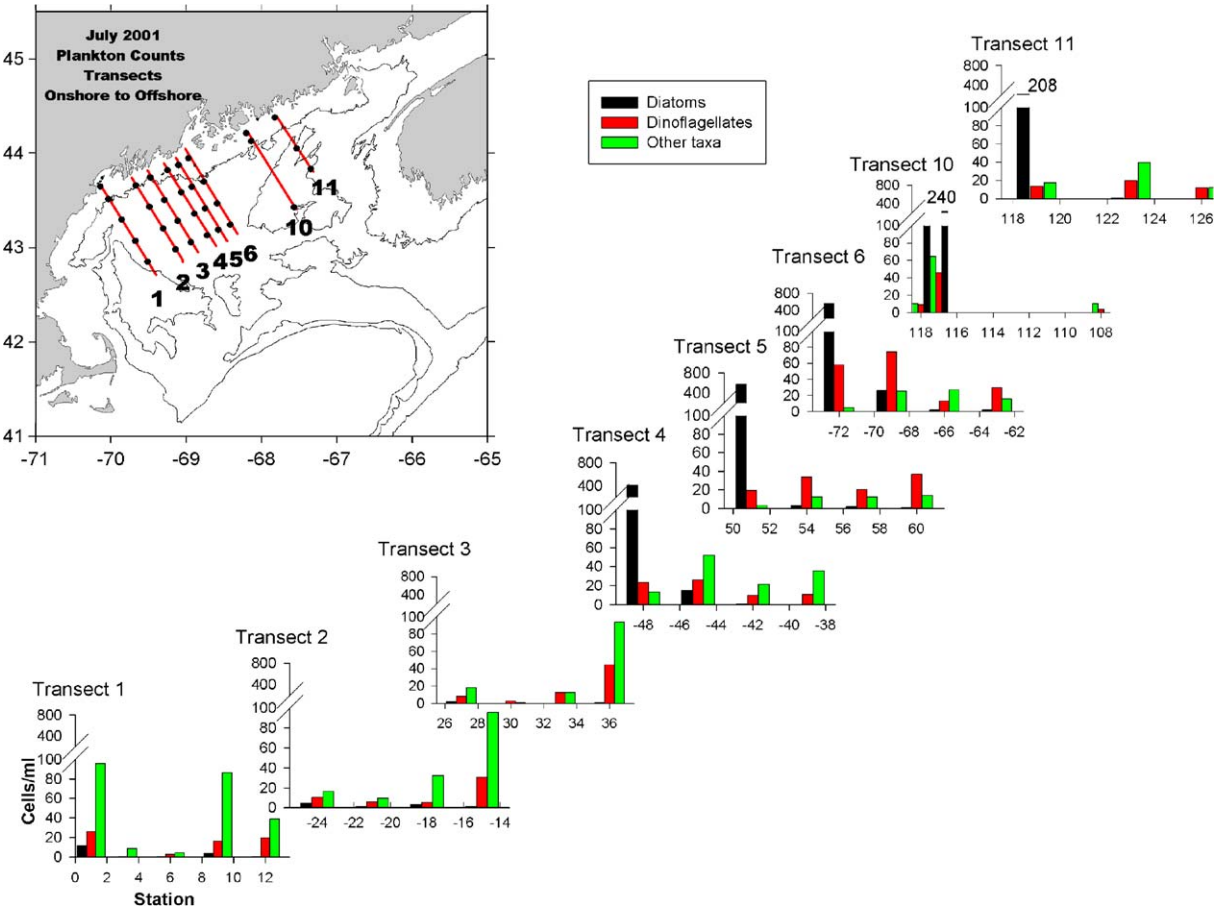

Fig. 12. Bar graphs of phytoplankton cell densities, by major taxonomic group, at the surface (1 m) along the stations and transects shown in Fig. 1 for samples collected July 2001 (see text for explanation).

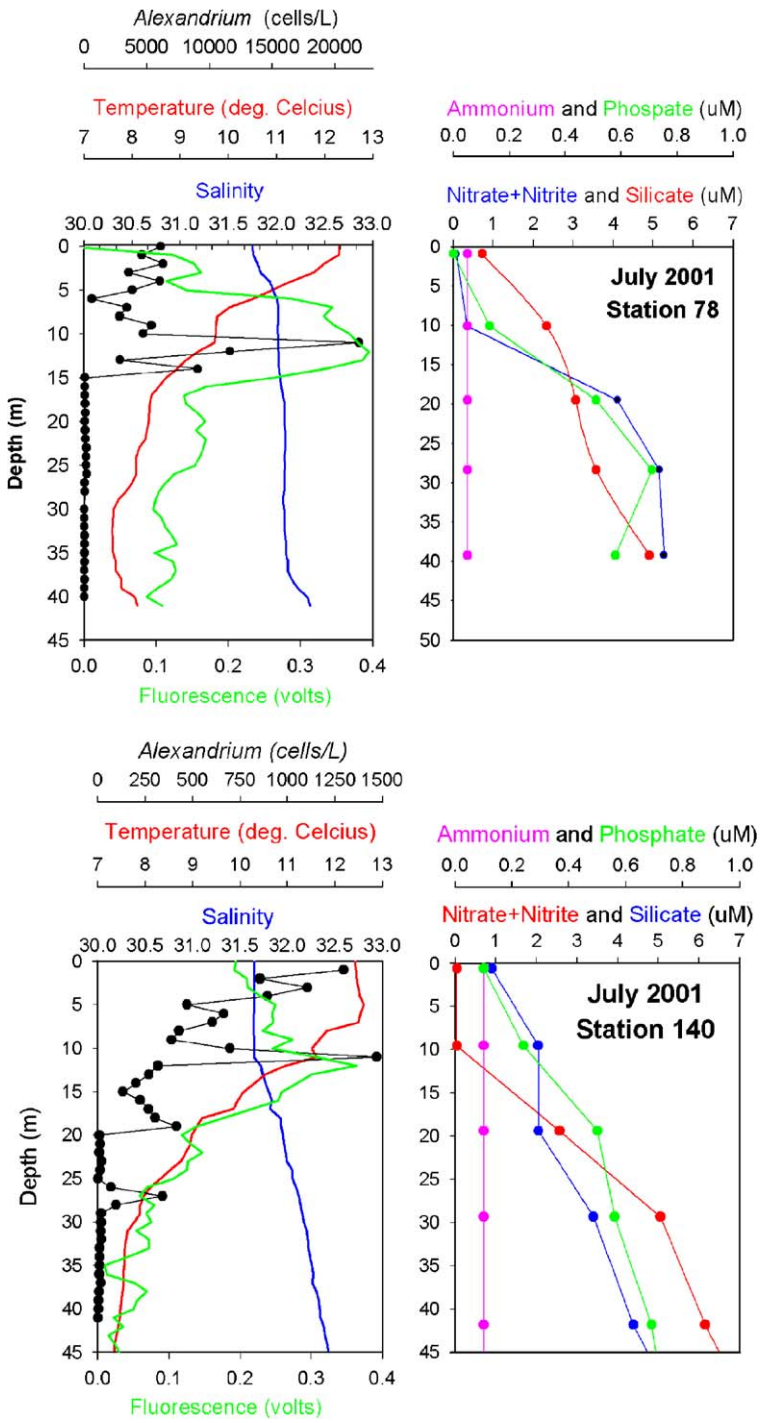

Fig. 13. Results of pump Stations 78 and 140 (Fig. 1), July 2001. Given are 1-m averaged CTD profiles of temperature, salinity, and in situ fluorescence, collected on the down cast, and dissolved inorganic nutrient concentrations in water samples collected on the up cast. *Alexandrium* cell densities in water samples collected on a separate pump profile are given at 1-m intervals from 1 to 40 m.

surface and about 10–15 m, and a halocline between about 3 and 8 m. There was a subsurface chlorophyll fluorescence maximum, as revealed by the in situ fluorometer, at the base of the thermocline, at about 13 m. *Alexandrium* cell densities were on the order of 4000–5000 cells L^{-1} in the top 10 m, but the

vertical profile exhibited a trend of decreasing cell densities between the surface and 6 m, before increasing again at 10 m where cell densities were once again equal to those at the surface. Cell densities sharply increased between 10 and 11 m in a thin layer of $>20{,}000$ cells L^{-1}, dropping to about 10,000 cells L^{-1} at 12 m, and 2500 cells L^{-1} at 13 m. The high densities of *Alexandrium* cells in the thin layer at 11 m was roughly coincident with the subsurface chlorophyll (fluorescence) maximum layer. However, the fluorescence and CTD data were collected on a separate hydrocast, as were the nutrient samples. That is, following an initial hydrocast on station, the CTD and pump hose were immediately lowered to 40 m to begin collecting *Alexandrium* cell samples as the CTD and hose nozzle were raised to the surface over a period of more than an hour. As such the data sets are likely aliased. With this caveat in mind, note also that the *Alexandrium*-cell thin layer and chlorophyll fluorescence maximum may have coincided with the depth of the nutricline, which was between 10 and 20 m.

The second pump sample was taken at Station 140 in vertically stratified waters in the mouth of the Bay of Fundy (Fig. 13). The thermocline and halocline were between 10 and 20 m, and the subsurface chlorophyll fluorescence maximum was between 10 and 15 m. The depth of the nutricline, as was the case with Station 78, could not be clearly identified given the coarse sample spacing. Cell densities of *Alexandrium* at Station 140 were an order of magnitude lower than at Station 78, but the vertical trend in cell densities was similar. Densities at the surface were on the order of 1000 cells L^{-1} dropping to approximately half that value at depths between 4 and 8 m, before abruptly increasing from about 700 cells L^{-1} at 10 m to 1500 cells L^{-1} in a thin layer at 11 m; cell densities then fell to about 200 cells L^{-1} at 12 m.

The two pump stations exhibited markedly dissimilar *Alexandrium* cell densities, but similar vertical distributions. Each exhibited relatively high cell densities at the surface, low cell densities a few meters beneath the surface, and a high-density thin layer coincident with the base of the pycnocline. The deep thin layer of high densities of *Alexandrium* is more than likely missed in discrete Niskin Bottle samples collected with standard hydrocasts, as done at all stations on our surveys, versus continuous pump samples. Neither the environmental conditions that favor such thin layers, nor survival or growth advantages afforded *Alexandrium* populations that occupy thin layers, are known. We expect that these layers probably do not result from a diel vertical migratory behavior, as shown in Townsend et al. (2005), but vertical migrations of frequencies longer than daily could perhaps give rise to such layers. McGillicuddy (personal communication) has shown, e.g., that the bimodal peaks in vertical distributions shown in Fig. 11 can be simulated in a simple model of vertical migrations with a period of several days. If such were the case, then we might expect that the deep thin layer might coincide with a sharp nutricline that is sought by the motile *Alexandrium* cells in alternation with shallow depths of high light for photosynthesis. One other explanation could be that dense aggregations in thin layers of cells are an important aspect of the life history and survival strategy of *Alexandrium*, in that such phenomena would increase the chance of encounter between gametes for sexual reproduction.

3.5. *Alexandrium* distributions in relation to nutrients and hydrography

Details of the distributions of *Alexandrium* cells in relation to nutrients and hydrographic regimes in July 2001 are summarized as vertical cross sections along every-other transect (Figs 14–19), beginning with the easternmost transect (see Fig. 1). Transect 12 (Fig. 14) crossed the upstream end of the eastern Maine coastal current, which is characterized by cold surface temperatures and relatively high nutrients, as seen in the vicinity of Stations 132 and 133. Note the sharp retrograde salinity and density fronts at Station 132, which constitutes the high-velocity core of the EMCC, and which encompasses the coldest surface-water temperatures. Elevated concentrations of dissolved inorganic nutrients extend to the surface at Stations 132 and 133, with concentrations of DIN in surface waters slightly exceeding those of silicate, by 0.8–1.0 μM. Excess concentrations of DIN over silicate may characterize relatively poor growth conditions for diatoms, especially if silicate concentrations fall below the half-saturation constant of about 2–4 μM Si(OH)4 for diatoms (Paasche, 1973; Martin-Jézéquel et al., 2000), which is commonly interpreted as the concentration below which that particular nutrient becomes limiting. Silicate concentrations were <5 μM at the inshore portion of this transect. These relative concentrations of DIN and silicate are to be expected, since the deep eastern Gulf of Maine source-water concentrations

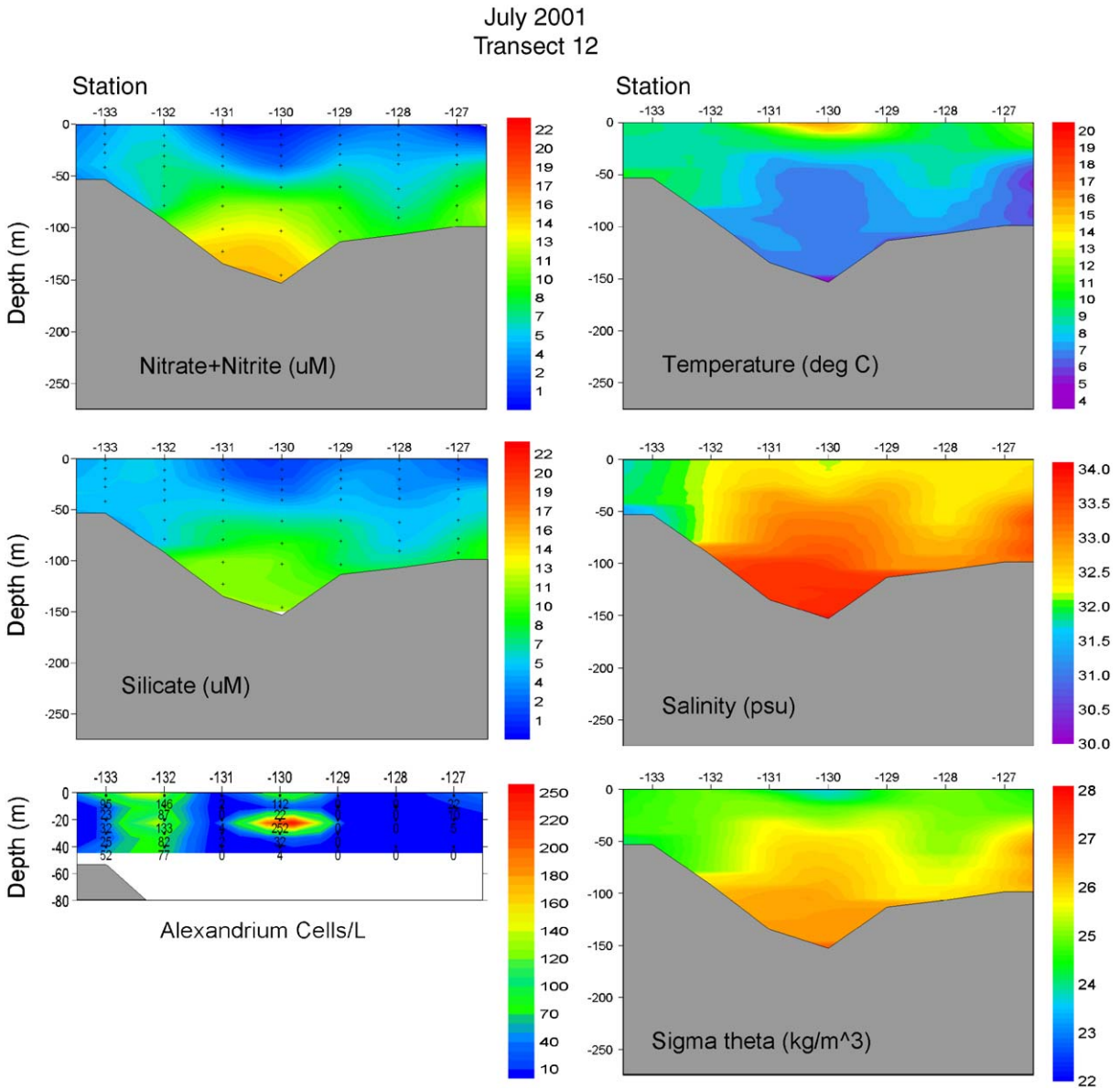

Fig. 14. Transect 12, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

are characteristically greater in DIN than silicate (Townsend et al., 2005); this is clearly evident in the deep waters shown in Fig. 14.

The densities of *Alexandrium* along Transect 12 are low (on the order of 50–150 cells L^{-1} in the top 40 m) and reflect the intense tidal mixing in these eastern Gulf of Maine coastal waters in that the cells are distributed vertically throughout the top 40 m within the core of the EMCC (at Station 132; Fig. 14). This eastern Gulf of Maine transect extends from the Maine coast through the core of the EMCC and across deeper stratified waters farther offshore, coming close to the Nova Scotian Shelf (see Fig. 1). There is also a subsurface maximum in *Alexandrium* cell densities (250 cells L^{-1} at 20 m depth) in those stratified offshore waters, which appears to be disconnected from the 20 m deep *Alexandrium* patch seen in Fig. 10.

Continuing downstream to transect 10 (Fig. 15), the water column becomes progressively more

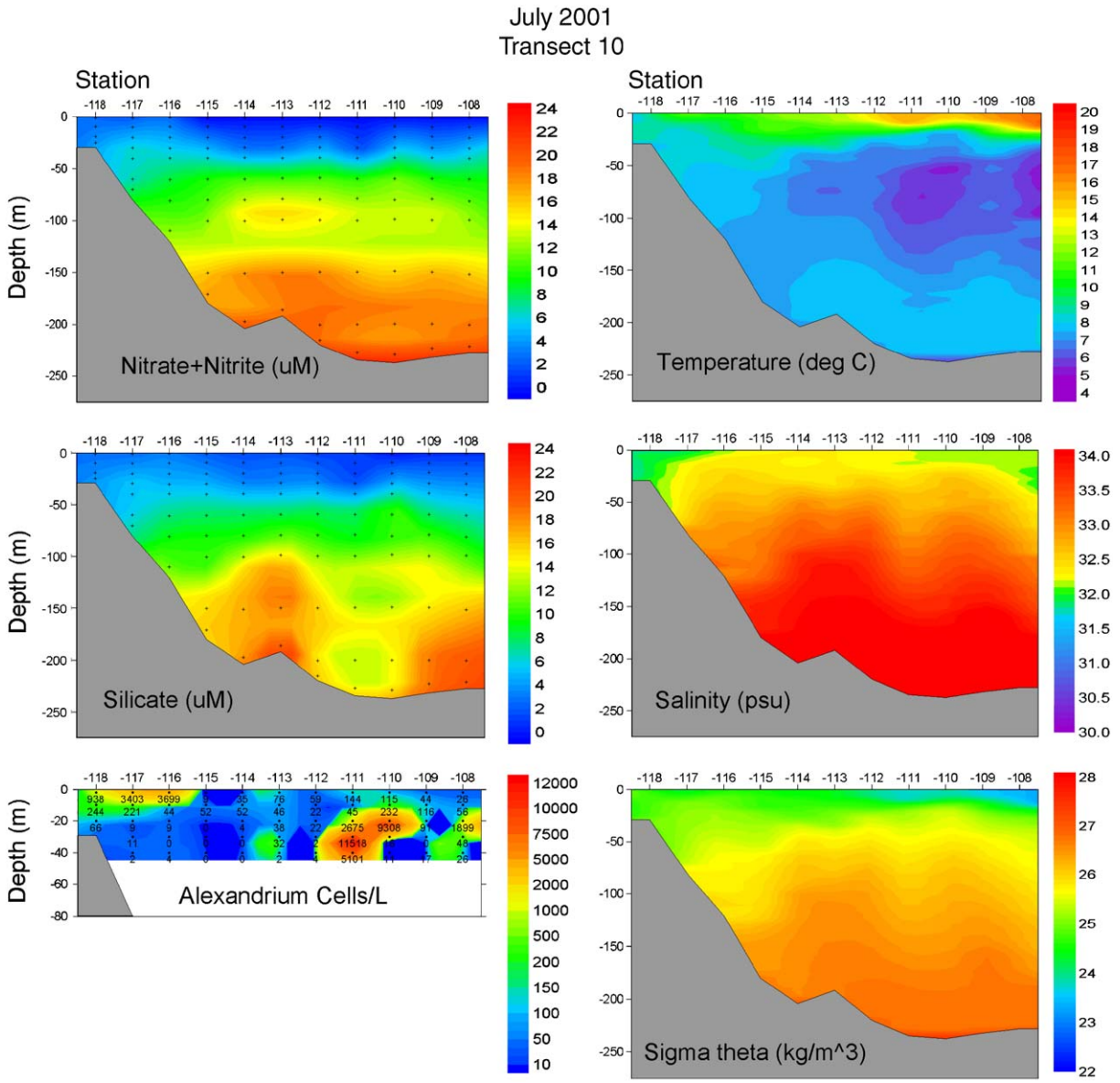

Fig. 15. Transect 10, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

vertically stratified, evidenced by warmer surface waters within the core of the EMCC as identified by the retrograde density and salinity fronts at stations 116 and 117. The satellite image of sea-surface temperature in Fig. 10 shows the approximate location of this warming of surface waters within southwestward-flowing EMCC. DIN concentrations continue to be greater than silicate in the EMCC waters (Stations 118–116), and near-surface silicate concentrations have fallen to less than 4 μM. Diatom cell densities (Fig. 12) are relatively high at the two innermost stations (Stations 117 and 118), and *Alexandrium* cell densities have increased from low concentrations upstream at Transect 12 to greater than 3000 cells L^{-1} within the surface stratified core region of the EMCC at Stations 116 and 117 on Transect 10, but dropping to less than 1000 cells L^{-1} at the innermost station (Station 118). Again, high subsurface densities of *Alexandrium* ($>10{,}000$ cells L^{-1}) farther offshore are clearly

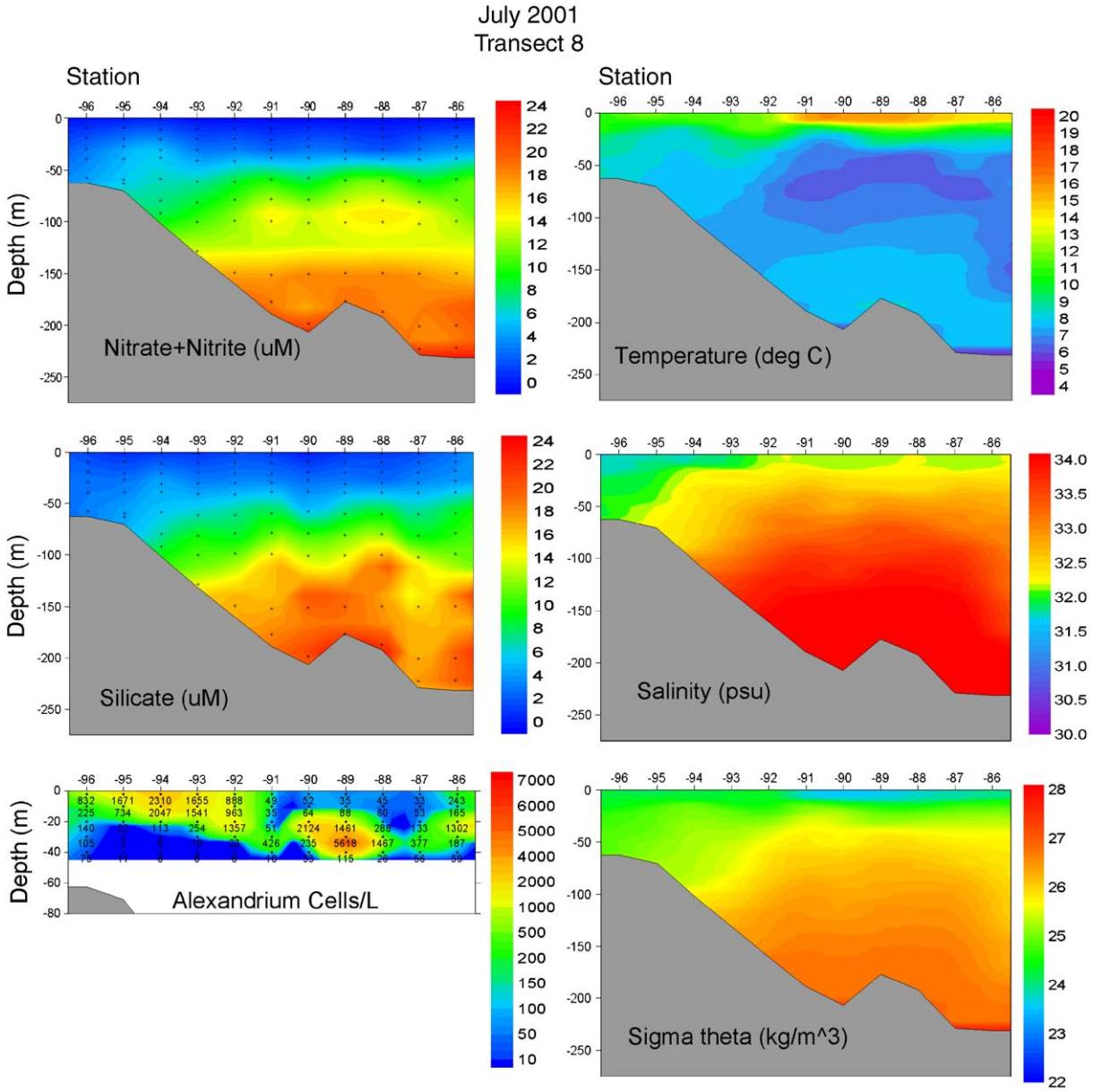

Fig. 16. Transect 8, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

evident in the a real contour plot in Fig. 10. As noted above, this deep population may represent a senescent population of older cells that have been advected back to the east in the offshore limb of the EMCC which has become part of a cyclonic gyre over Jordan Basin. The salinity and density fields in Fig. 15 show this eastward-flowing region at Stations 111 and 112.

The salinity and density fields at Transect 8 (Fig. 16) show the width of the EMCC becoming broader and extending farther offshore, encompassing Stations 92–96. This broadening would also reflect a slowing of current speeds in the EMCC. Nutrient concentrations are still elevated in the EMCC waters, with DIN concentrations continuing to exceed silicate. The surface-water

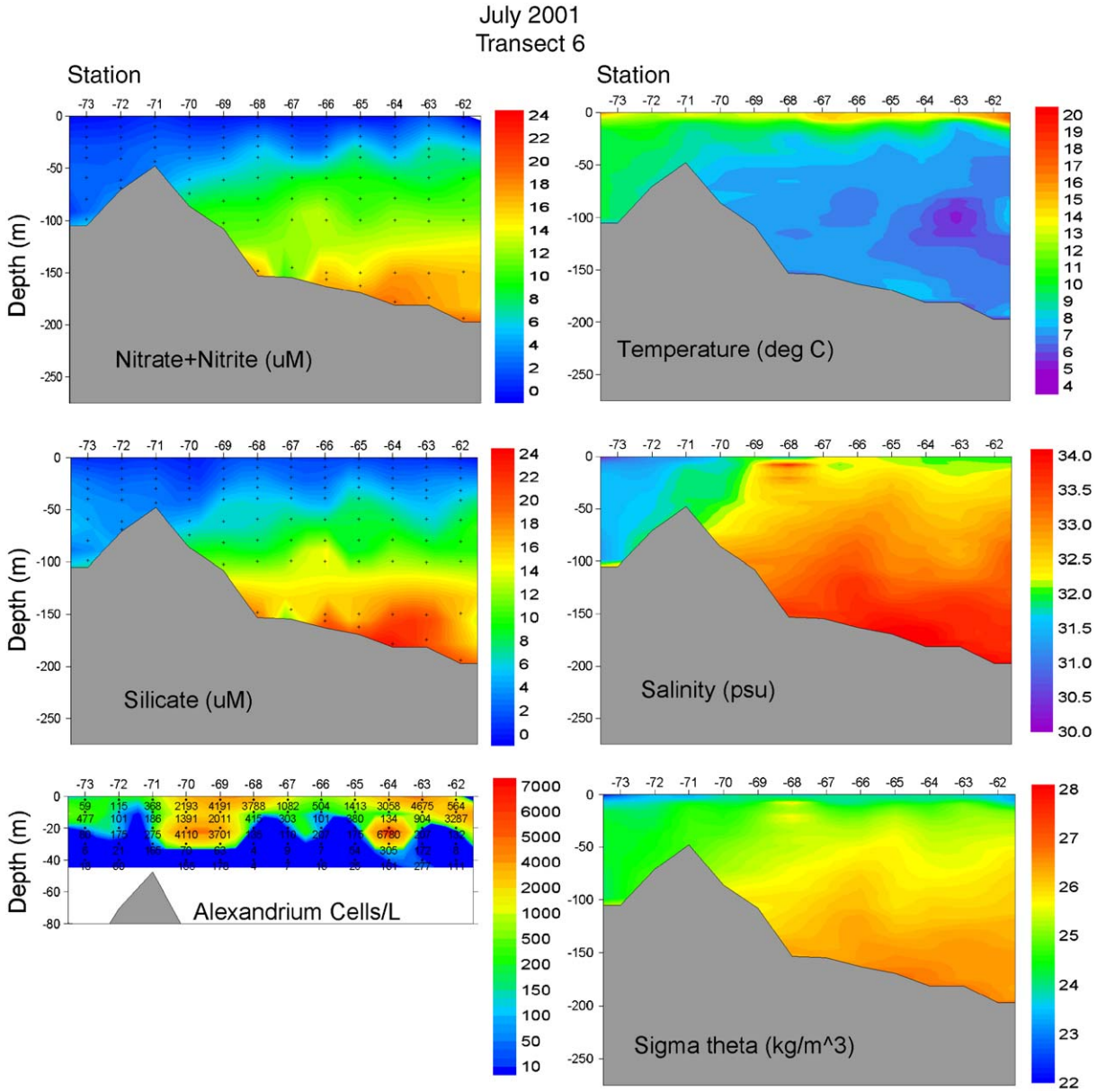

Fig. 17. Transect 6, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

distribution of *Alexandrium* cells continues to reflect the influence of the EMCC, and the patch of cells has become broader at this transect, extending from the innermost station near shore (Station 96) to Station 92. The offshore subsurface patch of *Alexandrium* cells appears to be more continuous with the surface population, and reappears at the surface at the outermost station (Station 86; Fig. 16).

The coastal current at Transect 6 (Fig. 17) has become bifurcated into an inshore limb, which appears to be driven by near-shore fresh waters from the Penobscot River, and a more offshore limb that is a continuation of the EMCC and thus reflects higher-salinity waters from upstream and offshore. The fresher inshore limb of the coastal current system is enriched with greater silicate than DIN concentrations, and consequently exhibits high cell

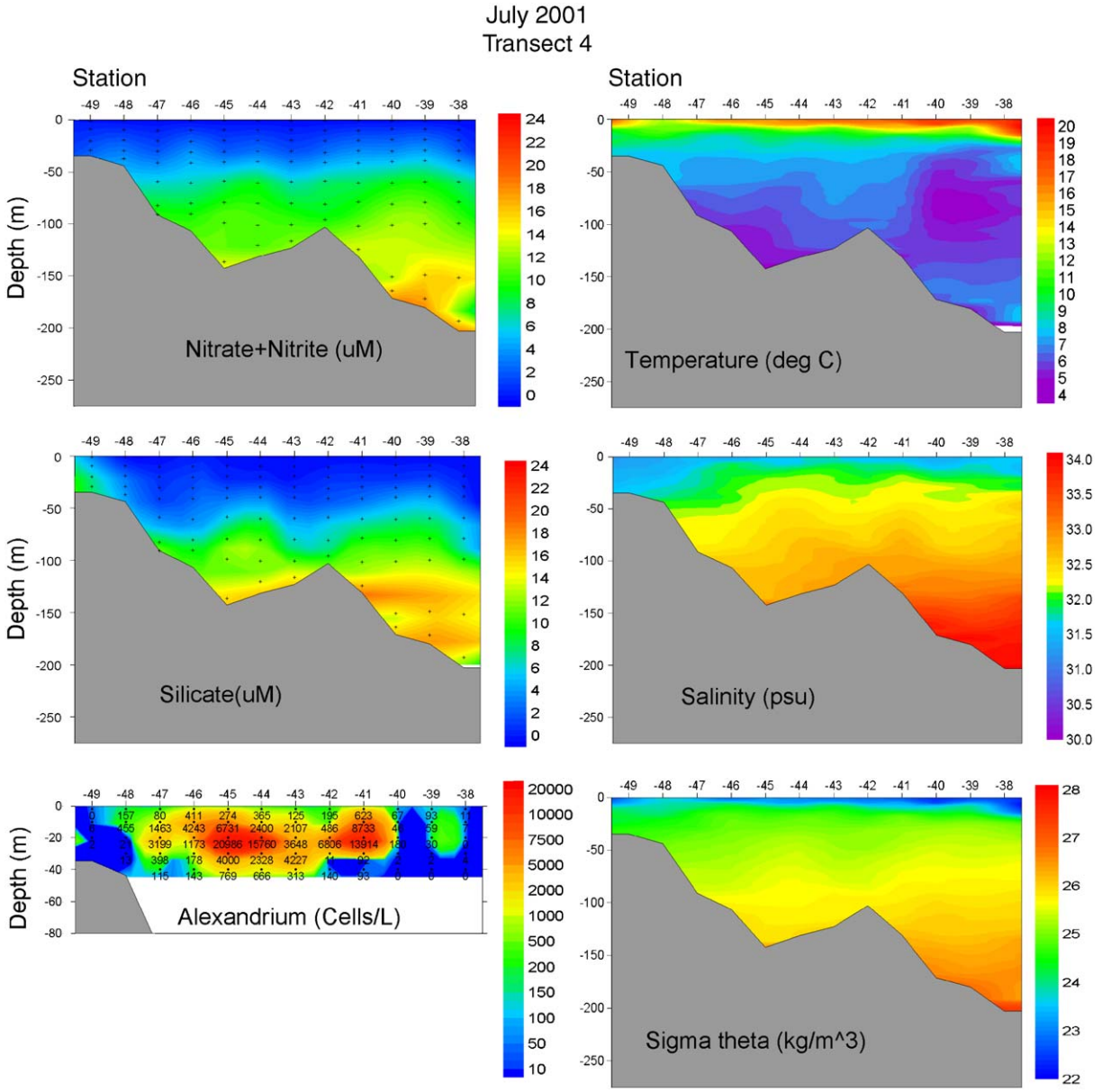

Fig. 18. Transect 4, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

densities of diatoms (>600 cells mL^{-1} at Station 72, Fig. 12). Those high densities of diatoms are also revealed in the chlorophyll distributions in Fig. 11. The distribution of *Alexandrium* cells along Transect 6 is split, with low cell densities in the near shore limb (<500 cells L^{-1} at Station 73; Fig. 17), and much higher cell densities in the offshore limb of the EMCC (Stations 67–70), where diatom densities have fallen to ca. 25 cells mL^{-1} (Fig. 12). We also see a bimodal vertical distribution of *Alexandrium* cells in the EMCC, with surface cell densities between 2000 and 4000 cells L^{-1} (at Stations 69 and 70) and a subsurface maximum with >4000 cells L^{-1}. Farther offshore, at Station 64, a subsurface *Alexandrium* patch (>6000 cells L^{-1}) appears to reside in easterly flowing waters, as indicated by the salinity and density fields. Another bimodal distribution of *Alexandrium* cells occurs

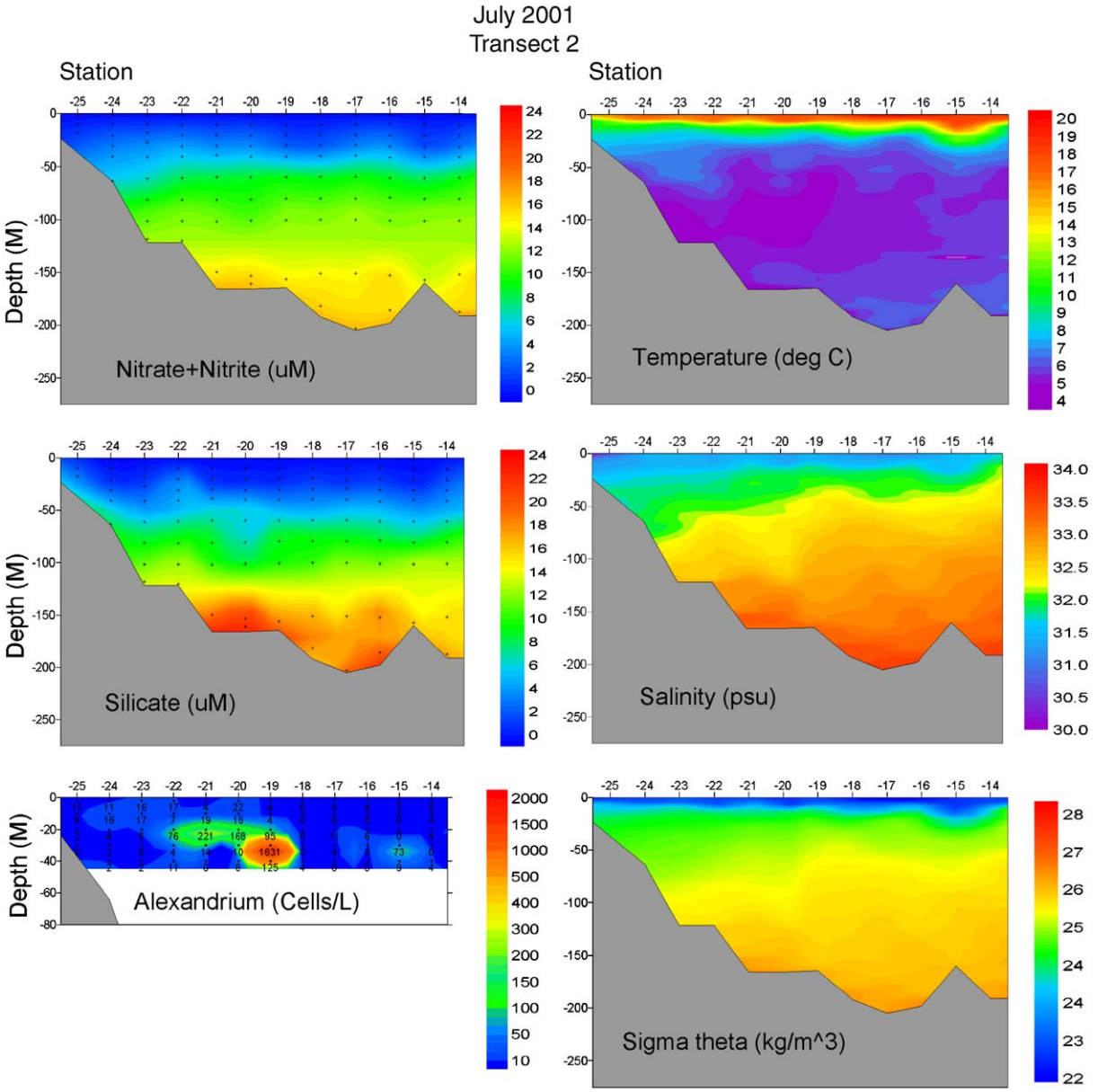

Fig. 19. Transect 2, July 2001 (labeled in Fig. 1). Contoured vertical cross sections of temperature, salinity and density anomaly based on 1-m averaged CTD data, and concentrations of nitrate (NO_3) plus nitrite (NO_2) and silicate ($Si(OH)_4$ in water samples collected at the stations and depths indicated, and *Alexandrium* cell densities; data for *Alexandrium* cell densities are given.

offshore at Station 64, where we see high surface-cell densities (>4000 cells L^{-1}), which is in a region of elevated nutrients; concentrations of DIN here exceed silicate.

Continuing west to Transect 4 (Fig. 18), the cross–shore hydrographic structure generally broadens and is accompanied by surface warming and freshening. As suggested by the AVHRR SST composite shown in Fig. 10, Transect 4 lies just beyond the frontal region that delineates the westward extent of the surface waters of the EMCC due to offshore retroflection of the main body of the current. The surface waters of this region arise primarily from the outflow of the Penobscot River, although there continues to be some through–flow of deeper waters (Pettigrew et al., 2005). Concentrations of silicate in the inshore waters on Transect 4 are significantly

greater than DIN; e.g., concentrations at 10 m at Station 49 are $>6\,\mu M$ silicate but DIN is $<0.6\,\mu M$. The source of this silicate flux is likely coastal freshwater runoff as discussed earlier. Consequently, there are relatively high diatom cell densities on the inshore end of this transect (>500 cells mL^{-1} at Station 48; Fig. 12), dropping to less than 20 diatom cells mL^{-1} at Station 44, which is in the vicinity of where we observed some of the highest densities of *Alexandrium* cells ($>20{,}000$ cell L^{-1}; Fig. 18). Those highest densities of *Alexandrium* cells were sampled at 30 m depth, and our diatom samples, shown in Fig. 12, were collected at the surface (1 m) only, and thus we have no measurements of diatom cell densities at the same subsurface depths where we observed the highest *Alexandrium* densities. However, there may be no reason to expect high diatom densities at depth. Note that at Stations 41–46 (Fig. 18) the concentrations of silicate from the surface down to 30 m depth are less than $1.4\,\mu M$, which may be below the silicate half-saturation constant for diatoms. Interestingly, the concentrations of silicate in the deepest waters, between 125 m and the bottom, exceed DIN, which is the opposite of what we observed in the deepest waters in the eastern Gulf (Figs. 14 and 15) where DIN exceeded silicate. We have already mentioned that the deep source waters, which enter the eastern Gulf of Maine along the bottom through the Northeast Channel, are generally higher in DIN than silicate, thus accounting for greater DIN in the deepest eastern Gulf of Maine waters. But as those bottom waters spread throughout the Gulf from east to west, sediment denitrification removes on the order of 2–4 μM DIN (Christensen et al., 1996), which helps to explain this apparent paradox.

Densities of *Alexandrium* cells along Transect 4 reached some of the highest values observed on this July 2001 cruise, especially at subsurface depths ($>20{,}000$ cell L^{-1}; Fig. 18). The highest *Alexandrium* cell densities were located in two subsurface patches in close association with doming of deep waters that held elevated nutrient concentrations. It is unclear whether those cells are being transported along subducting filaments of the EMCC, are actively growing in regions of high nutrient flux rates, or both. Regardless, the concentrations of DIN in each *Alexandrium* patch exceeded silicate concentrations, a pattern that is consistent in each of the transects to the east.

Finally, Transect 2 (Fig. 19) is the westernmost transect that encountered significant densities of *Alexandrium*, which were located in a subsurface patch of cells (1600 cells L^{-1} at 30 m depth at Station 19), in association with a region of slightly steeper slopes of isohalines and isopycnals.

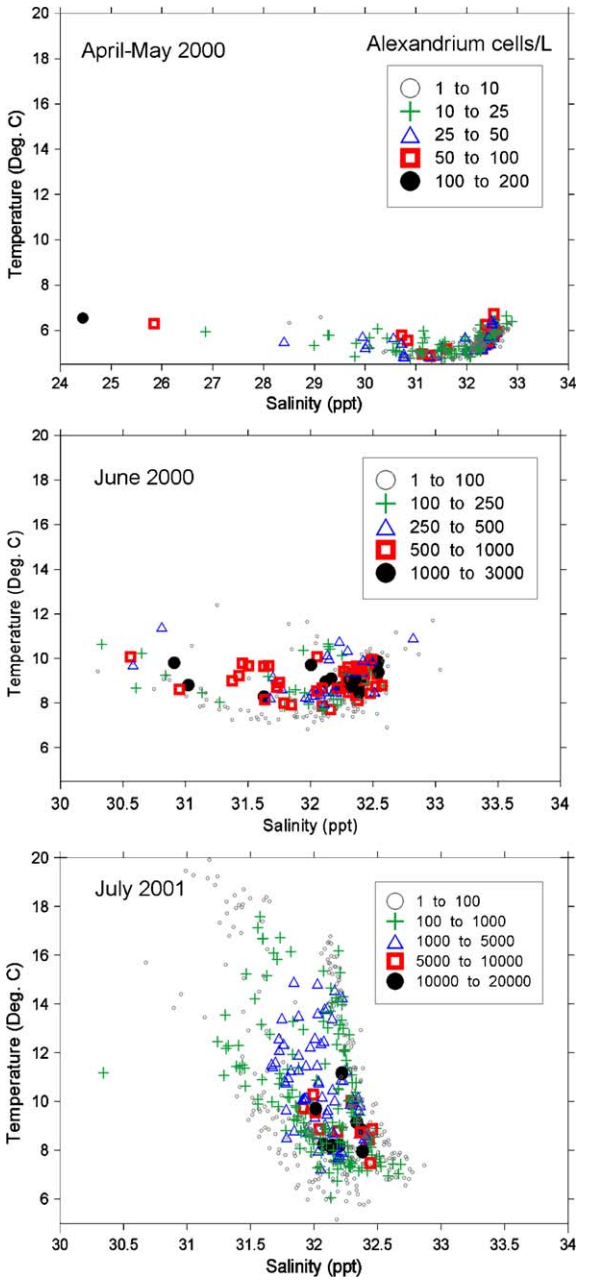

Fig. 20. *Alexandrium* cell densities, given for five density ranges, plotted on a temperature-salinity diagram, for all data collected on the three main cruises, April–May 2000, June 2000, and July 2001.

These six transects help to illustrate two key features of the summertime *Alexandrium* bloom in the Gulf of Maine: first, subsurface populations can reach relatively high cell densities in regions that can be traced back to a source population that has developed within the core region of the eastern Maine coastal current; and second, the distribution of DIN and silicate appears to explain some of the variability in distributions, and suggests the possibility of an allelopathic interaction between diatoms and *Alexandrium*. The alternative explanation that *Alexandrium* is confined to a particular water mass with specific temperatures and salinities, is not supported by the data. That is, we could find no evidence that *Alexandrium* populations were faring better in one water mass type over another, as determined by temperature and salinity (Fig. 20). Our analysis of all *Alexandrium* samples collected on our three main cruises show no consistent T-S envelope that accounts for greater growth rates in one water mass over another. In the early spring (April–May 2000) *Alexandrium* cells were apparently growing best in waters between 5 and 7 °C and salinity 24.5–32.5. In June 2000, the ranges were 7–11 °C and 30.5–32.5 salinity, and in July 2001, the highest cell densities were observed in waters ranging between 7 and 15 °C and 31.5–32.5 salinity (Fig. 20).

4. Conclusions

The important observations reported here can be summarized into a number of main points. We have confirmed earlier evidence from our 1998 work (Townsend et al., 2001) of high densities of *Alexandrium* in subsurface features throughout much of the offshore waters of the eastern Gulf of Maine. We observed in the present study that the annual *Alexandrium* bloom develops between early May and mid-June within the core waters of the nutrient-rich eastern Maine coastal current at its upstream end in the northeastern Gulf of Maine. The initial source of cells is likely from either cysts suspended in the water column, or resuspended from the sediments, or both (see Kirn et al., 2005, and Anderson et al., 2005), perhaps flowing out of the Bay of Fundy. Regardless of the source of the initial innoculum, our results indicate that an initially low cell density population grows while being transported downstream in the EMCC, as the upper layers of the water column warm and vertically stratify. By mid-June patches of relatively high densities of cells were evident extending into the central Gulf of Maine. In both June, and especially July, we observed high densities of *Alexandrium* cells in subsurface populations developing in frontal regions of the EMCC as it flowed to the central Gulf of Maine, a significant portion of which entered a cyclonic gyre-like circulation that brought cells out over Jordan Basin in the east-central Gulf.

Our vertical pump samples revealed subsurface thin layers, on the order of 1-m thickness or less, of high densities of *Alexandriu*m cells in association with pycnocline and nutricline depths, the nature of which is open to speculation. The thin layers may simply represent narrow depth ranges of highly favorable growth conditions, or, it could be that dense aggregations in two, versus three, dimensions are an important aspect of the life history and survival strategy of *Alexandrium*, in that such phenomena would increase the chance of encounter between gametes for sexual reproduction.

The late spring timing of the annual *Alexandrium* bloom in the offshore waters of the Gulf of Maine follows the annual spring diatom bloom, at a time when the concentrations of inorganic nutrients are already significantly drawn down from their wintertime levels. Our results suggest that coastal waters high in silicate from fresh water sources tended to support post-spring-bloom populations of diatoms, which in turn may have excluded the development of high densities of *Alexandrium* cells. The possibility of whether the presence of high densities of diatoms, or populations of high-growth-rate diatoms, could have expressed an allelopathic inhibition of *Alexandrium* remains unresolved, but nonetheless a possibility. The distributions of *Alexandrium* cells in the Gulf of Maine during our three main cruises (April–May and June 2000, and July 2001) did not correspond with any specific temperature–salinity envelope (Fig. 20). Instead, source water concentrations of dissolved inorganic nitrogen and silicate, which determine the dynamics of diatom populations, might explain to a large extent where developing patches of high densities of *Alexandrium* cell are likely to occur. We therefore suggest a hypothesis for future testing: that there is an allelopathic interference between diatoms and *Alexandrium* populations, and that high densities, or growth rates of diatoms impede the growth of *Alexandrium*.

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