

# Changes in copper-complexing organic ligands during spring blooms in the coastal waters of Nova Scotia, Canada

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**ABSTRACT:** Copper-complexing organic ligands were produced by actively-growing phytoplankton during spring blooms in the Bedford Basin and the Northwest Arm, Halifax, Nova Scotia. Iatroscan TLC/FID and TLC/AAS analysis showed the naturally occurring ligands to be qualitatively similar to those produced by phytoplankton grown in batch and continuous culture. The Bedford Basin bloom was interrupted by an extremely heavy rainfall, resulting in a large contribution of material, including organic ligands, from terrestrial sources. The ligand contributed from this source could be shown to be qualitatively different from that produced in situ by bloom organisms.

## INTRODUCTION

Many laboratory culture experiments have demonstrated that marine phytoplankton can produce significant amounts of organic ligands, during both the active growth phase in a cage culture turbidostat (Zhou & Wangersky 1985, 1989b), and in stationary phase in batch cultures (Swallow et al. 1978, McKnight & Morel 1979, Fisher & Fabris 1982, Imber et al. 1985, Zhou & Wangersky 1985, Seritti 1986). The extracellular production of dissolved organic matter (DOM) by phytoplankton could thus be an important source of organic ligands in seawater. In addition, distribution patterns and the correlation between organic ligand concentrations and other biochemical parameters suggested that phytoplankton activity is an important factor in controlling the quality and quantity of organic ligands in the sea (Hirose & Sugimura 1983, Buckley & van den Berg 1986, Kramer 1986, Zhou & Wangersky 1989b).

However, most of our knowledge of the dynamics of organic ligand production comes from laboratory culture experiments; few time-series studies have been done in the field (Foster & Morris 1971, Kremling et al. 1983, Anderson et al. 1984). The high cost of ship time makes it difficult to carry out a time-series experiment in the open ocean, while the relatively high background concentration and multiple terrestrial and

inshore sources make the contribution of organic ligands by phytoplankton difficult to recognize in inshore waters (Anderson et al. 1984).

In temperate waters, phytoplankton activity usually begins in the spring, due to increasing solar radiation, water column stability, and elevated nutrient concentrations in the surface waters resulting from winter storm mixing. Phytoplankton productivity increases rapidly to a peak which may be several times higher than normal for the other seasons, and then crashes as a result of depletion of nutrients. With such intense activity within a relatively short period of time, the spring phytoplankton bloom furnishes an excellent opportunity to study the dynamics of production of organic ligands in marine environments.

In this paper, we report observations taken during 2 spring blooms in the coastal waters of Nova Scotia. In this work we used a modified Sep-Pak C18 column extraction method (Zhou & Wangersky 1989a) to study the dynamics of organic ligand production by bloom phytoplankton.

## MATERIALS AND METHODS

**Sampling.** The first sampling program was carried out from early February to late May 1985. Seawater samples were taken twice a week from the Dalhousie University Aquatron seawater supply system. Seawater

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was drawn from a depth of 12 m in the Northwest Arm, Nova Scotia (Fig. 1, Stn A). Seawater was pumped by three 40 hp pumps through an underground fibreglass pipeline to a storage tank in the Aquatron. Sampling time was usually at high tide ( $\pm 1$  h).

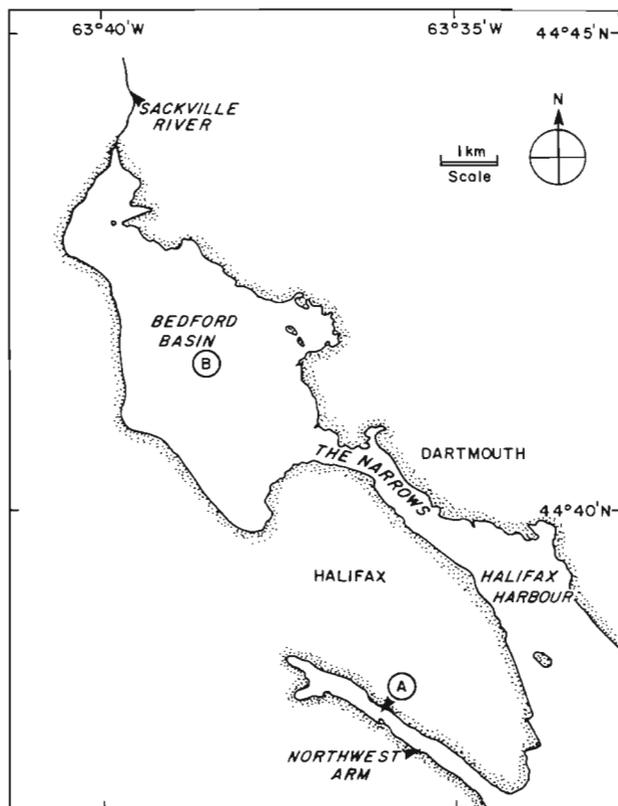


Fig. 1. Sampling sites in the Northwest Arm (A) with a pumping depth of 12 m, and in Bedford Basin (B)

The second sampling program, in Bedford Basin, Nova Scotia, extended from mid-February to mid-April 1987. Samples were collected at the center of the basin (Stn B, Fig. 1), from the MV 'Sigma T' using 5 l Go-Flo bottles. The sampling frequency was once a week at the beginning and 2 to 3 times a week during the peak period of the bloom. Seawater samples were brought back to the laboratory at the Department of Oceanography, Dalhousie University, filtered within 2 h, and completely processed within 5 to 6 h after sampling.

**Sample treatment.** Details of sample treatment have been described in Zhou & Wangersky (1989a), and will be summarized briefly here.

Usually 2 to 3 l of water were filtered through a glass fibre filter (Whatman GF/C for the first sampling program and Gelman AE for the second, precombusted and acid-rinsed) using a pressurized enclosed system. Between 0.5 and 2 l of filtrate was collected, spiked with  $1 \mu\text{M}$  of ionic copper, and processed through 2

Sep-Pak C18 cartridges (Waters Associates) connected in series. The cartridges were then removed from the system, washed with 20 ml of Super-Q water (Millipore) and eluted with 6 ml of methanol-water (1:1) solution (MW). The eluates were collected in 7 ml Teflon vials and stored in a freezer for further analysis and characterization. The copper-ligand concentration in the eluate, [L], is 'the concentration of extractable copper-complexing organic ligands'.

Reagents used for sample processing were Ultrex, Baker (HCl and  $\text{HNO}_3$ ), or OmniSolv, BDH (methanol). The plastic ware was cleaned by soaking in 5 M  $\text{HNO}_3$  for over 24 h and rinsing with Super-Q water. Sep-Pak C18 columns were precleaned by an elution of 10 ml of methanol, 10 ml methanol-HCl (1 M) (1:1), 10 ml of methanol and 20 ml of Super-Q water.

**Chromarod TLC characterization.** A Chromarod thin-layer chromatography technique has been developed to characterize the organic ligands isolated, coupled with flame ionization detector (TLC-FID) for carbon detection and atomic absorption spectroscopy (TLC-AAS) for copper analysis (Zhou & Wangersky 1989a). This technique was used to characterize 3 samples from Bedford Basin: 1 taken before the spring bloom started, 1 during the bloom and 1 at the time of lowest salinity and peak fresh water discharge.

With multi-step development in a series of solvent systems, organic matter was separated into different fractions: the 'base-mobile' in the solvent chloroform-methanol-ethylamine (70% aqueous solution) (70:18:12); the 'methanol-mobile' in methanol; and the 'acid-mobile' in methanol-formic acid-water (80:10:10). Organic carbon in each fraction was detected by partially or fully scanning through the flame ionization furnace (Iatronscan Th-10, Mk III). Copper concentration in the Sep-Pak C18 eluate was measured by graphite furnace atomic absorption spectroscopy (Perkin-Elmer 403 with HGH 2200 furnace and AS-1 auto-sampler) after proper dilution with 0.1 M  $\text{HNO}_3$  solution. The procedural blank, about 0.1 nM, was corrected in values reported here. Copper associated with organics in each fraction was rinsed off the rods with 1 ml of methanol- $\text{HNO}_3$  (1 M) (1:1) solution and measured directly.

**Other parameters measured.** Samples were collected from the Northwest Arm for particulate organic carbon (POC) and dissolved organic carbon (DOC) analyses and from Bedford Basin for POC, chlorophyll and phosphate analyses. POC was measured by a high-temperature combustion method (Wangersky 1976), DOC by a photo-oxidation method (Gershey et al. 1979), chlorophyll by a fluorometric method (Parsons et al. 1984) and phosphate by a spectrophotometric method (Parsons et al. 1984).

Salinity and temperature data in the Northwest Arm

were kindly provided by Dr. N. Balch, and the fresh water discharge rate from the Sackville River was kindly provided by the Inland Waters Directorate, Environment Canada.

## RESULTS AND DISCUSSION

The Northwest Arm is a small marine inlet along the southwestern boundary of the Halifax Peninsula, with a length of about 6 km, typical width of 300 m, and depth between 13 and 19 m. Fresh water input into the Arm is so small that it can hardly be considered as an estuary. This water body is not restricted by a sill and has considerable exchange with offshore waters (Gregory 1972). The effect of water displacement due to tidal movement was minimized by taking samples at high tide.

Results of observations are summarized in Fig. 2. If POC values were used as an indicator of bloom phytoplankton biomass in the Northwest Arm water, Fig. 2 suggests that the spring bloom started at Julian Day 90, and was disrupted by a storm at about Julian Day 109. The storm event was reflected by the influshing of offshore water with higher salinity and low temperature.

The bloom resumed right after the storm and developed rapidly, reaching its peak in about 1 wk. [L], generally followed the trend of POC (Fig. 2). During the first phase of POC increase, there was a slight but detectable increase in [L]. When the storm disrupted the bloom and brought about an intrusion of offshore water, [L] dropped to a minimum. As the bloom resumed after the storm, [L] recovered rapidly and increased from the minimum to the maximum value over a period of 2 wk, i.e. about 1 wk after the POC peak.

The time series of DOC was very similar to that of [L] (Fig. 2). There was a close correlation between [L] and DOC ( $r = +0.94$ ,  $n = 18$ ). This would suggest that organics responsible for changes in [L] and DOC had similar characteristics throughout the spring bloom and might be from the same source, i.e. the in situ production of dissolved organic matter (DOM) by bloom phytoplankton, as suggested by the significant correlation between [L] and POC ( $r = +0.68$ ,  $n = 17$ ). Even if the high [L] value on Julian Day 122 is discarded as an outlier, the correlation remains significant ( $r = +0.64$ ,  $n = 16$ ). The correlation between [L] and POC was not as good as that between [L] and DOC; this may be due to the necessary time-lag between increase of phytoplankton biomass and accumulation of copper-complexing DOM in seawater.

There was no obvious correlation between salinity and [L], which suggested that the influence of fresh

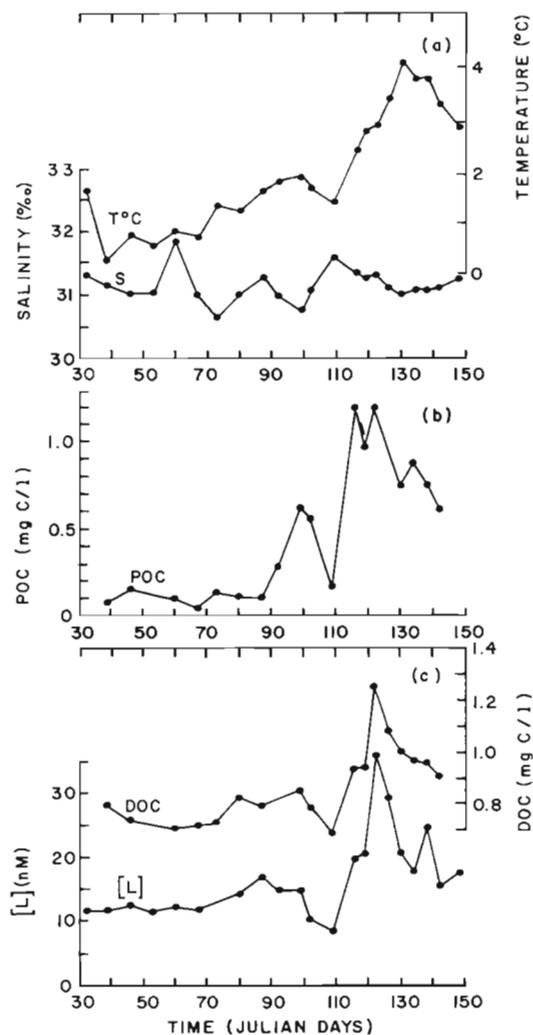


Fig. 2. Northwest Arm spring bloom, 1985. Time-series of temperature, salinity, POC, DOC and [L] in Northwest Arm seawater

water input on ligand production was not significant. While [L] and temperature show a positive correlation ( $r = +0.68$ ,  $n = 21$ ), inspection of Fig. 2 showed that peaks in [L] and DOC were reached fully 10 d before the temperature peak. The peak of the bloom was clearly over by Day 122, possibly as early as Day 115; the relationship between [L] and temperature before Day 122 reflects stabilization of the water column which is a necessary condition for the bloom.

### Bedford Basin, 1987

Bedford Basin is a small marine bay with area of about 17 km<sup>2</sup> and a maximum depth of 90 m, and is connected to the offshore region by a shallow inlet. There is a small river, the Sackville River, which is the

main source of fresh water to the basin. In spring, the high fresh water discharge rate from the Sackville River could significantly change the salinity in the central basin surface water, and may carry significant amounts of terrestrial humic materials. Thus, river-borne humic materials would be the major interference in the study of dynamics of in situ production of copper-complexing DOM by bloom phytoplankton.

Sample results to be discussed here were taken from 5 m depth, and are summarized in Fig. 3. Samples from

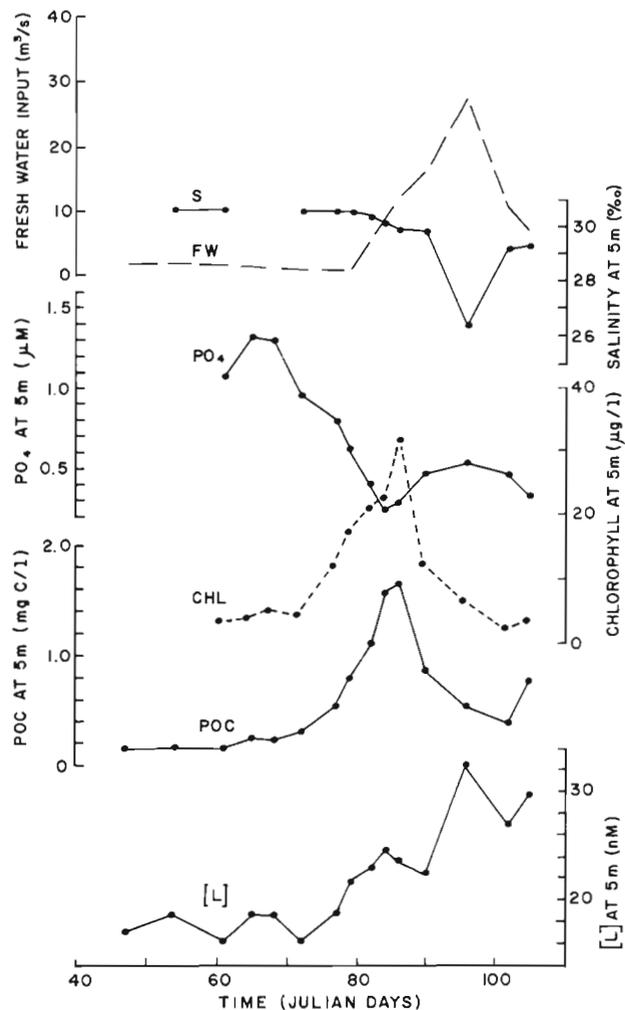


Fig. 3. Bedford Basin spring bloom, 1987 Time-series of fresh water discharge rate from the Sackville River, salinity, concentrations of phosphate, chlorophyll, POC and [L] at a depth of 5 m in the central Bedford Basin

other depths were also taken but only occasionally. Chlorophyll and POC data suggest that the bloom was a single peak, close to the classical concept of a spring bloom. Chlorophyll and POC started to increase rapidly at Julian Day 72, accompanied by the sharp drop in

nutrient phosphate. Peaks of chlorophyll and POC coincided with the minimum of phosphate. After reaching the peak, chlorophyll and POC values dropped off rapidly and phosphate recovered slightly, probably due to a combination of in situ regeneration, mixing of surface and deep waters, and a contribution from the fresh water input from the Sackville River.

[L] varied slightly, from 16.2 to 18.6 nM, before the inception of the bloom, possibly due to the effect of the tidal cycle. [L] increased gradually along the course of bloom development and reached a value of 24.4 nM at Julian Day 84, when chlorophyll and POC were close to their maxima. It was slightly lower in the next few days, even at the peak of the bloom, probably due to physical processes such as tidal movement and water mixing rather than biological activity. [L] increased sharply to a value of 32.3 nM at Julian Day 92, which was coincident with the peak of fresh water discharge of the Sackville River and the minimum of salinity of surface water. Similar coincidence between [L] and influence of fresh water discharge could also be seen in the data from a depth of 2 m (Fig. 4), with larger changes in both [L] and salinity. It is obvious that the input of terrestrial humic materials from river

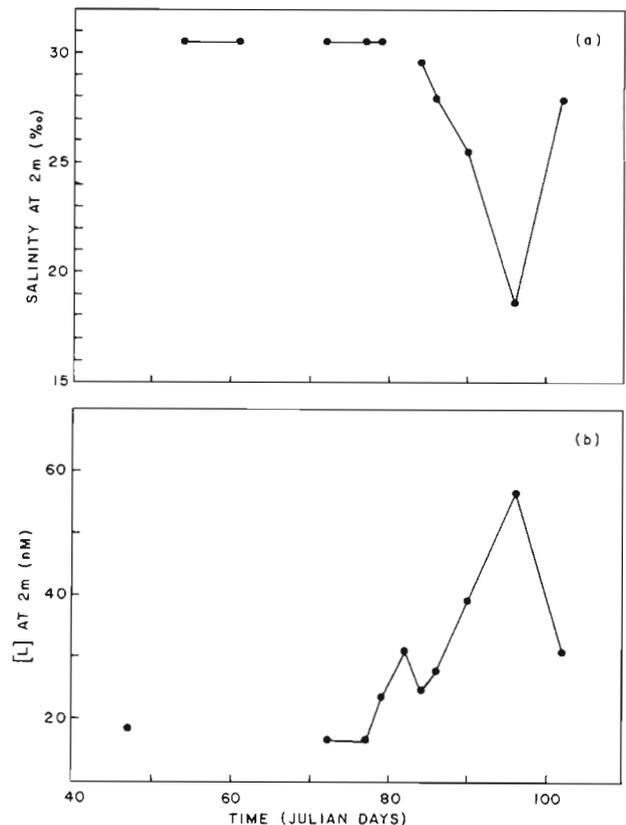


Fig. 4. Bedford Basin spring bloom, 1987 Time-series of salinity and [L] at 2 m in the central basin

water was the dominant source for the increase in organic ligand concentration at this stage.

### Comparison with the culture experiment

Many workers have observed the release of trace metal-complexing DOM by cultured phytoplankton, especially in the stationary or post-stationary phase of a batch culture (Swallow et al. 1978, McKnight & Morel 1979, Fisher & Fabris 1982, Imber et al. 1985, Zhou & Wangersky 1985, Seritti et al. 1986). With a cage culture turbidostat system and the modified Sep-Pak C18 column extraction method, we have found a significant production of copper-complexing organic ligands by the marine diatom *Phaeodactylum tricornutum* in log phase growth (Zhou & Wangersky 1989b). In a batch culture of the marine diatom designed to model a spring bloom, [L] was produced and accumulated from the early exponential growth phase, reaching a maximum value about 1 wk after the biomass peak.

The situation in the real marine environment is much more complicated than in laboratory cultures. Factors such as mixing between surface and deep water, and tidal movements of water masses would make observed parameters more variable. Other sources of organic ligands, e.g. river-borne humic materials, may sometimes be dominant over in situ production even during a spring bloom, in which phytoplankton biomass is still 1 to 2 orders of magnitude lower than that in the laboratory culture experiment. Thus, the in situ organic ligand production in the marine environment is much more difficult to recognize and study in laboratory culture experiments.

Even with these complexities in the field, observations in the Northwest Arm and Bedford Basin have similarities to results in batch cultures. First of all, there were noticeable amounts of organic ligands produced by phytoplankton and accumulated in the active growth phase in both the field study reported here and the batch culture experiment (Zhou & Wangersky 1989b), although the amount was smaller by about a factor of 10 in the field study than in the culture experiment. Secondly, there were time lags between the increases in phytoplankton biomass and [L]. The [L] maxima in the batch culture and in the Northwest Arm were reached about 1 wk after that of the phytoplankton biomass, as measured by POC. The situation in Bedford Basin, however, was complicated by the input of a large amount of terrestrial material from the Sackville River at a late stage. Similarities between field observations and the culture experiment would suggest that the increases in [L] in the Northwest Arm and in Bedford Basin in the early phases of the bloom were related to in situ phytoplankton productivity.

### Comparison with other studies

It is surprising to find that few time-series studies of organic ligand production during a spring bloom have been reported (Foster & Morris 1971, Anderson 1984). In an early study (Foster & Morris 1971), the organically bound copper concentration was found to vary seasonally, with higher values during spring and early summer, related to higher productivity in these seasons. This observation is in good agreement with the results of this study, especially with that in the Northwest Arm, where the fresh water input was insignificant.

On the other hand, Anderson et al. (1984) were unable to correlate changes in organic ligand concentration to in situ phytoplankton activity. Instead, they found a significant correlation between organic ligand concentration and salinity, suggesting the importance of terrestrial humic materials as a source of organic ligands in the waters they studied. This is similar to the situation in the late stages of the spring bloom in Bedford Basin, when the Sackville River input increased dramatically and terrestrial humics became the dominant source of organic ligands in surface water.

There may be several reasons for the difference between the results in this study and those of Anderson et al. (1984), such as higher bloom phytoplankton activity (5 to 15 times higher) and lower background ligand concentration in this study. Another important reason may be the different techniques used in the 2 studies. The previous authors used a bacterial bioassay technique with a detection limit around  $10^{-7}$  M, which was close to or even higher than the range of increase in [L] due to in situ production ( $10^{-8}$  to  $10^{-7}$  M). In addition, their method measured the total organic ligand concentration. With a high background value, it is difficult to detect a relatively small change with a less sensitive technique. On the other hand, the technique used in this study was a modified Sep-Pak C18 column extraction method which processed a larger volume of seawater sample and obtained a large concentration factor (100 to 300 times), thus providing a higher sensitivity ( $10^{-9}$  M). In addition, this technique does not isolate total organic matter, but only certain fractions of organic ligands, favoring phytoplankton exudates over bulk organics (Zhou & Wangersky 1989a). Our method measured a relatively large change of [L] against a relatively small background value. With this technique, we were able to observe the correlation of organic ligand concentration to in situ phytoplankton activity.

### Chromarod TLC characterization

Three typical samples from Bedford Basin were characterized by the Chromarod TLC technique, sam-

ples taken before and during the spring bloom, and at the peak of fresh water discharge. The TLC-FID chromatograms are shown in Fig. 5, and the quantita-

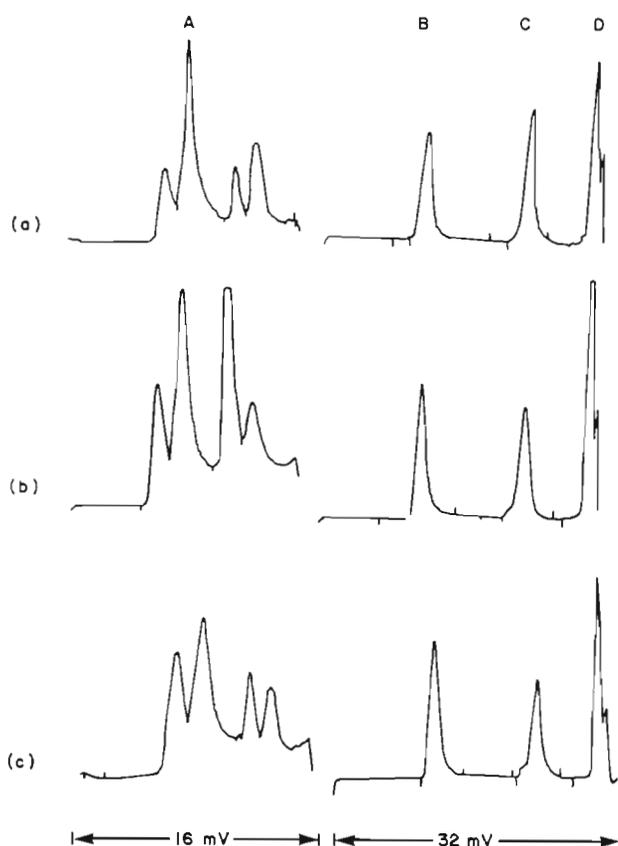


Fig. 5. Bedford Basin spring bloom, 1987. TLC-FID chromatograms of 3 typical samples. For each sample, there was a partial scan for the 'base-mobile' fraction (A) and a full scan for the 'methanol-mobile', 'acid-mobile' and 'residue' fractions (B, C and D respectively), resulting in 2 chromatograms per sample

tive results of the characterization are summarized in Table 1. The most striking characteristic of the chromatograms is the increase in the third peak in the base-mobile fraction at the height of the bloom. The second peak in the low-salinity sample was proportionally much higher than the first, presumably a result of additional humics of terrestrial origin. Identification of specific compounds by this technique, although theoretically possible, is not practical; some variation of HPLC/MS would be the indicated method. From pre-bloom to bloom and then to the peak of the river discharge, both total organic carbon and [L] increased. The specific copper-complexation capacity of the organics isolated ( $nM/\mu gC$ ) also increased, due to in situ organic ligand production by bloom phytoplankton and then to the input of terrestrial humic materials. The characteristics of the organics were quite different for the 3 types of samples. The proportion of the 'base-mobile' fraction for both organic carbon and copper-complexing sites and the specific complexation capacity of the organics in this fraction were the highest in the spring bloom sample, in the following order: (Bloom sample) > (Pre-bloom sample) > (Low salinity sample).

Since the proportion of the 'base-mobile' fraction and its specific complexation capacity were important indicators of phytoplankton contribution to organic ligands, the above order suggested that organic production by bloom phytoplankton was responsible for the increase in [L] from pre-bloom to bloom samples.

On the other hand, the proportion of organic carbon and copper-complexing sites in the 'acid-mobile' fraction were significantly higher in the low salinity sample than in the other two, increasing from 16 and 26% respectively in the bloom sample to 23 and 40% in the low salinity sample, implying that a significant number of complexing sites in the terrestrial humic materials

Table 1. Bedford Basin spring bloom, 1987. Characterization of samples taken at different times. Low salinity: sample with lowest salinity at 2 m, on Julian Days 90 and 96; Bloom: samples taken near the peak of bloom and with near-normal salinity, at 5 m, on Julian Days 82 and 84; Pre-bloom: sample taken before spring bloom at 5 m, on Julian Days 61 and 65

Sample type	Base-mobile	Methanol-mobile	Acid-mobile	Total
<b>Organic carbon, <math>\mu gC\ l^{-1}</math> (% of total)</b>				
Low salinity ( $n=5$ )	$92.2 \pm 8.5$ (56)	$35.0 \pm 1.7$ (21)	$37.5 \pm 2.8$ (23)	164.7
Bloom ( $n=4$ )	$59.1 \pm 4.0$ (65)	$16.5 \pm 0.5$ (19)	$14.9 \pm 0.7$ (16)	90.5
Pre-bloom ( $n=4$ )	$43.9 \pm 3.7$ (62)	$15.5 \pm 0.7$ (22)	$11.6 \pm 0.5$ (16)	71.0
<b>Organic ligands, <math>nM</math> (% of total)</b>				
Low salinity ( $n=4$ )	$5.6 \pm 0.6$ (12)	$23.4 \pm 0.7$ (48)	$19.0 \pm 0.4$ (40)	48.0
Bloom ( $n=4$ )	$5.6 \pm 0.5$ (23)	$12.1 \pm 0.4$ (50)	$6.3 \pm 0.6$ (26)	24.0
Pre-bloom ( $n=4$ )	$3.5 \pm 0.4$ (20)	$9.0 \pm 0.2$ (52)	$4.8 \pm 0.4$ (28)	17.3
<b>Copper-complexation capacity, <math>nM\ \mu g\ C^{-1}</math></b>				
Low salinity	0.060	0.67	0.49	0.30
Bloom	0.095	0.73	0.43	0.27
Pre-bloom	0.080	0.58	0.42	0.24

were in the 'acid-mobile' fraction. In addition, the specific complexation capacity of the organics in this fraction in the low salinity sample was the highest, with the following order: (Low salinity sample) > (Bloom sample) = (Pre-bloom sample), i.e. terrestrial materials in this fraction have higher specific complexation capacities than their counterparts in seawater and in phytoplankton exudates.

For the 'methanol-mobile' fraction, the order of specific copper-complexation capacities for the organics are: (Low salinity sample) < (Bloom sample) > (Pre-bloom sample). This suggests that the phytoplankton exudates in this fraction have higher specific complexation capacities than not only background organics in seawater but also terrestrial humics.

Since the characteristics of organics in the bloom sample were significantly different from those of the low salinity sample, it could be concluded that the increase in [L] from the pre-bloom to bloom sample was not due to the input of terrestrial humics. In fact, changes in the characteristics of organics in the interval from pre-bloom to bloom were quite similar to the differences between natural seawater and culture medium. Organic carbon and copper-complexing sites in the 'base-mobile' fraction and the specific copper-complexation capacity in this and the 'methanol-mobile' fractions increased with total organic ligands released by diatoms in a cage culture turbidostat (Zhou & Wangersky 1989b) and in the early, active growth phase of batch cultures. The release of organic ligands by these populations in culture was quantitatively similar, on a 'per organism' basis, to the releases by bloom populations. This thus provides evidence of the in situ production of organic ligands by bloom phytoplankton.

We were fortunate in having so clear a separation between the bloom peak and the contribution of terrestrial materials in the Bedford Basin samples. In a situation without such clear dominance of contributions from 1 source, some measure such as copper concentrations of specific Iatroscan peaks would have to be used to sort out the relative importance of the sources.

## CONCLUSION

Observations during spring blooms in the Northwest Arm and Bedford Basin demonstrated noticeable increases in concentration of extractable copper-complexing organic ligands during the course of bloom development. Close correlations between [L], DOC and POC in the Northwest Arm suggested that the bloom phytoplankton produced significant amounts of DOM capable of copper-complexation. Results of Chromarod TLC characterization of Bedford Basin samples showed that the characteristics of bloom sample were similar to

those of culture medium during the early phase of a batch culture, but different from those of the low salinity sample, providing evidence of in situ production of organic ligands by bloom phytoplankton.

The results of the Bedford Basin spring bloom study also suggested that in the coastal water where the fresh water runoff is significant, the terrestrial humic material input may at times become the dominant source of organic ligands.

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