

## Fluorescent dissolved organic matter in lakes: Relationships with heterotrophic metabolism

*W. K. Levi Cammack and Jacob Kalff*

Department of Biology, McGill University, 1205 avenue Docteur Penfield, Montréal, Québec H3A 1B1, Canada

*Yves T. Prairie<sup>1</sup> and Erik M. Smith*

Département des sciences biologiques, Université du Québec à Montréal, Case Postale 8888, succursale Centre-Ville, Montréal, Québec H3C 3P8, Canada

### Abstract

Characterizing dissolved organic matter (DOM) composition remains a major unresolved problem in aquatic ecology and is one of the key impediments to developing a good understanding of DOM production and consumption by heterotrophic bacteria. Fluorescence spectroscopy has been proposed as a promising method for characterizing DOM, but few links have been demonstrated between DOM fluorescence and DOM composition or the processes affected by DOM composition. In 28 southern Québec lakes, tryptophan-like DOM fluorescence (T-FDOM) was found to be a much better descriptor of rates of heterotrophic bacterial metabolism than dissolved organic carbon, describing 52%, 44%, 51%, and 55% of the variability in bacterial production, bacterioplankton respiration, total bacterial carbon consumption, and total plankton community respiration, respectively. In addition, evidence from a series of bacterial regrowth cultures suggests that T-FDOM represents a product of bacterial activity as well as, to a lesser extent, a bioavailable substrate. Our results instead raise the intriguing possibility that T-FDOM concentration reflects a balance between its production and consumption by bacteria. We demonstrate here that fluorescence spectroscopy can be used to identify a highly dynamic fraction of DOM related to bacterial metabolism in lakes.

Dissolved organic matter (DOM) is a heterogeneous mixture of compounds whose dynamics and characteristics strongly influence a number of key ecosystem processes, including the attenuation of solar radiation, control of nutrient availability, alteration of contaminant toxicity, and material and energy cycling (Williamson et al. 1999 and references therein). It is among the largest reservoirs of carbon on the planet (Hedges 1992) and an important, though poorly resolved, component in global carbon cycling models. Heterotrophic bacterioplankton are the main consumers of DOM, yet accurate quantification of their contribution has been impeded by a poor understanding of the factors that regulate the degree to which the DOM substrates they consume are respired or converted into biomass (e.g., del Giorgio and Cole 1998). In general, the source, as well as the subsequent processing, will affect the degree to which DOM is available to heterotrophic bacteria. Although nutrients and temperature have been shown to be important regulators of bacterial me-

tabolism, progress has been slower in understanding the likely large influence of substrate characteristics. A key difficulty in studying the relationship between substrate characteristics and bacterial metabolism is that DOM, the dominant substrate used by heterotrophic bacteria, is difficult to characterize.

Despite widespread recognition of the importance of variability in DOM composition and associated quality, DOM is most often described by bulk measures such as dissolved organic carbon (DOC) or stoichiometric ratios. Although it is possible to characterize DOM composition in terms of its molecular mass spectrum, stable carbon isotopic composition, and elemental composition, these measures are time consuming and typically require sample preparation that can modify DOM composition. Moreover, these techniques have had limited success in defining the relationship between compositional differences in DOM and the metabolic activity of heterotrophic bacteria.

In recent years, there has been a renewed interest in exploring the potential uses of fluorescence spectroscopy for characterizing DOM composition (e.g., Coble et al. 1990). Fluorescence spectroscopy is an ideal method for resolving the concentrations of compounds in a complex mixture because certain compounds fluoresce at characteristic emission wavelengths with an intensity that is proportional to their concentration (Coble 1996). Two main types of DOM fluorescence have been described in natural waters: a humic-like or gelbstoff signal (e.g., Coble 1996) and an amino acid-like signal attributed to tyrosine and tryptophan fluorescence (e.g., Coble 1996; Mayer et al. 1999).

Tryptophan-like DOM fluorescence (T-FDOM) generally

<sup>1</sup> Corresponding author (prairie.yves@uqam.ca).

### Acknowledgments

We thank I. Mak for assistance in the field, C. Côté for nutrient analyses, and the laboratory of R. Carignan for DOC analyses. Discussions with Paul del Giorgio and comments from Mary Ann Moran and from two anonymous reviewers greatly improved the manuscript. This research was supported by the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT) and Natural Sciences and Engineering Research Council of Canada (NSERC) research grants to J.K. and Y.T.P., as well as contributions from the Groupe de Recherche Interuniversitaire en Limnologie (GRIL) and the McGill University Limnology Research Centre.

dominates the amino acid fluorescence signal and, in contrast to tyrosine-like DOM fluorescence, is not subject to problems of Raman interference (Mayer et al. 1999). Although it is not yet possible to interpret T-FDOM as a precise indicator of free amino acids, dissolved proteins, or other amino acid metabolites, it can provide a rough estimate of amino acid concentration at levels found in nature (Mayer et al. 1999). Traganza (1969) was the first to suggest that T-FDOM might be useful for detecting and studying areas of recent biological activity after observing elevated levels of T-FDOM associated with a bloom of *Trichodesmium* sp. in the Sargasso Sea and in a laboratory culture of *Skeletonema costatum*. High levels of T-FDOM have also been observed in ocean sediment porewaters (e.g., Coble 1996; Mayer et al. 1999) and in oceanic surface waters (e.g., Determann et al. 1996), leading some to suggest this material to be a product of biological activity (e.g., Mopper and Schultz 1993).

Despite suggestions of a link between biological activity and T-FDOM, there have been few attempts to test directly whether such a relationship exists. Although the presence of phytoplankton (Determann et al. 1994), bacterial activity, and tryptophan bound to bacteria (Determann et al. 1996) have all been suggested as potential explanations for the observed T-FDOM, only two studies have examined the relationship between biological activity and T-FDOM in closer detail. Ferrari and Mingazzini (1995) found that T-FDOM (G. M. Ferrari pers. comm.) in algal cultures decreased over the course of many months from high initial levels, suggesting that the material was reabsorbed or transformed by algae, by the bacteria almost inevitably present in long-term cultures, or by both. In contrast, Moran et al. (2000) showed that T-FDOM increased after long-term incubation of bacterial cultures in the dark, suggesting this material to be produced by bacteria. However, the results of both studies are compatible with a number of alternative explanations because neither experiment directly measured whether the metabolism of these organisms was associated with the changes in levels of T-FDOM, and measurements were made infrequently over long-term incubations. Thus, although these experiments have provided some useful insights, the relationship between biological activity and T-FDOM remains ambiguous.

The principal goal of this study was to test whether T-FDOM is, across a productivity gradient, related to bacterial metabolism in lakes. We performed the among-system comparison of measurements of DOM and bacterial metabolism to test whether T-FDOM is a superior predictor of bacterial production (BP), bacterioplankton respiration (BR), total bacterial carbon consumption (TBCC), and total plankton community respiration (CR) than DOC. In addition, bacterial abundance (BA), BP, BR, bacterial growth efficiency (BGE), and T-FDOM were monitored in laboratory regrowth experiments to explore whether T-FDOM in nature is most likely to be a bacterial substrate or product.

## Methods

**Sampling**—Sampling was conducted during summer 2001 in 28 southern Québec (~45.20°N, 72.10°W) lakes in the St.

Lawrence Lowlands and Appalachian Mountains regions, a landscape dominated by temperate mixed wood forest and low-intensity agriculture. Depth-integrated epilimnetic water samples were collected with a tube sampler and pooled in an acid-washed 20-liter polyethylene container to allow for subsampling in the laboratory for chemical analyses and measurement of BP and total plankton CR. Water for measurements of BR was collected in an acid-washed 10-liter polyethylene container with a specialized attachment for the filtration apparatus (*see* “Field Sample Metabolic Measurements” below). Containers were placed in a cooler and transported to the laboratory within 2 h of collection. With the use of a similar protocol, epilimnetic water for bacterial regrowth experiments was collected from a subset of the study lakes in 2001, as was water from an overlapping group of 26 lakes during summer 2000. All procedures described are those used in 2001 unless otherwise indicated.

**Chemical analyses**—Total phosphorus (TP) was measured in triplicate spectrophotometrically by the ascorbic acid method following persulfate digestion (Griesbach and Peters 1991). Samples from 2000 were analyzed for TP and total dissolved phosphorus (TDP) in unfiltered and filtered (0.45- $\mu$ m surfactant-free cellulose acetate syringe filters [Nalgene]) water; total nitrogen (TN) and total dissolved nitrogen (TDN) concentrations were measured in unfiltered and 0.45- $\mu$ m-filtered samples as nitrate concentrations—measured after reduction through a cadmium coil—following alkaline persulfate digestion. pH was determined with an Oakton WD-356 pH meter with a combination ATC probe. In situ temperature profiles were collected with a YSI model 95 dissolved oxygen meter.

Triplicate water samples for both DOC and DOM fluorescence analysis were filtered through flushed sterile 0.45- $\mu$ m pore-size surfactant-free cellulose acetate syringe filters (Nalgene) into acid-washed 20-ml glass vials and stored at 4°C. Samples were analyzed within 2 weeks of collection for DOM fluorescence and within 4 weeks for DOC. In 2000, the single DOM fluorescence samples were analyzed within 4 months of collection. DOC was measured by high-temperature catalytic oxidation on a Shimadzu TOC-5000 analyzer, with a mean coefficient of variation of 7%. DOM fluorescence emission spectra were measured with a Shimadzu RF-5301PC spectrofluorometer with a 150-W Xenon lamp at 5 nm excitation wavelength intervals between 220 and 450 nm, with emission data collected at 2-nm intervals between 260 and 560 nm. Bandwidth was 5 nm for both excitation and emission. Samples were analyzed in a temperature-controlled cuvette chamber maintained at 21°C  $\pm$  1°C. All fluorescence data were normalized to the Raman peak of a Milli-Q water blank at 275 nm excitation and 304 nm emission (Coble et al. 1993), which was determined daily to account for secular variations in lamp intensity. A normalized Milli-Q water blank was subtracted from sample emission spectra to eliminate the Raman and Rayleigh scatter peaks of water. The T-FDOM value used was obtained from corrected emission spectra at 275 nm excitation by calculating the area under the curve from 346 to 356 nm emission. The among-replicate sample average coefficient of variation for T-FDOM was 6%, with a maximum of 16%.

Table 1. Mean physical and chemical characteristics and metabolic rates of the 28 lakes sampled in 2001: average epilimnetic temperature (T); dissolved organic carbon (DOC); dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission (T-FDOM); total phosphorus (TP); bacterial production (BP); bacterioplankton respiration (BR); total plankton community respiration (CR); bacterioplankton respiration as a percentage of total plankton community respiration (BR/CR); bacterial growth efficiency (BGE).

Lake	Date	T (°C)	pH	DOC (mg L <sup>-1</sup> )	T-FDOM (RFU)	TP (μg L <sup>-1</sup> )	BP (mg C L <sup>-1</sup> h <sup>-1</sup> )	BR (mg O <sub>2</sub> L <sup>-1</sup> d <sup>-1</sup> )	CR (mg O <sub>2</sub> L <sup>-1</sup> d <sup>-1</sup> )	BR/CR (%)	BGE (%)
Orford	25 Jun	20.2	7.25	3.1	4.01	5.1	9.71×10 <sup>-5</sup>	0.035	0.146	24	15
Fraser	26 Jun	21.0	6.96	5.0	5.80	5.5	1.41×10 <sup>-4</sup>	0.060	0.164	37	13
des Monts	27 Jun	24.1	7.02	5.5	7.59	10.3	9.38×10 <sup>-5</sup>	0.053	0.248	22	10
Boivin	28 Jun	24.0	7.19	6.0	11.62	91.8	2.08×10 <sup>-3</sup>	0.263	0.787	33	34
Bowker	1 Jul	20.4	6.94	2.5	3.94	3.0	3.41×10 <sup>-5</sup>	0.052	0.060	87	4
Brompton	3 Jul	19.4	7.04	5.2	7.32	8.8	1.01×10 <sup>-4</sup>	0.054	0.178	31	11
Lovering	10 Jul	20.6	7.00	6.0	6.96	11.5	2.04×10 <sup>-4</sup>	0.052	0.104	50	20
Stoke	11 Jul	20.7	7.37	3.7	5.08	14.9	1.58×10 <sup>-4</sup>	0.128	0.402	32	7
Desmarais	12 Jul	20.8	7.36	6.4	7.14	20.4	2.55×10 <sup>-4</sup>	0.058	0.131	44	22
Tomcod	14 Jul	19.9	7.62	6.3	10.96	117.1	1.89×10 <sup>-3</sup>	0.233	2.105	11	34
Massawippi	19 Jul	21.0	7.90	4.8	5.78	11.0	4.30×10 <sup>-4</sup>	0.092	0.204	45	23
Lyster	20 Jul	19.6	6.01	3.6	4.66	5.0	8.32×10 <sup>-5</sup>	0.035	0.103	34	13
Trois Lacs	21 Jul	23.9	—	9.0	8.34	41.6	2.10×10 <sup>-3</sup>	0.494	0.733	67	21
Montjoie	25 Jul	24.0	7.13	5.6	5.32	13.3	9.98×10 <sup>-5</sup>	0.021	0.032	66	23
Denison	26 Jul	24.3	7.35	5.6	8.90	27.4	8.14×10 <sup>-4</sup>	0.186	0.434	43	22
Simoneau	28 Jul	22.6	7.18	5.2	4.39	5.1	1.13×10 <sup>-4</sup>	0.032	0.062	52	18
Leclerc	29 Jul	21.6	7.30	4.7	5.15	7.9	4.18×10 <sup>-4</sup>	0.093	0.232	40	22
Parker	8 Aug	24.5	7.18	10.8	8.92	12.7	5.37×10 <sup>-4</sup>	0.092	0.244	38	27
St-Georges	9 Aug	25.7	7.54	7.1	9.56	29.1	8.26×10 <sup>-4</sup>	0.081	0.457	18	39
Libby	10 Aug	25.7	7.12	5.5	6.53	12.5	4.42×10 <sup>-4</sup>	0.066	0.197	33	30
Aylmer	11 Aug	22.8	7.13	9.5	7.04	10.3	3.14×10 <sup>-4</sup>	0.153	0.147	104	12
Drolet	13 Aug	22.8	7.32	4.3	5.13	18.0	6.22×10 <sup>-4</sup>	0.054	0.235	23	42
Stukely	17 Aug	23.6	6.86	4.4	4.81	5.1	1.05×10 <sup>-4</sup>	0.079	0.111	71	8
Lippé	22 Aug	21.1	7.41	5.3	6.95	18.2	7.56×10 <sup>-4</sup>	0.088	0.275	32	35
Brome	24 Aug	23.0	7.31	3.8	4.88	16.9	5.81×10 <sup>-4</sup>	0.061	0.256	24	28
Malaga	25 Aug	22.7	6.80	4.7	4.39	9.2	2.97×10 <sup>-4</sup>	0.046	0.129	36	29
Truite (Orford)	26 Aug	22.0	7.61	6.1	5.68	7.2	1.17×10 <sup>-4</sup>	0.082	0.063	131	8
Fitch Bay	29 Aug	21.5	7.79	8.2	8.54	23.9	7.17×10 <sup>-4</sup>	0.084	0.450	19	35

Spectra were not corrected for machine-specific characteristics because of the mismatch in the fluorescence properties of the standard reference material (quinine sulfate) and T-FDOM (Mayer et al. 1999). Our region of interest for T-FDOM (275 nm excitation, 346–356 nm emission) yielded essentially no fluorescence for quinine sulfate. As a result, we reported all our measures in relative fluorescence units (RFU). However, comparison of our results with those obtained on other instruments can still be achieved with the use of the following fluorescence intensities obtained for a 0.5-μmol L<sup>-1</sup> quinine sulfate solution in 0.5 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. At the peak excitation–emission wavelength coordinates (342 nm excitation, 450 nm emission), quinine sulfate yielded 630 RFU. At the T-FDOM 275-nm excitation wavelength, quinine sulfate yielded 83 RFU at a 450-nm emission wavelength.

*Field sample metabolic measurements*—All metabolic rates were estimated with the use of samples incubated in the dark in a water bath maintained at 20°C ± 1°C with a Lauda K2R thermostat, with the incubation temperature close to the epilimnetic temperature of the lakes (Table 1). Rates were measured at a standardized temperature, rather than at in situ temperatures, so that temperature effects would not obscure potential relationships between metabolic

and spectroscopic measurements. In using this particular experimental design, we assumed the metabolism of plankton communities not to be optimally adapted to any one specific temperature (e.g., Sampou and Kemp 1994).

BR was defined as the change in dissolved oxygen per day in 2-μm-filtered water, with estimates of total plankton CR providing a constraint on the maximum possible value of BR. Size-fractionated plankton samples have been used to estimate BR in a number of other studies, with filter pore sizes ranging from 0.8 to 3 μm (*see del Giorgio et al. 1997 and references therein*). Water from each lake was filtered following the procedure of Smith and Kemp (2001) with the use of an acid-washed, custom-built, reverse filtration system with 2-μm pore-size, 142-mm-diameter polycarbonate membrane filters (Poretics). A new filter was used for each lake. The filtrate was subsampled within 2 h of initiating filtration once a sufficient volume (i.e., ~3 liters) had been collected.

BR and CR were estimated from rates of dissolved oxygen consumption in sample water incubated in sealed biological oxygen demand (BOD) bottles. Dissolved oxygen was measured by precision Winkler titration with a Mettler DL21 automated titrator and a Mettler Toledo DM140-SC platinum ring redox electrode. After the addition of Winkler reagents and acidification, as prescribed in Carignan et al. (1998), aliquots of approximately 60 ml were poured from each

BOD bottle and titrated with  $0.025 \text{ mol L}^{-1}$  sodium thiosulfate. Sodium thiosulfate was kept at the water bath temperature ( $20^\circ\text{C} \pm 1^\circ\text{C}$ ), to avoid introducing error associated with temperature variation (Carignan et al. 1998). The mean coefficient of variation of this technique was 0.3% for BR and 0.2% for CR, with a maximum of 0.9% and 2.2%, respectively.

On return to the laboratory, sample water for CR was siphoned from the 20-liter carboy into 16 acid-washed 300-ml BOD bottles, allowing bottles to overflow with twice the sample volume; bottles were then sealed and placed in the water bath for 30 min to equilibrate to  $20^\circ\text{C}$ . Next, a set of five bottles was removed and fixed with Winkler reagents for measurements of the initial concentration of dissolved oxygen; an additional bottle was removed for determining the rate of bacterial [ $^3\text{H}$ ]-leucine incorporation. Dissolved oxygen was measured at an intermediate and a final time point in the two remaining sets of five bottles to test the assumption that oxygen concentrations decreased linearly over the course of the incubations (Pomeroy et al. 1994). BR was measured by an identical method, except that  $2\text{-}\mu\text{m}$ -filtered water was siphoned directly from the filtration system into 60-ml BOD bottles. With few exceptions, filtration yielded a sufficient quantity of filtered water for a minimum of four replicates at each of the three time points.

Most of the CR and BR samples were incubated for between 16 and 19 h, but for as much as 32 and 54 h for CR and BR, respectively, in the most oligotrophic lakes to allow sufficient time for metabolic activity to produce a measurable change in dissolved oxygen. In 13 lakes, BR was obtained from the intermediate time point measure of oxygen because the final measure deviated from linearity. Oxygen consumption was linear over the course of the CR incubations.

BP was estimated from the rate of [ $^3\text{H}$ ]-leucine incorporation in unfiltered water, following the method described by Smith and Azam (1992), with five replicate measurements at each time point. BP was calculated from these rates with the equation and parameter estimates provided by Kirchman (1993).

BGE was calculated by dividing BP by the sum of BP and BR (del Giorgio and Cole 1998). TBCC was calculated by adding BP and BR. In both calculations, BP in unfiltered water was assumed to be representative of rates in  $2\text{-}\mu\text{m}$ -filtered water. BR data were converted to carbon units with the use of a respiratory quotient of 1 (e.g., del Giorgio et al. 1997).

**Bacterial regrowth experiment**—For four of the study lakes (Brome, Brompton, Stukely, Tomcod), chosen to represent a wide range in lake trophicity (i.e., ambient TP of 5 to  $59 \mu\text{g L}^{-1}$ ), BA, BP, BR, BGE, and T-FDOM were monitored in dilution cultures prepared by reintroducing a natural bacterial inoculum to a particle-free ( $0.2\text{-}\mu\text{m}$ -filtered) lake water growth medium (Ammerman et al. 1984; see Smith and Prairie 2004 for specific details of the experiment). Bacterial abundance was determined with a Becton Dickinson FACScan flow cytometer as described in Smith and Prairie (2004). Lake water media were prepared by low-pressure ( $<7 \text{ psi}$ ) in-line filtration, first through a precombusted 142-

mm-diameter GF/F glass fiber filter (Whatman) and then through a deionized water-rinsed  $0.2\text{-}\mu\text{m}$  pore-size filter capsule (Gelman Maxi Culture Capsule). Inocula were prepared by filtering lake water through  $0.8\text{-}\mu\text{m}$  pore-size filters (Gelman pleated capsule filter). All media and inocula were filtered immediately after water collection and transported to the laboratory in cold, dark containers; media water was allowed to equilibrate to  $20^\circ\text{C}$  in a temperature-controlled environmental chamber in the laboratory subsequent to arrival. Next, duplicate dilution cultures were prepared by adding a  $0.8\text{-}\mu\text{m}$ -filtered water inoculum (5% final volume) to the  $0.2\text{-}\mu\text{m}$ -filtered lake water medium and dispensing the combined sample into replicate acid-washed incubation vessels before beginning dark incubation at  $20^\circ\text{C}$ . Cultures were subsampled immediately after the start of incubation to assess initial culture conditions and subsampled at intervals of  $\sim 4 \text{ h}$  (during the day) or  $\sim 8 \text{ h}$  (during the night) over the following 36 h.

**Data analyses**—Data were analyzed by ordinary least squares regression with JMP (SAS) software after log transformation of TP, BP, BR, TBCC, and CR to satisfy the assumptions of normality and equal variance. Multiple regression with stepwise variable selection was used to test whether additional variables significantly improved predictive models. The  $F$  statistic was used to judge significance ( $p < 0.05$ ).

## Results

The physical, chemical, and metabolic data for the 28 lakes sampled in 2001 are presented in Table 1 and are the data used unless otherwise indicated. Lakes were highly variable in trophic status and ranged from oligotrophic (e.g., Bowker and Lyster) to highly eutrophic (Petit St.-François and Boivin [total range in TP is from 3 to  $117 \mu\text{g L}^{-1}$ ]), and levels of DOC ranged from 2.5 to  $10.8 \text{ mg L}^{-1}$ . The morphometric characteristics of these lakes have been described elsewhere (del Giorgio and Peters 1994; Prairie et al. 2002), except for Lac des Monts, Lac Boivin, and Lac Denison, which have surface areas of 0.3, 3.0, and  $0.3 \text{ km}^2$ , respectively.

DOC explained little of the variability in BP, BR, or TBCC (=BP + BR) (25%, 22%, and 24%, respectively; Fig. 1a–c; Table 2) and was unrelated ( $p > 0.05$ ) to total plankton CR (Fig. 1d; Table 2). In contrast, T-FDOM explained substantially more variability in BP, BR, TBCC, and CR, explaining 52%, 44%, 51%, and 55%, respectively (Fig. 1e–h; Table 2), and was a much better ( $p < 0.005$ ) predictor of all metabolic rates than DOC. Although these results supported our original hypothesis, we exploited the spectroscopic data further to examine whether other regions of the fluorescence excitation–emission matrix (see Coble et al. 1990) were related to our measures of bacterial metabolism (e.g., Rochelle-Newall and Fisher 2002). This was accomplished by constructing a series of contour plots illustrating the strength of the correlation (as  $r$  values) between fluorescence intensity at all excitation–emission coordinates and each of the bacterial metabolism variables. T-FDOM was not the region of highest fluorescence intensity, which occurred

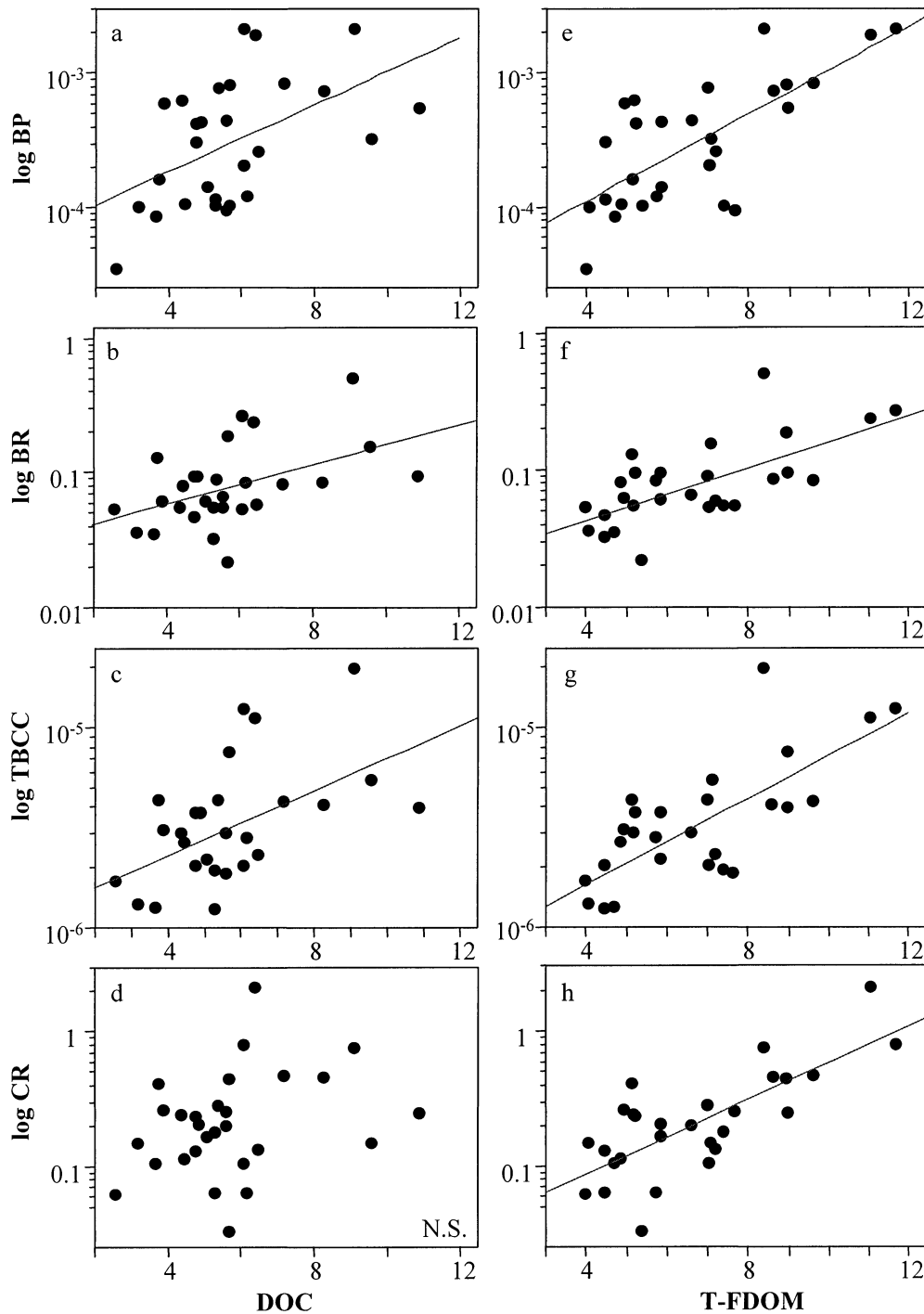


Fig. 1. (a–d) Bacterial production (BP,  $\text{mg C L}^{-1} \text{h}^{-1}$ ), bacterioplankton respiration (BR,  $\text{mg O}_2 \text{ L}^{-1} \text{d}^{-1}$ ), total bacterial carbon consumption (TBCC,  $\text{mol C L}^{-1} \text{d}^{-1}$ ), and total plankton community respiration (CR,  $\text{mg O}_2 \text{ L}^{-1} \text{d}^{-1}$ ) versus dissolved organic carbon (DOC,  $\text{mg L}^{-1}$ ) and (e–h) dissolved organic carbon (DOC,  $\text{mg L}^{-1}$ ) and dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission (T-FDOM, RFU). N.S., a nonsignificant relationship. Regression models and summary statistics are presented in Table 2.

at both  $\sim 265$  nm excitation and 445 nm emission and  $\sim 320$  nm excitation and 445 nm emission—regions well correlated with bulk DOM concentration (Cammack et al. unpubl. data); nevertheless, the resulting contour plots show that the

region for which the correlation between the fluorescence intensity and the bacterial metabolism was highest corresponded always to the T-FDOM region (i.e., DOM fluorescence intensity at 275 nm excitation and 346–356 nm emis-

Table 2. Linear regression models relating chemical and metabolic parameters. All relationships are between data collected in 2001 unless indicated. Standard errors of the regression parameter follow the  $\pm$  symbol. The proportion of variance explained ( $r^2$ ) and standard error of the estimate (SE) are given.

Model no.	Dependent variable	Regression equation	$r^2$	SE
1	log BP	$-4.23 \pm 0.25 + 0.12 \pm 0.04$ DOC	0.25**	0.41
2	log BP	$-4.61 \pm 0.21 + 0.16 \pm 0.03$ T-FDOM	0.52***	0.33
3	log BP	$-4.77 \pm 0.14 + 1.11 + 0.12$ log TP	0.77***	0.23
4	log BP	$-4.76 \pm 0.15 + -0.01 \pm 0.04$ T-FDOM + $1.16 \pm 0.22$ log TP	0.77***	0.24
5	log BR	$-1.53 \pm 0.16 + 0.07 \pm 0.03$ DOC	0.22*	0.27
6	log BR	$-1.74 \pm 0.14 + 0.10 \pm 0.02$ T-FDOM	0.44***	0.23
7	log BR	$-1.72 \pm 0.14 + 0.55 \pm 0.11$ log TP	0.47***	0.22
8	log BR	$-1.78 \pm 0.14 + 0.04 \pm 0.04$ T-FDOM + $0.33 \pm 0.21$ log TP	0.50***	0.22
9	log TBCC	$-5.96 \pm 0.17 + 0.08 \pm 0.03$ DOC	0.24**	0.28
10	log TBCC	$-6.22 \pm 0.14 + 0.11 \pm 0.02$ T-FDOM	0.51***	0.22
11	log TBCC	$-6.24 \pm 0.12 + 0.66 \pm 0.10$ TP	0.60***	0.20
12	log TBCC	$-6.28 \pm 0.13 + 0.03 \pm 0.03$ T-FDOM + $0.50 \pm 0.19$ log TP	0.62*	0.20
13	log CR	$-1.60 \pm 0.17 + 0.14 \pm 0.02$ T-FDOM	0.55***	0.26
14	log CR	$-1.64 \pm 0.14 + 0.85 \pm 0.12$ log TP	0.67***	0.23
15	log CR	$-1.69 \pm 0.15 + 0.03 \pm 0.04$ T-FDOM + $0.68 \pm 0.22$ log TP	0.68***	0.23
16	BR/CR	$0.75 \pm 0.16 - 0.27 \pm 0.13$ log TP	0.14*	0.26
17	BGE	$7.28 \pm 6.54 + 2.23 \pm 0.94$ T-FDOM	0.18*	10.18
18	BGE	$0.49 \pm 5.22 + 19.30 \pm 4.44$ log TP	0.42***	8.55
19	BGE	$3.46 \pm 5.44 - 2.18 \pm 1.41$ T-FDOM + $29.54 \pm 7.93$ log TP	0.47***	8.33
20	T-FDOM	$2.70 \pm 0.98 + 0.70 \pm 0.17$ DOC	0.40***	1.63
21	T-FDOM	$1.36 \pm 0.71 + 4.70 \pm 0.60$ log TP	0.70***	1.15
22	T-FDOM	$0.19 \pm 0.69 + 0.37 \pm 0.11$ DOC + $3.89 \pm 0.56$ log TP	0.80***	0.97
23	T-FDOM†	$-0.77 \pm 0.85 + 6.81 \pm 0.70$ log TP†	0.80***	1.28
24	T-FDOM†	$2.65 \pm 1.07 + 5.36 \pm 1.18$ log TDP†	0.46***	2.09
25	T-FDOM†	$3.35 \pm 0.69 + 0.01 \pm 0.001$ TN†	0.63***	1.74
26	T-FDOM†	$2.91 \pm 1.09 + 0.01 \pm 0.003$ TDN†	0.43***	2.16

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

† Data collected in 2000.

sion, T-FDOM; Fig. 2). This new approach provides strong evidence that T-FDOM is indeed associated with bacterial processes. Although DOM fluorescence values at higher emission wavelengths were weakly related to bacterial metabolism, they were no better ( $p > 0.05$ ) in their predictive power than bulk DOC concentrations. Thus, although the entire fluorescence spectrum of DOM might contain additional information about its composition, it appears that the region measured by T-FDOM is the single best predictor for describing those components of DOM that are related to bacterial metabolism.

Even though our findings show that T-FDOM provides important additional information about DOM composition to that revealed by bulk DOM measures, other variables were also strongly correlated with bacterial metabolism. For instance, TP was a stronger indicator ( $p < 0.05$ ) of CR, BP, and TBCC than T-FDOM, with the exception of BR, for which T-FDOM and TP were equally good ( $p > 0.05$ ) predictors (Table 2). As was the case with metabolic rates, variability in BGE was best described by TP ( $p < 0.001$ ). BGE and DOC were unrelated, and although T-FDOM was a weak indicator of BGE ( $r^2 = 0.18$ ,  $p < 0.05$ ; Fig. 3a; Table 2), there was no significant relationship ( $p > 0.05$ ) between BGE and T-FDOM when the latter was expressed as a proportion of total DOM (Fig. 3b). Interestingly, both T-FDOM and TP each served as good predictors of metabolic rates

and BGE but together explained no additional variability ( $p > 0.05$ ; models 4, 8, 12, 15, and 19; Table 2).

All regrowth experiments showed initial concomitant increases in both T-FDOM and BA, although the levels reached and the rates of increase varied considerably among lakes (Fig. 4). However, once bacteria had reached their stationary phase, several cultures exhibited a decrease in T-FDOM, suggesting that T-FDOM was being degraded or, more likely, consumed by the bacteria. Most importantly, the rates of increase of T-FDOM and BA were strongly related, suggesting that T-FDOM was either produced by a bacterial transformation of DOM or was the result of compounds secreted by bacteria (Fig. 5). In these experiments, the ratio of bacterial cell yield to increase in T-FDOM varied appreciably among cultures (Fig. 6), implying that the process(es) responsible for these patterns varies among lakes of different trophic status and might be influenced by the differing environmental conditions experienced by bacteria.

## Discussion

The main finding of this study was that T-FDOM is a significantly ( $p < 0.005$ ) better predictor of BP, BR, TBCC, and total plankton CR than DOC, the standard measure of bulk DOM concentration (Fig. 1; Table 2). This is consistent with the view that DOM is a highly heterogeneous mixture

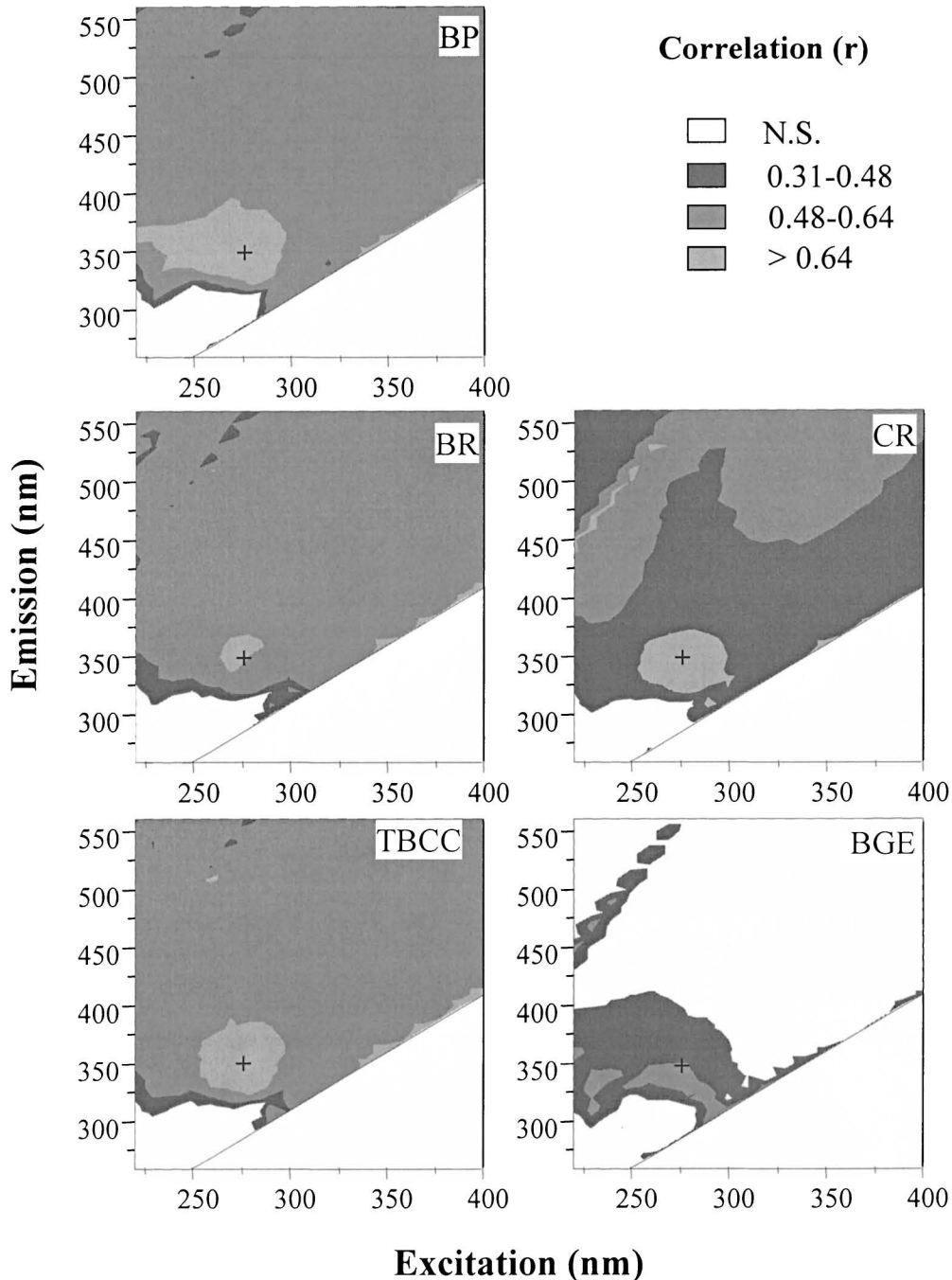


Fig. 2. Contour plot of the correlation ( $r$ ) between dissolved organic matter (DOM) fluorescence (RFU) and bacterial production (BP,  $\text{mg C L}^{-1} \text{h}^{-1}$ ), bacterioplankton respiration (BR,  $\text{mg O}_2 \text{L}^{-1} \text{d}^{-1}$ ), total plankton community respiration (CR,  $\text{mg O}_2 \text{L}^{-1} \text{d}^{-1}$ ), total bacterial carbon consumption (TBCC,  $\text{mg C L}^{-1} \text{d}^{-1}$ ), and bacterial growth efficiency (BGE, %). Shades of grey corresponding to ranges of correlation coefficient are presented in the legend, and nonsignificant results ( $p < 0.01$ ) are excluded. The + symbol indicates the fluorescence spectrum region measured by T-FDOM (i.e., 275 nm excitation and 346–356 nm emission). Minor interference from Rayleigh and Raman scatter by water is evident at emission values near the excitation wavelength and at  $\sim 500$ –560 nm emission.

composed of many fractions with different bacterial reactivities. In addition to providing a first systematic demonstration of a relationship between these parameters across a wide trophic range, our results clearly show that only a very spe-

cific region of the DOM excitation–emission matrix is associated with measures of bacterial activity (Fig. 2).

The results are especially noteworthy when compared with the lack of success of most alternative methods of DOM

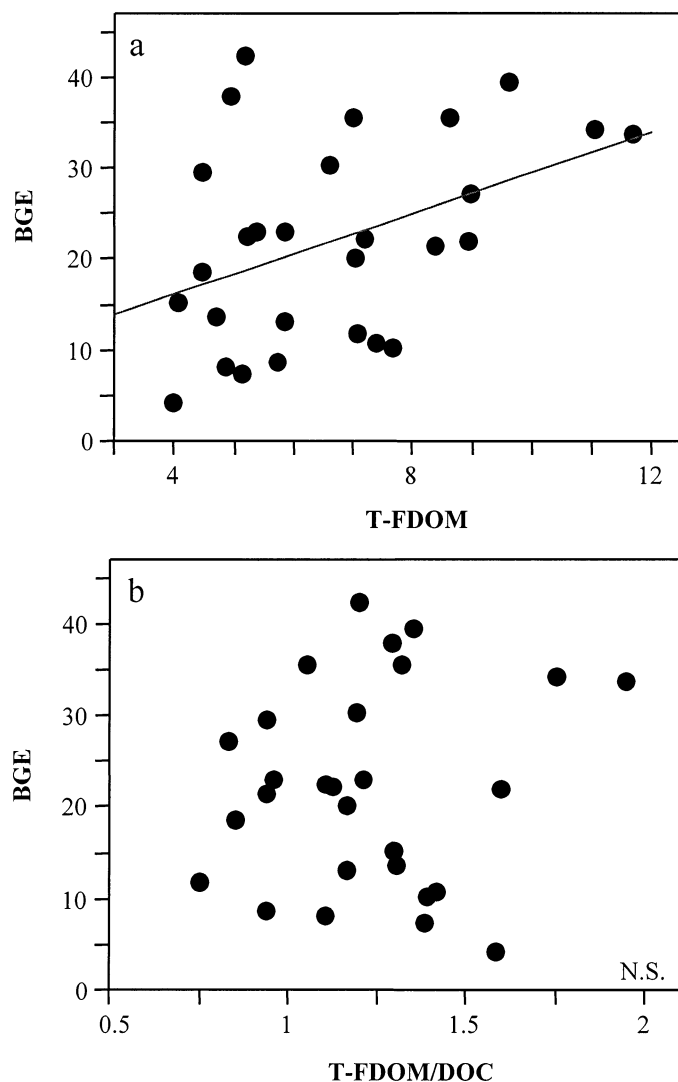


Fig. 3. Bacterial growth efficiency (BGE, %) versus (a) dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission (T-FDOM, RFU) and (b) T-FDOM as a proportion of total dissolved organic matter (T-FDOM/DOC). N.S., a nonsignificant relationship. The regression model and summary statistics are presented in Table 2.

characterization when relating DOM composition to bacterial function. For example, the relationship between bacterial metabolism and DOM of different molecular masses has remained ambiguous, and although low-molecular weight DOM in freshwater is commonly thought to be more available to bacteria (Kalff 2001), some studies have shown the large pool of high-molecular weight DOM to support higher metabolic rates than the much smaller pool of low-molecular weight DOM (e.g., Amon and Benner 1994). Attempts to relate DOM composition and bacterial metabolism by measuring DOM elemental composition have yielded more promising results (e.g., Hunt et al. 2000), as have approaches measuring the concentrations of specific compounds such as sugars and amino acids (e.g., Amon et al. 2001). Although such approaches provide important information about the chemical composition of DOM, they are, however, much

more time consuming than fluorescence spectroscopy. Fluorescence spectroscopy also has the advantage of allowing for fast, simple analysis without modifying DOM and should prove to be particularly useful in studies of DOM composition that require many measurements.

*Metabolic measurements*—BR was, on average, responsible for approximately one third of CR (Table 1), a finding consistent with values reported elsewhere (del Giorgio and Cole 1998 and references therein) and quite similar to those reported for a smaller but partially overlapping study in the same geographic region (Cimbleiris and Kalff 1998). The proportion of CR accounted for by BR (BR/CR) slightly exceeded 100% in two oligotrophic lakes (Table 1), suggesting that a minor experimental artifact was likely induced by the filtration procedure, either through stimulation of bacterial metabolism following filtration (Griffith et al. 1990) or following the removal of protozoan grazers (e.g., Hopkinson et al. 1989). Although picophytoplankton could have further contributed to error in the BR estimates in oligotrophic lakes (Geider 1997), where they are typically most important (Kalff 2001), their effect would be insufficient to obscure patterns over the large range of BR examined here (see also Biddanda et al. 2001). In any case, the estimates of BP, BR, CR, and BGE were well within the range of published lake values (del Giorgio and Cole 1998 and references therein), suggesting that there were no major sources of error associated with the manner in which they were obtained.

Dissolved organic carbon and nutrients strongly interact in modulating bacterial metabolism in lakes. Consequently, all metabolic rates were positively correlated not only with T-FDOM but also with TP, which alone explained 76%, 47%, 60%, and 67% of the variability in BP, BR, TBCC, and CR, respectively (Table 2). TP was also important as a predictor of the efficiency with which bacteria used organic matter (model 18; Table 2), a conclusion identical to that of a parallel study that used bacterial regrowth experiments in a largely overlapping set of lakes (Smith and Prairie 2004). Such a strong interaction between bacterial metabolism and nutrients—in particular phosphorus—has also been described elsewhere for BP (e.g., Cole et al. 1988), BA, BR, CR, BR/CR, and BGE (e.g., del Giorgio and Cole 1998; Biddanda et al. 2001 and references therein). We interpret these relationships as the result of the widespread phosphorus limitation of key metabolic processes in our lakes (e.g., Cimbleiris and Kalff 1998; Prairie et al. 2002).

*Relationships between T-FDOM, DOC, and nutrients*—To shed light on the interaction between DOM and nutrients, we examined relationships of T-FDOM with the standing stocks of DOC, TP, TDP, TN, and TDN. T-FDOM levels in 2001 were more strongly correlated with TP than DOC ( $p < 0.0005$ ; Fig. 7; Table 2) and best predicted by a multivariate model with TP and DOC ( $p < 0.005$ ; Table 2). This suggests T-FDOM abundance to be determined by those substances, processes, or both that are greater in more productive systems and not simply a function of the total amount of DOC (models 20, 21, and 22; Table 2). Furthermore, although T-FDOM increased with increasing DOC, it contributes a progressively smaller fraction of DOC in DOC-rich

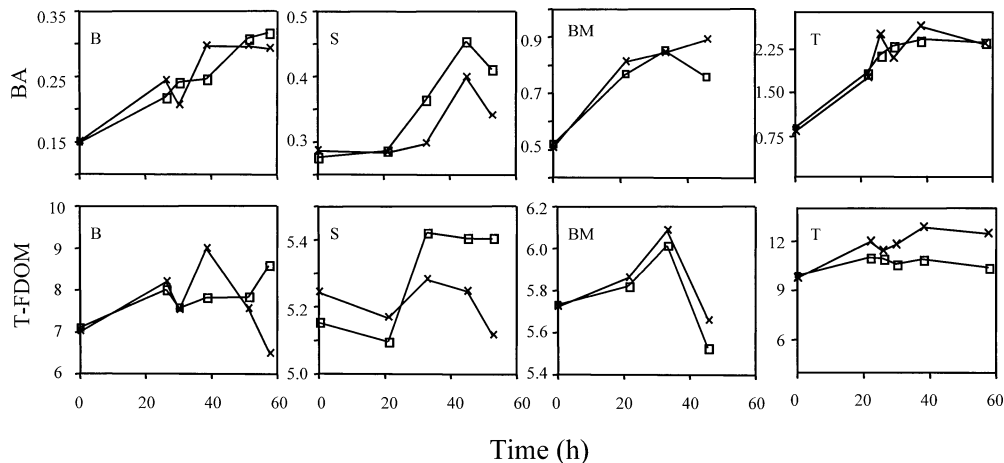


Fig. 4. Bacterial abundance (BA,  $10^6$  cells  $\text{ml}^{-1}$ ) and dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission (T-FDOM, RFU) versus time in bacterial regrowth cultures. Upper and lower panels illustrate changes in BA and T-FDOM, respectively, and symbols differentiate between replicate cultures. Cultures were grown from a natural bacterial inoculum on a natural dissolved organic matter growth medium obtained from Lac Brompton (B, ambient total phosphorus concentration [TP] of  $5 \mu\text{g L}^{-1}$ ), Lac Stukely (S, TP of  $6 \mu\text{g L}^{-1}$ ), Lac Brome (BM, TP of  $14 \mu\text{g L}^{-1}$ ), and Lac Tomcod (T, TP of  $59 \mu\text{g L}^{-1}$ ).

waters (slope = 0.7, model 20; Table 2). Exploratory data collected in the previous summer allowed for further insight into the more likely characteristics of T-FDOM because nutrient concentrations were measured in the same  $<0.45\text{-}\mu\text{m}$  fraction of water used to measure T-FDOM, as well as in unfiltered water. Interestingly, T-FDOM was significantly ( $p < 0.0025$ ) less well correlated with dissolved fractions of both nitrogen and phosphorus than with the TN and TP concentrations measured in unfiltered water (models 23, 24, 25, and 26; Table 2). Thus, T-FDOM appears more likely to

represent substances, metabolic processes, or both that are linked to trophic status than nutrient-rich DOM.

*T-FDOM as a substrate or product of bacterial metabolism?*—Although the empirical relationships indicate a tight coupling between T-FDOM and bacterial metabolism, they do not elucidate whether T-FDOM corresponds to a more labile fraction of the DOM being consumed by the bacteria or, conversely, whether it represents a bacterial metabolic product. The experimental work of Moran et al. (2000) sug-

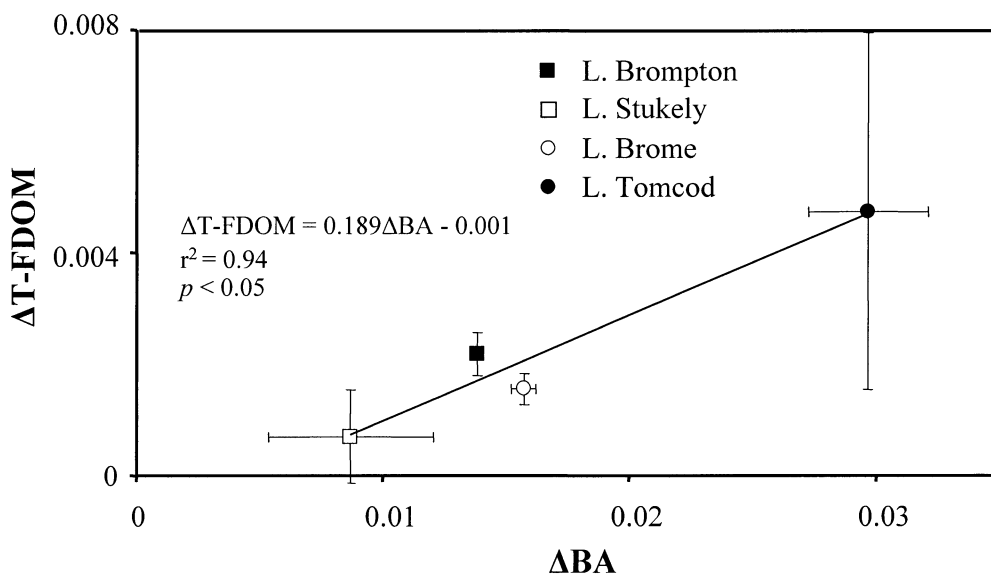


Fig. 5. Rate of change in dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission ( $\Delta\text{T-FDOM}$ ,  $\text{RFU h}^{-1}$ ) versus bacterial growth rate ( $\Delta\text{BA}$ ,  $10^6$  cells  $\text{ml}^{-1} \text{h}^{-1}$ ) from bacterial regrowth cultures for Lac Brompton, Lac Stukely, Lac Brome, and Lac Tomcod. Regression statistics derived from mean values.

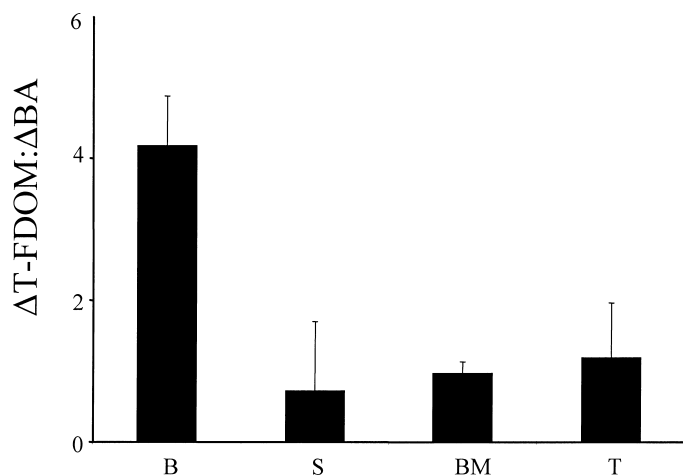


Fig. 6. Ratio of the magnitude of changes in dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission ( $\Delta$ T-FDOM, RFU) and bacterial abundance ( $\Delta$ BA,  $10^6$  cells  $\text{ml}^{-1}$ ) in bacterial regrowth cultures (i.e., for Lac Brompton [B], Lac Stukely [S], Lac Brome [BM], and Lac Tomcod [T]). Change in T-FDOM and BA were measured from the start of incubation until cultures reached stationary phase. Error bars indicate the standard deviation about the mean ratio for duplicate cultures from each lake.

gests that T-FDOM represents substances produced by the bacteria and is further supported by our own short-term bacterial regrowth experiments. In all four regrowth cultures, T-FDOM and BA increased in tandem until bacterial populations reached the stationary growth phase (Fig. 4), but the subsequent decline in T-FDOM strongly suggests it is consumed when nutrients are depleted (at stationary growth phase) and substrate quality might be less crucial.

The most likely fluorescent products that could have been generated by bacterial transformation of DOM and particulate organic matter in the water column include dissolved amino acids and proteins (e.g., Rosenstock and Simon 2001). In addition, the known bacterial secretion of polymers such as dissolved combined amino acids and ectoenzymes can also contribute to the observed coupling of T-FDOM production with increases in BA (e.g., Rosenstock and Simon 2001). Because tryptophan residues are present in almost all proteins and are therefore likely to be present in protein and organic matter hydrolysis products, it is quite plausible that the measured levels of T-FDOM reflect concentrations of ectoenzymes, as well as the other amino acid-containing substances secreted or produced by bacteria. Although T-FDOM can not provide strong estimates of total amino acid concentrations because the exact chemical compositions of the associated compounds is not known, we calibrated our field measures of T-FDOM with a bovine serum albumin solution to derive order of magnitude estimates (Mayer et al. 1999); the resulting estimates of 110–330  $\mu\text{g C L}^{-1}$  are well within the range of values found elsewhere (Mayer et al. 1999), lending further support to the claim that T-FDOM represents the amino acid-containing materials associated with metabolically active bacteria. Assuming these estimates are reliable, they suggest that amino acids might contribute between 2% and 5.5% of the total DOC pool.

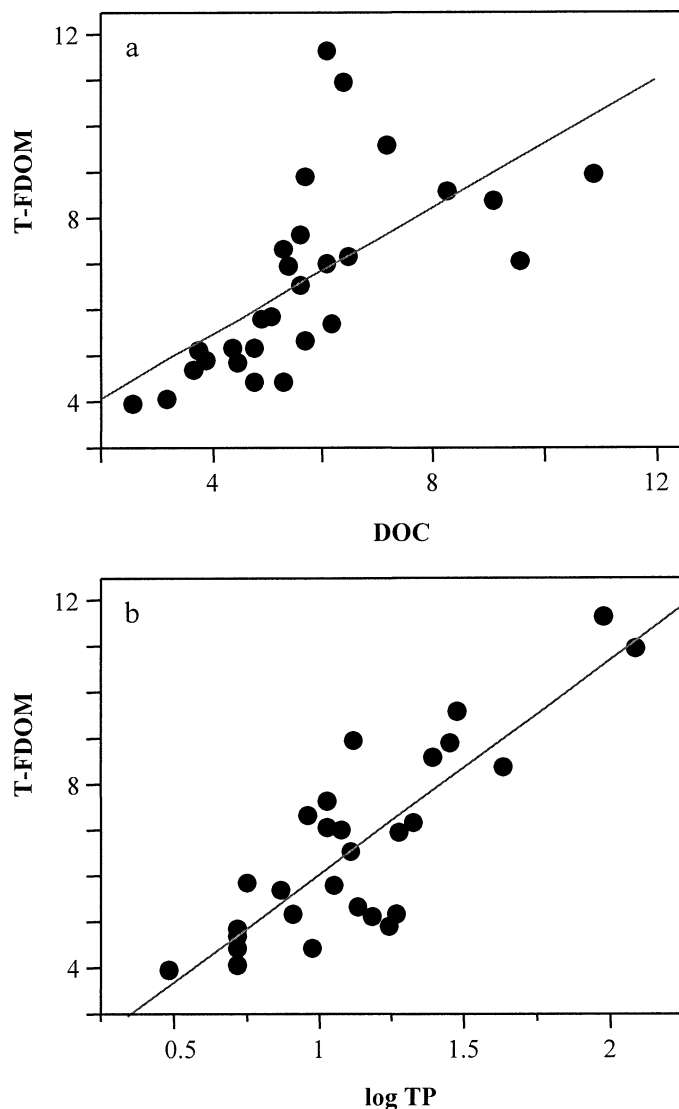


Fig. 7. Dissolved organic matter fluorescence intensity at 275 nm excitation and 346–356 nm emission (T-FDOM, RFU) versus (a) dissolved organic carbon (DOC,  $\text{mg L}^{-1}$ ) and (b) total phosphorus (TP,  $\mu\text{g L}^{-1}$ ). Regression models and summary statistics are presented in Table 2.

Although T-FDOM appears to be a by-product of biological activity, the possibility that it also constitutes a significant bioavailable substrate cannot be totally excluded. Amino acids have a high energy and nutrient content and have been proposed to be excellent indicators of the bioavailability of DOM (e.g., Amon et al. 2001). In addition, they have been shown to be an important substrate for bacteria, allowing for increased bacterial growth and BGE in laboratory experiments (*see del Giorgio and Cole 1998 and references therein*). Accordingly, amino acid-like DOM fluorescence has previously been suggested as a potential indicator of DOM bioavailability (e.g., Mayer et al. 1999). Because the present consensus holds that substrate quality is a major determinant of BGE (del Giorgio and Cole 1998), we used BGE to explore whether T-FDOM might represent a bioavailable substrate rather than, as postulated, a product of

bacterial metabolism. However, BGE was unrelated ( $p > 0.05$ ) to the relative amount of T-FDOM in total DOM, only weakly correlated to overall levels of T-FDOM (Fig. 3), and unrelated ( $p > 0.05$ ) to T-FDOM in the regrowth experiment (results not shown). This is further indicative of T-FDOM as a product of metabolism. Furthermore, T-FDOM and TP were strongly correlated, and the two together yielded no stronger prediction of BGE and metabolic rates than they did alone ( $p > 0.05$ )—something not expected if T-FDOM serves as a bioavailable substrate for bacteria in phosphorus-limited lakes (models 17, 18, 19, and 21; Table 2).

Considering all the available evidence, T-FDOM is more likely to be a product of bacterial metabolism than bioavailable DOM. However, our experimental work lends credibility to the interesting possibility that T-FDOM reflects the dynamic balance between bacterial consumption and production of a small fraction of the DOM pool. Even though T-FDOM was not strongly coupled with BGE and was found to increase initially in laboratory cultures—findings contrary to those expected if T-FDOM were to represent a good-quality substrate—T-FDOM decreased in all cultures after bacterial populations reached stationary growth phase (Fig. 4). Together, these results suggest that, during active microbial growth, T-FDOM production exceeds its degradation but that the reverse might be true during periods of relatively slow growth. If T-FDOM indeed represents a significant fraction of the carbon that bacteria recycle, its small proportion within the overall carbon pool (2–5%) implies that an overwhelming fraction of the DOC pool might not play a very dynamic role in bacterial carbon cycling. Figure 5 can be used to quantify roughly the potential of T-FDOM in carbon dynamics. On the basis of our bovine serum albumin calibration and assuming an average carbon content of 20 fg C per bacterial cell (Bratbak 1985), the slope of the relationship illustrated in Fig. 6 implies that for every gram of bacterial carbon biomass produced, an additional 0.25 g is potentially generated as T-FDOM compounds. If this is the case, it also suggests that up to 20% of BP might be released as dissolved compounds and therefore unaccounted for in BP estimates based on current leucine incorporation techniques. Whether this high value is realistic depends on several assumptions that necessitate further testing. Ultimately, quantifying the rates of T-FDOM production and consumption will be key to understanding how the balance between these two processes might vary among and within environments. Thus, although T-FDOM might not represent a direct measure of bacterial substrate quality, we suggest it is a strong indicator of the interactions between bacteria and a small and highly dynamic fraction of the DOM pool.

## References

- AMMERMAN, J. W., J. A. FUHRMAN, A. HAGSTROM, AND F. AZAM. 1984. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. *Mar. Ecol. Prog. Ser.* **18**: 31–39.
- AMON, R. M. W., AND R. BENNER. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* **369**: 549–551.
- , H. P. FITZNER, AND R. BENNER. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol. Oceanogr.* **46**: 287–297.
- BIDDANDA, B., M. OGDahl, AND J. COTNER. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnol. Oceanogr.* **46**: 730–739.
- BRATBAK, G. 1985. Bacterial biovolume and biomass estimates. *Appl. Environ. Microbiol.* **49**: 1488–1493.
- CARIGNAN, R., A. M. BLAIS, AND C. VIS. 1998. Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. *Can. J. Fish. Aquat. Sci.* **55**: 1078–1084.
- CIMBLERIS, A. C. P., AND J. KALFF. 1998. Planktonic bacterial respiration as a function of C:N:P ratios across temperate lakes. *Hydrobiologia* **384**: 89–100.
- COBLE, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. *Mar. Chem.* **51**: 325–346.
- , S. A. GREEN, N. V. BLOUGH, AND R. B. GAGOSIAN. 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature* **348**: 432–435.
- , C. A. SCHULTZ, AND K. MOPPER. 1993. Fluorescence contouring analysis of DOC intercalibration experiment samples: A comparison of techniques. *Mar. Chem.* **41**: 173–178.
- COLE, J. J., S. FINDLAY, AND M. L. PACE. 1988. Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Mar. Ecol. Prog. Ser.* **43**: 1–10.
- DEL GIORGIO, P. A., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.* **29**: 503–541.
- , AND R. H. PETERS. 1994. Patterns in planktonic P:R ratios in lakes: Influence of lake trophy and dissolved organic carbon. *Limnol. Oceanogr.* **39**: 772–787.
- , J. J. COLE, AND A. CIMBLERIS. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385**: 148–151.
- DETERMANN, S., R. REUTER, P. WAGNER, AND R. WILLKOMM. 1994. Fluorescent matter in the eastern Atlantic Ocean. Part 1: Method of measurement and near-surface distribution. *Deep-Sea Res. I* **41**: 659–675.
- , AND R. WILLKOMM. 1996. Fluorescent matter in the eastern Atlantic Ocean. Part 2: Vertical profiles and relation to water masses. *Deep-Sea Res. I* **43**: 345–360.
- FERRARI, G. M., AND M. MINGAZZINI. 1995. Synchronous fluorescence spectra of dissolved organic matter (DOM) of algal origin in marine coastal waters. *Mar. Ecol. Prog. Ser.* **155**: 305–315.
- GEIDER, R. J. 1997. Photosynthesis or planktonic respiration? *Nature* **388**: 132.
- GRIESBACH, S. J., AND R. H. PETERS. 1991. The effects of analytical variations on estimates of phosphorus concentration in surface waters. *Lake Res. Manag.* **7**: 97–106.
- GRIFFITH, P. C., D. J. DOUGLAS, AND S. C. WAINRIGHT. 1990. Metabolic activity of size-fractionated microbial plankton in estuarine, nearshore, and continental shelf waters of Georgia. *Mar. Ecol. Prog. Ser.* **59**: 263–270.
- HEDGES, J. I. 1992. Global biogeochemical cycles: Progress and problems. *Mar. Chem.* **39**: 67–93.
- HOPKINSON, C. S., B. SHERR, AND W. J. WIEBE. 1989. Size-fractionated metabolism of coastal microbial plankton. *Mar. Ecol. Prog. Ser.* **51**: 155–166.
- HUNT, A. P., J. D. PARRY, AND J. HAMILTON-TAYLOR. 2000. Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnol. Oceanogr.* **45**: 237–241.
- KALFF, J. 2001. *Limnology*. Prentice Hall.
- KIRCHMAN, D. L. 1993. Leucine incorporation as a measure of bio-

- mass production by heterotrophic bacteria, p. 509–512. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], *Handbook of techniques in aquatic microbial ecology*. Lewis.
- MAYER, L. M., L. L. SCHICK, AND T. C. LODER III. 1999. Dissolved protein fluorescence in two Maine estuaries. *Mar. Chem.* **64**: 171–179.
- MOPPER, K., AND C. A. SCHULTZ. 1993. Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. *Mar. Chem.* **41**: 229–238.
- MORAN, M. A., W. M. SHELDON, JR., AND R. G. ZEPP. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnol. Oceanogr.* **45**: 1254–1264.
- POMEROY, L. R., J. E. SHELDON, AND W. M. SHELDON, JR. 1994. Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory rates in seawater by the precision Winkler method. *Appl. Environ. Microbiol.* **60**: 328–332.
- PRAIRIE, Y. T., D. F. BIRD, AND J. J. COLE. 2002. The summer metabolic balance in the epilimnion of southeastern Quebec lakes. *Limnol. Oceanogr.* **47**: 316–321.
- ROCHELLE-NEWALL, E. J., AND T. R. FISHER. 2002. Production of chromophoric dissolved organic matter fluorescence in marine and estuarine environments: An investigation into the role of phytoplankton. *Mar. Chem.* **77**: 7–21.
- ROSENSTOCK, B., AND M. SIMON. 2001. Sources and sinks of dissolved free amino acids and protein in a large and deep mesotrophic lake. *Limnol. Oceanogr.* **46**: 644–654.
- SAMPOU, P., AND W. M. KEMP. 1994. Factors regulating plankton community respiration in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **110**: 249–258.
- SMITH, D. C., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using <sup>3</sup>H-leucine. *Mar. Microb. Food Webs* **6**: 107–114.
- SMITH, E. M., AND W. M. KEMP. 2001. Size structure and the production/respiration balance in a coastal plankton community. *Limnol. Oceanogr.* **46**: 473–485.
- , AND Y. T. PRAIRIE. 2004. Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability. *Limnol. Oceanogr.* **49**: 137–147.
- TRAGANZA, E. D. 1969. Fluorescence excitation and emission spectra of dissolved organic matter in sea water. *Bull. Mar. Sci.* **19**: 899–904.
- WILLIAMSON, C. E., D. P. MORRIS, M. L. PACE, AND O. G. OLSON. 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnol. Oceanogr.* **44**: 795–803.

*Received: 6 August 2003*  
*Accepted: 30 March 2004*  
*Amended: 28 May 2004*