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PRIMARY RESEARCH PAPER

# The elusive nature of fluorescent organic matter in Lake Kinneret, Israel

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**Abstract** The dynamics of fluorescent organic matter (FOM) in lakes have generally been studied over extended periods of weeks to months rather than short time scales. We employed spectrofluorometry to examine whether short-term in situ variations in the three components of FOM (protein-like, terrestrial, and marine fluorescence) were related to changes in production in Lake Kinneret, Israel. Protein-like fluorescence correlated strongly with phytoplankton biomass, chlorophyll a, and Secchi depth in situ, suggesting it might serve as an indicator of aquatic production. In vitro dark incubations of filtered and unfiltered surface lake water (without added nutrients or organic matter) revealed considerable protein-like and marine fluorescence production in the unfiltered samples, suggesting that FOM is linked to particulate matter dynamics and to microbial processing of this suspended material. Terrestrial fluorescence did not significantly change in incubation, indicating that this component is probably produced deeper in the lake. Moreover, the lack of marine FOM accumulation in surface waters, despite producing in vitro, suggests that FOM photobleaching may regulate this component.

**Keywords** Particulate organic matter · Spectrofluorometry · Excitation–emission matrices · Limnology · Marine humics · Protein fluorescence

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## Introduction

Chromophoric dissolved organic matter (CDOM) is the optically active fraction of the dissolved organic matter (DOM) pool that absorbs light at ultraviolet and visible spectral range. A variety of studies (see below) examined the correlation between CDOM or fluorescent organic matter (FOM) and algal or bacterial production, but did not find a clear relationship between these; we believe that this may be due to the time scales employed and this paper addresses this point. CDOM has been described as a fast-cycling pool of DOM with additional ecologically important properties such as the absorption of ultraviolet and photosynthetically active radiation (PAR) (Ferrari & Tassan, 1991; Miller, 2000). As the dominant regulator of UV penetration in the open ocean (Tedetti & Sempere, 2006), CDOM is also photoreactive, degrading to an array of compounds with varying levels of bioavailability, including labile bacterial substrates, inorganic nutrients, reactive oxygen species, and

compounds that affect the availability of micronutrient metals (Blough & Del Vecchio, 2002). Thus, CDOM degradation, accomplished by microbial and photochemical processes, can both support and inhibit algal and bacterial production in aquatic systems. Moreover, the global CDOM carbon pool is so large that changes in its dynamics could profoundly affect global carbon cycling and atmospheric carbon concentrations (Jiao et al., 2010). Understanding the ecological role of CDOM, including the dynamics of production and degradation, is critical to our understanding of global carbon budgets, nutrient cycling, and aquatic trophic structures.

Quantification of CDOM was initially accomplished based on its light absorption spectrum, but researchers have increasingly chosen to use spectrofluorometry as a more sensitive method of accurately quantifying and characterizing CDOM in small volumes at ambient concentrations. A range of studies have found robust linear (Ferrari & Tassan, 1991; Hoge et al., 1993; Ferrari et al., 1996; Vodacek et al., 1997; Ferrari & Dowell, 1998; Ferrari 2000; Kowalczuk et al., 2003) or non-linear (Zhao et al., 2009) relationships between CDOM absorption and fluorescence, which may vary as a function of productivity and ecosystem type, suggesting that fluorescence can serve as a good indicator of CDOM concentration. Three primary classes of fluorescent organic components were identified according to the location of their fluorescence emission peaks (Coble, 1996). Peaks centered around excitation/emission values of 220/303 (B peak) and 275/345 (T peak) represent a protein-like component, which includes the fluorescent amino acids tyrosine and tryptophan. Two additional classes of fluorescence peaks are associated with aromatic humic acids; one class representing humics of primarily terrestrial origin (C peak) and one class representing humics produced within the water column (M peak), also known as "marine" humics in early ocean-based studies (Donard et al., 1989; Coble et al., 1990; De Souza Sierra et al., 1994).

In situ studies have found that these three classes of FOM differ in their distribution and reactivity in natural waters. The terrestrial humic-like fluorescent organic matter component (C-comp) of the FOM pool has been used to track riverine or estuarine input into gulfs, bays, and deltas (Coble, 1996; Stedmon et al., 2003; Murphy et al., 2008; Kowalczuk et al., 2009), while the "marine" humic-like fluorescing component (M-comp) has been related to biological production within the water column (Chen & Bada, 1992; Stedmon et al., 2003; Chen et al., 2004; Nieto-Cid et al., 2006; Yamashita et al., 2007, 2008; Murphy et al., 2008). The protein-like fluorescing component (T-comp), which varies linearly with the concentration of total hydrolysable amino acids (Yamashita & Tanoue, 2003), has been shown to correlate with phytoplankton and bacterial production (Rochelle-Newall & Fisher, 2002; Chen et al., 2004; Cammack et al., 2004; Stedmon & Markager, 2005a; Nieto-Cid et al., 2006; Hudson et al., 2008), though the relationship is not yet clear. Notably, fluorescing OM represents an unknown portion of the bulk OM pool. Thus, while changes in specific component fluorescence may indicate trends in OM production or degradation, fluorescence data alone cannot be used as quantitative measures of the concentration of any specific OM compounds. This ambiguity may be related to a paucity of seasonal studies to track FOM component dynamics in situ, especially in freshwater systems. Freshwater FOM research has largely focused on spatial studies and incubation experiments. Thus, to date, Borisover et al.'s (2009) study of Lake Kinneret is the only study that tracks these three FOM components year-round in a freshwater lake.

A number of biological pathways have been proposed for FOM production, consumption, and overall regulation, and these have been tested in laboratory incubations and cultures. In situ studies have found conflicting relationships between FOM/CDOM and phytoplankton productivity, reporting either no consistent correlation between FOM and chlorophyll a (chl *a*) (Chen et al., 2004; Borisover et al., 2009), a positive correlation in eutrophic waters (Zhang et al., 2009), or suggesting that a relationship may only be discernable during high-production algal blooms (Stedmon & Markager, 2005a; Zhao et al., 2009). Blooms induced in nutrient-amended laboratory algal cultures (Rochelle-Newall & Fisher, 2002) did not show FOM enhancement related to increases in chl *a* or phytoplankton biomass. On the contrary, these studies suggest that microbial decomposition of phytoplankton-produced DOM is the factor that determines FOM concentration.

Lake Kinneret, Israel, is a warm mesotrophic lake (Berman et al., 1995) that receives most of its water inflow in the winter months. Carbon input to the lake is

dominated by an early springtime algal bloom which is quickly processed by the microbial community (Hart et al., 2000). Hart et al. (2000) reported that bacterial production closely tracks detrital carbon loading, and that Kinneret bacterial communities are primarily carbon limited (Berman et al., 1994). A study of FOM in Lake Kinneret and its catchment basin by Borisover et al. (2009) utilized parallel factor analysis (PARAFAC; Stedmon & Bro, 2008) to identify three major FOM components in the Kinneret: (1) a protein-like component with consistent surface distribution and decreasing fluorescence with depth (T-comp), (2) a marine humic-like component (M-comp), and (3) a terrestrial humic-like component (C-comp). Excitation/emission matrices (EEM) show that while spectral plots of these three components do not necessarily show sharp peaks with high slopes, their EEM locations are aligned with those of the T (tryptophan), M (marine humic), and C (terrestrial humic) peaks first identified by Coble (1996).

This study tracked FOM, phytoplankton biomass, and microbial productivity during the winter–spring production period in Lake Kinneret (Israel) to test the hypothesis that phytoplankton and microbial production are the primary regulators of in situ FOM concentration and composition. Samples were collected more often during the winter/spring period so as to capture rapid, in situ changes in production (blooms), and unamended lake water incubations were employed to track FOM change during natural organic matter decomposition processes.

## Methods

### Study site

Water samples were collected from 'Station A' (32′49.350″N; 35′35.487″E), located at one of the deepest points in the lake (mean yearly depth—39 m), where seiche influence on thermocline depth is minimal and horizontal mixing is maximal. Surface water at this location was assumed to be laterally well-mixed and accurately represent an average of overall lake characteristics in the upper mixed layer, due to the powerful mixing and advection effects of the Mediterranean sea breeze and central lake gyre (Pan et al., 2002).

### Timecourse sampling

Sampling began in January 2009, shortly after the initiation of total mixing. An 8-month (January–August) sampling period was selected to capture seasonal changes, including: the anticipated high-volume runoff events of January–March, the high productivity of early spring (February–April), and the post-stratification period (May–July). Samples were collected more frequently during the winter–spring production period to capture the effect of changes in lake productivity on the OM pool and on FOM. Summer lake conditions were characterized by an anoxic hypolimnion below 15 m depth, and a well-mixed epilimnion. Throughout the study, the photic zone, as determined by Secchi depth (measured in triplicate), ranged from 6 to 13.5 m in depth.

Station A was accessed aboard the motorized Kinneret Limnological Laboratory research vessels *Lillian* and *Hermona* and by tying up to a fixed-chain buoy marking the station. Each day of sample collection represents an individual *timepoint*. Lake-water samples were drawn from a depth of 1 m using an acid-washed 5L PVC water sampler (Hydro-Bios). Surface water was selected since intensive wind-driven mixing produces a uniform upper mixed layer in the Kinneret which is mixed on timescales of several hours (Marti & Imberger, 2008). Thus, near-surface samples are a representative measure of overall epilimnetic FOM in this water body. This assumption is supported by the finding that bacterial production did not vary considerably within the epilimnion from 2000 to 2007 (Berman et al., 2010). Three replicate water samples were taken on each sampling date, with a separate cast for each of the three sample bottles per timepoint. Secchi disk depth measurements (Tyler, 1968) were performed immediately after sampling, between 8 a.m. and 10 a.m. on the shady side of the vessel, without sunglasses, using a standard 20 cm Secchi disk.

Samples for FOM and bacterial production measurement were transported to the laboratory in brown-tinted, 130 ml glass bottles, which had been acid-washed and rinsed with HPLC grade $H_2O$ (Sigma). Twice per month, two 1.9 ml subsamples were taken from each of three sampling bottles for bacterial productivity assays (see incubation section below). Samples were kept on ice in darkness until

fluorometry, which was carried out 3–7 h after sampling unless otherwise noted.

To examine the relationship between algal biomass and FOM, in situ fluorescence data were tested for linear regression with phytoplankton biomass and chlorophyll a data (data provided by the Kinneret Limnological Laboratory). To examine the effect of external sources on Kinneret FOM, Jordan river flow volume data (provided by the Israel Water Authority) and lake level data (provided by the Kinneret Limnological Laboratory) were obtained and compared with this study's FOM data.

## Fluorescent organic matter (FOM) determination

Fluorescence excitation/emission matrices were produced by exciting water samples in a 3-ml quartz cuvette using a Fluorolog 3-22 spectrofluorometer equipped with a xenon lamp set at 90° to the fluorescence sensor (Jobin–Yvon, Horiba, Longjumeau, France). EEMs were produced by excitation at 270–350 nm and emission measurement at 290–462 nm with a band pass width of 5 nm. To control for machine variation in excitation light intensity, emission signal values were divided by lamp intensity. All FOM data are therefore presented as (unit-less) fluorescence units, FU (signal/Lamp). HPLC grade $H_2O$ (Sigma) was used as a comparative control for fluorescence and to track signal/noise ratio over the course of the study.

Three FOM components were tracked over the course of this study. Emission peaks were selected based on Borisover et al.'s (2009) PARAFAC analysis of Kinneret water. Borisover et al.'s PARAFAC analysis was used because that study benefitted from a much larger dataset collected at multiple depths and in all four seasons, and because fluorescent peaks were not visually differentiable in this dataset. The following peaks were extracted from excitation emission matrices and tracked over time in incubations and in situ: (1) a protein-like peak (T-comp) produced by excitation (Ex) at 280 nm, and emission (Em) within the range 322–382 nm. Since the shape of this peak changed both seasonally and as a result of filtration, T-comp fluorescence was calculated as the average area under the curve (AUC) in this excitation range (the sum of emission intensities in 16 consecutive 4 nm bins divided by 16) (see Fig. 1c), (2) a marine humic-like component (M-comp): Ex: 310 nm, Em: 394 nm, (3) a terrestrial humic-like component (C-comp): Ex: 350 nm, Em: 458 nm.

## Lake water incubations

Unaltered lake water samples were incubated in the laboratory for a period of 3–4 weeks to follow natural microbial processing of the particulate and dissolved organic matter therein and the temporal dynamics of FOM. Based on Ostapenia et al.'s (2009) study of the relative lability of DOM/POM in a number of lakes (including Lake Kinneret), it was assumed that the amount of bioavailable organic substrate present in a sample (total labile organic matter; $TOM_L$) is the sum of the labile POM ($POM_L$) and DOM ($DOM_L$), respectively. Incubation of unfiltered water simulated the in situ degradation of $TOM_L$, while incubation of filtered water simulated the degradation of $DOM_L$. The degradation of $POM_L$ was determined as the difference of these two fractions ($TOM_L - DOM_L$). Based on these assumptions, the relative contribution of labile DOM and POM to microbial production of FOM in the lake was examined.

Samples were collected for incubation experiments on four dates (5.1.09, 9.3.09, 19.4.09, 25.5.09) at station A. For each experiment, two 5 l water samples were collected and transported to the laboratory in acid-washed plastic jerrycans. One sample was filtered through precombusted (450°C, 3 h) Whatman GF/C glass fiber filters to remove fine particulate matter, POC, and algae while retaining small microbes in the sample. The other sample remained unfiltered. To correct for filtration-related reductions in oxygen concentration, filtered and unfiltered samples were left open to ambient air in high-surface-area decanters overnight in darkness. Samples were then bottled for incubation. The first (January) incubation took place in two 250 ml glass BOD bottles kept in the dark at 20°C for 17 consecutive days. Bottles were sampled four times (about twice per week) for FOM over the course of the incubation. The March incubation followed the same protocol and was sampled six times over 26 days for FOM, heterotrophic bacterial production, and cell counts. March and April incubations were performed in duplicate, with one pair of samples (filtered and unfiltered) treated as above, and the other pair bottled in 12 individual 100 ml glass BOD bottles. Within the 12-bottle sample series, sampling was conducted by opening one pair of BOD bottles (filtered and

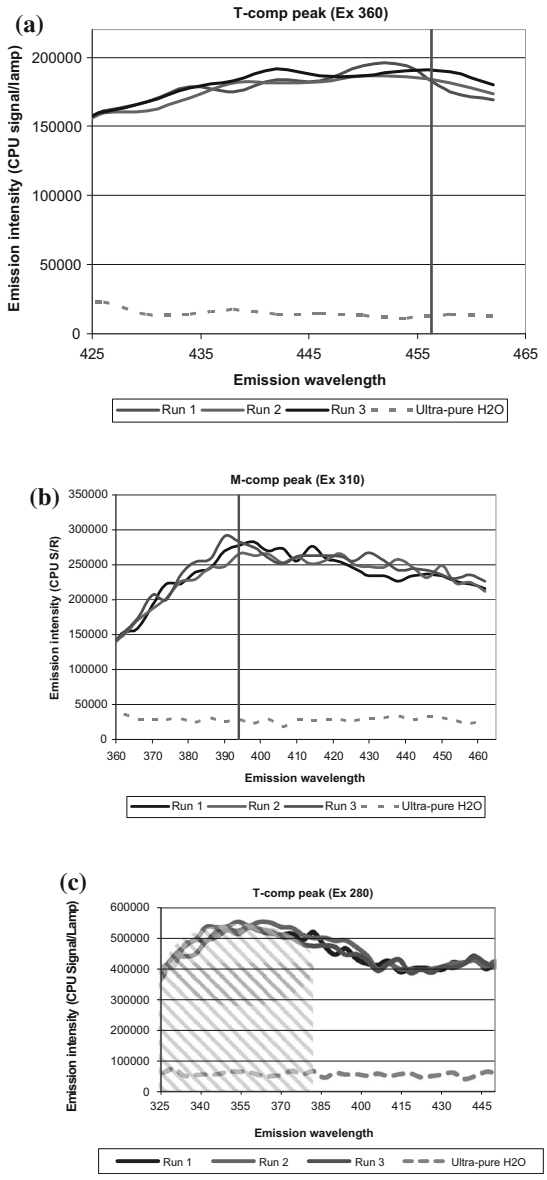



**Fig. 1** Example of emission spectra and peak shapes for **a** C-comp (terrestrial humic-like fluorescent component), **b** M-comp (marine humic-like fluorescent component) and **c** T-comp (protein-like fluorescent component) in Kinneret lake water collected on 19/4/09. *Green-dashed lines* are control fluorescence of ultra-pure HPLC grade water. *Red lines* in **a** and **b** are the wavelengths used to quantify these components. *Red-hatched area* in **c** is the area under the curve (AUC) used to quantify this component

unfiltered) at each sampling timepoint, ensuring that the remaining bottles remained airtight. The third (April) and fourth (May) incubations comprised six samples over 32 days, and four samples over 22 days, respectively. The fourth incubation was conducted using the 12-bottle, airtight method only. Subsamples of about 10 ml were decanted into acid-washed tinted glass bottles for transport to the spectrofluorometer. Analysis of the March and April incubations, which were performed in duplicate, showed very good agreement in spectrofluorometric readings between the two incubation methods (2-bottle and 12-bottle). The 12-bottle incubation data for these two months were used for subsequent data analysis. Oxic conditions were maintained throughout the incubations; $O_2$ levels did not fall below the limiting concentration of 4 mg/l over the course of any incubation, except for days 18–25 of the April incubation. It is possible that the hypoxic conditions that occurred during the April incubation affected the aerobic metabolism of organic matter, but this was not tested.

For each incubated timepoint in the March–May time series (including initial lake samples), three triplicate subsamples (three filtered, three unfiltered, three killed controls) were taken from each of three replicate sampling bottles and inoculated with 10 µl $^3$H radiolabelled leucine (Amersham Biosciences corp) for bacterial productivity assays (Simon & Azam, 1989). One triplicate series was killed and proteins were precipitated with 100 µl of 100% trichloroacetic acid (TCA) solution (Sigma). Live and killed leucine-amended samples were incubated in the dark at room temperature (20°C) for 4–16 h, after which TCA was added to the remaining samples. Samples were stored an additional 24–72 h at room temperature (20°C) before analysis. Previous experience has shown that triplicate scintillation counts were more precise after waiting 24–72 h. Prior to analysis, samples were centrifuged for 10 min at 14,000 RPM to settle out cells and the supernatant was decanted to eliminate unbound tritiated leucine. Pellets were washed with 1 ml of 5% TCA solution, centrifuged, decanted, and 1 ml scintillation fluid (Perkin Elmer Ultima Gold) was added to pellets. Samples were counted in a Packard Tri-Carb 2100TR liquid scintillation counter. Incorporated leucine was calculated by comparing the radioactivity of samples killed pre and post incubation, and this value was converted to daily carbon production (Simon & Azam, 1989). This method was also used to quantify in situ bacterial productivity from lake samples.

To test for significance in fluorescent component changes over the course of laboratory incubations, a repeated measures ANOVA was performed, with the

repeated measure defined as successive samples within an incubation, and groups defined as independent incubations (January, March–May). This analysis could not be used to compare leucine uptake data from incubations because each incubation bottle was sampled for bacterial production on a different incubation day, i.e., consecutive leucines running within each incubation were not comparable between incubations. Instead, differences in maximum and integrated leucine uptake peaks between incubations were tested using t-tests. Statistical analyses were performed using SPSS software v.12.

## Results

### Fluorescent organic matter (FOM)

The excitation/emission ranges of the organic matter fluorescence peaks tracked in this study do not fall within the range of Raman and Raleigh scattering ridges, therefore scattering corrections were not carried out. Mean fluorescence (calculated for the entire 7-month study period) of the marine (M-comp) and terrestrial (C-comp) humic-like components were 61 and 39% of mean fluorescence of the protein-like component (T-comp), respectively. All three components responded linearly to 3-point dilution with significant regressions between concentration and specific fluorescence (T-comp: $R^2 = 0.922$, $P < 0.001$, $a = 3.14E5$, $b = 7.2E4$; M-comp: $R^2 = 0.947$, $P < 0.001$, a = 2.39E5, b = 4.6E4; C-comp: $R^2 = 0.994$, $P < 0.001$, $a = 1.51E5$, $b = 1.7E4$). This indicates that the changes in fluorescence recorded over time in this study correspond to the changes in FOM concentration.

From early January until the development of a strong thermocline and subsequent hypolimnetic anoxia in late April, T-comp fluorescence was highly variable, with a maximum difference (between contiguous sampling days) of 48% over 14 days (Fig. 2). Local T-comp fluorescence maxima were recorded on 19.1.09, 15.3.09, and 19.4.09.

The levels of M-comp and C-comp fluorescence remained relatively stable throughout the winter production period. These two components were tightly coupled in situ, demonstrating a significant positive linear relationship between them ($R^2 = 0.71$, $P < 0.000$, $N = 16$) for the duration of the study period. All three components (T-comp, M-comp, and C-comp) showed a gradual reduction in fluorescence following lake stratification (roughly mid-May to mid-June), with T-comp rising again in late summer (Fig. 2).

No discernable relationship was found between fluorescence and either Jordan River inflow or lake level during the highly dynamic winter period, though the largest rise in T-comp fluorescence began immediately after the dramatic seasonal peak in river inflow on 2.3.2009 (Fig. 2).

### FOM and lake productivity

Several algal biomass peaks were documented during the winter period, expressed as increases in total phytoplankton biomass and chl *a*. Total phytoplankton biomass data demonstrated three peaks of increasing intensity between lake turnover and stratification (Fig. 3). These peaks, recorded on 15.2.09, 15.3.09, and 19.4.09, were concurrent to (or immediately following) the three peaks in T-comp (described above). The seasonal chl *a* timecourse was characterized by two local maxima on 15.2.09 and 19.4.09, concurrent to the first and third peaks in phytoplankton biomass (Fig. 4). Chl *a* and phytoplankton biomass were positively correlated (One-tailed Pearson $R = 0.674$, $N = 11$, $P = 0.012$).

A significant positive linear relationship was found between total phytoplankton biomass (data from the Kinneret Limnological Laboratory) and T-comp fluorescence for the period ranging from 2 weeks post-turnover to the end of the study period ($R^2 = 0.445$, $P = 0.035$, $N = 9$). Because the T-comp fluorescence and algal biomass data were not obtained concurrently, some data reduction was necessary to align data from these two measures, decreasing the sample size in the process. A significant linear relationship was also found between chl *a* and T-comp when examining all data after and including 29.3.09 ($R^2 = 0.60$ $P = 0.042$, $N = 7$). T-comp data preceding this date were excluded from the regression due to problems of data reduction.

Secchi depth decreased from 4.2 m in January to 2 m in mid-March, changing in close relation to T-comp (Fig. 2). A significant negative correlation was found between Secchi depth and in situ protein-like fluorescence over the course of the study (Pearson $R = 0.68$), and linear regression showed that 46% of the variance in protein-like fluorescence could be

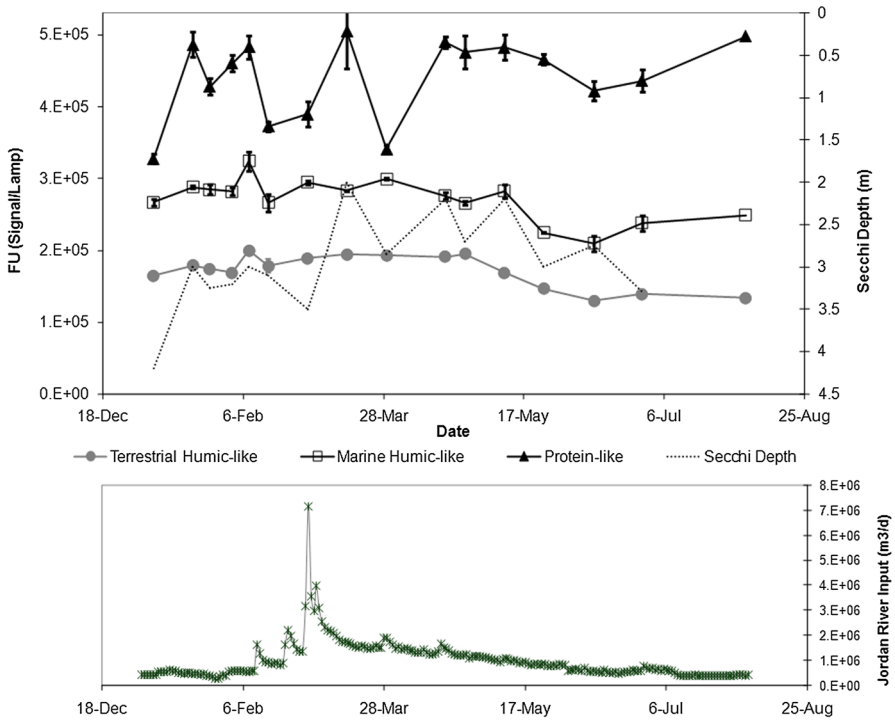



**Fig. 2** Lake Kinneret in situ fluorescence intensity time series for protein-like component (T-comp), marine humic-like and terrestrial humic-like components. *FU* raw fluorescent units, adjusted for lamp intensity. *Dashed line* is Secchi depth in m. A significant linear relationship was found between T-comp and Secchi depth. *Stars* are volumes of Jordan River input in $m^3$/day

explained by changes in turbidity as measured by Secchi depth ($N = 16$, $R^2 = 0.48$, $F = 11.2$, $P = 0.005$).

In vitro lake water incubations

Lake water samples showed significant changes in protein-like and marine humic-like fluorescence over an incubation period of 17–22 days (Figs. 5, 6, 7, 8). Repeated measures ANOVA using 4-point incubation curves showed a significant main effect of incubation time for total T-comp ($P = 0.006$) and total M-comp ($P < 0.001$) components. When testing the dissolved and particulate fractions separately, T-comp showed a significant main effect of incubation time for both the dissolved ($P < 0.001$) and particulate ($P = 0.014$) fractions. M-comp showed a significant main effect of incubation time for the dissolved fraction ($P = 0.013$) but not for the particulate fraction. C-comp did not exhibit any significant changes over the course of the incubations, either in unfiltered samples (total C-comp) or in the particulate fraction (unfiltered

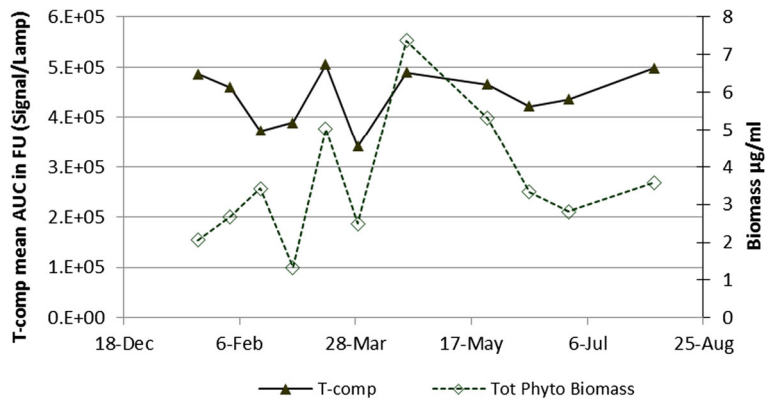



**Fig. 3** Time series of protein-like fluorescence (T-comp) and total phytoplankton biomass in Lake Kinneret

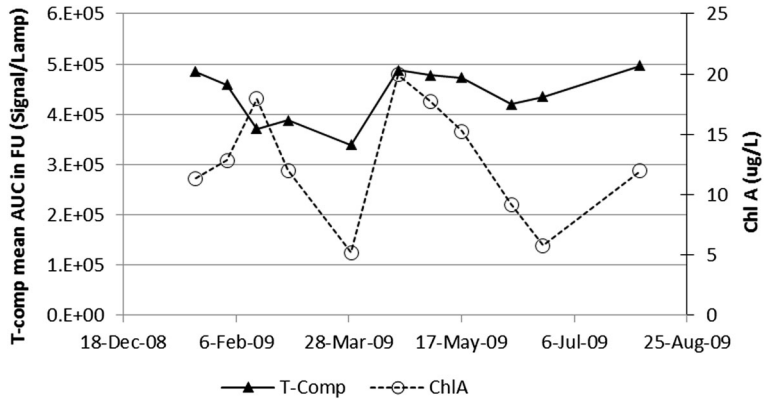



**Fig. 4** Time series of protein-like fluorescence (T-comp) and chlorophyll a (chl *a*) in Lake Kinneret

minus filtered), though a significant main effect of incubation time was found for the dissolved component (filtered samples) ($P = 0.001$).

A significant interaction effect between incubation time and month of initial sampling was found for the total ($P < 0.001$), dissolved ($P < 0.001$), and particulate ($P < 0.001$) fractions of T-comp, suggesting a seasonal effect. The same effect was observed in the total ($P < 0.001$) and dissolved ($P < 0.001$) fractions of M-comp, but not in the particulate fraction. No significant interaction effects were found for the total or particulate fractions of C-comp, though a significant interaction effect (incubation time *x* month) was found for the dissolved component ($P = 0.001$). A significant between-subject effect of incubation month was observed in the total ($P = 0.006$), dissolved ($P = 0.005$), and particulate ($P = 0.022$) fractions of T-comp. A significant between-subject effect of incubation month was observed in the total ($P = 0.001$) and particulate ($P = 0.018$) fractions of M-comp, and a trend-level effect of month was found for the dissolved component ($P = 0.067$). To help compare FOM production/consumption between incubation months, the total change (from baseline) in T-comp and M-comp over the course of each incubation ($\Delta$T-comp and $\Delta$M-comp) is reported, and contrasts are summarized in Table 1. No significant effect of incubation month was found with respect to the terrestrial humic-like component (all fractions).

### Bacterial production in in vitro incubations

Bacterial carbon production, as measured by the uptake of radiolabelled leucine, showed a roughly 200% increase in total productivity (AUC of incubation) from the March to the April incubation. The majority of this increase was attributed to the particulate fraction of the lake water (Fig. 9). Total bacterial production remained high through the end of May. A comparison of production timecourses within the April and May incubations reveals an early spike in unfiltered samples during the May incubation, and faster ramping-up of production in both filtered and unfiltered treatments as compared with the April incubation.

## Discussion

This study attempts to relate changes in primary production and microbial activity to FOM concentrations in mesotrophic Lake Kinneret, Israel. Data were collected throughout a nine-month period at a finer temporal resolution than has previously been documented in this water body. Short sampling intervals were chosen in an attempt to identify rapid responses of the in situ microbial community to increases in phytoplankton biomass. Between February and May, there were a number of spikes in phytoplankton biomass (Fig. 3), and to a lesser extent in chl *a* concentration (Fig. 4). While biomass and Secchi depth captured three major bloom events prior to stratification (Figs. 3, 5), chlorophyll data (from the Kinneret Limnological Laboratory) revealed only one major rise. This disagreement may be an artifact of the low temporal resolution of chl *a*, since in a previous twenty-year study of the Kinneret, there was a strong positive correlation between chl *a* and algal biomass within a given year (Berman et al., 1992). The microbial community responded to two of the

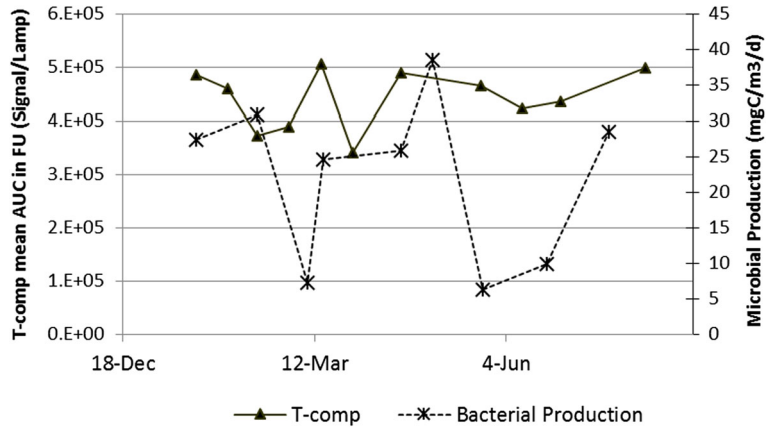



**Fig. 5** Time series of protein-like fluorescence and bacterial production in Lake Kinneret

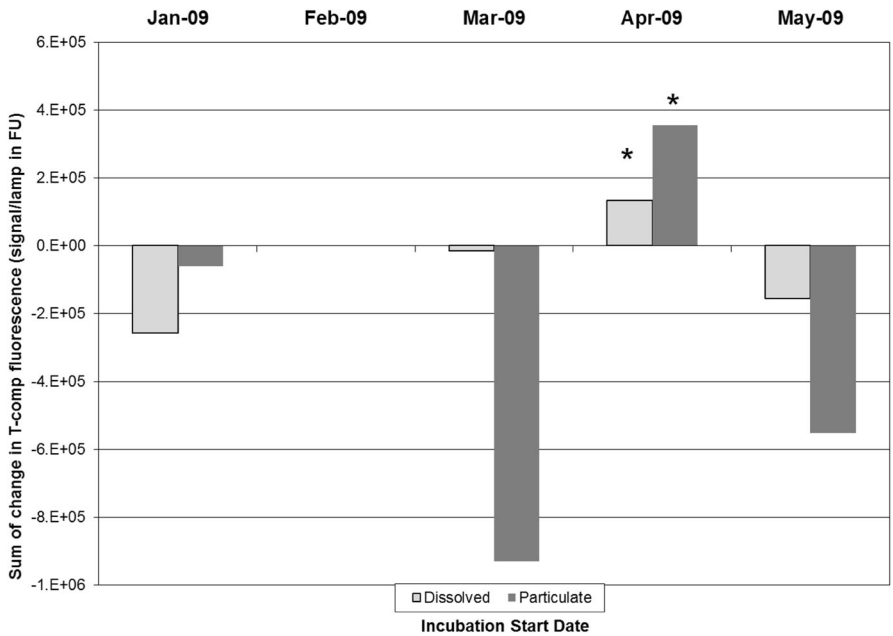



**Fig. 6** Change (AUC minus baseline) in protein-like fluorescent component (T-comp) over the course of incubation for four independent 17–22 day incubations beginning in Jan, Mar–May. Production/consumption attributable to dissolved (filtered incubation) and particulate (unfiltered incubation minus filtered) fractions are shown separately. T-comp was consumed in all incubations apart from the April incubation, in which T-comp was produced. Post hoc tests of repeated measures ANOVA show that values for April are sig. different from values in all other months

phytoplankton bloom events, with a 240% rise in carbon production in April and a further 50% increase in early May 2009, though the May peak in microbial production lagged approximately 2 weeks behind the largest of the spring production peaks (Figs. 4, 5). Subtraction of filtered incubation production from unfiltered incubation production suggests that up to 52% of the integrated microbial carbon production originated in the particulate fraction, though this value does not differentiate between fresh POM and POM that was degraded over the course of the incubation.

While T-comp fluctuated continuously throughout the winter, T-comp values deviated by more than one standard deviation from the mean only five times, exhibiting three maxima and two minima (Fig. 2). This suggests that there were three major T-comp

production/addition events in the lake, and two major consumption/reduction events during winter. Algal production peaks were accompanied by increases of up to 48% in the T-comp of the FOM pool, suggesting that T-comp is produced in the water column during fast-paced phytoplankton growth. This finding reinforces the results of Stedmon & Markager (2005b), who showed that increases in protein-like fluorescence were related to exponential phytoplankton growth in seawater mesocosms. The relationship between T-comp and primary producers is strengthened by significant positive linear relationships between T-comp and total phytoplankton biomass and chl *a*, and a negative correlation with Secchi depth. In addition, a decreasing post-stratification (June–July) and increasing midsummer (July–August) trend was apparent in T-comp, chl *a*, phytoplankton biomass, and microbial productivity (Figs. 3, 4, 5, 6).

This study suggests that in situ protein-like FOM responds fairly rapidly to changes in phytoplankton biomass. While at least two previous incubation studies have found dramatic fine scale increases in FOM, these involved nutrient amendments or additions of phytoplankton laboratory cultures (Stedmon & Markager, 2005b; Zhang et al., 2009) and in situ studies have primarily reported significant FOM variability on timescales of weeks to months (Stedmon & Markager, 2005a; Borisover et al., 2009). These findings suggest that further studies of in situ FOM dynamics should strive to minimize sampling intervals, in an effort to recognize the true dynamics of FOM concentrations in natural systems. At high temporal resolutions, protein-like fluorescence may prove useful as an additional marker for changes in primary producer biomass in aquatic systems. In a series of 28 Canadian lakes representing a range of trophic conditions, Cammack et al. (2004) found that tryptophan fluorescence (similar to the protein-like fluorescence measured in this study) was an excellent predictor of bacterial growth, bacterial respiration, and

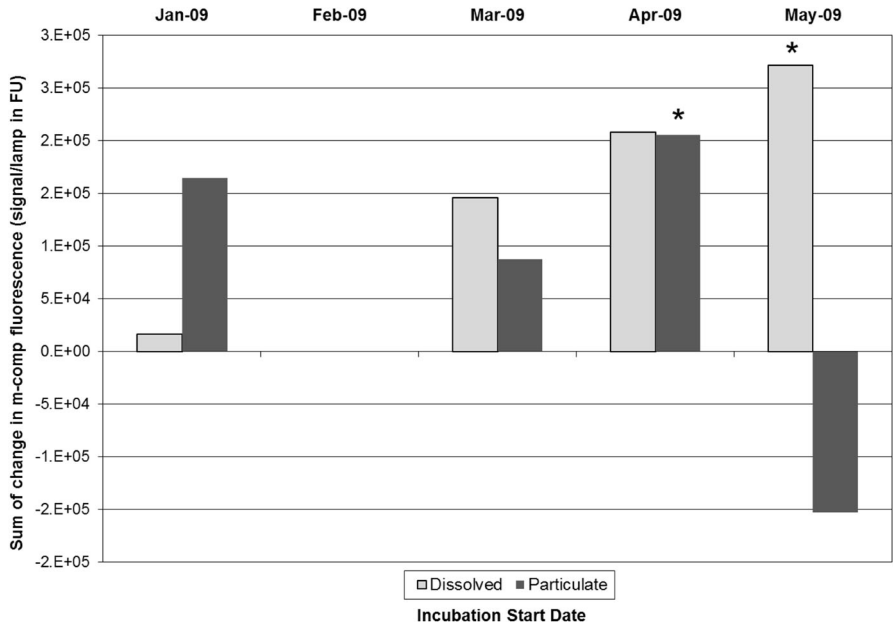
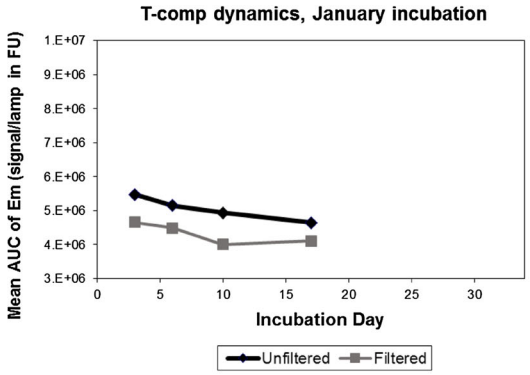
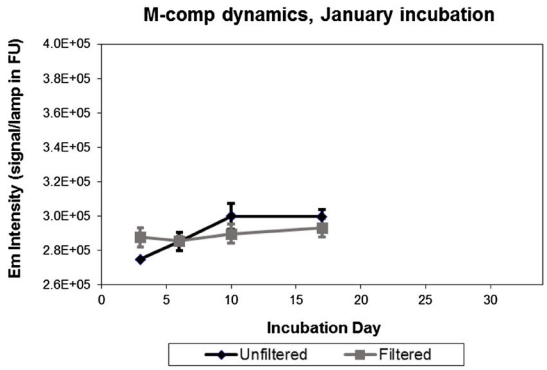
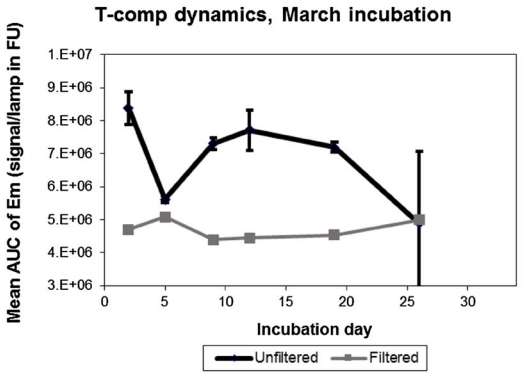


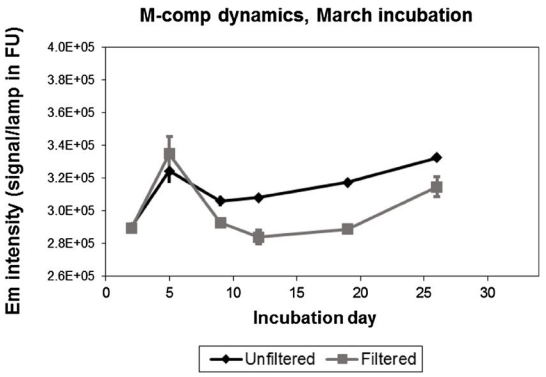

**Fig. 7** Change (AUC minus baseline) in marine humic-like fluorescent component (M-comp) over the course of four independent 17–22 day incubations. Production/consumption attributable to dissolved (filtered incubation) and particulate (unfiltered incubation minus filtered) fractions are shown separately. M-comp was produced in all incubations. Post hoc tests of repeated measures ANOVA show that values of total change for April are sig. different from values in all other months, and particulate values for April and dissolved values for May are sig. different from corresponding (particulate or dissolved) values for May and January, respectively

**Fig. 8** Raw fluorescence measures for protein-like (T-comp) and marine humic-like (M-comp) FOM in four separate incubations starting in January, March, April, and May. *Black lines* represent particulate unfiltered fractions and *gray lines* filtered fractions. Values are means ± standard deviations


T-comp dynamics, January incubation
Mean AUC of Em (signal/lamp in FU)
1.E+07
9.E+06
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
0
5
10
15
20
25
30
Incubation Day
Unfiltered
Filtered


M-comp dynamics, January incubation
Em Intensity (signal/lamp in FU)
4.0E+05
3.8E+05
3.6E+05
3.4E+05
3.2E+05
3.0E+05
2.8E+05
2.6E+05
0
5
10
15
20
25
30
Incubation Day
Unfiltered
Filtered


T-comp dynamics, March incubation
Mean AUC of Em (signal/lamp in FU)
1.E+07
9.E+06
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
0
5
10
15
20
25
30
Incubation day
Unfiltered
Filtered


M-comp dynamics, March incubation
Em intensity (signal/lamp in FU)
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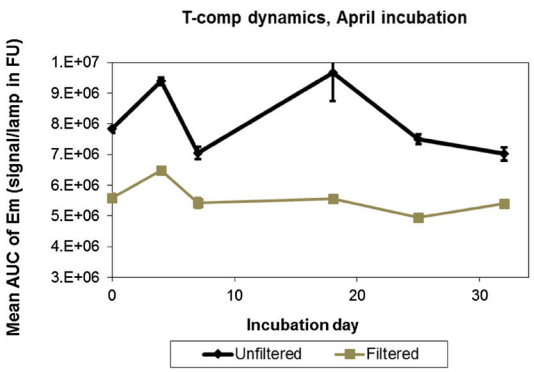
3.6E+05
3.4E+05
3.2E+05
3.0E+05
2.8E+05
2.6E+05
0
5
10
15
20
25
30
Incubation day
Unfiltered
Filtered


T-comp dynamics, April incubation
Mean AUC of Em (signal/lamp in FU)
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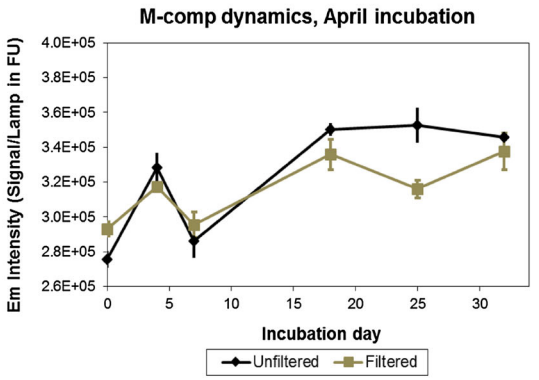
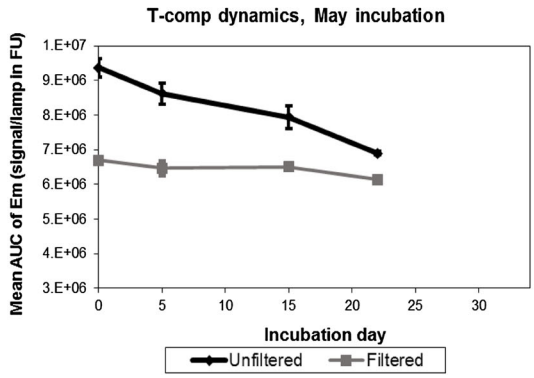
9.E+06
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
0
10
20
30
Incubation day
Unfiltered
Filtered


M-comp dynamics, April incubation
Em Intensity (Signal/Lamp in FU)
4.0E+05
3.8E+05
3.6E+05
3.4E+05
3.2E+05
3.0E+05
2.8E+05
2.6E+05
0
5
10
15
20
25
30
Incubation day
Unfiltered
Filtered


T-comp dynamics, May incubation
Mean AUC of Em (signal/lamp in FU)
1.E+07
9.E+06
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
0
5
10
15
20
25
30
Incubation day
Unfiltered
Filtered

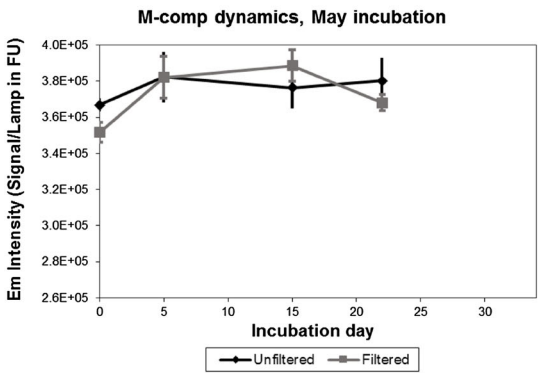

M-comp dynamics, May incubation
Em Intensity (Signal/Lamp in FU)
4.0E+05
3.8E+05
3.6E+05
3.4E+05
3.2E+05
3.0E+05
2.8E+05
2.6E+05
0
5
10
15
20
25
30
Incubation day
Unfiltered
Filtered

**Table 1** Results of post hoc comparisons (Tukey's test) between incubation months, grouped by fluorescent component and filtration fraction

| Component | Fraction | Comparison | *P* value |
|---|---|---|---|
| ΔT-comp | | | |
| | Total (unfiltered) | Apr > Mar | 0.006 |
| | | Apr > May | 0.016 |
| | Dissolved | Apr > Jan | 0.005 |
| | | Apr > May | 0.025 |
| | Particulate | Apr > Mar | 0.02 |
| | | Apr > May | 0.098 |
| ΔM-comp | | | |
| | Total (unfiltered) | Apr > Jan | 0.003 |
| | | Apr > Mar | 0.012 |
| | | Apr > May | 0.001 |
| | Dissolved | May > Jan | 0.056[a] |
| | Particulate | Apr > May | 0.018 |

T-comp and M-comp represent protein-like and marine humic-like fluorescent components, respectively

[a] Trend-level significance ($0.75 > P > 0$)

total plankton community respiration, performing better than the concentration of bulk DOM.

Unlike T-comp fluorescence, the terrestrial humic-like fluorescent component (C-comp) remained steady throughout the winter production period (Fig. 2), dropping only after stratification set in. In this study, C-comp concentrations did not change significantly in incubation, suggesting that this fluorescent component is neither consumed nor produced in the water column of Lake Kinneret. While oceanic and estuarine FOM studies (Coble, 1996; Kowalczuk et al., 2003, 2009; Stedmon et al., 2003; Murphy et al., 2008) have successfully used terrestrial fluorescence (identified by PARAFAC or peak picking) to track riverine inputs into bays and estuaries, C-comp was not found to relate with terrestrial input, as measured by Jordan River inflow, in this study. This suggests that the majority of C-comp inputs to Lake Kinneret may be the result of FOM production within lake sediments as opposed to terrestrial runoff rich in soil leachates or plant detritus. Borisover et al. (2009) identified an accumulation of near-bottom C-comp especially during summer stratification, suggesting a sedimentary source for this component in the Kinneret. Indeed, our finding that surface water C-comp levels decline with the onset of stratification, reinforces this hypothesis. The unmonitored subsurface saline springs which feed into the Kinneret are another possible source of terrestrial DOM, though water and solute input from these springs appears to track lake level (Rimmer & Gal, 2003), which was unrelated to C-comp fluorescence in this study.

Despite the suggestion that filtration does not significantly affect fluorescence in Kinneret samples (Borisover, pers. communication), we occasionally found substantial differences in FOM levels between filtered and unfiltered samples. Therefore, future studies should measure the fluorescence of both filtered and unfiltered in situ samples to separate between the FOM signatures of POM and DOM.

The winter/spring season of 2009, did not include a major *P. gatunense* bloom. Instead, between February and May, a number of smaller phytoplankton growth events provided stepwise boosts to phytoplankton biomass. This production period (February–May) was dominated by chlorophytes, which contributed about 50% of the total wet-weight biomass, as well as *Peridiniopsis* (20–30%) and several species of cyanobacteria (mostly *Microcystis aeruginosa*). *Cryptomonas* sp. also made a significant contribution to algal biomass in January (34%) and February (10–28%) but then declined to 3–5% of the total biomass (Zohary, personal communication). While these various taxa may differ in the composition of their photosynthetic pigments and protein contents (factors that may impact fluorescence), no clear relationships between species composition and fluorescence were observed in this study.

## FOM and the microbial community

Whereas phytoplankton growth may be tracked by T-comp in Lake Kinneret, the primary producers may not be the primary source of protein-like FOM in this system. Several studies have related CDOM absorption (Nelson et al., 2004a), as well as protein-like and marine humic-like fluorescence to microbial processing of organic matter. In nutrient-amended seawater incubations, CDOM absorption was correlated with rising microbial cell densities (Nelson et al., 2004b), and T-comp has been shown to accurately track the fraction of microbially bioavailable DOM in freshwater, as measured by biological oxygen demand (Hudson et al., 2008). Cammack et al. (2004) found that T-comp accounted for 44–55% of in situ variability in

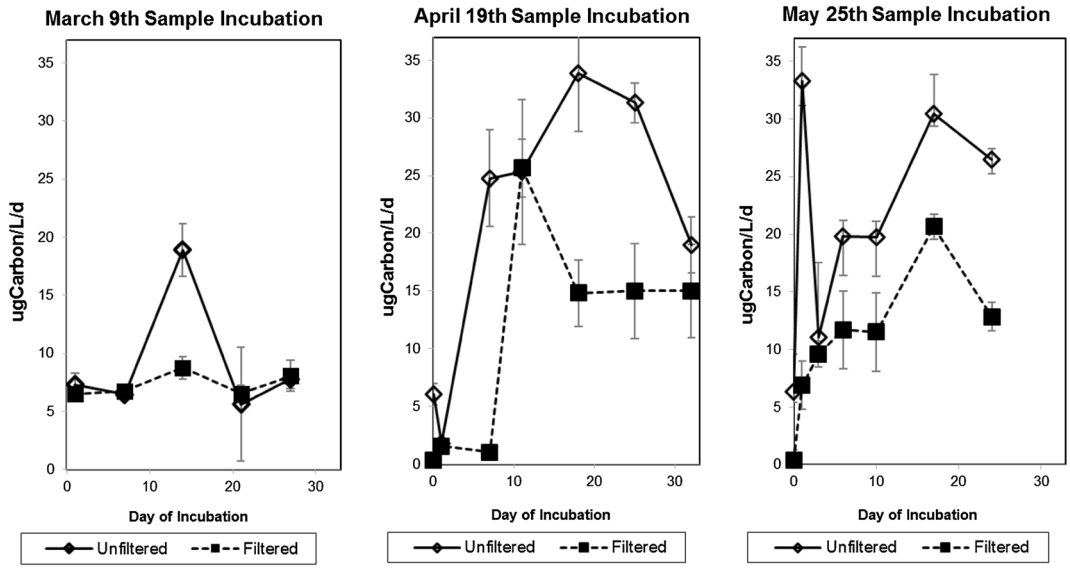



**Fig. 9** Bacterial carbon production rate (μg C produced per liter sample volume per day) for dark-incubated lake water sampled on three separate days over the course of the spring season. Bacterial production during incubation of unfiltered (*solid line*) and filtered (*dashed line*) water samples are shown separately. Values are means ± standard deviations

bacterial production, respiration, and carbon consumption, as well as total plankton community respiration in a series of lakes. A number of incubation studies have suggested that T-comp and M-comp are both produced and consumed by aquatic microbes, and that individual components only accumulate when high concentrations of DOM from phytoplankton or zooplankton sources are available (Nelson et al., 2004a; Nieto-Cid et al., 2006; Zhang et al., 2009).

In this study, incubation results suggested that T-comp dynamics reflect microbial processing of organic matter. Moreover, within the photic zone, the organic carbon pool is roughly composed of 75% DOC and 25% POC, with the labile fraction representing about 12% of total DOC and 9% of total organic carbon (Ostapenia et al., 2009). A recent review of Lake Kinneret ecology reports mean levels of seston, POC, and DOC as 4.16, 1.56, and 3.91 mg $l^{-1}$, respectively for the period 2000–2010 (Parparov et al., 2014). The present study's seasonal time series suggests that microbial production responds quickly to increases in phytoplankton biomass, which are also tracked by T-comp fluorescence (Figs. 4, 5, 6). Since turnover rates for the most labile fraction of both DOC and POC are around 4 days (Ostapenia et al., 2009), the microbial community can conceivably convert newly produced phytoplankton biomass into protein-like FOM within just a few days.

A study by Berman et al. (2010) has found that between the years 2000–2007 in Lake Kinneret, monthly bacterial growth efficiency fluctuated widely (from 15 to 81%), but that when data were averaged over 6-month period this variability diminished considerably. These authors hypothesize that this difference in apparent microbial growth rates may be caused by short-term fluctuations in bacterial substrate availability. In a similar fashion, we found that while T-comp fluorescence showed no general trend over the course of the 7-month sampling period, T-comp fluctuated widely on shorter timescales during the winter production period (Fig. 2). If microbial activity regulates the concentration of T-comp in the Kinneret, then rapid cycles of T-comp production and removal may indeed reflect these hypothesized fluctuations in the availability *or lability* of microbial substrates. Further study is required to examine the relationship of the rapid fluctuations in T-comp fluorescence to the accumulation and degradation of protein-like DOM. Moreover, it remains to be seen whether these rapid fluctuations are found in other lakes with varying rates of OM loading and microbial production.

Further evidence of microbial processing of FOM was obtained in dark incubations, where T-comp and

M-comp fluorescence exhibited changes over the course of 3 weeks. Significant changes in M-comp and T-comp were recorded over time for incubations of filtered samples, representing a highly variable fraction of the total changes in T-comp and M-comp fluorescence in unfiltered samples of the same water. Changes in filtered water accounted for as little as 2% and as much as 100% of the total changes in these components, depending on the month of incubation. These values suggest that the relative importance of DOM concentrations during FOM production and/or consumption is variable, and may change in response to changing DOM/POM composition and loading. In the May incubation, for example, the fraction of total M-comp production attributable solely to DOM was dramatically greater than in January (see Fig. 9). Specifically, the ratio

$$\Delta\text{M-comp}_{\text{filtered}} : \Delta\text{M-comp}_{\text{unfiltered}}$$

was much higher for May than for January. This increase in the relative contribution of DOM to M-comp production may reflect fresh DOM inputs related to the spring bloom.

Subtraction of filtered incubation samples' ΔFOM values from unfiltered values suggests that microbial decomposition of POM is highly variable, accounting for 19–98 and 38–91% of the summed changes in T-comp and M-comp fluorescence, respectively. These values should be treated with caution, since microbial processing of dissolved and particulate organic matter may not be additive processes (Parparov, personal communication) as was previously assumed (Ostapenia et al., 2009). In the May incubation, for example, filtered samples demonstrated a greater accumulation of M-comp than unfiltered samples. This suggests that the relative lability of POM and DOM, at least with respect to the fluorescent fraction, may vary widely over the course of the year.

M-comp and T-comp fluorescence was found to successively increase and decrease over the course of some incubations, suggesting that both components' concentrations are controlled by opposing processes of production and consumption or decomposition. This reinforces the results of previous studies, which showed that protein-like fluorescent components could be either produced or consumed in incubation, depending on the level of primary production (Nieto-Cid et al., 2006) or the availability of labile DOM (Nelson et al., 2004b). Accumulation of both components was significantly higher in April than in other months, and integrated microbial production also increased threefold in April relative to March. Chlorophyll a and phytoplankton biomass concentrations (Figs. 3, 4) both reached their seasonal maximum in late April, implying that the April incubation contained the highest concentration of phytoplankton biomass. For M-comp, which accumulated in every incubation the relative importance of DOM over POM in fluorescence accumulation (as measured by the ratio of fluorescence increase in *filtered* versus *unfiltered minus filtered* samples) increased from January to May. For T-comp, DOM was a relatively small influence on FOM change in every month except for January. Accumulation and removal of T-comp in incubation were primarily driven by the influence of the particulate fraction (see Fig. 7).

Whereas M-comp accumulated in all of the incubations, seasonal changes in lake M-comp levels were negligible, indicating that M-comp production in incubation is not reflected in situ. This discrepancy may result from an unidentified process of M-comp removal in situ, rather than a lack of production. Stedmon et al. (2003) found that photodegradation is an important sink for microbially derived humic material, which suggests that, in the Kinneret, newly produced M-comp may be photodegraded before it can accumulate in the upper mixed layer. Borisover et al. (2009) found that humic concentrations were greater at depth regardless of the distance from the lake bottom. Since photochemical processes decrease uniformly with depth, this finding strengthens the hypothesis that light-dependent processes control the concentration of M-comp in the Kinneret. These processes may include simple photobleaching, or post-irradiative increases in lability leading to rapid FOM bio-degradation (Moran & Zepp, 1997). Nieto-Cid et al. (2006) found that in certain coastal waters, marine humic FDOM concentrations increased with depth, while surface waters were enriched in protein-like FDOM. Using a combination of short (1 day) unamended dark and light incubations, those authors found a positive correlation between photodegradation of dissolved marine humic substances and bacterial production. This was interpreted as evidence that humic substances produced during bacterial metabolism in the dark were quickly degraded and consumed after exposure to sunlight. This further supports our hypothesis that photodegradation effectively

quenched the in situ M-comp production signal in this study, despite the fact that M-comp was produced in every incubation. Future incubation studies would benefit from parallel treatments of dark and sunlight-exposed incubations to examine the effect of photodegradation on the fluorescence and bacterial metabolism of Kinneret FOM. Moreover, further study is required to elucidate the drivers of FDOM accumulation, specifically those factors that determine or alter FDOM composition and lability under natural conditions.

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