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Flood events can reduce key fatty acid content of early-stage benthic algal assemblages in an urban stream

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Abstract

Effects of urbanization on stream chemistry and biota have been widely examined. However, few studies quantify the effect of urban stream characteristics on the biochemical properties of basal food resources, such as benthic algae, which can affect aquatic consumers and food webs. A common feature of urban streams is the high frequency of short floods, which may disrupt aquatic communities and their biotic controls. These disturbances can create algal assemblages low in biomass and which remain in early successional stages. This study examined the effects of frequent flood events and macroinvertebrate grazing on biomass, elemental stoichiometry, and essential fatty acid (EFA) concentration of early colonizing benthic algal assemblages in an urban stream. Over a 3-week period in which two flood events occurred, algal biomass (chlorophyll-*a*) and EFA profiles were each affected by floods, but the responses were weakly correlated. Concentrations of EFA molecules essential for aquatic consumers, such as arachidonic acid (ARA) 20:4 ω 6, eicosapentaenoic acid (EPA) 20:5 ω 3, and docosahexaenoic acid (DHA) 22:6 ω 3 were greatly reduced after floods, potentially lessening algal nutritional quality. There were no statistically significant differences in biomass or stoichiometry of benthic algae in assemblages under different grazing regimes, suggesting hydrological factors played a greater role in their production and nutritional quality. This study provides evidence that flood-prone streams in urban landscapes may be susceptible to greatly altered biochemical profiles in early colonizing benthic algal assemblages. Results also suggest that biochemically important compounds, such as EFAs, offer valuable information on the quality of benthic algal assemblages under anthropogenic stress.

Key words: benthic algae; biofilm; fatty acids; grazing; flood events; urban stream.

1. Introduction

In stream ecosystems, benthic algae are important primary producers and a key basal food resource for lotic food webs (Lamberti 1996; Wetzel 2001). Algae colonize a diversity of benthic surfaces, but exist within a benthic algae matrix, which is a complex biofilm that also includes detritus, bacteria and fungi. However, it is the algal cells in that matrix that are an especially rich source of nutrients and essential fatty acids (EFAs) for consumers (Brett and Müller-Navarra 1997; Torres-Ruiz et al. 2007, 2010). The rate at which algal assemblages colonize rocks and other surfaces over time (their temporal succession) varies with

both physical and biological conditions in a river, which affect both biomass and nutritional composition (Biggs 1996; Murdock et al. 2004; Torres-Ruiz et al. 2007).

Early stage benthic algal assemblages become established (the colonization phase) through immigration and growth, and with time develop greater taxonomic and physical complexity, unless losses occur due to flooding and/or grazing activity (Biggs 1996). Changes in benthic algal assemblage biomass and composition can occur over very short time-scales. One study of early-colonizing benthic algae found that assemblages develop and diversify in as short a period as 10 days (Jordan and Staley

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1976). Hillebrand and Sommer (2000) quantified measurable biovolume of algal cells after as little as 5 days of growth. Korte and Blinn (1983) documented diatom colonization on bare substrata within the first week in one forest stream, with succession proceeding from simple, adnate forms to more complex and diverse assemblages over three weeks. Most colonization studies indicate that the biomass, taxonomic diversity, and physical structure of early colonizing algal assemblages increase rapidly within 1–2 weeks, with rates differing by season, nutrient conditions, flooding, and other disturbances (Hoagland et al. 1982; Peterson and Stevenson 1990; Stevenson 1990; Biggs 1996).

In urban streams, rates of benthic algal development may be more severely affected by hydrological factors than biotic interactions, due to more frequent and abrupt floods (Paul and Meyer 2001). Urban streams are characterized by increased nutrient concentrations, reduced stream channel structure, altered riparian habitat, and in particular, highly variable hydrological regimes (Paul and Meyer 2001; Meyer et al. 2005; Walsh et al. 2005). During wet periods, urban streams can experience a severe flood event as often as once a week, in contrast to well-buffered streams within intact watersheds (Roy et al. 2005). These flood events predominantly affect loosely attached and filamentous algal taxa in later stages of colonization, and select for adnate and prostrate taxa typical of early stages (Francoeur and Biggs 2006). Hydrological conditions in urban streams thus create benthic algal assemblages typified by early successional assemblages and a lack of climax assemblages (Murdock et al. 2004).

Nearly all studies on early stage benthic algal assemblages thus far have focused on taxonomic and/or biomass changes over time. Recently, biochemical properties of some early successional assemblages have also been recorded. Photosynthetic activity and biochemically active components of algae can be quantified after just 1 week of in situ incubation (Frost et al. 2007; Whorley and Francoeur 2013). However, no studies are known which have examined early stage benthic algal assemblages in urban streams with regard to their nutritional quality.

Early studies on nutritional quality of benthic algal assemblages have largely been based on elemental stoichiometry (e.g. C:N:P), and these findings are now well established (Stelzer and Lamberti 2002; Fink et al. 2006; Arts et al. 2009; O'Brien and Wehr 2010). A few studies indicate that the nutritional quality of algae may be more effectively assessed by measuring essential fatty acid (EFA) concentrations (Torres-Ruiz et al. 2007; Brett et al. 2009). These data show that macroinvertebrate consumers receive greater nutritional benefit from algal than terrestrial matter, due to greater concentrations of essential lipids (Brett et al. 2009; Torres-Ruiz et al. 2010). Lipids in the form of EFAs from algae are particularly critical for macroinvertebrate reproduction, development, and growth (Arts et al. 2009). But thus far, studies measuring EFAs have examined only established algal assemblages (Boëchat et al. 2011; Cashman et al. 2013; Boëchat et al. 2014; Honeyfield and Maloney 2015); thus little is known of benthic algal fatty acid composition in early-stage assemblages. In urban streams, stressors such as extreme and abrupt changes in discharge and altered light availability can render assemblages in a perpetual state of early colonization, which may alter nutritional quality of basal food sources for the ecosystem (Hillebrand et al. 2004; Barbee 2005; Cashman et al. 2013).

It has been widely shown that macroinvertebrate grazing can also limit algal growth in streams (Jacoby 1987; Biggs 1996) as well as alter nutritional quality, as shown by increased C:N and C:P ratios (Liess and Kahlert 2007). These biotic effects can vary with assemblage composition and successional stage.

Villanueva and Modenutti (2004) demonstrated that later successional stages with larger over-story algal taxa (e.g. filamentous green algae or cyanobacteria) are more strongly affected by grazing than were earlier successional assemblages composed of tightly adherent diatom taxa. Furthermore, in some cases, nutrient enrichment can moderate or minimize biomass losses due to grazing activity (McCormick and Stevenson 1991; Hillebrand and Kahlert 2001). Given the common conditions in urban streams of both elevated nutrients and frequent floods, the relative importance of biotic versus hydrological factors on algal nutritional composition needs to be assessed.

We suggest that due to frequent floods, early successional benthic algal assemblages typical of urban streams may provide a limited source of EFAs for macroinvertebrate consumers. This study examines the quantity and nutritional quality of early successional benthic algal assemblages developing in an urban stream subject to frequent flood events, and whether these properties are affected by macroinvertebrate grazing pressure. To determine the impact of these stressors on algal biomass and nutritional composition, we employed a manipulative field experiment using natural substrata to follow benthic algal early succession with high-frequency sampling in an urban stream. We hypothesized that (1) flood events will maintain early successional algae to assemblages with lower nutritional quality (as determined by EFA concentrations), while (2) assemblages relieved from grazing pressure will have a greater nutritional quality than grazed assemblages.

2. Methods

2.1 Site description

The Bronx River flows through an urbanized watershed (155 km² area) in Westchester County, NY (USA), and is surrounded by medium to high-density residential and commercial development, with impervious surfaces averaging 34.4 per cent of the watershed land area (Westchester County Department of Planning 2007). The river receives inputs from multiple sources along its course, including siltation, non-point source nutrients, and municipal/industrial wastes (Smith et al. 2015). Although a major roadway parallels the river along most of its length, there is a narrow riparian habitat that includes deciduous trees, parks, and wetlands, which create intermittently shaded and open canopy sections. This study was conducted in a 25-m long reach of the river in Bronxville, NY (40.943671N, 73.837453W), which is also within the Bronx River Parkway Reservation, which contains planted vegetation and intact forest tracts maintained and protected by the county (Frankel 1999). In this region, the stream width/depth ratio averages ~18 (= moderate to high; Rosgen 1994), the entrenchment ratio varies due to sections that have been stabilized with concrete embankments, and sinuosity is low to moderate. In our study reach, most of the streambed is sandy, with occasional riffles composed mostly of cobbles, and has an average canopy cover of 38.6 per cent. The hydrology is typified by sharp and intense peaks in discharge following rain events (U.S. Geographical Survey 2012), typical of urban watersheds (Paul and Meyer 2001). In the year of this study (2010), 28 flood events (defined as more than a 5-fold increase in discharge) were recorded. During the study period, floods occurred on days 11–12 (13–14 July), and days 15–18 (17–20 July) (judged by field observations and USGS hydrograph). Stage height data from a USGS stream gauge (no. 01302020) 11 km downstream indicated that discharge levels during two of these events were > 11 m³/s (vs. mean base flow ~0.8 m³/s). Colonization time was defined as the

period between floods, which incorporates both flood frequency and duration.

2.2 Field Procedures

Ninety-four smooth, cleaned cobbles (~15 cm in diameter) were collected from the river to be used as experimental substrata for periphyton colonization. Prior to their incubation in the river, each cobble was scrubbed with a stiff brush under running water and dried for 72 h at 80 °C to remove attached periphyton and other organisms. Half were rimmed with petroleum gel to exclude grazing invertebrates (after Dudley and D'Antonio 1991). Petroleum gel was re-applied after floods and rocks were examined daily for evidence or presence of macroinvertebrates during the study. Each was identified with a unique number and a random number generator was used to create an eight-by-twelve placement grid of the cobbles spaced ~1 m apart in the stream channel (Fig. 1).

Cobbles were placed in the stream on 2 July 2010. Daily sampling began 4 days later to capture early-stage algal colonization and proceeded until 21 July. Each day, eight rocks were selected by random number (four with petroleum gel, four without) and scraped with a razor blade and toothbrush to remove attached algae. Sampled cobbles were not replaced in the river. Cobble dimensions were measured following algal collection based on an assumed spheroid shape where the colonizable area was defined as the upper 50 per cent of surface area of each (after O'Brien and Wehr 2010). Qualitatively, grazing activity was noted by the presence of macroscopic scrape marks or presence of denuded areas in the biofilm. The volume of the collected algal material was measured and stored in acid-washed 150 ml polypropylene containers and stored on ice until return to the laboratory (after O'Brien and Wehr 2010).

Temperature, pH, dissolved oxygen (DO), and specific conductance were measured in situ using a Hach Hydrolab water sonde (Loveland, CO, USA) (Table 1). Stream water was collected for water chemistry analyses and filtered through a 0.2 µm pore-size syringe filter, stored in 10 per cent HCl acid-washed polypropylene test tubes, preserved with 12.5 per cent H₂SO₄ to pH < 2.0 and stored cold (4 °C (USEPA 1987)).

2.3 Laboratory Procedures

Biofilm samples were processed on the same day as collection for chlorophyll-*a*, EFA content, and C:N stoichiometry.

Chlorophyll-*a* samples were extracted in MgCO₃-neutralized 90 per cent acetone and processed on a Shimadzu UV-1800 spectrophotometer (Columbia, MD, USA), and corrected for phaeopigments using 0.1 M HCl (Lorenzen 1967; Jeffrey and Humphrey 1975). C and N concentrations were determined from collected material dried at 80 °C in tin capsules and analyzed on a Thermo Scientific Flash 2000 Organic Elemental Analyzer (Pittsburg, PA, USA). EFA samples were filtered onto ashed GF/F filters (GE/Whatman, Buckinghamshire, UK) and stored in chloroform-washed borosilicate test tubes at -20 °C. Samples were then extracted using chloroform:methanol (2:1), methylated using BF₃, and transferred to a hexane solvent (Parrish 1999; Torres-Ruiz et al. 2007; Cashman et al. 2013). An internal standard of nonadecanoic acid (19:0) was used as test of methylation efficiency and to assess constancy among sample runs along with blank hexane samples. Samples were analyzed and quantified using a Shimadzu GC-2014 fitted with a capillary column (Omegawax320, 30 m × 0.32 mm × 0.25 µm film thickness; Supleco, Bellefonte, PA, USA). The temperature program has an initial injection into a splitless inlet at a temperature of 220 °C and oven temperature of 100 °C, followed by 1-h ramping to 260 °C in increments of 10 °C/10 min with helium as the carrier gas to an FID (flame ionization detector). The Supelco 37 component FAME mix was used as a standard for analysis and standard curves of the EFAs. Compounds with ≥18C were identified and measured, since many biologically important fatty acids are derived from 18C as a base molecule. Measurements of EFA compounds were classified and summed into four groups; Total, Σω3, Σω6, and ΣOther; the latter defined as all molecules that were neither Σω3 or Σω6. The GC identified signal-peaks that correspond to a minimum concentration of 0.011–0.024 mg/m², depending on the size of the molecule. Peaks below this detection limit could not be reliably distinguished from background noise. Water chemistry samples were analyzed for SRP, NO₃⁻/NO₂⁻, and NH₄⁺ on an Astoria-Pacific A2 flow-through spectrophotometric nutrient analyzer (Clackamas, OR, USA) using standard methods (USEPA 1987) (Table 1).

2.4 Data analysis

After checking for homogeneity of variance and normality, data were log-transformed to meet assumptions of subsequent tests (Shapiro-Wilk analysis). Measurement results presented are untransformed. We specifically tested the effects of (1)

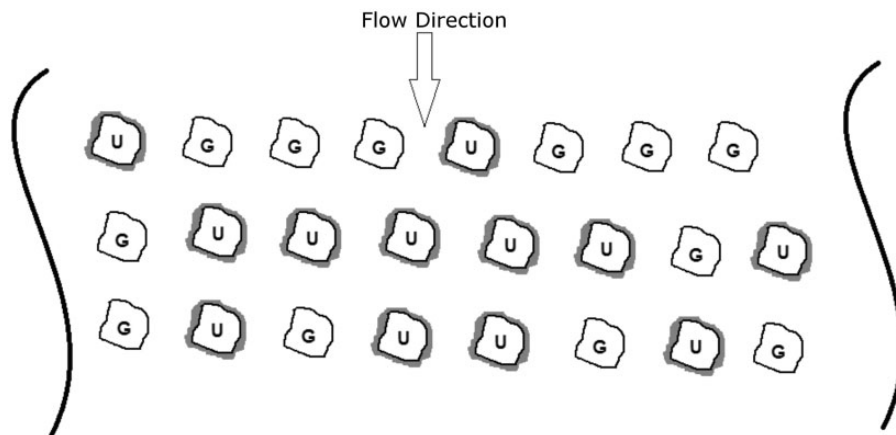


Figure 1. Schematic diagram of the experimental cobble layout in the central stream riffle region of the Bronx River (repeated for 94 cobbles), with random placement of cobbles of each treatment (G, grazed; U, ungrazed). Gray border around 'U' cobbles represents petroleum gel barrier. Four cobbles were destructively sampled from each treatment per day.

Table 1. Summary of environmental measurements made during days when benthic algal assemblages were collected. Flood events occurred on 13–14 July and 17–20 July.

Factor	6 July	7 July	8 July	9 July	11 July	12 July	15 July	16 July	21 July
Temp (°C)	26.73	27.76	27.24	26.23	25.26	26.64	23.58	25.65	25.56
Velocity (m/s)	0.38	0.37	0.21	0.34	0.36	0.37	0.66	0.22	0.38
Cond. (mS/cm)	1.15	1.16	1.16	1.17	1.16	1.13	0.41	0.57	0.46
pH	7.69	7.69	7.63	7.62	7.44	7.56	7.43	7.41	7.33
DO (mg/L)	6.39	6.06	5.57	5.49	5.55	5.99	7.24	6.08	6.16
NH ₄ ⁺ (mg-N/L)	0.21	0.18	0.16	0.21	0.26	0.25	0.24	0.30	0.23
NO ₃ ⁻ (mg-N/L)	0.54	0.50	0.48	0.51	0.40	0.35	0.66	0.64	0.59
SRP (μg-P/L)	31.3	41.7	36.6	179.4	32.4	383.5	392.4	402.6	46.7

colonization time between floods and (2) grazing-pressure on algal variables (biomass, C,N, and fatty acids) using single and two-way MANOVAs for the entire duration and also for each inter-flood growth period (Mertler and Vannatta 2013). Further effects of colonization time and grazing on nutritional quality were tested using two-way ANOVA (Sokal and Rohlf 1995) for each interval. For samples collected in the final interval (following the second flood event), a two-sample t-test was used. Pearson's correlations were used to determine the relationship between chlorophyll-*a* and EFA concentrations across all dates, and between elemental C, N and C:N. The *a priori* α level for all tests was set at $\alpha = 0.05$. All statistical analyses were conducted using SYSTAT 13 (Systat Software, Inc., Chicago, IL, USA).

3. Results

3.1 Benthic algal biomass

Two major flood events were recorded in the Bronx River during the study, on days 11–12 (13–14 July), and days 15–18 (17–20 July). Overall, colonization time (defined as the age of the algal assemblage between floods) had a significant influence ($F_{8,50} = 2.813$, $P = 0.012$) on algal biomass (chlorophyll-*a* concentration), while differences due to grazing treatment were largely non-significant ($F_{2,50} = 3.463$, $P = 0.069$). During the first colonization period (6–12 July), colonization time was a significant factor in differences between chlorophyll-*a* levels ($F_{5,32} = 5.280$, $P = 0.001$), while grazing pressure had no significant effect ($F_{1,32} = 3.042$, $P = 0.090$). Benthic algae experienced a 37 per cent decrease in biomass following the first flood event. Following this decline (the second colonization period: 15–16 July), neither colonization time nor grazing had a significant effect on benthic algal biomass ($P >> 0.05$). Following the second flood event, algal biomass declined by 20 per cent. Overall, algal biomass (as chlorophyll-*a*) on most dates was modestly greater (~17–52 per cent) on grazed cobbles than on those protected from invertebrate grazers (Fig. 2), although no statistically significant difference between grazed and ungrazed treatments was detected ($P > 0.05$). No visible evidence of grazing was noted (presence of scraped or denuded areas on each rock) on petroleum gel-rimmed cobbles, but denuded areas and egg clusters were observed on untreated cobbles.

3.2 Biofilm nutrient stoichiometry

Biofilms on ungrazed cobbles had between 4 and 60 per cent greater concentrations of C, and 4–46 per cent greater N content than assemblages on grazed cobbles. In the youngest

assemblages (4–6-days old), biofilms in the grazed treatments had C:N ratios 20–50 per cent less than ungrazed assemblages. This difference changed over time. Older ungrazed biofilms (≥ 7 -days old) had a C:N ratio averaging of ~2.6 per cent less than those exposed to benthic grazers (Table 2). C:N stoichiometry also varied before and after flood events. C and N concentrations generally increased following early flood events, but this trend differed with grazing treatment. For example, average C content increased by 55 per cent, and N by 60 per cent during the first flood event on grazed cobbles, but only by 17 per cent (C) and 14 per cent (N) on ungrazed cobbles. There were lesser effects of the first flood, with C and N increases of only ~5 per cent. Later, following the second flood, C and N content decreased by ~15 per cent (C) and ~20 per cent (N) across both grazing treatments.

3.3. EFA profiles

Colonization time (periods between floods) also had a significant effect on the essential fatty acid (EFA) concentration of biofilms over the duration of the study ($F_{8,50} = 2.606$, $P = 0.019$). On ungrazed substrata, the first flood resulted in a decrease in total EFAs by 56 per cent (1.8 mg/m²), and the second by 50 per cent (3.4 mg/m²). Following the first flood, EFA concentration also declined by 32 per cent (3.7 mg/m²) on cobbles exposed to grazers, but increased by 38 per cent (2.0 mg/m²) after the second flood (Fig. 3). Quantities of major fatty acid categories were also affected by floods. Within the EFAs, colonization time (between floods) explained most of the differences in concentrations of total omega-3 ($\Sigma\omega 3$) FA in the assemblage ($F_{8,50} = 3.006$, $P = 0.017$), $\Sigma\omega 6$ ($F_{8,50} = 5.660$, $P < 0.001$), as well as FA classified as Σ Other ($F_{8,50} = 8.480$, $P < 0.001$) (Fig. 3). Grazing treatments exerted no overall significant effect on total EFA concentration ($F_{1,50} = 0.306$, $P = 0.582$). There was no consistent pattern or statistically significant effects detected in levels of $\Sigma\omega 3$, $\Sigma\omega 6$ or Σ Other EFA categories between grazing treatments (all tests: $P > 0.05$) (Fig. 3).

Concentrations of key individual EFA compounds varied among both treatments and colonization time (Figs 4 and 5). Most saturated fatty acids, such as 18:0 (which was most consistently present), varied little among biofilm assemblages over time. But unsaturated, biochemically important compounds, including α -linolenic acid (ALA 18:3 $\omega 3$ (ALA)) ($F_{8,50} = 2.404$, $P = 0.028$), (ARA 20:4 $\omega 6$) ($F_{8,50} = 6.460$, $P < 0.001$), (EPA 20:5 $\omega 3$) ($F_{8,50} = 9.247$, $P < 0.001$), and (DHA 22:6 $\omega 3$) ($F_{8,50} = 4.197$, $P = 0.001$), all varied significantly with colonization time. Linoleic acid (LIN 18:2 $\omega 6$) was not significantly different over time ($F_{8,50} = 2.877$, $P = 0.010$). Concentrations of these compounds were never

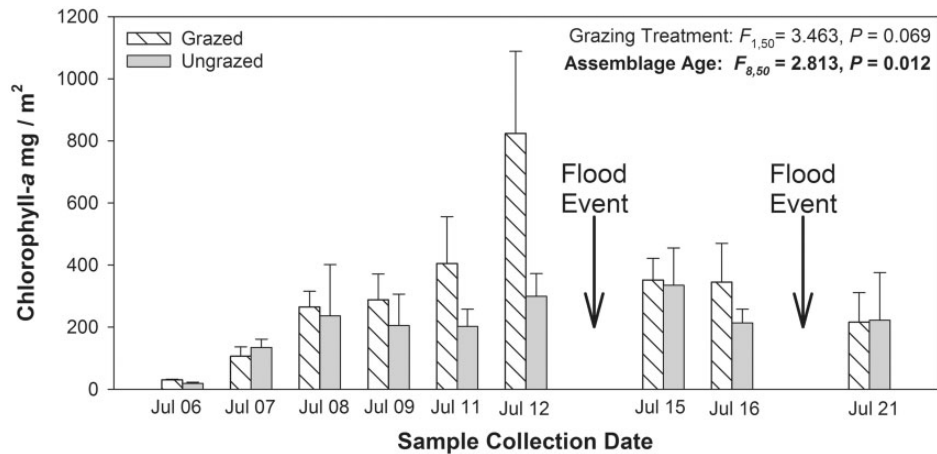


Figure 2. Mean concentrations chlorophyll-*a* over time and by grazing treatment. Error bars are ± 1 SE. Arrows between sets of bars indicate flood events (time scale is non-linear). Each mean is composed of four cobbles that were destructively sampled from each treatment per day. Statistics in bold are significant.

Table 2. Daily averages for algal C, N, and C:N measured in each treatment group, with SE

	Day	6 July		7 July		8 July		9 July	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
C $mmol/m^2$	Grazed	138.1	62.6	305.1	105.7	147.8	11.3	310.2	41.8
	Ungrazed	352.2	120.2	317.9	62.3	342.2	52.8	340.2	74.8
N $mmol/m^2$	Grazed	7.5	3.1	21.7	7.4	18.3	1.5	42.5	5.1
	Ungrazed	11.7	3.9	17.7	3.6	33.7	7.6	49.7	9.8
C:N	Grazed	16.0	5.6	14.4	2.3	8.2	1.0	7.3	0.2
	Ungrazed	31.7	7.0	18.0	0.3	11.7	3.0	6.8	0.3

	Day	11 July		12 July		15 July		16 July		21 July	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C $mmol/m^2$	Grazed	482.0	66.8	314.5	46.4	326.6	67.3	251.9	42.5	210.6	54.6
	Ungrazed	397.8	64.6	415.8	69.5	302.0	87.3	279.8	69.7	244.6	21.7
N $mmol/m^2$	Grazed	67.8	8.5	49.0	5.7	46.9	8.9	40.1	6.9	33.3	10.6
	Ungrazed	56.8	8.2	65.5	10.0	43.8	11.5	44.6	10.1	34.9	1.6
C:N	Grazed	7.1	0.2	6.4	0.2	6.9	0.2	6.4	0.6	29.1	22.9
	Ungrazed	7.0	0.2	6.4	0.4	6.7	0.3	6.3	0.5	7.1	0.9

Four cobbles were analyzed in each treatment per day.

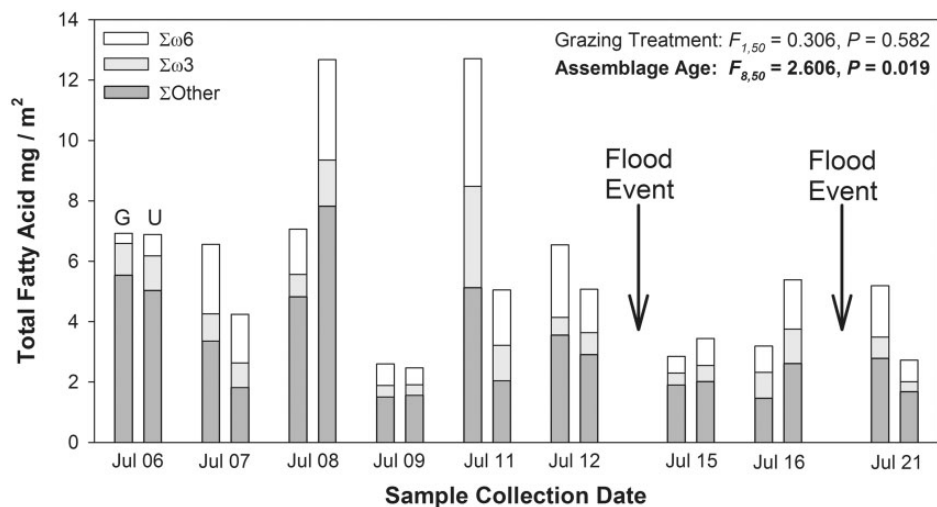


Figure 3. Mean amount of total algal EFA compounds with $>18C$, classified by structural category: $\Sigma\omega 3$, $\Sigma\omega 6$, and Σ Other compounds. Bars are paired by grazing treatment: G, Grazed; U, Ungrazed. Arrows between sets of bars indicate flood events. Time scale is not linear. Each mean is composed of four cobbles that were destructively sampled from each treatment per day. Statistics in bold are significant.

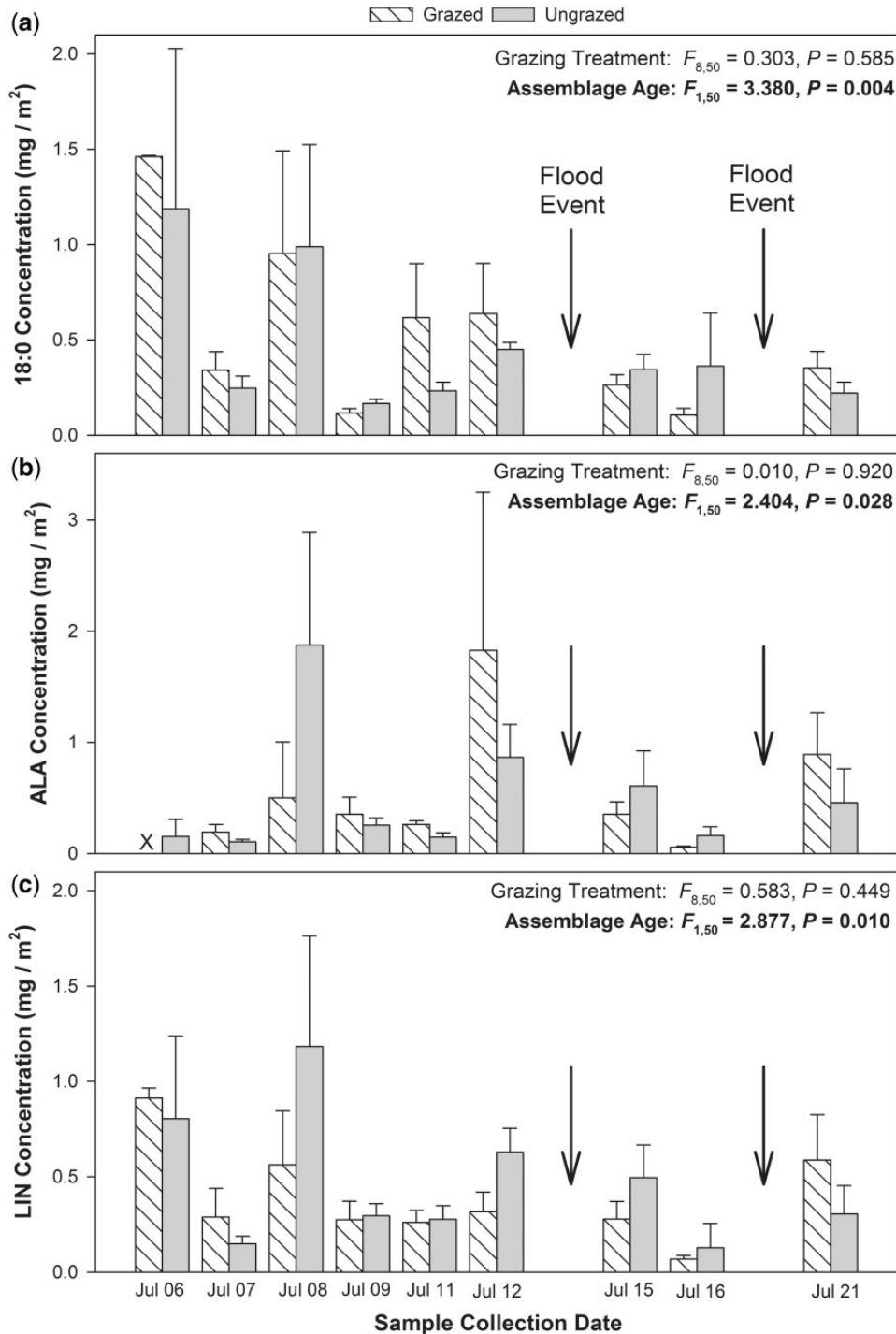


Figure 4. Mean amount of key EFA compounds (a) stearic acid (18:0), (b) ALA (18:3 ω 3), and (c) LIN (18:2 ω 6). Bars are paired by grazed and ungrazed cobbles. Error bars are ± 1 SE. Arrows between sets of bars indicate flood events. Time scale is not linear. Each mean is composed of four cobbles that were destructively sampled from each treatment per day. Statistics in bold are significant.

significantly affected by the presence or absence of invertebrate grazers ($P > 0.05$).

Due to very low algal biomass in the first few days of growth on bare substrata, individual EFAs were detectable only after a period of continued colonization (Fig. 4). The base molecule stearic acid (18:0) was detected in all assemblages over time, but varied in concentration over the course of the study, with peak concentrations in the youngest assemblages and having

variable, lower concentrations in progressively older assemblages ($F_{8,50} = 3.380, P = 0.004$). ALA was present in low concentrations, however LIN was present in greater concentrations in biofilms collected at the earliest stages of colonization (6 July), but were present in larger quantities in remaining days. Concentrations of highly unsaturated fatty acids derived from those compounds (ARA derived from LIN, and EPA and DHA from ALA) had inconsistent temporal patterns (Fig. 5). The first

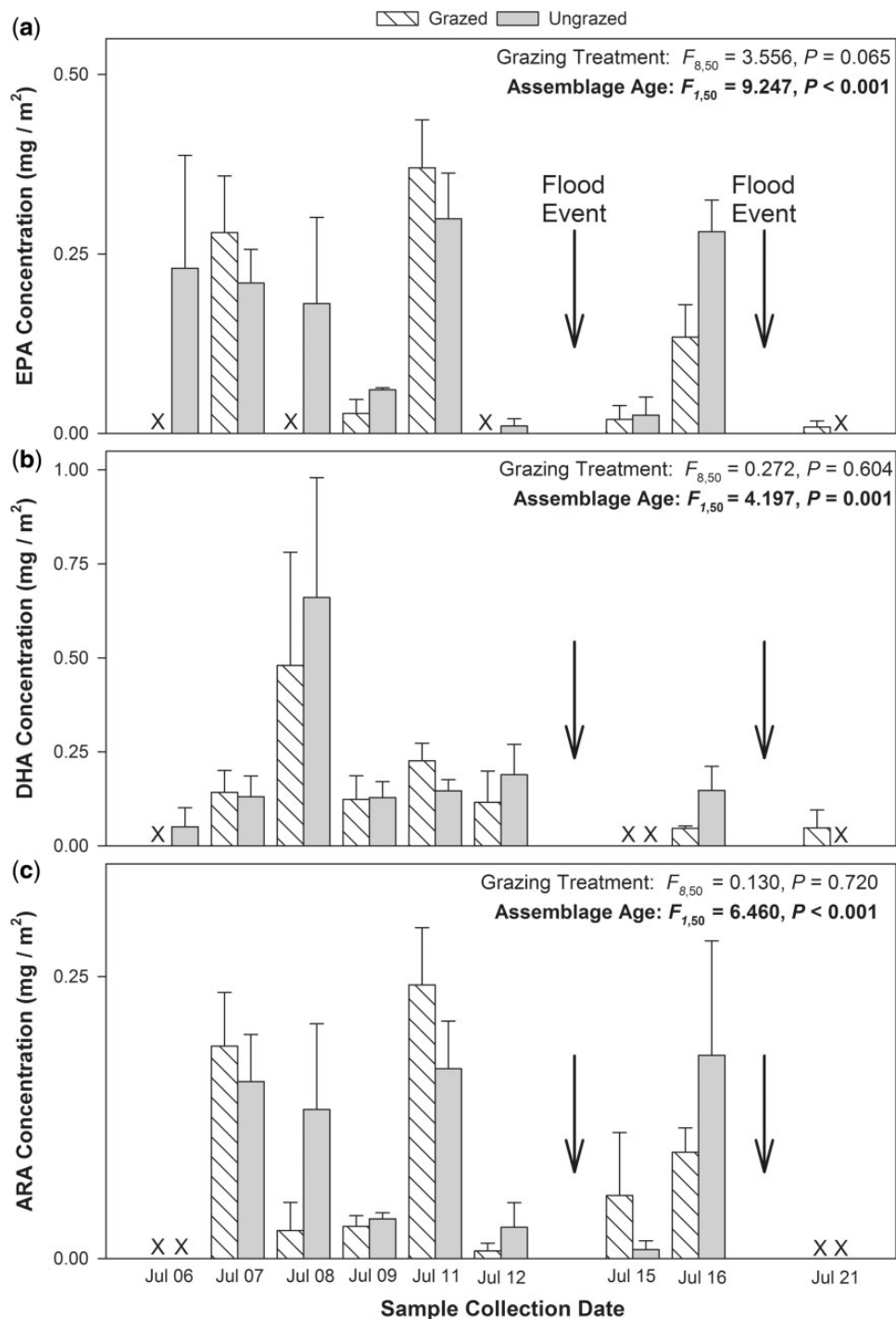


Figure 5. Mean amount of key EFA compounds (a) (EPA 20:5 ω 3), (b) (DHA 22:6 ω 3), and (c) (ARA 20:4 ω 6). Bars are paired by grazed and ungrazed cobbles. Error bars are ± 1 SE. Arrows between sets of bars indicate flood events. Time scale is not linear. Each mean is composed of four cobbles that were destructively sampled from each treatment per day. Statistics in bold are significant.

and second flood events resulted in significantly altered concentrations of several FA compounds, but with contrasting patterns. During the first flood event, average concentrations of 18:0 (-0.24 mg/m^2), ALA (-0.87 mg/m^2), LIN (-0.09 mg/m^2), and DHA (-0.15 mg/m^2) declined, although only DHA was found to be significantly less (t -test, $P = 0.005$). EPA ($+0.02 \text{ mg/m}^2$) and ARA ($+0.01 \text{ mg/m}^2$) increased in concentrations during this time

period, though the difference was not significant. During the second flood, concentrations of nearly all EFA molecules decreased significantly (all $P < 0.025$) with the exception of 18:0 ($P > 0.327$) (Figs 4 and 5). Overall, the general trend exhibited by these specific molecules was that simple compounds, such as 18:0, ALA, and LIN, were consistently detectable despite flood disturbance, while more complex, essential compounds, like

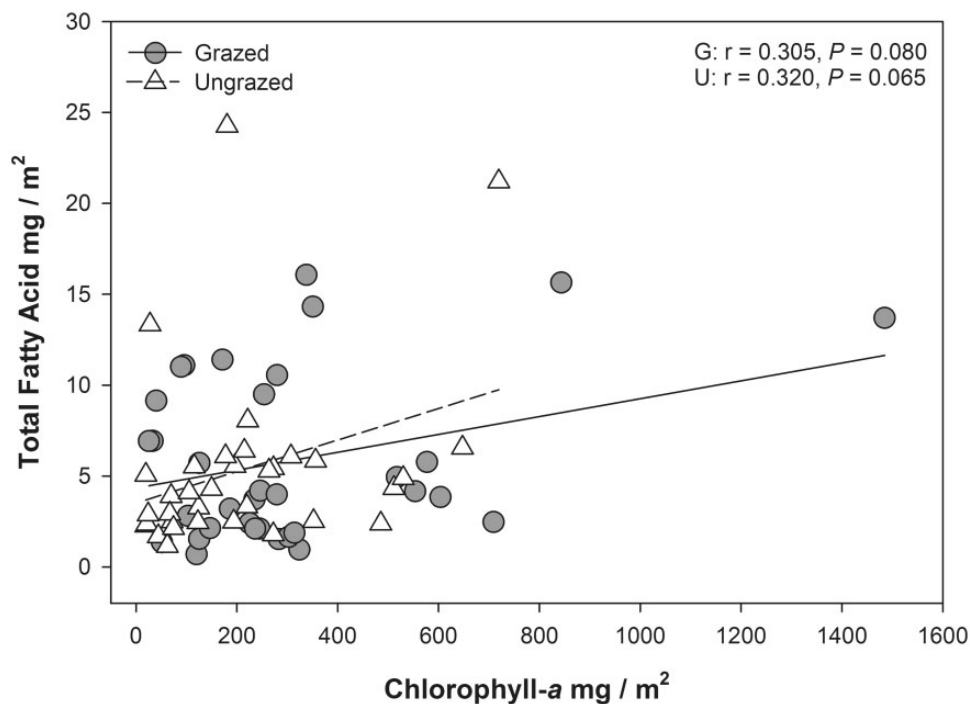


Figure 6. Pearson correlations between chlorophyll-*a* and fatty acid content of benthic algae collected from experimental cobbles in the Bronx River. Data outliers are included in this data presentation, though they were removed for statistical analysis. Trend lines are shown (solid, Grazed; dashed, Ungrazed). Statistics in bold are significant.

ARA, EPA, and DHA, decreased in concentration following severe flood events.

3.4 Relationships between Biomass, nutrient stoichiometry, and EFAs

There were weak, non-significant correlations between benthic algal biomass (as chlorophyll-*a*) and EFA concentration of the biofilm (Fig. 6). Both grazed ($r=0.305$, $P=0.08$) and ungrazed ($r=0.32$, $P=0.065$) assemblages lacked a clear trend. Therefore, a two-way MANOVA was used to determine if any underlying relationships existed between algal biomass and EFAs as a grouped variable (where the maximum variable distances of both biomass and EFA are re-calculated as a single new variable, Mertler and Vannatta 2013) and effects from grazing and colonization time (both separately and combined). The combination of colonization time and grazing treatment had no effect on the combined result of chlorophyll-*a* plus EFA (two-way MANOVA Wilks' Lambda, $\lambda=0.191$). However, MANOVAs examining the singular effect of colonization time ($\lambda < 0.001$) or grazing treatment ($\lambda < 0.001$) did indicate a significant effect on combined chlorophyll-*a* and EFA response. During the first post-flood growth period (6–12 July), there was similarly no significant combined effect of colonization time + grazing treatment ($\lambda=0.310$), but the analysis found separate significant effects of grazing treatment ($\lambda < 0.001$) and colonization time ($\lambda=0.008$) on the combination of the chlorophyll-*a* plus EFA. Following the second flood event, (15–16 July), time and grazing treatment had a significant effect on the combined chlorophyll-*a* and EFA response ($\lambda < 0.001$), while separately, neither time ($\lambda=0.179$) nor grazing treatment ($\lambda=0.094$) had a significant individual effect.

Relationships observed between biofilm nutrient stoichiometry and algal biomass were complex. Carbon content (mmol) and chlorophyll-*a* (mg) concentrations per m^2 of substratum co-

varied positively but correlated significantly only on cobbles exposed to grazers (Fig. 7a). In contrast, N concentrations in biofilms correlated positively and significantly with chlorophyll-*a* in both the grazed and ungrazed treatments (Fig. 6b). Across grazing treatments, total EFAs positively correlated only with concentrations of C, but the relationship with N was non-significant (Fig. 7d and e). The C:N ratio in periphyton was weakly correlated with chlorophyll-*a* in the ungrazed treatments, but not with fatty acid content (Fig. 7c and f).

4. Discussion

4.1 Emerging role of biochemical properties in urban streams

Several studies confirm that the EFA, nutrient stoichiometry (lower C:N), and protein content of algae make them a superior food source to terrestrial detritus for aquatic consumers (Lamberti 1996; Frost et al. 2002; Torres-Ruiz et al. 2007; Brett et al. 2009). In urban streams subject to frequent flooding, reduced riparian buffers, and altered terrestrial inputs, algal sources, especially when present in limited amounts, may be a critical food resource for aquatic consumers. Compounds specifically critical for consumers are the $\omega 3$ fatty acids commonly or solely found in algal cells, including, α -linoleic acid (ALA 18:3 $\omega 3$), (EPA 20:5 $\omega 3$), and (DHA 22:6 $\omega 3$), and the $\omega 6$ fatty acids, linoleic acid (LIN 18:2 $\omega 6$), and (ARA 20:4 $\omega 6$) The present study demonstrated that the availability of these essential compounds can be reduced by frequent and severe floods, which are typical of urban streams.

This examination of early colonizing and flood-reduced assemblages indicates several significant effects of flood events on both algal biomass and biochemistry of benthic biofilms.

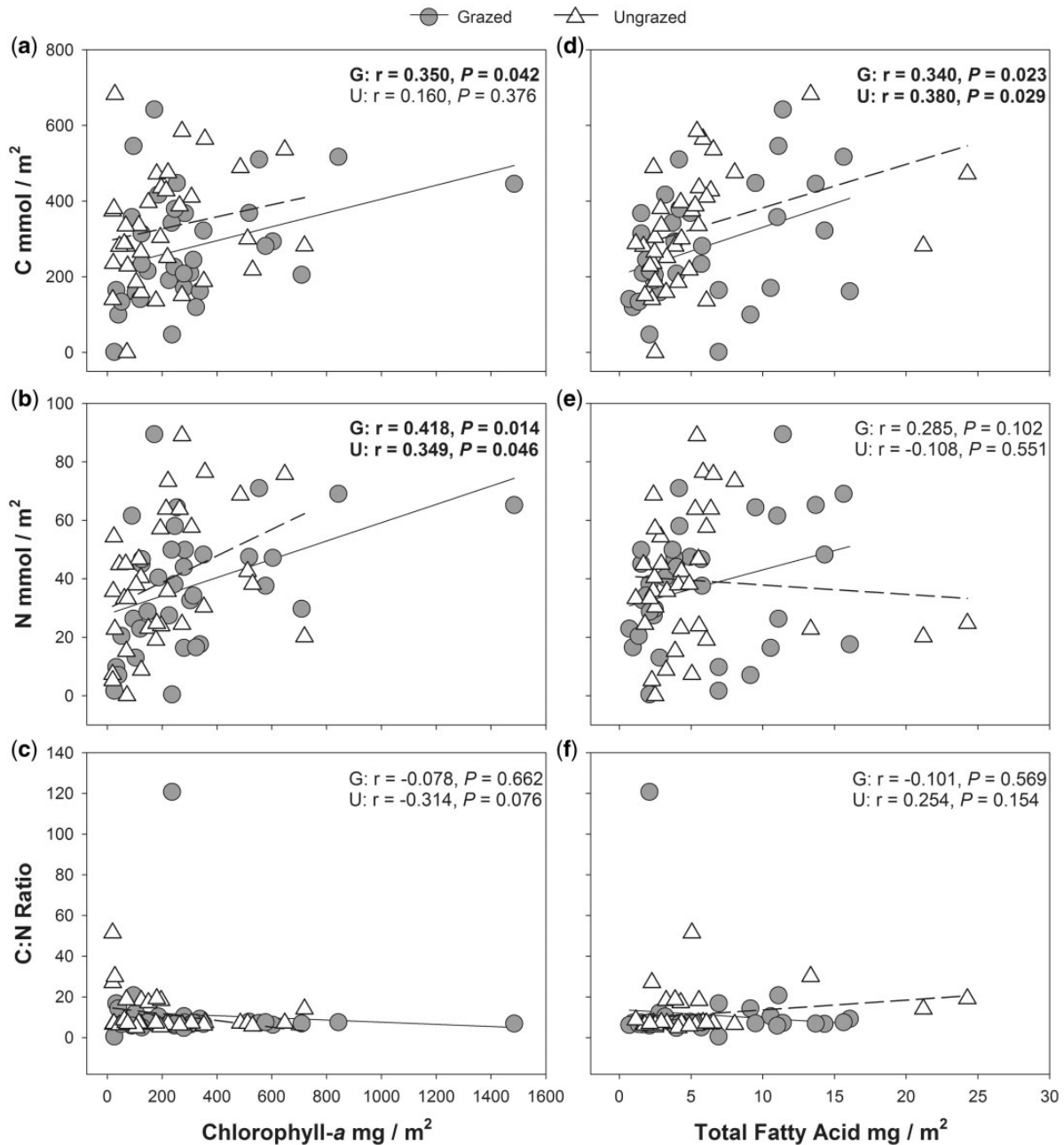


Figure 7. Pearson correlations between algae nutrient stoichiometry (C, N, C:N) and chlorophyll-a (a–c), and fatty acid concentration (d–f). Data outliers are included in this data presentation, though they were removed for statistical analysis. G, Grazed; U, Ungrazed. Trend lines are shown (solid, Grazed; dashed, Ungrazed). Statistics in bold are significant.

More complex EFAs that are primarily produced by algal species tended to require longer time periods to be produced than the time periods between the observed flood events. We also documented a few correlations between algal biomass and complex biochemical compounds, such as EFAs, although benthic algal nutritional quality never strongly tracked variation in algal biomass. The highly stochastic and flood-prone nature of urbanized streams likely affected this relationship. Our data suggest that although in the days immediately after an intense flood, consumers may depend on lower quality detrital and/or microbial food sources, several algal-based EFAs are detectable in the assemblages within a week of a severe flood event. These patterns also suggest that algal biomass alone may not be a

sufficient indicator of an adequate food supply for consumers. Scouring is known to greatly reduce benthic algal biomass in many streams (Peterson and Stevenson 1990; Stevenson 1990; Francoeur et al. 1998; Francoeur and Biggs 2006). It may be that in an urban system characterized by frequent disturbances, early colonist taxa will persist at low levels over longer time periods, unlike that in some pristine streams that undergo longer-term succession to other larger algal taxa (Power and Stewart 1987; Konrad and Booth 2005).

Benthic algal biomass measurements are routinely used to assess anthropogenic disturbances, including urban flood events (Porter-Goff et al. 2010), but few studies have examined algal nutritional composition in response to such disturbances.

Larson et al. (2013) observed that EFA concentrations in lake phytoplankton were positively correlated with degree of urbanization in the watershed. Extrapolation of this pattern to lotic ecosystems is however problematic. A few stream studies have identified how nutrient loading or reduced canopy may alter EFA composition in stream assemblages, but the link to urbanization is less clear (Cashman et al. 2013). Our study suggests that while flood events may not greatly affect benthic algal biomass, floods nonetheless alter and perhaps adversely affect important biochemical properties of this basal food source. We documented changes in the availability of essential compounds, particularly ARA, EPA, and DHA, following flood events. Additionally, while grazer-exclusion treatments and flood events did not significantly affect algal biomass, we measured greater concentrations of EFAs in grazed versus ungrazed assemblages. Taken as a whole, our data suggest that benthic algal biomass and biochemical properties are not closely coupled in streams. Despite this uncoupling, temporal dynamics of algal fatty acid content in response to floods likely has a significant effect on higher trophic levels. Although not considered here, this variation in nutritional composition is likely paralleled by changes in algal assemblage species composition. It has been documented that assemblages with a high proportion of green algae (e.g. streams with more stable flow regimes) can have greater concentrations of ALA and LIN, while assemblages dominated by diatoms (e.g. early stages) have greater concentrations of EPA (Honeyfield and Maloney 2015).

Very few studies have examined the impact of an urban landscape on the EFA composition of biofilms in streams. One recent study observed that altered canopy cover and nutrients affect the biochemical properties of established assemblages (colonized for > 30 d) in one small suburban stream (Cashman et al. 2013). Another demonstrated that increased nutrient inputs can result in greater EFA concentrations (Boëchat et al. 2014). However, neither examined the influence of urban hydrological patterns on nutritional properties of benthic algae. Our data suggest that while biomass periodically can be low, greater fatty acid composition in combination with lower C:N values that are typical of benthic algae can create a high quality food source for stream macroinvertebrates in urban streams.

4.2 Importance of stoichiometry

In this study, biofilms on both grazed and ungrazed cobbles had high amounts of elemental C and N, and low C:N ratios (= high N source), indicating a potentially high-quality food source for invertebrate grazers (Hillebrand and Sommer 1999; O'Brien and Wehr 2010). The C:N values determined in this study (most values between 5 and 40) are consistent with those measured in other urban streams (O'Brien and Wehr 2010; Tsoi et al. 2011; Newcomer et al. 2012). C:N values of 5–10 are suggested to be optimal for algal growth (Hillebrand and Sommer 1999), which is consistent with findings in this study. We also documented that assemblages protected from macroinvertebrate grazing pressure showed a consistent increase in nutrient (nitrogen) content. The lack of a negative effect of grazers on algal biomass is not unusual. Wellnitz and Poff (2006) also observed increases in algal growth under grazed conditions, while Leiss and Hillebrand (2004) have described several mechanisms by which grazing can increase algal accumulation. Furthermore, recent surveys of the macroinvertebrate community along the Bronx River found very low densities of macroinvertebrates, predominantly of pollution tolerant midge-larvae, crustaceans, and worms (Smith et al. 2015). There simply may not be enough

invertebrates in the Bronx River to produce an effect. However, if restoration efforts of urban streams are to be successful, then all levels of food web biota must be considered in evaluation efforts. In this study, it was nutrient content rather than biomass that was most affected.

Several studies have found biochemical differences are likely due to nutrient loading (Ramírez and Pringle 2006; Singer and Battin 2007; Ventura et al. 2008), although the role of nutrient loading and its effect on algal stoichiometry in urban streams has not been adequately studied (Tsoi et al. 2011). In a study of landscape features of urban areas that may influence stream and algal characteristics, O'Brien and Wehr (2010) observed a strong correlation between increasing human population density and greater benthic algal nutrient content and stoichiometry, although that study did not take into account temporal changes due to floods. Previously, a meta-analysis of USGS stream data confirmed that high values of algal chlorophyll were related to increasing levels of urbanization (Dodds et al. 2002). However, increases in aqueous nutrient concentration did not correspond with changes in benthic algal stoichiometry or EFAs. In this study, stoichiometric C:N values for all benthic algal assemblages decreased under the stress of the most severe flood event, despite an increase in aqueous nutrient concentrations.

4.3 Early colonizing benthic algal assemblages support urban stream food webs

Our data indicate that flood events can result in significantly altered EFA profiles (composition and concentration). Variable food sources can have profound effects on consumers. Although these young assemblages are not rich in biomass, they remain an important source of EFAs in the Bronx River. In those studies that have examined various effects on early-colonizing algal assemblages, most assemblages have been incubated for 2–3 weeks before measurements are made (Frost et al. 2007; Kominoski et al. 2007; Rothlisberger et al. 2008). These studies may have missed critical periods when recolonization takes place. By taking daily measurements shortly after initial colonization, we have documented important effects on the biochemical composition of early-colonizing assemblages, which may be periodically common in urban streams and therefore an important food source in urbanized landscapes. Further work is therefore needed, as early colonists may be typical in many urban, flood-prone systems. This study suggests that flood events in urban streams significantly alter the nutritional quality of these early colonizing assemblages, even while changes in biomass may be more resistant to frequent flood events.

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