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Author manuscript

*Freshw Biol.* Author manuscript; available in PMC 2020 January 05.

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Published in final edited form as:

*Freshw Biol.* 2019 January 5; 64(5): 984–996. doi:10.1111/fwb.13279.

## Urbanisation alters fatty acids in stream food webs

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### Abstract

1. Fatty acids are essential to macroinvertebrate growth and reproduction and can indicate food web structure and nutritional quality of basal resources. However, broad-scale examinations of how catchment land cover and associated stressors affect the proportions of fatty acids (FAs) in stream food webs are few.
2. Here, we: (1) examine relationships among proportions of FAs among benthic periphyton and macroinvertebrate collector/gatherers, shredders, and predators; and (2) test if relationships between periphytic and macroinvertebrate FAs were altered due to the intensity of urban development in catchments.
3. Proportions of the 20-C eicosapentaenoic acid (EPA 20:5 $\omega$ 3), arachidonic acid (ARA 20:4 $\omega$ 6), and docosahexaenoic acid (22:6 $\omega$ 3) indicated collector/gatherers had a diet richer in periphyton than in shredders, which had significantly lower proportions of these FAs. Collector/gatherers were in turn likely to be high-quality sources of  $\omega$ 3 and 20-C FAs for predators, which also had significantly greater EPA and ARA proportions than those in shredders. Linoleic (18:2 $\omega$ 6) and  $\alpha$ -linolenic acid (18:3 $\omega$ 3) comprised the greatest proportions of FAs in shredders, which suggested a diet dominated by leaf litter and associated hyphomycetes.
4. As catchment urbanisation increased, proportions of total  $\omega$ 3 FAs and EPA in periphyton were significantly greater. This pattern also was seen through macroinvertebrate consumers and predators, given that proportions of these FAs in macroinvertebrates also were significantly correlated with factors associated with catchment urbanisation. The significant increase in total  $\omega$ 3 FAs and EPA proportions within shredders indicated that periphyton growth, and their FAs, increased on leaf

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Conflict of Interest

The authors have no relationships, financial or otherwise, that could be perceived as influencing their objectivity. The authors have no conflicts of interest to declare.

litter, probably due to greater nutrient concentrations associated with catchment urbanisation. Proportions of total  $\omega 6$  FAs in biota were not significantly correlated with factors associated with urban development, which could indicate that they were of sufficient abundance for consumers regardless of urban intensity or possible changes in their sources.

5. Our study provides an informative first step that identified notable differences in proportions of FAs among macroinvertebrates in urban streams and an increase in proportions of total  $\omega 3$  FAs and EPA in periphyton, consumers, and predators as catchment urbanisation increases. Identifying how FA relationships within food webs change in response to catchment alterations and stressors could inform land use and management decisions by linking environmental changes to measures important to ecosystem outcomes.

## Keywords

algae; macroinvertebrates; land cover; nutrients; watershed

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## 1. Introduction

Identifying how human activities in catchments affect downstream habitats and ecological communities is important to informing the protection and management of stream ecosystems (Booth, Roy, Smith, & Capps, 2016; Parr, Smucker, Bentsen, & Neale, 2016), particularly as human populations and development continue to increase and expand (Seto, Fragkias, Guneralp, & Reilly, 2011). Humans depend on water resources, but ongoing catchment development and population growth negatively affect these ecosystems and associated biota by altering geomorphology, hydrologic regimes, and water quality (Booth et al., 2016; Paul & Meyer, 2001; Walsh et al., 2005). Catchment urbanisation: (1) increases nutrient concentrations in streams due to greater amounts of impervious surface runoff, stream bank erosion, and wastewater from human sources; (2) reduces channel complexity and riparian habitat; and (3) alters base flows in streams, resulting in flashy hydrology during storm events (Bhaskar et al., 2016; Meyer, Paul, & Taulbee, 2005; Smucker & Detenbeck, 2014). These changes in water quality and habitat subsequently affect periphyton and benthic macroinvertebrate communities in urban streams (Smucker, Detenbeck, & Morrison, 2013; Wallace & Biastoch, 2016; Walsh & Webb, 2016).

Benthic periphyton contributes the majority of primary production in streams, making them important in nutrient and organic matter cycling, as well as for the dietary needs of higher trophic levels (Battin, Kaplan, Newbold, & Hansen, 2003; Costello, Rosi-Marshall, Shaw, Grace, & Kelly, 2016; Dodds et al., 2004). Degradation of stream habitat and increased nutrient concentrations decrease periphyton diversity and increase the abundance of disturbance-tolerant species and those that dominate under high nutrient availability (Murdock, Roelke, & Gelwick, 2004; Passy & Blanchet, 2007). Changes in benthic periphyton assemblages can lead to increases in periphyton nitrogen and phosphorus content (O'Brien & Wehr, 2010), which contribute to shifts in macroinvertebrate consumers toward taxa with faster growth rates and lower C:P ratios (Evans-White, Dodds, Huggins, & Baker,

2009). Changes in periphyton assemblages and nutrient concentrations also affect macroinvertebrate consumers due to differences in fatty acid (FA) production by periphyton, especially changes in high quality essential FAs produced by the algal component (Guo, Kainz, Sheldon, & Bunn, 2016a; Hill, Rinchar, & Czesny, 2011; Torres-Ruiz, Wehr, & Perrone, 2010), even in detritus-based food webs (Danger et al., 2013; Guo et al., 2016a). For example, greater agricultural intensities in catchments can increase abundances of motile diatoms while reducing those of prostrate diatoms, chlorophytes, and chrysophytes, leading to significant differences in the diversity of periphyton FAs produced and their overall quantities due to greater amounts of nutrients (Whorley & Wehr, 2016a).

The ecological importance of FAs is receiving increased recognition because of their critical biochemical roles in animals, their use in describing food webs, and their potential effects at the ecosystem-scale (Twining, Brenna, Hairston, & Flecker, 2016). In urban streams, altered light availability and elevated nutrients can change the FA and stoichiometric content of basal resources, such as periphyton, for macroinvertebrate consumers (Cashman, Wehr, & Truhn, 2013; Guo, Kainz, Sheldon, & Bunn, 2016b; Hill et al., 2011). Benthic macroinvertebrates depend heavily on FAs from periphyton because of their inability to synthesise the long chain, polyunsaturated FAs required for growth, neurotransmission, and regulation of physiology, hormone production, and behaviour (Arts, Brett, & Kainz, 2009; Ravet, Brett, & Müller-Navarra, 2003; Stanley-Samuelson, 1994). As a result, the FA content of stream organisms can indicate how anthropogenic stressors affect nutritional quality of periphyton and subsequently the diets of consumers and predators (Boëchat, Krüger, Chaves, Graeber, & Gücker, 2014; Larson et al., 2013; Torres-Ruiz, Wehr, & Perrone, 2007). Characterising relationships between land use and FA content could also provide biologically relevant information regarding how development, management, and restoration in catchments affect streams. However, broad surveys examining these relationships are limited to only a few examples for seston in rivers (Boëchat et al., 2014; Larson et al., 2013).

In this study, we collected benthic periphyton, primary consumer macroinvertebrates, and predatory macroinvertebrates from second- to fourth-order streams spanning highly forested to suburban and highly urban catchments. Our aim was to document the effects of urban development intensity in catchments on the FAs in benthic periphyton and macroinvertebrates and to examine if their FAs more broadly reflect changes to the nutritional qualities of food webs as urban development increases. We hypothesised that: (1) proportions of FAs would differ among macroinvertebrate functional feeding groups due to dissimilarity of diets; and (2) FA profiles would indicate decreased nutritional quality of food sources as catchment urbanisation increases due to associated nutrient enrichment and greater severities of multiple stressors, for which impervious cover and chloride concentrations are proxies.

## 2. Methods

### 2.1 Study streams within the Narragansett Bay catchment

We used a random sampling design to select 74 stream sites along a gradient of catchment development intensity in the Narragansett Bay catchment (Smucker et al., 2016), which is in

the north-eastern U.S.A. (Figure 1). This 4,421 km<sup>2</sup> catchment is one of the most densely populated in the U.S.A. with 442 people/km<sup>2</sup> and approximately 35% developed land cover (Kuhn et al., 2018; NBEP 2017). The catchment is comprised of 39% forested land, 15% saltwater and freshwater wetlands, and 6% agricultural lands (cultivated crops or hay/pasture). This catchment has a coastal location in the mid-latitudes that experiences cold winter and warm summer airmasses from the continental interior and the moderating and moistening influence of the western Atlantic Ocean. The annual average precipitation is approximately 125 cm (Kuhn et al., 2018; NBEP, 2017).

The catchment of each sampled stream was delineated using NHDPlus Basin Delineator Software ([www.horizon-systems.com](http://www.horizon-systems.com)) and checked for accuracy using U.S. Geological Survey 7.5-min quadrangles (1:24,000). Land cover in catchments was generated from photo-interpreted aerial imagery with 0.6-m and 0.5-m resolution for Rhode Island and Massachusetts, respectively ([www.edc.uri.edu/rigis](http://www.edc.uri.edu/rigis); [www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis](http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis)). All land cover characterisations were conducted using ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA, U.S.A.). Catchments of sampled streams ranged from 15.2 to 100% forest, 0 to 39.5% impervious cover, and 3–1,519 people/km<sup>2</sup>.

## 2.2 Sample collection in streams

Streams were sampled between late July and early October 2012 during typical base flow conditions. At each stream, water for chemical analysis was collected in an acid-washed (10% HCl) 1-L polypropylene container. Benthic periphyton, which is comprised of algae, microbes, protozoans, detritus, and extracellular polysaccharides, was collected from each stream by selecting six cobbles, approximately 10–15 cm in diameter, evenly distributed within a 50-m reach. Attached benthic periphyton was removed from all above streambed surfaces of the selected cobbles using a firm-bristled brush (Stevenson & Bahls, 1999). The pooled volume of periphyton material was measured and stored in acid-washed (10% HCl) polypropylene containers. Benthic macroinvertebrates were qualitatively sampled by conducting at least five 2-minute kick-net collections. Additional macroinvertebrates were collected from periphyton scrapings, a visual survey of an additional six cobbles, and from leaves and woody debris when observed. Stream water, periphyton, and macroinvertebrate samples were stored on ice in the dark until being processed within 24 hr after collection.

## 2.3 Laboratory analyses

Stream water was filtered through a 0.2- $\mu$ m syringe filter and analysed for soluble-reactive phosphorus, nitrate/nitrite ( $\text{NO}_3^- / \text{NO}_2^-$ ), ammonium ( $\text{NH}_4^+$ ), dissolved organic carbon, and chloride. Unfiltered samples were persulfate digested for analysis of total nitrogen and total phosphorus. Nutrient concentrations were determined using a Lachat flow-injection analyzer (Lachat Instruments, Milwaukee, WI, U.S.A.). Dissolved organic carbon was determined by UV-promoted, persulfate oxidation on an organic carbon analyser (Tekmar–Dohrmann Model Phoenix 8000, Teledyne Tekmar, Mason, OH, U.S.A.). Chloride was measured using ion chromatography (Dionex DX 600, Dionex Corporation, Sunnyvale, CA, U.S.A.; APHA 1998; US EPA 1987).

Macroinvertebrates were identified to family taxonomic level and categorised into functional feeding groups (FFG): collector/gatherers, shredders, and predators (Table 1; Cummins & Klug, 1979). In general, scrapers were rarely observed and, when present at sites, their low abundance rendered them unavailable for FA analysis after being used for other aspects of this research. Macroinvertebrates were blotted dry and periphyton samples were filtered onto ashed GF/F filters (GE/Whatman, Buckinghamshire, UK). All macroinvertebrates and periphyton were stored at  $-20^{\circ}\text{C}$  in chloroform-washed borosilicate test tubes after being flushed with  $\text{N}_2$  gas until FA analysis.

To extract FAs, periphyton and macroinvertebrate samples were homogenised using a tissue tearor and extracted in chloroform–methanol (2:1). Extracted FAs were methylated using BF<sub>3</sub>, and transferred to a hexane solvent (after Parrish, 1999; Torres-Ruiz et al., 2007; Whorley & Wehr, 2018). Nonadecanoic acid (19:0) was used as an internal standard to test methylation efficiency and to assess consistency among sample runs along with blank hexane samples. Samples were analysed and quantified using a Shimadzu GC-2014 fitted with a capillary column (Omegawax320, 30 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$  film thickness; Supelco®, Bellefonte, PA, USA). The temperature programme has an initial injection into a splitless inlet at a temperature of  $100^{\circ}\text{C}$ , followed by 1-hr ramping to  $260^{\circ}\text{C}$  in increments of  $10^{\circ}\text{C}/10$  min with helium as the carrier gas to an FID detector. A standard dilution series made from a Supelco® 37 component FAME mix was used for standard curves and analysis of the FA compounds. Quantification focused on compounds with 18C, because many biologically important FAs are derived from 18C base molecules, and while algal and non-algal sources of 18C FAs exist, algae are primarily responsible for elongating and desaturating lipids beyond 18C (Olsen, 1999; Stanley-Samuelson, 1994; Torres-Ruiz et al., 2010). Detection limits of the GC were set to identify peaks that correspond to an average concentration minimum of 0.011–0.024 mg/m<sup>2</sup> (for benthic periphyton samples) depending on the size of the molecule, although well-cleaned samples can yield lower detectable values (Supporting Information Table S1).

## 2.4 Statistical Analysis

We examined the content of 18 FAs in periphyton and macroinvertebrates with a focus on proportions of total 18C FAs of  $\Sigma\omega 3$ ,  $\Sigma\omega 6$ ,  $\Sigma\text{other}$ , and the most important and commonly reported essential FAs:  $\alpha$ -linolenic acid (ALA 18:3 $\omega 3$ ), eicosapentaenoic acid (EPA 20:5 $\omega 3$ ), docosahexaenoic acid (DHA 22:6 $\omega 3$ ), linoleic acid (LIN 18:2 $\omega 6$ ), and arachidonic acid (ARA 20:4 $\omega 6$ ). These five FAs have high biological importance and are essential because macroinvertebrates are probably unable to desaturate and elongate from shorter-chained FAs, except in rare instances and with limited ability (Arts et al., 2009; Desvillettes & Bec, 2009). We quantified data as proportions of FAs because they represent changes of individual FAs relative to the others, thus indicating important nutritional changes in diets.

Non-parametric Kruskal–Wallis ANOVAs were used to determine if proportions of FA content were significantly different among periphyton and FFGs regardless of catchment % impervious cover; Dunn's tests were used to examine significant differences of medians. We used site means of each FFG for examining FA relationships with environmental variables. Ternary plots were used to compare distributions of periphyton and macroinvertebrates

based on their proportions of  $\Sigma\omega 3$ ,  $\Sigma\omega 6$ , and  $\Sigma$ other FAs. To aid with interpretation of these ternary plots, we used non-parametric Mann–Whitney U tests to examine if proportions of these three major groups of FAs in periphyton and FFGs differed significantly between the least disturbed sites with <5% catchment impervious cover and more urban sites with > 5% catchment impervious cover (Sokal & Rohlf, 1995). This criterion is frequently reported as a level beyond which substantial changes in biota occur (King, Baker, Kazyak, & Weller, 2011; Smucker et al., 2013; Utz, Hilderbrand, & Boward, 2009). Non-parametric Spearman's Rank correlations were used to examine relationships of periphyton and macroinvertebrate FA content with stream nitrate and chloride concentrations, catchment population density (people km<sup>-2</sup>), and percent impervious cover. The a priori  $\alpha$  level for all tests was set at  $\alpha = 0.05$ . All statistical analyses were conducted using SYSTAT 13 and SigmaPlot 13 (Systat Software, Inc., Chicago, IL, U.S.A.).

### 3 Results

#### 3.1 Summary of FAs in stream periphyton and macroinvertebrates

Of the 74 sites sampled, both periphyton and macroinvertebrates were able to be collected from 52 sites, with 15 sites having collector/gatherers, 25 with shredders, and 38 with predators (Table 1). Darner dragonfly larvae (Aeshnidae) and dobsonfly larvae (Corydalidae) were the most common predators, larval crane flies (Tipulidae) and case-building caddisflies (Limnephilidae) were the most common shredders, and the most common collector/gatherers were net-spinning caddisflies (Hydropsychidae) and small crayfish (Cambaridae).  $\Sigma$ other FAs, typically dominated by stearic acid (18:0) and oleic acid (18:1 $\omega$ 9), comprised the greatest proportions of FAs in periphyton, shredders, and predators (Table 2), with  $\Sigma\omega 6$  being intermediate and  $\Sigma\omega 3$  being least abundant; collectors had approximately equal proportions of these three major groups of FAs (Figure 2).

Major structural groups of FA compounds were significantly different among the organisms analysed (Figure 2). Proportions of  $\Sigma\omega 3$  FAs in periphyton (mean  $\pm$  SE,  $0.13 \pm 0.01$ ) were significantly lower than those in macroinvertebrate FFGs ( $H_3 = 60.747$ ,  $p < 0.001$ ), which were higher and similar to each other (shredders  $0.26 \pm 0.03$ ; predators  $0.27 \pm 0.02$ ; collector/gatherers  $0.31 \pm 0.03$ ). The proportions of  $\Sigma\omega 6$  FAs among periphyton and FFGs were not significantly different ( $H_3 = 5.055$ ,  $p = 0.168$ ; periphyton  $0.30 \pm 0.01$ ; collector/gatherers  $0.35 \pm 0.03$ ; shredders  $0.36 \pm 0.04$ ; predators  $0.33 \pm 0.02$ ). Proportions of  $\Sigma$ other FAs in periphyton ( $0.58 \pm 0.01$ ) were significantly greater than those in macroinvertebrate FFGs ( $H_3 = 68.637$ ,  $p < 0.001$ ), which were similar to each other (collector/gatherers  $0.35 \pm 0.03$ ; shredders  $0.38 \pm 0.04$ ; predators  $0.40 \pm 0.02$ ). Macroinvertebrate FFGs had similar variation in the three major groups of FAs (Figure 3a).

Quantities of three key essential  $\omega 3$  FA compounds, ALA, EPA, and DHA were significantly different among periphyton and macroinvertebrate FFGs ( $H_3 > 21.0$ ,  $p < 0.001$ ; Table 2, Figure 4a-c). Proportions of ALA were the greatest and most variable in shredders, intermediate in predators, and least in collector/gatherers and periphyton. Collector/gatherers had the greatest proportions of EPA and DHA (Table 2). The important essential  $\omega 6$  FA compounds linoleic acid (LIN;  $H_3 = 23.966$ ,  $p < 0.001$ ) and arachidonic acid (ARA;  $H_3 = 42.367$ ,  $p < 0.001$ ) were significantly different among periphyton and macroinvertebrate

FFGs (Figure 4d-e). Proportions of LIN were greatest in shredders and predators, intermediate in collector/gatherers, and least in periphyton. Collector/gatherers and predators had the greatest proportions of ARA.

### 3.2 Fatty acid relationships with urban stressors

Land cover characteristics and stream water chemistry ranged greatly among streams (Table 3). The proportions of  $\Sigma\omega 3$  FAs in all macroinvertebrates, regardless of feeding group, were greater at urban sites with >5% catchment impervious cover than in those at least disturbed sites with < 5% impervious cover (Figure 3b; Mann–Whitney U test,  $p < 0.05$ ), as were the proportions of  $\Sigma\omega 3$  of collector/gatherers and predators (Mann–Whitney U test,  $p < 0.05$ ; shredders  $p = 0.07$ ). The proportions of collector/gatherer EPA and predator EPA were greater in urban sites than in least disturbed sites (Mann–Whitney U test,  $p < 0.05$ ). Proportions of  $\Sigma\omega 6$  and  $\Sigma\text{other}$  in FFGs were not significantly different between least disturbed and urban sites. Proportions of major groups of FAs in periphyton did not significantly differ between urban and least disturbed sites (Figure 3c).

The proportions of  $\Sigma\omega 3$  in macroinvertebrate FFGs increased with greater impervious cover in catchments, chloride, and nitrate concentrations (Figure 5, Table 4). Proportions of  $\Sigma\omega 3$  in shredders and predators were most strongly correlated with increasing impervious cover ( $r_s = 0.42, 0.39$ , respectively;  $p < 0.05$ ), nitrate concentrations ( $r_s = 0.44, 0.32$ , respectively;  $p < 0.05$ ), and chloride concentrations ( $r_s = 0.55, 0.39$ , respectively;  $p < 0.05$ ). Periphyton  $\Sigma\omega 3$  proportions increased with higher population densities in catchments and chloride concentrations ( $r_s = 0.24, 0.26$ , respectively;  $p < 0.05$ ). Variables associated with urbanisation were not correlated with proportions of  $\Sigma\omega 6$  in periphyton and macroinvertebrate FFGs. The proportions of  $\Sigma\text{other}$  FAs in periphyton, shredders, and predators decreased with greater catchment percent impervious cover ( $r_s = -0.29, -0.41$ , and  $-0.32$ , respectively;  $p < 0.05$ ). Increases in population densities were correlated with decreases in the proportions of  $\Sigma\text{other}$  FAs in periphyton and collectors ( $r_s = -0.37, -0.60$ , respectively;  $p < 0.05$ ).

The proportion of EPA in periphyton increased with greater catchment percent impervious cover, concentrations of chloride, and population density ( $r_s = 0.37, 0.39, 0.35$ , respectively;  $p < 0.01$ ). Collectors had few significant relationships, with only ALA increasing along with catchment percent impervious cover ( $r_s = 0.60$ ,  $p < 0.05$ ). Shredder EPA proportions were positively associated with concentrations of nitrate, chloride, and impervious cover ( $r_s > 0.45, 0.48, 0.37$ , respectively;  $p < 0.05$ ), whereas their proportions of DHA were negatively associated with catchment impervious cover and population density ( $r_s = -0.43, -0.40$ , respectively;  $p < 0.05$ ). The proportion of EPA in predators increased with greater catchment percent impervious cover ( $r_s = 0.51$ ,  $p < 0.01$ ), chloride ( $r_s = 0.49$ ,  $p < 0.01$ ), and population density ( $r_s = 0.37$ ,  $p < 0.05$ ).

## 4 Discussion

### 4.1 Comparisons of FAs among periphyton and macroinvertebrates

Quantifying the proportions of FAs among periphyton and FFGs can provide information on the structure and nutritional qualities of food webs, but studies including an examination of predators and how environmental changes affect FAs of multiple consumers in natural ecosystems are uncommon, particularly at large scales (Guo et al., 2016a; Twining et al., 2016). In our study of a large catchment, macroinvertebrates had greater proportions of  $\Sigma\omega 3$  FAs and lower proportions of  $\Sigma$ other FAs than those in periphyton, whereas their proportions of  $\Sigma\omega 6$  were similar. These patterns probably resulted from macroinvertebrates having the ability to selectively consume and retain periphyton-derived FAs even if food sources have low amounts of them, especially ALA, EPA, and ARA (Brett et al., 2017; Crenier et al., 2017; Guo, Bunn, Brett, & Kainz, 2017). These compounds also indicate higher food quality than shorter-chained or saturated FAs (Guo et al., 2016a; Torres-Ruiz et al., 2007). Although proportions of  $\Sigma\omega 3$ ,  $\Sigma\omega 6$ , and  $\Sigma$ other FA groups did not significantly differ among shredders, collector/gatherers, and predators, their proportions of the five essential FAs did. These differences probably resulted from dissimilar diets among feeding groups, given that FA content and proportions differ among basal resources in streams, and most macroinvertebrates are unlikely, or are at least greatly limited in their ability, to alter their content of these FAs via desaturation and elongation (Guo et al., 2016a, 2017; Torres-Ruiz et al., 2007, 2010).

Of the five essential FAs, LIN had, on average, either the greatest or second greatest proportion in periphyton and each macroinvertebrate feeding group. EPA was measured in the greatest proportion in collector/gatherers, ALA was the second greatest proportion in shredders, and EPA was the second greatest proportion in predators. Collector/gatherers probably consume a variety of basal resources, but their proportions of longer-chained FAs, especially EPA, ARA, and DHA, were significantly greater than those in shredders, which suggests that they benefited from a more periphyton-rich diet (Taipale et al., 2013; Whorley & Wehr, 2018). Our results also indicate that diatoms probably comprised a substantial portion of their diets, given that diatoms are the most common algal group in streams that produces EPA, DHA, and ARA, whereas chlorophytes and cyanobacteria produce greater amounts of shorter-chained ALA and LIN (Galloway & Winder, 2015; Harwood & Guschina, 2009; Richoux, Bergamino, Moyo, & Dalu, 2018; Taipale et al., 2013). Collector/gatherers (excluding Cambaridae) were probably high-quality sources of EPA and ARA for predators. However, shredders may have been the dominant prey in predator diets, given that proportions of EPA and ARA in predators were greater than those in shredders and less than those in collector/gatherers. These essential FAs have been reported to increase in secondary consumers (Guo et al., 2016a; Persson & Vrede, 2006), and proportions in predators would be expected to be greater than those in collector/gatherers, if these were their dominant prey.

A study of stable isotope ratios of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in this same system provided results complimentary to the FA data in the present study, indicating a dominance of detrital pathways in these stream food webs across the urban gradient (Smucker, Kuhn, Cruz-Quinones, Serbst, & Lake, 2018). Although not quantified, most sites observationally had an



abundance of riparian trees and in-stream leaf litter. Many terrestrial plants have undetectable amounts of highly unsaturated FAs and are prolific producers of ALA and LIN (Mills, McArthur, Wolfe, Aho, & Rader, 2001; Simopoulos, 1999), which had the greatest proportions in shredders. The high proportions of LIN, and even stearic acid (18:0) and oleic acid (18:1 $\omega$ 9), in periphyton and all feeding groups also indicated that detrital food sources were probably quite abundant, even as a component of periphyton, which can be comprised of >30% allochthonous material (Rasmussen, 2010). In addition, fungi, especially hyphomycetes, can enhance the nutritional quality of detritus and were probably important contributors to the high proportions of LIN, ALA, oleic acid, and stearic acid (18-C FAs) in shredders and even in other FFGs (Arce-Funck, Bec, Perrière, Felten, & Danger, 2015; Taube, Ganzert, Grossart, Gleixner, & Premke, 2018; Vonk, van Kuijk, van Beusekom, Hunting, & Kraak, 2016). Quantification of major periphyton groups could provide additional insights in the future (e.g., Whorley & Wehr, 2016a), given that increased abundances of chlorophyte and cyanobacteria taxa associated with greater amounts of nutrients also could contribute to greater  $\Sigma\omega$ 6 FAs, LIN, and the  $\omega$ 3 ALA (Galloway & Winder, 2015; Guo et al., 2016a; Hill et al., 2011). However, macroscopic filaments or mats of these types of algae appeared to be uncommon in periphyton at our sites.

#### 4.2 Responses of FAs to urban stressors

Proportions of  $\Sigma\omega$ 6 FAs, LIN, and the  $\omega$ 3 ALA were not significantly correlated with factors affected by urbanisation, which could indicate that they were of sufficient abundance for consumers regardless of urban intensity or possible changes in their sources. Even given the significant differences in proportions of essential FAs among FFGs, proportions of  $\Sigma\omega$ 3 FAs and EPA in periphyton, shredders, and predators increased with factors associated with greater amounts of urbanisation, particularly catchment impervious cover, nitrate, chloride, and human population density (Booth et al., 2016; Walsh et al., 2005). Similar increases in  $\omega$ 3 FAs of sestonic algae in large rivers affected by urban development have been observed (Boëchat et al., 2014; Larson et al., 2013), but our study is the first report for benthic periphyton, along with macroinvertebrate consumers and predators, from a large survey of low-order streams affected by a gradient of catchment development.

Although not correlated with nutrients in our study, increases in proportions of  $\Sigma\omega$ 3 FAs and EPA in periphyton could have been associated with increased nutrients in more urban catchments, given their well-documented mechanistic links to increased FA production (Dalu, Galloway, Richoux, & Froneman, 2016; Guo et al., 2016b; Whorley & Wehr, 2018), although high levels of nutrients also can lead to reduced proportions of 20-C FAs in algae (Cashman et al., 2013). Within agricultural systems, biofilms contained greater concentrations of important FA compounds as nutrient availability increased, despite exhibiting decreased taxonomic diversity and more eutrophic indicative taxa (Whorley & Wehr, 2016a, 2018). Stable isotope ratios of  $\delta^{15}\text{N}$  in periphyton and macroinvertebrates from our study sites indicated that human-related sources of nutrients were increasingly assimilated into biota as catchment urbanisation became greater (Smucker et al., 2018). The strong correlations of  $\Sigma\omega$ 3 FAs and EPA proportions with chloride concentrations could indicate nutrient effects as well, because chloride is a conservative tracer of water delivered to streams from sources affected by human activities, and is less affected than nutrients by

biological uptake and transformation. Canopy cover by riparian trees, which was qualitatively abundant at most sites, may have further promoted higher proportions of periphyton EPA and  $\omega$ 3 FAs by reducing oxidative damage to their carbon double bonds and by reducing the amount of surplus carbon stored as saturated, monounsaturated, and shorter 18-C FAs under high light conditions (Cashman et al., 2013; Hill et al., 2011; Twining et al., 2016).

In our study catchment, as urbanisation increased, greater proportions of  $\Sigma\omega$ 3 FAs and EPA in basal resources appeared to propagate through macroinvertebrate consumers and predators, as evidenced by their correlations with factors affected by catchment urbanisation. Collector/gatherers were an exception to this pattern, although their marginally non-significant correlations were probably due in part to their smaller sample size. The significant increase in  $\Sigma\omega$ 3 FAs and EPA proportions in shredders indicated that periphyton growth, and their FAs, increased on leaf litter and/or transported matter, as catchment urbanisation increased (Guo, Kainz, Valdez, Sheldon, & Bunn, 2016). An increase in proportions of  $\Sigma\omega$ 3 FAs and EPA of periphyton and macroinvertebrates may seem like beneficial responses to urbanisation, but these possibly could be outweighed by the negative and well-documented effects of altered habitat, hydrology, and other stressors on the biomass and diversity of periphyton and macroinvertebrate communities (Hoyle, Kilroy, Hicks, & Brown, 2017; Moore & Palmer, 2005; Pearson et al., 2017; Roy, Rosemond, Paul, Leigh, & Wallace, 2003; Smucker & Detenbeck, 2014). Sensitive periphyton and macroinvertebrate taxa that thrive in minimally-impacted systems are replaced by those with faster growth rates or higher P content under greater nutrient availability and by those more tolerant to altered habitat and deteriorated water quality (King et al., 2011; Morse, Wollheim, Benstead, & McDowell, 2012; Stevenson, Hill, Herlihy, Yuan, & Norton, 2008; Tsoi, Hadwen, & Fellows, 2011). Further research could determine if increased FA availability along urban gradients contributes to community turnover in a manner similar to that of increased nutrient availability, especially given the importance of FAs in growth, behaviour, emergence, and reproduction of macroinvertebrates.

### 4.3 Conclusions

Our catchment-scale approach was useful for examining differences in FA profiles within a portion of stream food webs and how they changed in response to catchment urbanisation. However, future efforts would benefit from combining FA profiles with quantitative measures of biomass, densities of individuals, and finer taxonomic resolution of periphyton and macroinvertebrates, along with additional characterisation of basal resources. We collected the most common families of macroinvertebrates in the catchment (Gould, 1993), but some intra-family differences in feeding strategies among species and changes in species' diets during larval growth can exist. Additionally, consumers may exhibit more selective small-scale feeding on biofilm material and leaf litter than previously considered. Taxa-specific FA profiles are particularly sparse for streams, and future assessment of food web changes could benefit from evaluating temporal and developmental variation, as well as among and within species variability in their dietary needs, uptake, and content of FAs, which could provide insights on mechanistic links to macroinvertebrate community structure (Cavaletto & Gardner, 1999; Torres-Ruiz et al., 2007; Whorley & Wehr, 2016b).

Even given the likely sources of variability, our study provides an informative first step that identified: (1) notable differences in proportions of FAs among FFGs in urban streams; and (2) an increase in proportions of  $\Sigma\omega 3$  FAs and EPA in periphyton, consumers, and predators as catchment urbanisation became greater. These findings provide insights into the dietary, biochemical, and nutritional changes of biota and into potential trophic relationships. Given the importance of FAs to macroinvertebrate growth and reproduction, to higher trophic levels, and to terrestrial ecosystems (Twining et al., 2016), identifying how FA relationships within food webs change in response to catchment alterations and stressors could inform land use and management decisions by linking environmental changes to measures important to ecosystem outcomes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

Jonathan Serbst, Carlos Cruz-Quinones, Emily Seelen, and Joe Bishop assisted with sampling and laboratory processing. We thank Colleen Elonen and Terri Jicha for conducting water chemistry analyses and Michael Charpentier for generating land cover data and assisting with stream selection. Ken Fritz and Brent Johnson provided helpful comments on an earlier draft. This manuscript, tracking number ORD-020571, has been reviewed by the Systems Exposure Division and approved for publication. Approval does not signify that contents necessarily reflect the views and policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## References

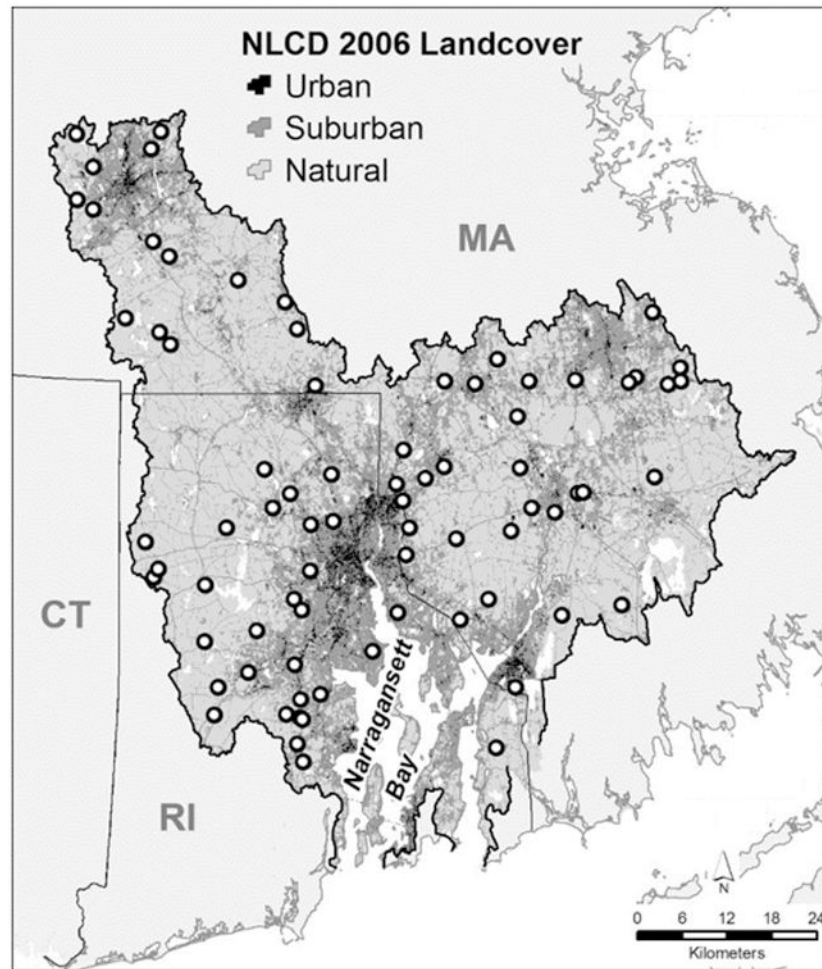
- American Public Health Association. ( 1998). In Clesceri LS, Greenberg LE & Eaton AD (Eds.). Standard methods for the examination of water and wastewater ( 20th ed.). Washington, DC: American Public Health Association.
- Arce-Funck J, Bec A, Perrière F, Felten V, & Danger M ( 2015). Aquatic hyphomycetes: A potential source of polyunsaturated fatty acids in detritus-based stream food webs. *Fungal Ecology*, 13, 205–210. 10.1016/j.funeco.2014.09.004
- Arts MT, Brett MT, & Kainz M (Eds.). ( 2009) *Lipids in aquatic ecosystems* ( 377 pp.). New York, NY: Springer.
- Battin TJ, Kaplan LA, Newbold JD, & Hansen CME ( 2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*, 426, 439–442. 10.1038/nature02152 [PubMed: 14647381]
- Bhaskar AS, Beesley L, Burns MJ, Fletcher TD, Hamel P, Oldham CE, & Roy AH ( 2016). Will it rise or will it fall? Managing the complex effects of urbanization on base flow. *Freshwater Science*, 35, 293–310. 10.1086/685084
- Boëchat IG, Krüger A, Chaves RC, Graeber D, & Gücker B ( 2014). Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. *Science of the Total Environment*, 482, 62–70. 10.1016/j.scitotenv.2014.02.111 [PubMed: 24636887]
- Booth DB, Roy AH, Smith B, & Capps KA ( 2016). Global perspectives on the urban stream syndrome. *Freshwater Science*, 35, 412–420. 10.1086/684940
- Brett MT, Bunn SE, Chandra S, Galloway AWE, Guo F, Kainz MJ, ... Wehr JD ( 2017). How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biology*, 62, 833–853. 10.1111/fwb.12909
- Cashman MJ, Wehr JD, & Truhn K ( 2013). Elevated light and nutrients alter the nutritional quality of stream periphyton. *Freshwater Biology*, 58, 1447–1457. 10.1111/fwb.12142

- Cavaletto JF, & Gardner WS ( 1999). Seasonal dynamics of lipids in freshwater benthic invertebrates In Arts MT, & Wainman BC (Eds.), *Lipids in freshwater ecosystems* (pp. 109–131). New York, NY: Springer 10.1007/978-1-4612-0547-0
- Costello DM, Rosi-Marshall EJ, Shaw LE, Grace MR, & Kelly JJ ( 2016). A novel method to assess effects of chemical stressors on natural biofilm structure and function. *Freshwater Biology*, 61, 2129–2140. 10.1111/fwb.12641
- Crenier C, Arce-Funck J, Bec A, Billoir E, Perrière F, Leflaive J, ... Danger M ( 2017). Minor food sources can play a major role in secondary production in detritus-based ecosystems. *Freshwater Biology*, 62, 1155–1167. 10.1111/fwb.12933
- Cummins KW, & Klug MJ ( 1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, 10, 147–172. 10.1146/annurev.es.10.110179.001051
- Dalu T, Galloway AWE, Richoux NB, & Froneman PW ( 2016). Effects of substrate on essential fatty acids produced by phytobenthos in an austral temperate river system. *Freshwater Science*, 35, 1189–1201. 10.1086/688698
- Danger M, Cornut J, Chauvet E, Chavez P, Elger A, & Lecerf A ( 2013). Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: A case of aquatic priming effect? *Ecology*, 94, 1604–1613. 10.1890/12-0606.1 [PubMed: 23951720]
- Desvillettes C, & Bec A ( 2009). Formation and transfer of fatty acids in aquatic microbial food webs: Role of heterotrophic protists In Arts MT, Brett MT, & Kainz MJ (Eds.), *Lipids in aquatic ecosystems* (pp. 25–42). New York, NY: Springer 10.1007/978-0-387-89366-2
- Dodds WK, Mart E, Tank JL, Pontius J, Hamilton SK, Grimm NB, ... Gregory S ( 2004). Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. *Oecologia*, 140, 458–467. 10.1007/s00442-004-1599-y [PubMed: 15179578]
- Evans-White MA, Dodds WK, Huggins DG, & Baker DS ( 2009). Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society*, 28, 855–868. 10.1899/08-113.1
- Galloway AWE, & Winder M ( 2015). Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *PLoS ONE*, 10, e0130053 10.1371/journal.pone.0130053 [PubMed: 26076015]
- Gould M ( 1993). Establishment and field testing of a rapid bioassessment screening of Rhode Island freshwater benthic macroinvertebrates. Providence, RI: Rhode Island Department of Environmental Management.
- Guo F, Bunn SE, Brett MT, & Kainz MJ ( 2017). Polyunsaturated fatty acids in stream food webs – High dissimilarity among producers and consumers. *Freshwater Biology*, 62, 1325–1334. 10.1111/fwb.12956
- Guo F, Kainz MJ, Sheldon F, & Bunn SE ( 2016a). The importance of high-quality algal food sources in stream food webs—current status and future perspectives. *Freshwater Biology*, 61, 815–831. 10.1111/fwb.12755
- Guo F, Kainz MJ, Sheldon F, & Bunn SE ( 2016b). Effects of light and nutrients on periphyton and the fatty acid composition and somatic growth of invertebrate grazers in subtropical streams. *Oecologia*, 181, 449–462. 10.1007/s00442-016-3573-x [PubMed: 26883960]
- Guo F, Kainz MJ, Valdez D, Sheldon F, & Bunn SE ( 2016). High-quality algae attached to leaf litter boost invertebrate shredder growth. *Freshwater Science*, 35, 1213–1221. 10.1086/688667
- Harwood JL, & Guschina IA ( 2009). The versatility of algae and their lipid metabolism. *Biochimie*, 91, 679–684. 10.1016/j.biochi.2008.11.004 [PubMed: 19063932]
- Hill WR, Rinchar J, & Czesny S ( 2011). Light, nutrients and the fatty acid composition of stream periphyton. *Freshwater Biology*, 56, 1825–1836. 10.1111/j.1365-2427.2011.02622.x
- Hoyle JT, Kilroy C, Hicks DM, & Brown L ( 2017). The influence of sediment mobility and channel geomorphology on periphyton abundance. *Freshwater Biology*, 62, 258–273. 10.1111/fwb.12865
- King RS, Baker ME, Kazyak PF, & Weller DE ( 2011). How novel is too novel? Stream community thresholds at exceptionally low levels of catchment. *Ecological Applications*, 21, 1659–1678. 10.1890/10-1357.1 [PubMed: 21830709]

- Kuhn A, Leibowitz SG, Johnson ZC, Lin J, Massie JA, Hollister JW, ... Compton JE (2018). Performance of national maps of watershed integrity at watershed scales. *Water*, 10, 604-616. 10.3390/w10050604
- Larson JH, Richardson WB, Knights BC, Bartsch LA, Bartsch MR, Nelson JC, ... Vallazza JM (2013). Fatty acid composition at the base of aquatic food webs is influenced by habitat type and watershed land use. *PLoS ONE*, 8, e70666. 10.1371/journal.pone.0070666 [PubMed: 23940619]
- Meyer JL, Paul MJ, & Taulbee WK (2005). Stream ecosystem function in urbanizing landscapes. *Journal of the North American Benthological Society*, 24, 602–612. 10.1899/04-021.1
- Mills GL, McArthur JV, Wolfe C, Aho JM, & Rader RB (2001). Changes in fatty acid and hydrocarbon composition of leaves during decomposition in a southeastern blackwater stream. *Archiv für Hydrobiologie*, 152, 315–328.
- Moore AA, & Palmer MA (2005). Invertebrate biodiversity in agricultural and urban headwater streams: Implications for conservation and management. *Ecological Applications*, 15, 1169–1177. 10.1890/04-1484
- Morse NB, Wollheim WM, Benstead JP, & McDowell WH (2012). Effects of suburbanization on foodweb stoichiometry of detritus-based streams. *Freshwater Science*, 31, 1202–1213. 10.1899/12-004.1
- Murdock J, Roelke D, & Gelwick F (2004). Interactions between flow, periphyton, and nutrients in a heavily impacted urban stream: Implications for stream restoration effectiveness. *Ecological Engineering*, 22, 197–207. 10.1016/j.ecoleng.2004.05.005
- Narragansett Bay Estuary Program (NBEP). (2017). State of Narragansett Bay and its Watershed-technical report. Retrieved from <http://nbep.org/the-state-of-our-watershed/>
- O'Brien PJ, & Wehr JD (2010). Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. *Hydrobiologia*, 657, 89–105. 10.1007/s10750-009-9984-5
- Olsen Y (1999). Lipids and essential fatty acids in aquatic food webs: What can freshwater ecologists learn from mariculture. In Arts MT, & Wainman BC (Eds.), *Lipids in freshwater ecosystems* (pp. 161–202). New York, NY: Springer 10.1007/978-1-4612-0547-0
- Parr TB, Smucker NJ, Bentsen CN, & Neale MW (2016). Potential roles of past, present, and future urbanization characteristics in producing various stream responses. *Freshwater Science*, 35, 436–443. 10.1086/685030
- Parrish CC (1999). Determination of total lipid, lipid classes, and fatty acids in aquatic samples. *Lipids in freshwater ecosystems*. In Arts MT, & Wainman BC (Eds.), *Lipids in freshwater ecosystems* (pp. 4–20). New York, NY: Springer 10.1007/978-1-4612-0547-0
- Passy SL, & Blanchet FG (2007). Algal communities in human-impacted stream ecosystems suffer beta-diversity decline. *Diversity and Distributions*, 13, 670–679. 10.1111/j.1472-4642.2007.00361.x
- Paul MJ, & Meyer JL (2001). Streams in the urban landscape. *Urban Ecology*, 32, 333–365.
- Pearson RG, Christidis F, Connolly NM, Nolen JA, St Clair RM, Cairns A, & Davis (2017). Stream macroinvertebrate assemblage uniformity and drivers in a tropical bioregion. *Freshwater Biology*, 62, 544–558. 10.1111/fwb.12884
- Persson J, & Vrede T (2006). Polyunsaturated fatty acids in zooplankton: Variation due to taxonomy and trophic position. *Freshwater Biology*, 51, 887–900. 10.1111/j.1365-2427.2006.01540.x
- Rasmussen JB (2010). Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for  $\delta^{13}\text{C}$ . *Journal of Animal Ecology*, 79, 393–402. 10.1111/j.1365-2656.2009.01648.x [PubMed: 20039981]
- Ravet JL, Brett MT, & Müller-Navarra DC (2003). A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. *Limnology and Oceanography*, 48, 1938–1947. 10.4319/lo.2003.48.5.1938
- Richoux NB, Bergamino L, Moyo S, & Dalu T (2018). Spatial and temporal variability in the nutritional quality of basal resources along a temperate river/estuary continuum. *Organic Geochemistry*, 116, 984–12. 10.1016/j.orggeochem.2017.11.009
- Roy AH, Rosemond AD, Paul MJ, Leigh DS, & Wallace JB (2003). Stream macroinvertebrate response to catchment urbanisation (Georgia, USA). *Freshwater Biology*, 48, 329–346. 10.1046/j.1365-2427.2003.00979.x

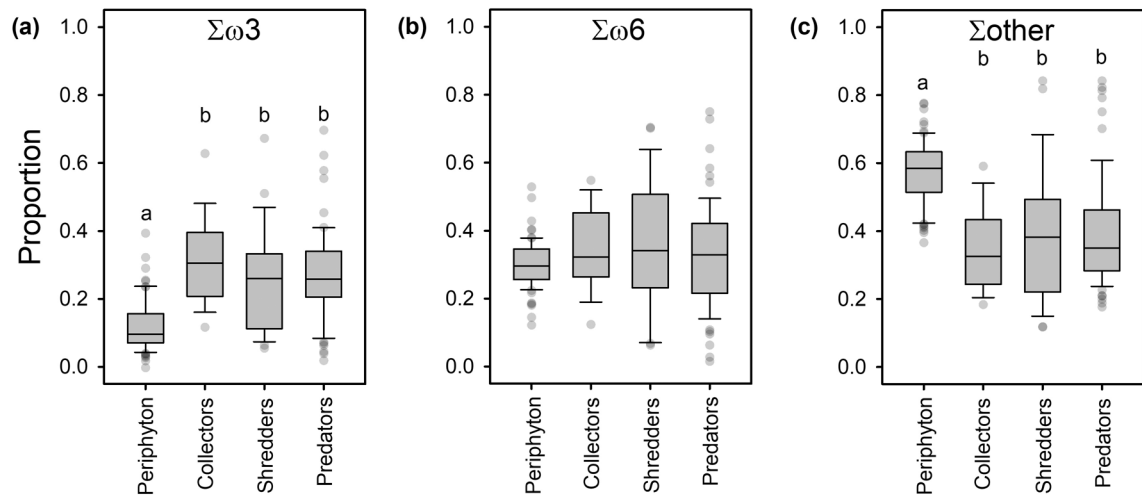
- Seto KC, Fragkias M, Guneralp B, & Reilly MK ( 2011). A meta-analysis of global urban land expansion. *PLoS ONE*, 6, e23777 10.1371/journal.pone.0023777 [PubMed: 21876770]
- Simopoulos AP ( 1999). Evolutionary aspects of omega-3 fatty acids in the food supply. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 60, 421–429. 10.1016/s0952-3278(99)80023-4
- Smucker NJ, & Detenbeck NE ( 2014). Meta-analysis of lost ecosystem attributes in urban streams and the effectiveness of out-of-channel management practices. *Restoration Ecology*, 22,741–748. 10.1111/rec.12134
- Smucker NJ, Detenbeck NE, & Morrison AC ( 2013). Diatom responses to watershed development and potential moderating effects of near-stream forest and wetland cover. *Freshwater Science*, 32, 230–249. 10.1899/11-171.1
- Smucker NJ, Kuhn A, Charpentier MA, Cruz-Quinones CJ, Elonon CM, Whorley SB, ... Wehr JD ( 2016). Quantifying urban watershed stressor gradients and evaluating how different land cover datasets affect stream management. *Environmental Management*, 57, 683–695. 10.1007/s00267-015-0629-3 [PubMed: 26614349]
- Smucker NJ, Kuhn A, Cruz-Quinones CJ, Serbst JR, & Lake JL ( 2018). Stable isotopes of algae and macroinvertebrates in streams respond to watershed urbanization, inform management goals, and indicate food web relationships. *Ecological Indicators*, 90, 295–304. 10.1016/j.ecolind.2018.03.024 [PubMed: 29805317]
- Sokal RR, & Rohlf FJ ( 1995). *Biometry* ( 3rd ed.). New York, NY: W.H. Freeman and Company.
- Stanley-Samuelson DW ( 1994). Assessing the significance of prostaglandins and other eicosanoids in insect physiology. *Journal of Insect Physiology*, 40, 3–11. 10.1016/0022-1910(94)90106-6
- Stevenson RJ & Bahls LL ( 1999) Periphyton protocols In Barbour MT, Gerritsen JB & Snyder D (Eds.), *Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, benthic macroinvertebrates, and fish* ( 2nd ed., pp. 6-1–6-22). Washington, DC: EPA 841-B-99-002 United States Environmental Protection Agency.
- Stevenson RJ, Hill BH, Herlihy AT, Yuan LL, & Norton SB ( 2008). Algae-P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of the North American Benthological Society*, 27, 783–799. 10.1899/07-077.1
- Taipale S, Strandberg U, Peltomaa E, Galloway AWE, Ojala A, & Brett MT ( 2013). Fatty acid composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, 71, 165–178. 10.3354/ame01671
- Taube R, Ganzert L, Grossart H, Gleixner G, & Premke K ( 2018). Organic matter quality structures benthic fatty acid patterns and the abundance of fungi and bacteria in temperate lakes. *Science of the Total Environment*, 610, 469–481. 10.1016/j.scitotenv.2017.07.256 [PubMed: 28818662]
- Torres-Ruiz M, Wehr JD, & Perrone AA ( 2007). Trophic relations in a stream food web: Importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society*, 26, 509–522. 10.1899/06-070.1
- Torres-Ruiz M, Wehr JD, & Perrone AA ( 2010). Are net-spinning caddisflies what they eat? An investigation using controlled diets and fatty acids. *Journal of the North American Benthological Society*, 29, 803–813. 10.1899/09-162.1
- Tsoi WY, Hadwen WL, & Fellows CS ( 2011). Spatial and temporal variation in the ecological stoichiometry of aquatic organisms in an urban catchment. *Journal of the North American Benthological Society*, 30, 533–545. 10.1899/10-085.1
- Twining CW, Brenna JT, Hairston NG, & Flecker AS ( 2016). Highly unsaturated fatty acids in nature: What we know and what we need to learn. *Oikos*, 125, 749–760. 10.1111/oik.02910
- US EPA, United States Environmental Protection Agency ( 1987) *Handbook of methods for acid deposition studies Laboratory analysis of surface water chemistry*. EPA, 600/4-87/026 Section 21.0 . Washington, DC: US Environmental Protection Agency.
- Utz RM, Hilderbrand RH, & Boward DM ( 2009). Identifying regional differences in threshold responses of aquatic invertebrates to land cover gradients. *Ecological Indicators*, 9, 556–567. 10.1016/j.ecolind.2008.08.008

- Vonk JA, Kuijk BF, Beusekom M, Hunting ER, & Kraak MH ( 2016). The significance of linoleic acid in food sources for detritivorous benthic invertebrates. *Scientific Reports*, 6, 35785 10.1038/srep35785 [PubMed: 27767068]
- Wallace AM, & Biastoch RG ( 2016). Detecting changes in the benthic invertebrate community in response to increasing chloride in streams in Toronto, Canada. *Freshwater Science*, 35, 353–363. 10.1086/685297
- Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, & Morgan RP ( 2005). The urban stream syndrome: Current knowledge and the search for a cure. *Journal of the North American Benthological Society*, 24, 706–723. 10.1899/04-028.1
- Walsh CJ, & Webb A ( 2016). Interactive effects of urban stormwater drainage, land clearance, and flow regime on stream macroinvertebrate assemblages across a large metropolitan region. *Freshwater Science*, 35, 324–339. 10.1086/685105
- Whorley SB, & Wehr JD ( 2016a). Connecting algal taxonomic information to essential fatty acid content in agricultural streams. *Phycologia*, 55, 531–542. 10.2216/15-123.1
- Whorley SB, & Wehr JD ( 2016b) Flood events can reduce key fatty acid content of early-stage benthic algal assemblages in an urban stream. *Journal of Urban Ecology*, 2, juw002 10.1093/jue/juw002
- Whorley SB, & Wehr JD ( 2018). Multi-year patterns in benthic algal fatty acid compounds under agricultural stress. *Freshwater Science*, 37( 3), 534–550. 10.1086/699201



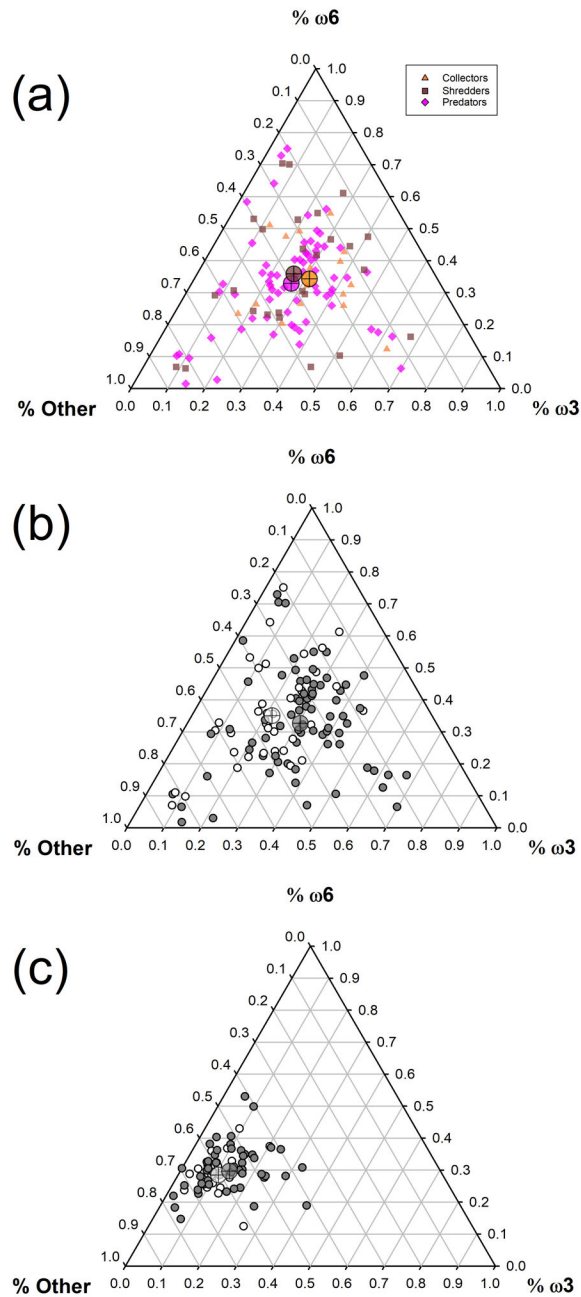
**Figure 1.** Map showing development intensities and the distribution of sampled streams in the 4,421 km<sup>2</sup> Narragansett Bay catchment located in north-eastern U.S.A. Natural land cover includes forest, vegetated, and wetland land cover. CT, Connecticut; MA, Massachusetts; NLCD, National Land Cover Database; RI, Rhode Island





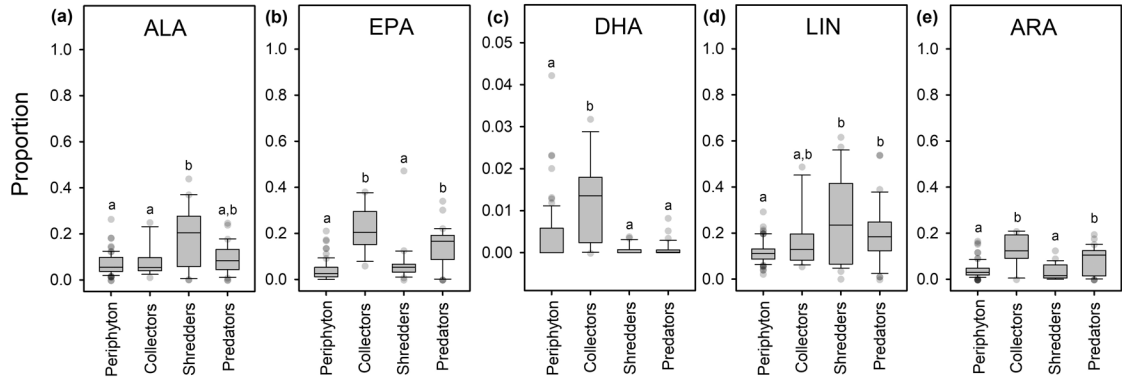
**Figure 2.**

Box plots showing proportions of (a)  $\Sigma\omega3$ , (b)  $\Sigma\omega6$ , and (c)  $\Sigma\text{other}$  fatty acids of all periphyton and invertebrate collectors, shredders, and predators collected from all sites. Boxes are interquartile ranges with lines showing medians and whiskers showing 10<sup>th</sup> and 90<sup>th</sup> percentiles. Boxes not sharing any letters are significantly different (Kruskal–Wallis ANOVA with Dunn's test for significant differences among medians)



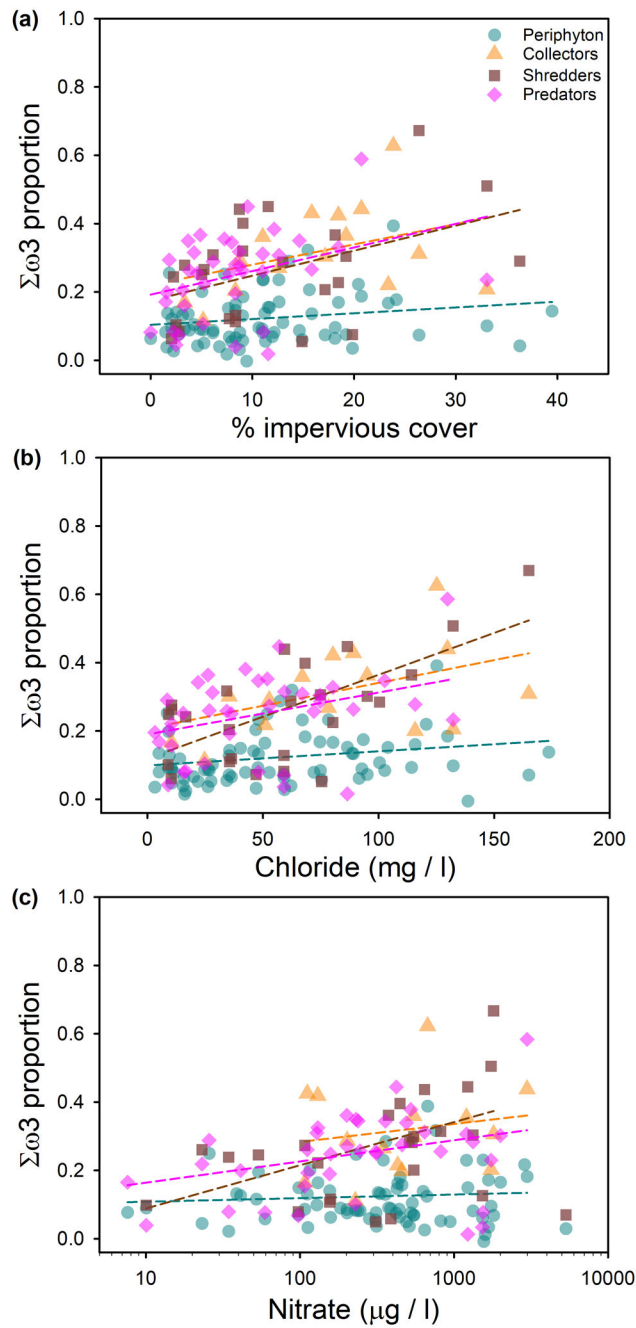
**Figure 3.**

Ternary plots based on proportions of  $\Sigma\omega 3$ ,  $\Sigma\omega 6$ , and  $\Sigma$ Other fatty acids for (a) all macroinvertebrates collected in the study distinguished by functional feeding group and for (b) all macroinvertebrates and (c) periphyton distinguished by being collected from least disturbed streams with <5% catchment impervious cover (white) or from urban streams with >5% catchment impervious cover (grey). Larger crossed symbols indicate group mean values



**Figure 4.**

Box plots showing proportions of five essential fatty acids of all periphyton and invertebrate collectors, shredders, and predators collected from all sites including: (a)  $\alpha$ -linolenic acid (ALA), (b) eicosapentaenoic acid (EPA), (c) docosahexaenoic acid (DHA; note different scale), (d) linoleic acid (LIN), and (e) arachidonic acid (ARA). Boxes are interquartile ranges with lines showing medians and whiskers showing 10th and 90th percentiles. Boxes not sharing any letters are significantly different (Kruskal–Wallis ANOVA with Dunn's test for significant differences among medians)



**Figure 5.**

The  $\Sigma\omega 3$  proportions of periphyton and invertebrate functional feeding groups plotted against (a) percent catchment impervious cover, (b) chloride concentrations, and (c) nitrate concentrations. Lines are shown only to highlight relationships. See Table 4 for a breakdown of within-group correlations. An outlier of 5,327  $\mu\text{g}$  nitrate/L was excluded from fitted lines due to being nearly twice that of the next highest observed concentration

**Table 1:**

Mean proportions ( $\pm SE$ ) of main structural fatty acid groups and counts for each family of benthic invertebrate included in the analysis. Where no standard error is indicated, there was only one organism. Each Family (\*Order) is listed by its functional feeding group (FFG) and for each impervious cover (% IC) category

FFG	Family	Mean proportion FA ( $\pm SE$ )			Number of sites	
		$\Sigma\omega 3$	$\Sigma\omega 6$	$\Sigma\text{other}$	<5% IC	5% IC
Collector/Gatherers	Asellota*	0.181 (n/a)	0.477 (n/a)	0.343 (n/a)		1
	Cambaridae	0.277 (0.041)	0.269 (0.019)	0.454 (0.045)	1	5
	Gammaridea	0.346 (0.035)	0.393 (0.062)	0.261 (0.050)		4
	Hydropsychidae	0.350 (0.089)	0.365 (0.071)	0.285 (0.024)	1	4
	Philopotamidae	0.556 (n/a)	0.188 (n/a)	0.256 (n/a)		1
Shredders	Limnephilidae	0.292 (0.048)	0.264 (0.045)	0.445 (0.068)	4	9
	Tipulidae	0.228 (0.037)	0.458 (0.046)	0.314 (0.029)	3	10
Predators	Aeshnidae	0.252 (0.028)	0.349 (0.030)	0.399 (0.030)	9	16
	Calopterygidae	0.312 (0.072)	0.375 (0.084)	0.314 (0.012)		2
	Corydalidae	0.221 (0.018)	0.339 (0.038)	0.440 (0.041)	14	10
	Gomphidae	0.268 (n/a)	0.353 (n/a)	0.379 (n/a)		1
	Libellulidae	0.305 (0.025)	0.365 (0.020)	0.331 (0.034)		4
	Perlidae	0.322 (0.032)	0.280 (0.026)	0.399 (0.042)	6	4
	Rhyacophilidae	0.434 (0.168)	0.267 (0.095)	0.299 (0.073)		3

**Table 2:**Mean proportions ( $\pm SE$ ) of each fatty acid compound quantified in this analysis

Lipid formula	Periphyton	Collectors	Shredders	Predators	<i>H</i> ( <i>p</i> )
18:0	0.143 (0.009)	0.122 (0.012)	0.123 (0.026)	0.158 (0.016)	<b>11.806 (0.008)</b>
18:1 $\omega$ 9	0.346 (0.008)	0.136 (0.031)	0.140 (0.033)	0.175 (0.015)	<b>71.885 (&lt;0.001)</b>
18:2 $\omega$ 6 (LIN)	0.117 (0.005)	0.174 (0.030)	0.259 (0.036)	0.192 (0.015)	<b>23.966 (&lt;0.001)</b>
18:3 $\omega$ 6	0.141 (0.009)	0.038 (0.010)	0.061 (0.023)	0.045 (0.010)	<b>76.289 (&lt;0.001)</b>
18:3 $\omega$ 3 (ALA)	0.071 (0.006)	0.079 (0.017)	0.184 (0.025)	0.103 (0.008)	<b>21.780 (&lt;0.001)</b>
20:0	0.010 (0.001)	0.015 (0.006)	0.027 (0.007)	0.016 (0.003)	2.998 (0.392)
20:1	0.010 (0.001)	0.015 (0.004)	0.033 (0.012)	0.007 (0.002)	<b>24.426 (&lt;0.001)</b>
20:2	0.008 (0.001)	0.014 (0.003)	0.005 (0.002)	0.004 (0.0005)	<b>17.298 (0.001)</b>
20:3 $\omega$ 6	0.001 (0.0002)	0.004 (0.001)	0.006 (0.002)	0.005 (0.001)	<b>44.383 (&lt;0.001)</b>
20:4 $\omega$ 6 (ARA)	0.039 (0.004)	0.128 (0.017)	0.034 (0.007)	0.089 (0.007)	<b>42.367 (&lt;0.001)</b>
20:3 $\omega$ 3	0.012 (0.003)	0.005 (0.001)	0.008 (0.003)	0.014 (0.004)	1.498 (0.683)
20:5 $\omega$ 3 (EPA)	0.039 (0.005)	0.214 (0.023)	0.068 (0.017)	0.150 (0.011)	<b>77.867 (&lt;0.001)</b>
22:0	0.015 (0.001)	0.026 (0.013)	0.033 (0.009)	0.030 (0.008)	<b>18.719 (&lt;0.001)</b>
22:1 $\omega$ 9	0.006 (0.001)	0.003 (0.001)	0.008 (0.005)	0.010 (0.005)	<b>9.257 (0.026)</b>
22:2	0.008 (0.001)	0.001 (0.001)	0.005 (0.004)	0.001 (0.0003)	<b>32.027 (&lt;0.001)</b>
23:0	0.002 (0.001)	0.003 (0.001)	0.003 (0.001)	0.001 (0.001)	<b>10.083 (0.018)</b>
24:0	0.025 (0.002)	0.006 (0.002)	0.001 (0.0003)	0.0003 (0.0001)	<b>109.565 (&lt;0.001)</b>
22:6 $\omega$ 3 (DHA)	0.003 (0.001)	0.013 (0.003)	0.001 (0.0002)	0.001 (0.0002)	<b>28.207 (&lt;0.001)</b>

Note

Comparisons among trophic levels by Kruskal–Wallis ( $df = 3$ ). Significant results across functional feeding groups ( $p < 0.05$ ) are in bold. Essential fatty acid compounds are indicated by their three-letter abbreviations and bold numbers. Totals will not equal 1.0 due to averaging and rounding.

**Table 3.**

Summary of water chemistry and GIS land cover categories across all streams surveyed

	TC (ppb)	TP (ppb)	TN (ppb)	NO <sub>2</sub> / NO <sub>3</sub> <sup>-</sup> (µg/L)	NH <sub>4</sub> <sup>+</sup> (ppb)	PO <sub>4</sub> <sup>-</sup> (ppb)	Cl <sup>-</sup> (mg/L)	% IC	% Wetland	% Forest	% Agriculture
Min	1.583	1.148	100.442	7.599	7.300	3.310	3.212	0.0	0.0	15.201	0.0
Max	40.664	247.712	4,227.00	5,327.37	467.151	186.619	173.604	39.460	26.236	100.0	15.751
Mean (SE)	7.961 (0.601)	37.351 (3.273)	940.678 (50.390)	605.683 (56.681)	35.434 (3.542)	13.398 (1.178)	54.679 (2.870)	10.556 (0.592)	8.819 (0.566)	58.161 (1.397)	3.600 (0.253)
CV (%)	1.019	1.195	0.731	1.276	1.363	1.199	0.716	0.765	0.874	0.328	0.957

TN, total nitrogen; TP, total phosphorus

**Table 4.**

Spearman correlations of the proportions of major fatty acid (FA) groups in periphyton and invertebrate feeding groups with urban-related environmental variables

	Impervious cover	Population density	Nitrate	Chloride
$\Sigma\omega 3$				
Periphyton	0.21 (0.08)	<b>0.24 (&lt;0.05)</b>	-0.01 (0.91)	<b>0.26 (0.02)</b>
Collectors	0.48 (0.07)	0.46 (0.10)	0.30 (0.28)	0.48 (0.07)
Shredders	<b>0.42 (0.04)</b>	0.35 (0.09)	<b>0.44 (0.03)</b>	<b>0.55 (&lt;0.01)</b>
Predators	<b>0.39 (0.01)</b>	0.28 (0.09)	<b>0.32 (0.05)</b>	<b>0.33 (0.04)</b>
$\Sigma\text{other}$				
Periphyton	<b>-0.29 (0.01)</b>	<b>-0.37 (0.001)</b>	-0.08 (0.48)	-0.14 (0.24)
Collectors	-0.43 (0.11)	<b>-0.60 (0.02)</b>	-0.21 (0.46)	-0.28 (0.31)
Shredders	<b>-0.41 (0.04)</b>	-0.23 (0.27)	-0.18 (0.39)	-0.33 (0.11)
Predators	<b>-0.32 (0.05)</b>	-0.09 (0.59)	-0.22 (0.19)	-0.20 (0.23)

Note

$\Sigma\omega 6$  had no significant correlations. Significant results ( $p < 0.05$ ) are in bold.