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ARTICLE

A Molecular and Stable Isotopic Approach to Investigate Algal and Detrital Energy Pathways in a Freshwater Marsh

Laura L. Belicka · Eric R. Sokol · J. Matthew Hoch · Rudolf Jaffé · Joel C. Trexler

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Abstract The relative importance of algal and detrital energy pathways remains a central question in wetlands ecology. We used bulk stable isotope analysis and fatty acid composition to investigate the relative contributions of periphyton (algae) and floc (detritus) in a freshwater wetland with the goal of determining the inputs of these resource pools to lower trophic-level consumers. All animal samples revealed fatty acid markers indicative of both microbial (detrital) and algal origins, though the relative contributions varied among species. Vascular plant markers were in low abundance in most consumers. Detritivory is important for chironomids and amphipods, as demonstrated by the enhanced bacterial fatty acids present in both consumers, while algal resources, in the form of periphyton, likely support ephemeropteran larvae. Invertebrates such as amphipods and grass shrimp appear to be important resources for small omnivorous fish, while *Poecilia latipinna* appear to strongly use periphyton and Ephemeroptera larvae as food sources. Both *P. latipinna* and *Lepomis spp.* assimilated small amounts of vascular plant debris, possibly due to unintentional ingestion of floc while foraging for invertebrates and insect larvae. Physid snails, *Haitia spp.*, were characterized by considerably different fatty acid compositions than other taxa examined, and likely play a unique role in Everglades' food webs.

Keywords Fatty acids · Stable isotopes · Food webs · Detritus · Algae · Everglades

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L. L. Belicka (✉) · R. Jaffé
Department of Chemistry and Biochemistry and Southeast Environmental Research Center, Florida International University, 3000 NE 151st Street, Marine Sciences Building, North Miami, FL 33181, USA
e-mail: lbelicka@fiu.edu

E. R. Sokol · J. M. Hoch
Southeast Environmental Research Center, Florida International University, 3000 NE 151st Street, Marine Sciences Building, North Miami, FL 33181, USA

J. C. Trexler
Department of Biological Sciences, Florida International University, 3000 NE 151st Street, Marine Sciences Building, North Miami, FL 33181, USA

Present Address:
E. R. Sokol
Department of Biological Sciences, Virginia Polytechnic Institute and State University, 2011 Derring Hall, Blacksburg, VA 24061, USA

Introduction

Wetlands are commonly identified as environments where detritus plays an important role in energy cycling and trophic dynamics. Determining the relative contribution of detrital resources towards sustaining aquatic food webs is an important goal in characterizing the origins of trophic structure and biodiversity (Moore et al. 2004); however, challenges remain in separating detrital resources from more conventional algal energy pathways. Recent evidence has shown that an algal/detrital dichotomy oversimplifies the complex relationships between habitat and feeding ecology in wetlands (Taylor and Batzer 2010) and that the importance of detrital processing of organic matter through a microbial loop remains a key question for wetland systems (Williams and Trexler 2006). While direct assimilation of

detritus may not be that common because consumers often lack the digestive enzymes necessary to process it, assimilation of microbial epifauna that coats detritus may be a major route of energy and limiting nutrient transfer (Bowen 1984; Smoot and Findlay 2010).

Tools for understanding algal and detrital energy pathways in ecosystems have evolved from more traditional methods of food web analysis (i.e., stomach content examination) to molecular approaches such as stable isotope and lipid biomarker analysis. These newer approaches provide information on food consumption integrated over longer periods of time compared to the "snap-shot" provided by stomach contents and can also provide estimates of dietary components in complex systems with a variety of resources (Dalsgaard et al. 2003; Iverson et al. 2004). Bulk $\delta^{13}C$ and $\delta^{15}N$, for example, have been widely used for food web studies (Fry et al. 1978; McCutchan et al. 2003). Carbon isotopes undergo a minimal fractionation of approximately 0.4‰ during dietary transfer from prey to consumer; therefore, carbon isotopic composition mostly reflects the source of organic matter in food webs (McCutchan et al. 2003). Nitrogen isotopes, on the other hand, undergo an enrichment of about 2.3‰ during trophic transfer, providing useful information on trophic level (McCutchan et al. 2003). Fatty acid signatures are also particularly useful for dietary studies because fatty acids from triacylglycerol storage lipids in a prey item are taken into consumer tissue with relatively minor or predictable modifications (Iverson et al. 2004). Changes in distributions of fatty acids or their stable carbon isotopic composition can provide information on spatial and/or temporal variation in diets, while certain unique source-specific fatty acids, termed biomarkers, can provide detailed tracking of a particular carbon substrate up the food chain (Budge et al. 2006; Williams et al. 2009).

The complex wetland system of the Florida Everglades provides an ideal environment to address the relative contributions of detrital and algal energy resources to consumers. Particulate organic matter is largely absent from the water column, but instead present as a flocculent detrital layer just above the sediment-water interface. This flocculent material, hereafter referred to as "floc", is composed of decaying vascular plant and algal debris with associated microbial biota, carbonates, and the remains of aquatic organisms, and is the predominant pathway of detrital energy sources in the Everglades (Neto et al. 2006; Williams and Trexler 2006). Furthermore, it has been argued that floc may be the most bioavailable organic matter substrate in wetland detrital food-chains (Hart and Lovvorn 2003; Neto et al. 2006). Algal energy pathways are dominated by abundant floating and submerged calcareous periphyton mats (Browder et al. 1994) composed mainly of cyanobacteria, diatoms, and green algae, which thrive in the oligotrophic environment of the Everglades by rapidly sequestering phosphorus (P) that becomes available (Gaiser et al. 2004). Enigmatically, increased P eventually causes disaggregation of the mat-forming matrix of the periphyton, resulting in an overall loss of biomass (Gaiser et al. 2006).

Here, we apply bulk stable isotope analysis and molecular distribution determinations of fatty acids to examine algal and detrital energy pathways in food webs. We focus on the two dominant basal resources, periphyton and floc, and selected invertebrate and fish consumers, with the goal of determining the inputs of these resource pools to lower trophic-level consumers in a freshwater wetland system. We expect this study to provide important information on methods to chemically distinguish algal and detrital contributions to higher trophic levels for future larger-scale food web studies.

Methods

Study Area and Sampling

The inputs of algal and detrital material to consumers were examined in the wet-prairie slough ecosystem near Taylor Slough in the Everglades, Florida, USA during the dry season (late January) of 2010. In this region, periphyton largely occurs on the surface of limestone and is the dominant primary producer, with estimates suggesting periphyton reaches 80% of total biomass in this region (Gaiser et al. 2006). Two sites were chosen–a site adjacent to the C-111 canal (~25.314°N, 80.521°W; hereafter "adjacent site") that experiences enhanced phosphorus (P) concentrations as a result of agricultural run-off via canal flow, and a site approximately 200 m into the marsh perpendicular to the canal (~25.312°N, 80.521°W; hereafter "far site"). See map in Light and Dineen (1997: Figure 4.12); sites were located on the southern boundary of the C-111 canal between water control structures S-18C and S-197. We selected these sites as representative of many P-enriched sites downstream from canals in the Everglades (adjacent site) and oligohaline sites unaffected by anthropengic nutrient enrichment (far site). At each site, three sub-sites were chosen at the initial point and approximately 50 m to the east and west of the initial sampling point. Sub-sites were approximately 15 m^2, however sampling beyond the perimeter of the sub-site was occasionally necessary (i.e., to find water deep enough for minnow traps).

Periphyton was collected with forceps from six random locations around the 15 m^2 sub-site and pooled into a composite periphyton sample for each sub-site. Similarly, six cores (7.5 cm in diameter) of floc were obtained at submerged locations adjacent to where periphyton samples were taken. Floc in the core tube was allowed to settle, the overlying water was decanted, and floc samples were

poured into glass jars and combined into a composite sample for each sub-site.

We qualitatively sampled consumers by collecting representatives of the dominant taxa at each site. This effort was not meant to be a quantitative estimate of consumer community composition. The following consumers were collected visually from the sub-sites (where present) with throw-traps, dip-nets, and minnow traps until enough biomass was achieved for analysis: Chironomidae larvae (midges), Ephemeroptera larvae (mayflies), *Hyalella azteca* (amphipods), *Palaemonetes paludosus* (grass shrimp), *Haitia spp.* (physid snails), *Gambusia holbrooki* (eastern mosquitofish), *Lucania goodei* (bluefin killifish), *Heterandria formosa* (least killifish), *Jordanella floridae* (flagfish), *Enneacanthus gloriosus* (blue spotted sunfish), *Poecilia latipinna* (sailfin molly), *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish). No chironomid or ephemeropteran larvae were found at the sub-sites far from the canal. Throw traps were 1 m^2, constructed out of copper pipe, and covered with 1.5 mm mesh. Traps were sampled with a bar seine of the same mesh size, periphyton was broken-up manually, and organisms were removed with forceps. Dip-nets were also used both inside and adjacent to the throw-trap sampling region to collect organisms. Minnow traps were deployed for 2–4 hours at each sub-site (occasionally overnight). Vertebrates were euthanized in a solution of MS-222 (tricaine methanesulfonate) in marsh water following humane animal care guidelines (Nickum et al. 2004) and all animal samples were placed on ice in the field and frozen upon return to the laboratory. *Haitia spp.* were removed from shells after 24 h of freezing to eliminate interferences from inorganic carbon present in shell material.

Sample Preparation

Animal samples were rinsed with ultrapure (milli-Q) water and lyophilized at –47°C for a minimum of 24 h. Periphyton and floc were examined microscopically and conspicuous metazoans were removed with forceps. Additionally, obvious vascular plant material was removed from periphyton samples. Samples were then homogenized with a mortar and pestle. For smaller organisms, including chironomid and ephemeropteran larvae, *Haitia spp.*, and *H. azteca*, 10–50 individuals were pooled to obtain sufficient mass (>10 mg dry weight) for analysis. All other organisms were analyzed individually. The whole bodies of organisms processed singly or in groups were used for this study because most prey species at the lower trophic levels studied here are consumed whole–as such, this data reflects both consumption and assimilation of organic matter sources. Although inclusion of stomach contents might increase within-species variability for fatty acid composition (Budge et al. 2006) and possibly also for stable carbon isotopic composition (Hill and McQuaid 2011), within-species variability of fatty acid signature is still considerably smaller than among-species variability (Budge et al. 2006) and an effect of gut contents on the composition of $\delta^{15}N$ has not been observed (Hill and McQuaid 2011). Similarly, annual variation in consumer tissue fatty acid content is also presumed to be minor compared to between-species variation (Budge et al. 2006). Bulk analysis of macroinvertebrate groups, particularly the diverse Chironomidae, provide results representative of the diets of consumers that feed indiscriminately at lower taxonomic levels of their prey.

Stable Isotope and Bulk Parameter Analysis

Homogenized samples were split for bulk chemical and isotopic and lipid analyses. Total organic carbon (TOC), total nitrogen (TN), and isotopic composition were measured concurrently with a Carlo Erba NA 1500 Elemental Analyzer coupled to a Finnigan MAT Delta isotope ratio mass spectrometer. Unacidified samples were analyzed for TN and $\delta^{15}N$ and TOC and $\delta^{13}C$, except for those samples which required acidification to remove interferences from carbonates (periphyton, floc, and *Haitia spp.*). Periphyton, floc, and *Haitia spp.* (somatic tissue) were treated with 10% HCl for 24 h to remove inorganic carbon, then rinsed to neutral pH with ultrapure H_2O and analyzed for TOC and $\delta^{13}C$. All isotopic values are presented using the standard δ notation with Pee Dee Belemnite (PDB) and atmospheric air as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The average isotopic lab error for replicate glycine internal standards treated identically as the samples was 0.11‰ for $\delta^{13}C$ and 0.12‰ for $\delta^{15}N$. Total phosphorus (TP) content was measured for periphyton and floc samples with colorimetric analysis following dry oxidation and an acid hydrolysis extraction (Fourqurean et al. 1992). We report TN (%) and TOC (%) based on the mass of each per dry mass of sample matrix and TP as μg per gram dry mass of sample matrix.

Lipid Extraction and Analysis

Lipids were extracted from periphyton, floc, and whole-body organisms or composites following a modification of Folch et al. (1957). Briefly, samples were ultrasonically extracted with a 2:1 mixture (v/v) of methylene chloride:methanol (CH_2Cl_2:MeOH). Ultrapure (milli-Q) water was added to achieve a final ratio of 2:1:0.7 CH_2Cl_2:MeOH:H_2O, the samples were strongly agitated, and the lower organic phase was removed to an evaporation flask. Fresh organic solvent was added and the extraction was repeated two more times. The three extracts were combined and excess solvent was removed by rotary evaporation. Total lipid extracts were flushed with nitrogen and stored in CH_2Cl_2 at –20°C.

Total lipid extracts were then saponified with 0.5 N methanolic KOH at 70°C for 30–60 min. Neutral lipids were

partitioned three times with a mixture of hexane:diethyl ether (9:1) after addition of water and archived for subsequent analysis. Samples were then acidified to pH <2 with HCl, and free fatty acids were partitioned into 9:1 hexane:diethyl ether three times and combined. Fatty acids were methylated to corresponding methyl esters with freshly distilled diazomethane.

Fatty acids were identified and relative abundances were determined using gas-chromatography-mass spectrometry (GC/MS) with an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer operating in electron ionization (EI) mode at 70 eV. The gas chromatograph was equipped with a Restek Rtx®-5MS capillary column, and column and temperature parameters followed those reported by Jaffé et al. (2001). Identification of fatty acids was performed by comparison of chromatographic retention times with authentic standards and mass spectra of standard and previously reported compounds. Overall, 69 different fatty acids were identified and the fatty acids are expressed here as a percentage of the total identified.

Data Analysis

To explore the distribution and variance of the bulk parameter data, Levene's homogeneity of variance tests were employed using SPSS 17.0 software. If data were normally distributed and had equal variance, t-tests were used to compare bulk parameters between adjacent and far sites. If data failed to meet the assumption of homogeneity of variance, a Mann–Whitney *U* test was performed. For graphical purposes, lipids were reduced into three categories (algal, bacterial, and higher plant) based on source assignments in the literature, with odd-chain branched and straight chain fatty acids and 18:1ω7 characteristic of bacteria; C_{16}, C_{18}, C_{20}, and C_{22} polyunsaturated fatty acids representing algal inputs; and long-chain saturated fatty acids as indicators of vascular plant material (Volkman et al. 1980; Ahlgren et al. 1992; Napolitano 1999; Dalsgaard et al. 2003). Average-linkage hierarchical cluster analysis and multidimensional scaling were performed based on Bray-Curtis dissimilarity coefficients on untransformed fatty acid data using the statistical packages R and PRIMER 5.0. K-means clustering with the Hartigan-Wong algorithm was used to define clusters. The ANOSIM (analysis of similarities) and SIMPER (similarity percentage breakdown) functions in PRIMER was used to investigate groupings (Clarke 1993).

Results

Bulk Sample Parameters

Organic carbon and total nitrogen content were similar in periphyton and floc at the two sites; however, total phosphorus (TP), was, on average, higher adjacent to the canal than at the far site (Table 1). Organic carbon in basal resources ranged between 40% and 47% (based on dry mass of basal resource material) at both sites while total nitrogen content in periphyton and floc averaged 0.8 and 1%, respectively. Mean TP, used in this region as a proxy for nutrient enrichment status, was 220 μg g^{-1} dry mass of periphyton and 438 μg g^{-1} in floc at sub-sites near the canal (Table 1) and neared the threshold set for defining enriched environments (500 μg g^{-1} (Gaiser et al. 2004)). Far from the canal, TP was lower in periphyton and floc, averaging 52 μg g^{-1} and 187 μg g^{-1}, respectively (Table 1). However, because of the high variance in TP collected from the adjacent sites, mean TP in periphyton near and far from the canal was not significantly different (Mann–Whitney *U* test, $p=0.100$). Mean TP in floc was not significantly different between sites ($p=0.100$), nor was mean floc TP significantly

Table 1 Descriptive statistics of bulk parameters in basal resources at sites adjacent to and far removed from the C-111 canal ($n=3$). OC, TN, and TP reported per dry weight of material

Parameters	Location			
	Adjacent		Far	
	Range	Mean (SD)	Range	Mean (SD)
Periphyton OC (%)	40.0–46.2	42.2 (3.5)	41.2–41.5	41.3 (0.1)
Floc OC (%)	40.3–46.9	42.9 (3.5)	42.7–46.1	44.6 (1.8)
Periphyton TN (%)	0.68–1.07	0.82 (0.22)	0.82–0.91	0.87 (0.05)
Floc TN (%)	1.2–1.5	1.34 (0.17)	0.7–1.3	1.01 (0.26)
Periphyton TP (μg g^{-1})	100–455	220 (204)	50–55	52 (2.9)
Floc TP (μg g^{-1})	305–525	438 (117)	130–250	187 (60)
Periphyton $\delta^{13}C$ (‰)	–27.1 to –31.0	–28.4 (2.2)	–28.6 to –31.1	–29.7 (1.3)
Floc $\delta^{13}C$ (‰)	–29.0 to –31.2	–30.1 (1.1)	–28.1 to –29.8	–29.0 (0.9)
Periphyton $\delta^{15}N$ (‰)	12.2–15.5	13.3 (1.9)	3.8–5.3	4.5 (0.8)
Floc $\delta^{15}N$ (‰)	12.5–14.3	13.1 (1.0)	6.4–7.1	6.7 (0.4)

different from mean periphyton TP at either site (p=0.700 for adjacent site, p=0.100 for far site).

Stable carbon isotopic composition was similar in periphyton near and far from the canal, ranging from –31 to –27‰ (Table 1). Floc $\delta^{13}C$ was similar to periphyton $\delta^{13}C$, with a minimum of –31 and a maximum of –28‰ (Table 1). In contrast, $\delta^{15}N$ in both periphyton and floc differed between sites adjacent and far from the canal, averaging ~13‰ adjacent to the canal but only 4.5–6.7‰ away from the canal (Table 1). Bulk $\delta^{13}C$ of animal tissue was highly variable, with most invertebrates and fish displaying more depleted $\delta^{13}C$ signatures than periphyton or floc (Fig. 1). Although $\delta^{15}N$ of most fish species and *P. paludosus* were similar between adjacent and far sites, $\delta^{15}N$ of *H. azteca* at the sites far from the canal were approximately twice as depleted as signatures of *H. azteca* adjacent to the canal (Fig. 1).

Fatty Acid Distributions in Basal Resources and Consumers

A diverse suite of fatty acids, including both ubiquitous and source-specific structures, was identified in C-111 canal basal resources and consumers (Electronic Appendix). Fatty acids are named here as A:BωC, where A refers to the number of carbon atoms in the molecule, B refers to the number of double bonds present, and C, listed where

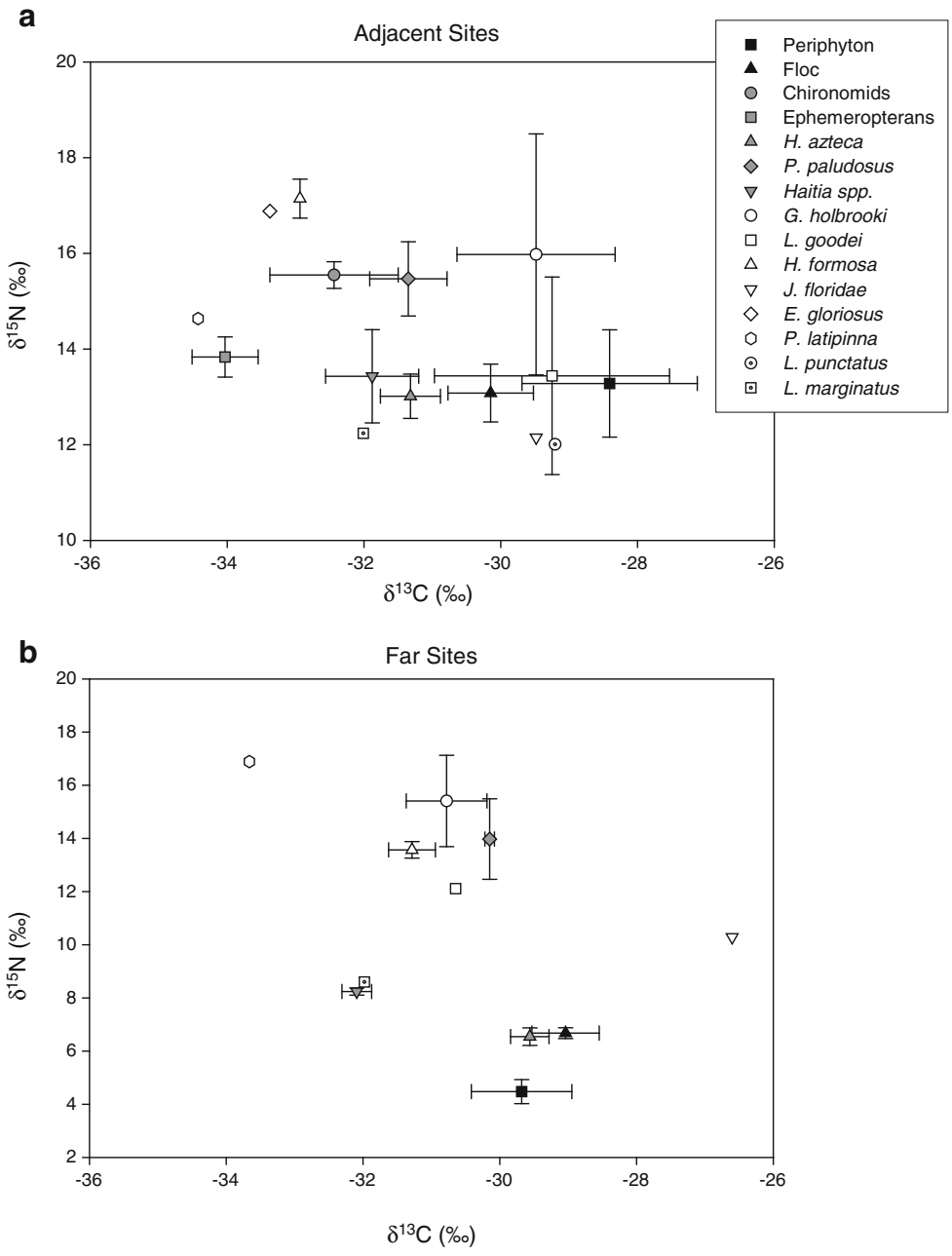

Fig. 1 Mean stable carbon and nitrogen isotope values (‰) of basal resources and consumers at locations adjacent to (**a**) and far from (**b**) the C-111 Canal. Note difference in y-axis scale between (**a**) and (**b**). Error bars indicate ±1 standard error, n=3 where plotted; otherwise, n=1 or 2

confirmed, indicates the position of the first double bond counted from the terminal methyl group, with additional double bonds separated by a single methylene group. Periphyton and floc shared the same six major fatty acids, although in differing proportions, including 16:0, 16:1ω7, 18:1ω9, 18:1ω7, 18:3ω3, and 18:2ω6 that accounted for approximately 80% and 65% of the periphyton and floc total fatty acids, respectively (see Electronic Appendices). The fatty acid composition of periphyton from both locations was very similar; in contrast, floc located far from the canal contained much higher proportions of saturated fatty acids and odd-chain branched fatty acids compared to floc adjacent to the canal (Fig. 2). Important differences between fatty acids in periphyton and floc were demonstrated by the higher abundances of typically algae-derived even-chain polyunsaturated fatty acids (PUFA) in periphyton, particularly compared to floc located far from the canal, and the higher abundances of long-chain fatty acids (C_{22}–C_{28} saturates; vascular-plant derived) and odd-chain saturated and monounsaturated fatty acids (bacterially-derived) in floc compared to periphyton (Fig. 2). Floc had the highest vascular plant fatty acid content of all basal resources and consumers ($p < 0.05$ for all pair-wise comparisons following ANOVA on arcsine square root transformed data).

The composition of major groups of fatty acids (algal, vascular plant, and bacterial; see Table 2 for the subset of source-specific fatty acids used in this figure) was similar for invertebrates between adjacent and far locations,

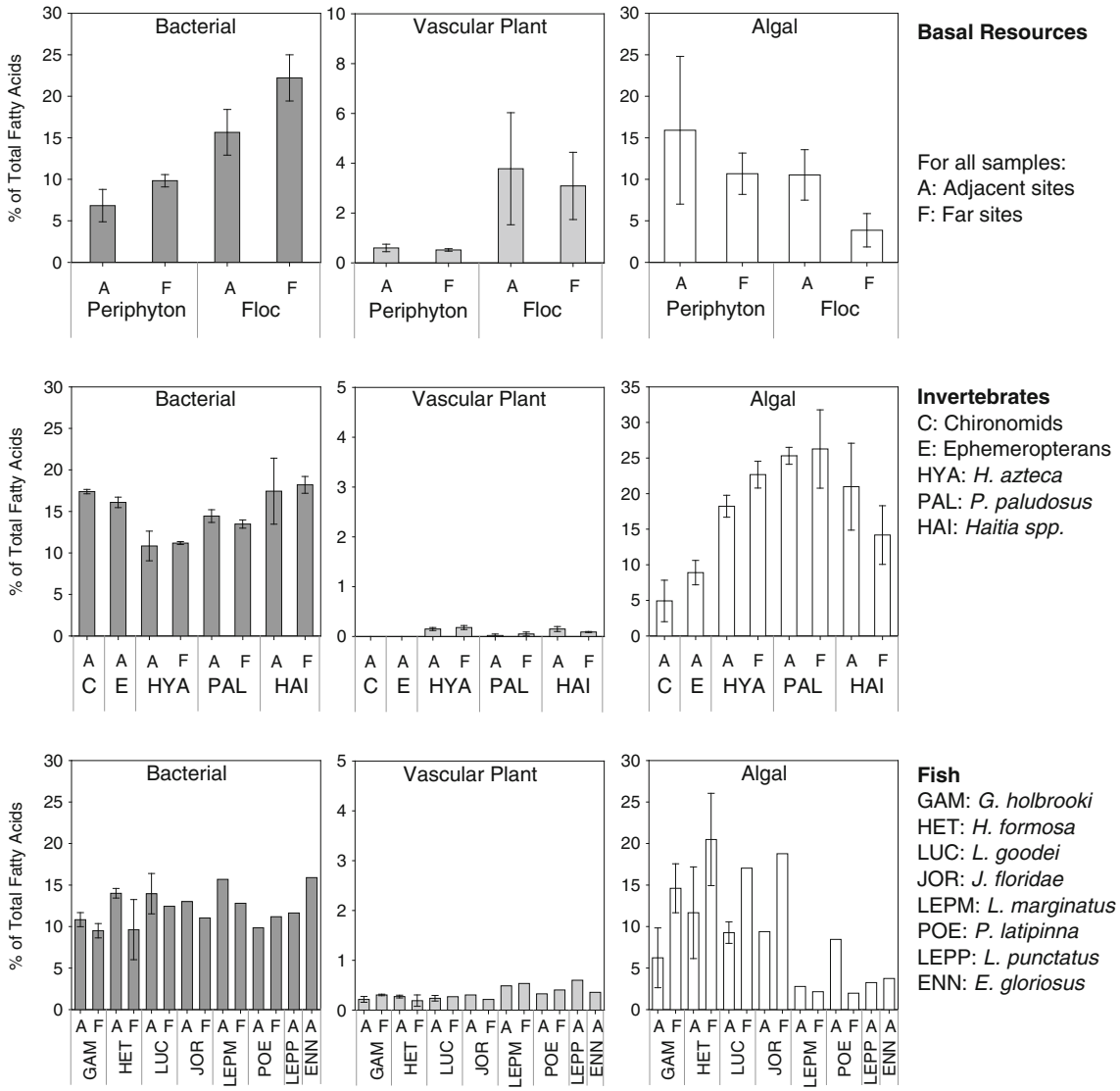

Fig. 2 Relative percentages of algal, bacterial, and vascular plant fatty acids in (**a**) periphyton and floc, (**b**) invertebrates, and (**c**) fish at locations adjacent to and far from the C-111 canal. Note axis scale differences for vascular plant fatty acids. For the subset of source-specific individual fatty acids included in each group, see Table 2. Error bars indicate ±1 standard deviation, $n=3$ where plotted; otherwise, $n=1$ or 2

Table 2 Source assignments of individual and groups of fatty acids reported in the literature

Carbon Source (Grouped fatty acids used in this study)	References
Bacteria (15:0i, 15:0a, 15:0n, 17:0i, 17:0a, 17:0n, 18:1ω7, 19:1)[a]	
Odd carbon number fatty acids, 15:0i, 15:0a, 17:0i, 17:0a, 18:1ω7	Findlay and Dobbs (1993); Napolitano (1999) and references therein; Volkman et al. (1980)
Vascular Plants (22:0n, 24:0n, 26:0n, 28:0n)[a]	
C_{22}–C_{32} saturated fatty acids	Eglinton and Hamilton (1967)
Algae (16:3, 18:3ω3, 18:4, 18:3ω6, 20:4ω6, 20:5ω3, 20:4, 22:4ω6, 22:5ω3, 22:5ω6, 22:6ω3)[a]	
14:0, 16:1ω7: multiple sources, but high in diatoms and some cyanobacteria	Napolitano (1999) and references therein
C_{16} PUFA: green algae and diatoms	Kates and Volcani (1966); Cranwell et al. (1990); Napolitano (1999)
18:3ω3: green algae, cyanobacteria	Ahlgren et al. (1992); Dalsgaard et al. (2003)
18:3ω6: cyanobacteria	Napolitano (1999)
18:4ω3, 18:5ω3, 22:6ω3: dinoflagellates	Ahlgren et al. (1992); Dalsgaard et al. (2003)
20:5ω3, ratio of 20:5ω3 to 22:6ω3: diatoms	Napolitano (1999); Dalsgaard et al. (2003)

[a]Groups of fatty acids used in this study to partition bacterial, vascular plant, and algal organic matter, respectively, in Figs. 2 and 3

especially for grass shrimp, *P. paludosus* (Fig. 2). Despite differing proportions of bacterial and algal fatty acids, all invertebrates were characterized by extremely low abundances of fatty acids from vascular plant sources. Interestingly, fish species contained slightly higher proportions of vascular plant fatty acids than invertebrates, with also greater differences noted between fatty acids from the same species of fish adjacent to and far from the canal (Fig. 2). Of all food sources and consumer tissues analyzed here, floc contained the greatest proportion of vascular plant fatty acids.

An average-linkage hierarchical cluster analysis based on the Bray-Curtis dissimilarity matrix of relative abundance of consumer and food source fatty acids suggests the presence of multiple groupings of organisms using a dissimilarity threshold of 0.20 (Fig. 3). With minor exceptions, the groups contained (a) *Haitia* spp. snails, (b) periphyton and ephemeropteran larvae, (c) floc, (d) chironomidae larvae, (e) most fish species, excepting *P. latipinna*, and (f) the crustaceans *P. paludosus* and *H. azteca* (Fig. 3). Most species and food sources were resolved into individual clusters at less than 15% dissimilarity, with the exception of *G. holbrooki, L. goodei, H. formosa*, and *J. floridae*, which were mixed and appeared to group more by location (adjacent versus far). The fatty acid composition of periphyton adjacent to

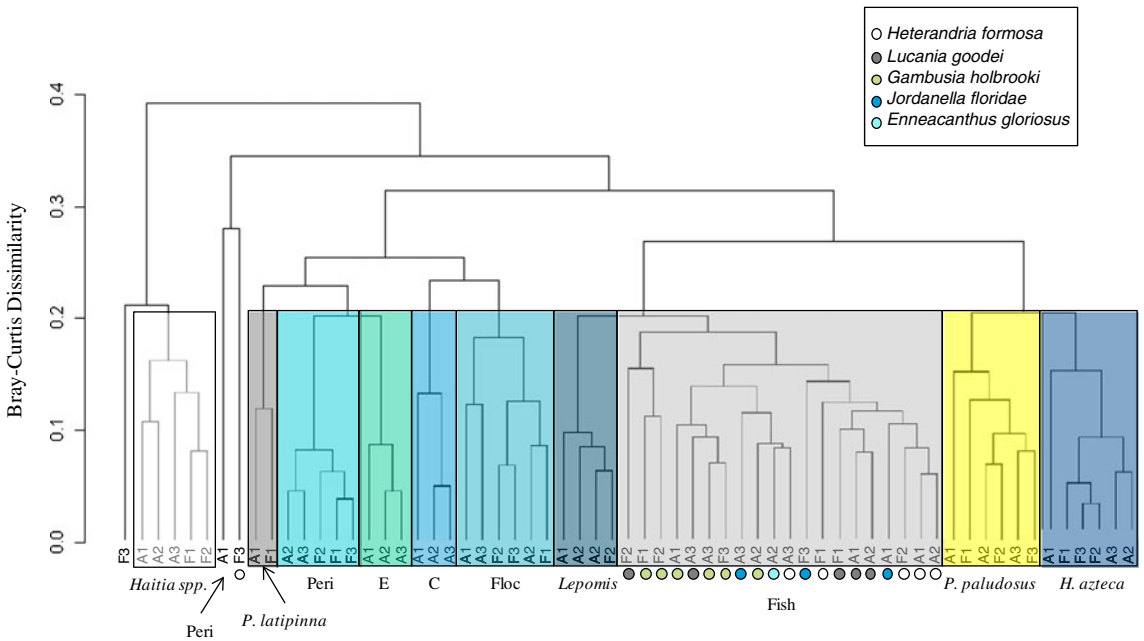

Fig. 3 Average-linkage hierarchical cluster analysis of the Bray-Curtis dissimilarity matrix based on the relative abundance of fatty acids in all food sources and consumers. Shaded rectangles denote clusters defined at a < 20% dissimilarity level. *A* adjacent to canal; *F* far from canal; *E* ephemeropterans; *C* chironomids; Fish species noted in figure legend

the canal at the first sub-site (PERI A1) was distinctively different from the rest of the periphyton samples, as was that of *H. formosa* from the third sub-site far from the canal (Fig. 3). The spatial distribution of organisms demonstrated by the MDS (Fig. 4) was similar to the cluster analysis, again highlighting the associations between *P. paludosus* and *H. azteca*; floc, periphyton, and larvae of Chironomidae and Ephemeroptera; as well as the differences in *Haitia* snails compared to the rest of the food resources and consumers investigated (analysis not shown for brevity). Species had high within-group similarities based on SIMPER, ranging from 83% for *H. formosa* to 93% for ephemeropteran larvae. The greatest dissimilarity among species was found between *Haitia spp.* snails and the following: *P. latipinna*, periphyton, *L. punctatus*, floc, chironomid larvae, and *G. holbrooki*, followed by *P. latipinna* and *P. paludosus*. Conversely, high similarity was demonstrated between fish species.

Discussion

Fatty acid composition in food resources and consumers revealed complex trophic relationships, with both detrital (floc) and algal (periphyton) organic matter fueling productivity for higher trophic levels in this wetland ecosystem. Although the remains of periphyton are a large component

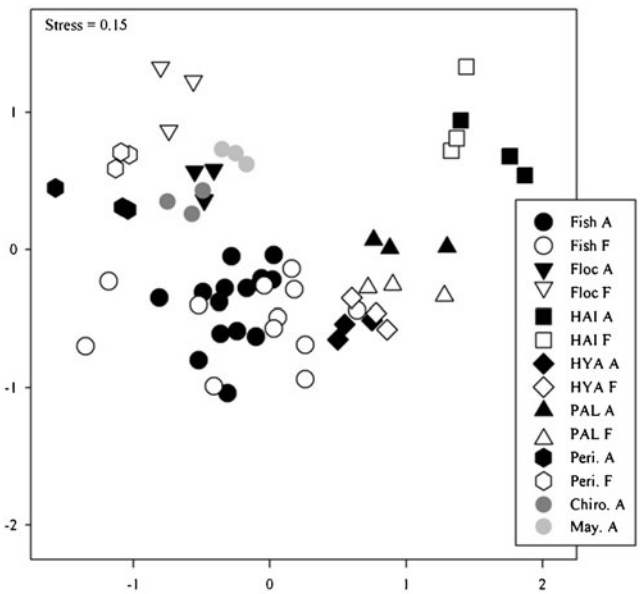

Fig. 4 Multidimensional scaling (MDS) of the Bray-Curtis dissimilarity matrix based on the relative abundance of fatty acids in all food sources and consumers. Filled symbols indicate samples collected adjacent to the canal (A); open symbols show samples collected far from the canal (F). *Fish* all fish species pooled; *Floc* flocculent material; *HAI* Haitia spp.; *HYA* Hyalella azteca; *PAL* Palaemonetes paludosus; *Peri*. periphyton; *Chiro*. Chironomidae larvae; *May*. Emphemeroptera larvae

of floc (Neto et al. 2006), resulting in considerable similarities in their stable isotopic composition and predominant fatty acids, molecular analysis suggests that floc contained greater amounts of bacterial organic matter compared to periphyton, as well as detritus from vascular plant material (Fig. 2). Higher proportions of the C_{15} and C_{17} branched and normal fatty acids typically derived from gram-positive bacteria (Findlay and Dobbs 1993; Napolitano 1999) indicate bacterial re-working of floc organic matter. The presence of vascular plant inputs in floc (Neto et al. 2006), such as those from *Cladium spp.* which is one of the most common vascular plants in the region, were further supported by the more depleted $\delta^{13}C$ values of floc compared to periphyton at sites adjacent to the canal (Table 1) and the presence of long-chain fatty acids (C_{24}, C_{26}, and C_{28}), which originate from epicuticular waxes of higher plants (Eglinton and Hamilton 1967). In contrast, periphyton fatty acid composition contained high proportions of fatty acids indicative of fresh algal material, specifically markers of diatoms, cyanobacteria, and green algae (Table 2, Fig. 2), which closely matches regional periphyton compositional analysis (Gaiser et al. 2006).

Based on similarities of fatty acid composition revealed by hierarchical cluster analysis, algal energy pathways (periphyton) appear to dominate for ephemeropteran larvae and *P. latipinna* (Fig. 3). Ephemeropteran larvae tissue contained high abundances of 14:0, 16:1ω7, 20:5ω3, and 18:3ω3, implying in particular that fresh diatomaceous and cyanobacterial organic matter is a dominant food source assimilated by consumers in the freshwater marshes of the Everglades (Table 2). The close association between *P. latipinna* and periphyton and mayflies in the cluster analysis, suggests that, ultimately, algal basal resources make up its food supply. Our data cannot rule out direct feeding on mayfly larvae, and in fact, the highly depleted carbon isotopic composition of *P. latipinna* (Fig. 1), with similar values to ephemeropteran larvae, readily suggests it. These findings are consistent with results from gut content analysis of this species that indicate they are primarily herbivores, but also consume some invertebrate prey (Harrington and Harrington 1961; Loftus 1999). The highly depleted carbon isotopic composition of *P. latipinna* and ephemeropteran larvae may also suggest contributions from methane-derived carbon in Everglades' food webs, as was demonstrated by highly depleted $\delta^{13}C$ values for *Daphnia longispina* feeding on methane-oxidizing microbes in humic lakes (Kankaala et al. 2006). Laboratory feeding experiments and focused fatty acid analysis on phospholipid markers of methane oxidizing microbes would help constrain the importance of methane-derived carbon in this system.

Fatty acid composition suggests that detrital energy pathways are important for chironomid larvae. Chironomids

contained the 14:0 and 16:1ω7 acids that were also found in ephemeropteran larvae, but also contained elevated levels of bacterial fatty acids (especially iso- and anteiso-C_{15} branched acids). Bacteria living on decaying organic matter (i.e., microbial conditioning of allochthonous organic matter (Barlocher and Kendrick 1975)) likely contribute considerably to the diet of chironomids, along with smaller amounts of algal material either directly from periphyton or periphyton detritus in the floc. Because whole organisms were analyzed in this study, we cannot ignore that a fraction of the bacterial fatty acids present in the animal tissue derive from the digestive tract of the organism, and not from dietary sources. However, the pattern of C_{15} fatty acids in the chironomids matches that of floc, with abundant proportions of the iso branched form, followed by the anteiso form, and lowest proportions of the normal (unbranched) fatty acid. No other invertebrates analyzed here contained C_{15} fatty acids at the same abundances, and the distribution of C_{15} fatty acids varied in the other invertebrates, with the normal chain either dominant or equal to the iso form (see Electronic Appendix). These findings strongly support the presence of the C_{15} fatty acids in the chironomid larvae as indicators of floc contributions to their diet. Amorphous detritus has previously been shown to dominate chironomid diets in saline wetlands, although this finding was site-specific, with diatoms also as dominant food sources for these consumers (Hart and Lovvorn 2003). The low levels of C_{16} polyunsaturated fatty acids and 20:5ω3 in chironomids found in this study suggest minor consumption of diatomaceous organic matter at the site where we collected them. It is important to note that our samples were a mixture of chironmid taxa found at the site when we visited; future studies should attempt to separate these at least at the subfamily level, though it will be challenging to do this and obtain enough homogeneous material for analysis.

Despite the strong similarities in fatty acid composition between periphyton and ephemeroptera larvae and floc and chironomid larvae, stable carbon isotopic analysis revealed that the consumers were considerably more isotopically depleted than their food sources (Fig. 1). It is possible that an important dietary component (i.e., one that is much more deplete in ^{13}C than the basal resources) not evaluated in this study is being assimilated in both mayfly and chironomids. Higher plants are one potential source of organic matter that was not investigated in this system, and those plants that use the C_3 photosynthetic pathway do tend to have highly depleted carbon isotopic compositions. However, given that the range of $\delta^{13}C$ for sawgrass in the Everglades is –24.5‰ to –30.1‰ and for cattail is –26.4‰ to –28.7‰ (Chang et al. 2009), and that macrophyte inputs to detritivores in saline wetlands were estimated to be minimal (Hart and Lovvorn 2003), this suggests that higher plant input to insect diets cannot account for the strongly depleted isotopic composition of their tissue. Furthermore, no long-chain fatty acids, typical of higher plant leaf waxes, were present in the insects, suggesting that even if vascular plant material is part of their diet, ephemeropteran and chironomid larvae lack the necessary enzymes to assimilate this long-chain organic matter. Selective feeding of specific fractions of periphyton or floc may partially explain the highly depleted isotopic values found for chironomids and mayflies. For example, Mead et al. (2005) determined that $\delta^{13}C$ of *n*-alkanes in *Utricularia spp*., a submerged aquatic plant found widely in freshwater marshes of this region, were highly depleted, ranging from –39.1‰ to –41.9‰. Similarly, the $\delta^{13}C$ of a C_{20} highly-branched isoprenoid, a biomarker for periphyton and possibly the cyanobacterial portion of Everglades periphyton, was also quite deplete, ranging from –36.7‰ to –36.9‰ (Jaffé et al. 2001). Although there is some degree of fractionation between bulk organic matter and hydrocarbons, it is possible that selected portions of periphyton or other plant material could be accounting for the strongly deplete carbon isotopic signature of the emphemeropterans and chironomids.

The invertebrates *H. azteca* and *P. paludosus* contained similar distributions of the ubiquitous 16:0, 18:0, and 18:1ω9, bacterial markers, and algal polyunsaturated fatty acids, yet were not found to be closely associated with periphyton or floc, or the two types of insect larvae, in the statistical analysis (Figs. 3, 4). In spite of this, several specific fatty acids point to periphyton herbivory and floc detritivory for these consumers. High levels of 20:5ω3 in *P. paludosus* suggest diatoms as a major source of organic matter, either directly from periphyton, or through detritivory of the decaying algal components in floc. *H. azteca* contained greater abundances of bacterial and vascular plant fatty acids relative to *P. paludosus*, implying a greater contribution of floc to the amphipod diet. Selective feeding on specific fractions of floc and detritus, as has been shown for amphipods and chironomids feeding on diatoms and amorphous detritus (Hart and Lovvorn 2003), or preferential retention of specific lipids based on metabolic requirements may cause this apparent discrepancy between the overall basal resource fatty acid composition and the consumer fatty acid composition. The dissimilarity is mainly driven by increased relative abundances of C_{20} polyunsaturated fatty acids (20:4ω6 and 20:5ω3), 18:1ω9, and 18:0n, and decreased abundances of 16:0n and 16:1ω7 in the consumers compared to the basal resources. These fatty acids derive from multiple sources, with 16:0 and 18:0 being non-specific and ubiquitious, 16:1ω7 and 20:5ω3 generally considered as diatom markers, 18:1ω9 typically high in green algae and dinoflagellates, although also ubiquitous, and 20:4ω6 attributed to a variety of sources in freshwater systems, including green algae and aquatic insects (Napolitano 1999). Considering that the two major diatom fatty acid

markers (20:5ω3 and 16:1ω7) exhibit contrasting behavior from food source to consumer, it is likely that preferential retention of 20:5ω3, instead of selective feeding on diatomaceous fractions of periphyton, is accounting for the variability between overall lipid compositions of basal resources and *P. paludosus*. Two of the fatty acids that were present in increased abundances in the consumer tissue, 20:5ω3 and 20:4ω6, together with 22:6ω3, 18:2ω6, and 18:3ω3 are considered essential for proper growth, reproduction, and survival (Arts et al. 2001). Additionally, 20:5ω3 and 20:4ω6 are precursors to the eicosanoids, a class of hormones that play important roles in inflammation, immunity, energy allocation, and reproductive success (Koussoroplis et al. 2011). As such, these fatty acids may be preferentially retained or converted in de novo synthesis through carbon chain elongation and desaturation (Hall et al. 2006). Koussoroplis et al. (2011) demonstrated that proportions of C_{20} polyunsaturated fatty acids in basal resources and consumers increased with increasing trophic level, although they were unable to determine if invertebrates were undergoing selective feeding on essential fatty acid-rich diet components or enhanced retention and/or bioconversion.

The other primary consumer investigated in this study were *Haitia spp.* snails, which with the exception of the ubiquitous 16:0 and 18:0 fatty acids, had a unique distribution of fatty acids compared to other consumers, characterized by relatively high abundances of 4,8,12-trimethyltridecanoic acid (trimethyl-13:0), 18:1ω7, 20:4ω6, 20:1ω9, C_{22} polyunsaturated fatty acids (see Electronic Appendix). The trimethyl-13:0 fatty acid has been shown to be a produced by the bacterial breakdown of phytol, a side chain of chlorophyll (Rontani et al. 1999). The 20:1ω9 fatty acid, although frequently used as a marker for herbivorous zooplankton in the marine environment (Dalsgaard et al. 2003), is also an important constituent of plant seed oils (Hopkins and Swingle 1967). Together, these two fatty acids suggest that plant material is therefore the likely source of organic matter for *Haitia* snails, and further characterization of plant and algal material in the Everglades freshwater marshes is necessary to provide a more rigorous analysis of this snail's diet.

In contrast to the basal resources and invertebrates, secondary consumers (fish) were not resolved by statistical analysis to the genus or species level based on fatty acid composition, with the exception of *P. latipinna*, as discussed above, and *L. marginatus* and *L. punctatus*, which formed a unique branch among the fish group at a similarity of ~90% (Fig. 3). Fatty acid composition was similar between *G. holbrooki, L. goodei, H. formosa,* and *J. floridae*, and species-level as well as site distinctions were not apparent (Figs. 3 and 4). In general, an invertebrate diet was suggested for these omnivorous fish, based on their close association to *P. paludosus* and *H. azteca*. The small proportions of vascular plant-derived fatty acids present in these fish coupled with the general lack of these fatty acids in the insects and invertebrates analyzed here suggests that some grazing, perhaps unintentional, of vascular plant material or floc occurs. This floc/plant foraging was most pronounced for *P. latipinna* and fish genus *Lepomis*, which contained the highest levels of vascular plant-derived fatty acids in all consumers. For some fish species, particularly at sites adjacent to the canal, $\delta^{15}N$ values were lower than those found for primary consumers and basal resources (Fig. 1). It is possible that the isotopic composition of primary consumers and basal resources do not represent the spatial variability encompassed in the isotopic composition of higher, more mobile consumers which may have fed in a completely different environment prior to arriving at the sampling location. However, because this trend was largely restricted to species where only one or two individuals were captured (*J. floridae, L. marginatus, L. punctatus*), it is more likely that intraspecies variability is the cause of this anomaly. Clearly a greater sample number is necessary for interpreting the trophic level of individual fish species based on $\delta^{15}N$. Similarly, temporal variability in basal resource isotopic composition, an issue greater than the scope of this study, should also be investigated.

Sub-sites near the canal were enriched in P, as noted by periphyton TP as opposed to water column TP (Gaiser et al. 2004), compared to the far sub-sites (Table 1). At the adjacent sites, TP in periphyton and floc approached or exceeded the enrichment threshold (Gaiser et al. 2004) and was, on average, higher than periphyton and floc from the interior sites (Table 1). Nutrient enrichment at the sites adjacent to the canal was also demonstrated by the 6–9‰ difference in $\delta^{15}N$ between basal resources and primary consumers (amphipods) found adjacent to the canal versus far from the canal (Fig. 1). P enrichment led to increased $\delta^{15}N$ signatures for macrophytes and soils in the northern Everglades due to the increased N demand and subsequently, reduced N fractionation (Inglett et al. 2007). The strong difference in resource and primary consumer $\delta^{15}N$ between adjacent and far sites found here likely represents a similar process and lends further support for P enrichment due to the canal. Although P enrichment enhances the nutrient content (quality) of periphyton, enrichment also results in a loss of periphyton biomass as the community shifts from calcifying cyanobacteria to more filamentous green algae and diatoms (McCormick et al. 2001; Gaiser et al. 2006).

Despite the nutrient enrichment from the canal, site differences (adjacent versus far sub-sites) in periphyton and floc composition were less obvious than the biochemical compositional differences between periphyton and floc (Fig. 2), although hierarchical cluster analysis did separate periphyton from adjacent and far sites (Fig. 3). The first sub-

site adjacent to the canal (A1) appears to have experienced the most nutrient enrichment, with increased abundances of 18:3ω3 and 20:5ω3 compared to the other two adjacent sites, and the highest TP content, which implies the highest nutritional quality of periphyton. Strong differences in the fatty acid compositions of consumers, especially fish, between adjacent and far sites were not apparent; this implies that consumer mobility may be great enough to overcome spatial differences in resource quality.

Conclusions

Assessing the relative importance of algal and detrital energy pathways in freshwater ecosystems remains challenging. Although our data generally suggest that mixed algal and detrital organic matter fuel secondary productivity in this freshwater marsh, analysis of fatty acid composition revealed that detritus, in the form of flocculent material, appears to be most important for chironomids and amphipods in this wetland ecosystem. Furthermore, the similarity of microbial markers in the chironomids and floc suggests that microbes likely form the link between the floc and consumers. Algal energy pathways appeared most important for *P. latipinna*, with ephemeropterans as a possible trophic link in addition to grazing. The similarity in fatty acid distribution between periphyton and floc precluded quantitative estimates of the relative importance of each basal resource to higher trophic levels. Future work incorporating controlled feeding studies together with techniques such as compound-specific stable carbon isotopic composition (Copeman et al. 2009; Williams et al. 2009) may help further clarify the roles of algal and detrital energy pathways in larger food-web studies.

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