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Broad sampling and diverse biomarkers allow characterization of nearshore particulate organic matter

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Suspended particulate organic matter (POM) is a primary food source for benthic and pelagic consumers in aquatic and marine ecosystems. POM is potentially composed of many sources including phytoplankton, bacteria, zooplankton and macrophyte (seaweed and seagrass) and terrestrial detritus. The relative importance of these sources to POM consumers is debated, in large part due to differing interpretations of stable isotope and fatty acid biomarkers. We investigated POM composition in a nearshore marine ecosystem using multiple methods including visual quantification of living and detrital components, multiple stable isotope (MSI) and fatty acid (FA) analyses. Sampling was conducted at multiple temporal and spatial scales to 1) determine the range of variability in POM biomarkers, 2) quantitatively evaluate $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and FA biomarkers with proportional abundance of putative sources and 3) determine the availability of phytoplankton, macrophytes and terrestrial carbon in nearshore POM. Variation of total FA concentration and proportions, and $\delta^{13}C$ and $\delta^{34}S$ were strongly correlated to phytoplankton abundance, at tidal and seasonal timescales. Using multivariate multiple regressions, MSI and FA explained 59.6% and 89.7% of the variation in POM composition, respectively. As phytoplankton abundance increased, total FA concentration increased concurrent to $\delta^{13}C$ and $\delta^{34}S$ enrichment. In high detritus samples, bacterial FA and saturated FA were proportionally higher, corresponding to depletion of $\delta^{13}C$ and $\delta^{34}S$ and enrichment of $\delta^{15}N$. We identify MSI and FA biomarkers that are good predictors of diatom, dinoflagellate and detrital contributions to the POM. The results of this multi-scale study show that POM composition is highly dynamic and largely driven by phytoplankton abundance, with minor contributions from terrestrial or macrophyte subsidies. This quantitative approach provides novel and critical empirical information linking POM compositional dynamics to specific biomarkers that are commonly used for tracking energy subsidies and biogeochemical cycling in aquatic ecosystems.

Suspended particulate organic matter (POM) is a major energetic pathway in aquatic environments, linking primary producers and detritus to benthic and pelagic suspension feeders and detritivores in a broad range of habitats (Polis et al. 1997, Kreeger and Newell 2001). POM is potentially composed of phytoplankton, zooplankton, bacteria, detritus from benthic macrophytes (Duggins et al. 1989, Bustamante and Branch 1996, Kaehler et al. 2006), and detritus of terrestrial origin (Pace et al. 2004, Yamanaka et al. 2013). Effective methods for quantifying the relative contribution of each of these disparate resources to consumers are of great importance for determining connectivity among ecosystems, as energy subsidies from neighboring environments strongly influence ecosystem dynamics (Polis et al. 1997). Owing to the heterogeneous composition of POM and the wide variety of particle sizes available to consumers, researchers commonly use biomarkers such as multiple stable isotopes (MSI) and fatty acids (FA) to identify the contribution of various energy sources to freshwater and marine food webs (Peterson and Fry 1987, Kelly and Scheibling 2012). This approach is based on each primary producer having characteristic proportions, or specific ratios of MSI and FA that can be traced to higher trophic levels. Using MSI and FA in combination allows tracking of carbon subsidies as well as FA only synthesized by primary producers and required by heterotrophic organisms (essential fatty acids). Many studies have investigated the trophic role of detrital carbon and FA, however there is debate over the importance of terrestrial and macrophyte detrital sources versus phytoplankton to POM consumers in freshwater (Brett et al. 2009) and marine ecosystems (Miller and Page 2012).

A fundamental challenge for tracking basal resource utilization in aquatic food webs is defining the biomarker signatures and quantifying natural variation of all potential sources available to consumers. This is particularly important when using biomarker mixing models to estimate source contribution (Dethier et al. 2013). In nearshore marine ecosystems, POM can originate from marine, freshwater and terrestrial sources connected by coastal rivers. MSI and FA signatures of primary producers in all of these environments

are influenced by nutrients, growth rate, growth substrate (e.g. DIC or DOC), bacterial degradation and other environmental factors that make quantifying precise signatures difficult (Degens et al.1968, Savoye et al. 2003, Piepho et al. 2012). While the average biomarker signatures of potential contributors of organic matter often differ enough to enable identification of unique sources (Fry and Sherr 1984, Budge and Parrish 1998, Kelly and Scheibling 2012), the ranges of MSI and FA for many of the components of the POM can overlap. Under these circumstances, differentiation is impossible (Cloern et al. 2002); thus studies with limited scale may not be able to account for the variability of source signatures (Fry and Sherr 1984, Vuorio et al. 2006, Guest et al. 2010). A factor contributing to this challenge is that sampling of end-member sources often occurs in one season even though considerable temporal variation exists in many primary producers (Cloern et al. 2002, Dethier et al 2013). If left unaddressed, the contributions of disparate energy sources cannot be resolved using biomarkers.

Another challenge for studies of POM is differentiating biomarker signals of detrital sources from in situ phytoplankton production (Vuorio et al. 2006). Phytoplankton are the dominant pelagic primary producers, contributing considerable living and detrital biomass to the POM, but are extremely difficult to physically separate from other suspended particulates of similar size and density. Therefore, phytoplankton endpoints are often determined with alternative methods, such as cultures, size or density-dependent fractionation or collecting samples offshore at a distance sufficient to reduce the influence of benthic macrophytes and river runoff. Values determined under these conditions may not reflect pure phytoplankton from the nearshore growing environment and may bias food web models (Miller and Page 2012). Previous studies have provided presence–absence information for a given phytoplankton taxon and putative biomarkers, however, few have quantified the relationship between phytoplankton abundance or composition and stable isotopes ($\delta^{13}C$, $\delta^{15}N$; Savoye et al. 2003, Miller et al. 2013) or FA (16:4, Claustre et al. 1989) in natural settings.

Studies exploring carbon isotopic signatures in phytoplankton long ago observed temperature-dependent fractionation (Sackett et al. 1965), especially as a function of carbon availability and growth rate (Degens et al. 1968, Deuser et al. 1968). Observations of bloom succession have qualitatively related MSI and FA of POM to diatom or dinoflagellate abundance (Jeffries 1970, Budge and Parrish 1998, Ramos et al. 2003). Similar qualitative comparisons linked the presence of kelp-derived particles to $\delta^{13}C$ enrichment of POM (Bustamante and Branch 1996, Kaehler et al. 2006). While the combination of MSI and FA is common in biomarker studies (Rothlisberg et al. 1994, Volkmann et al. 2008), to our knowledge, no quantitative investigation of the relationships among variation in POM constituent composition (including detritus), MSI ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) and FA signatures has been conducted. The general lack of empirical comparisons of biomarkers with putative sources across the natural range of environmental and biological variation limits the effective application of biomarkers in consumer studies. Quantifying variation in a suite of MSI and FA that are related to changes in the relative abundance of POM components allows investigators to determine the food sources available to consumers. These patterns can then be compared to consumers in a specific area. The combination of techniques comparing multiple biomarkers offers a method for validating hypotheses based on a single biomarker, while increasing the resolution of sources used in mixing models (Connelly et al. 2004, Volkmann et al. 2008, Dethier et al. 2013).

The goals of the current study were to investigate variation of living and detrital POM components over multiple temporal and spatial scales to determine the relative availability of autochthonous (phytoplankton, macrophytes) and allochthonous (terrestrial material) energy sources to a nearshore marine system. We collected detailed POM composition data to empirically evaluate MSI and FA biomarkers commonly reported in the literature for sources of POM. This study took place in the San Juan Archipelago (SJA) and Pacific coast of Washington, a temperate marine environment influenced by freshwater inputs from coastal rivers. The region has diverse and abundant macrophyte assemblages (Galloway et al. 2012) and phytoplankton blooms in spring and summer. Sampling was designed to incorporate variability associated with tidal, seasonal and interannual changes in the oceanographic conditions (Moore et al. 2008) and the expected abundance of terrestrial and marine POM sources. We tested the hypothesis that marine macrophytes and terrestrial detritus provide seasonally important energy subsidies to the nearshore environment. An additional goal of the research was to 'dissect' the POM using a combination of microscope and biomarker-based approaches to evaluate the use of various source-tracking biomarkers commonly utilized in food web mixing models (Duggins et al. 1989, Hondula and Pace 2014). We show that, when applied across the range of natural variation and compared across ecosystems, these simple techniques provide valuable information regarding energy subsidies.

Methods

Sample collection

Seasonal POM samples were collected in summer (August), winter (December) and spring (April–May) from summer 2010 through winter 2011. Sampling occurred at three sites within the San Juan Archipelago, WA: Pillar (PIL), Skipjack Island (SKP) and Pt. Caution (PTC) (Fig. 1). The sites experience differing influences of freshwater (primarily from the Fraser River in British Columbia, Canada) and oceanic water from the Strait of Juan de Fuca. SKP is most heavily influenced by freshwater runoff. PIL is more characteristic of the Strait of Juan de Fuca water mass and PTC is intermediate between the other two based on surface temperature and salinity. To verify this assumed relationship, we measured in situ temperature with Hobo data loggers, suspended with a subsurface buoy at ~6 m depth at each site: average temperature and standard deviation during Summer 2011 and 2012 were lowest at PIL (10.24 ± 0.80°C), intermediate at PTC (10.76 ± 1.14°C) and highest at SKP (10.94 ± 1.33°C). Seasonal sampling was structured to capture spatial and temporal variability on multiple scales. During each season, samples were collected from each site in each of four days

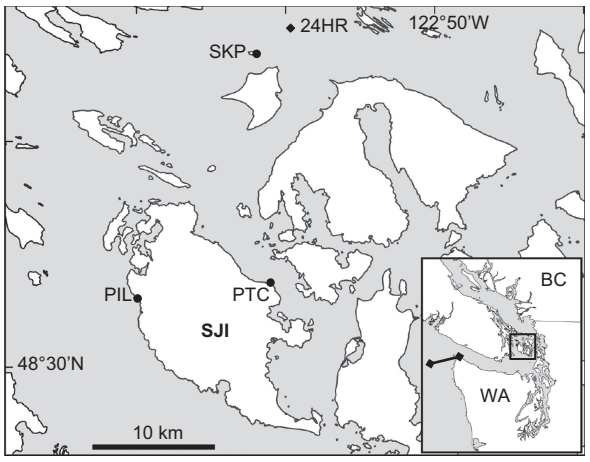

Figure 1. Sampling was conducted primarily within the San Juan Archipelago, WA. Seasonal samples were collected at Pillar (PIL), Skipjack Island (SKP) and Pt. Caution (PTC) during the same day. The 24-h (24HR) sampling was conducted aboard an anchored vessel north of Skipjack Island. The path of the offshore transect is shown in the regional inset and extended 40 miles offshore from Cape Flattery, WA.

evenly distributed over a spring and neap tide cycle (~14 days). During each sampling day, all three sites were sampled in the same order at approximately the same time of day. The time of sampling was fixed at each site in order to sample across multiple tide stages. This resulted in an even distribution of samples collected in slack, ebb and flood tides.

At each site, an oblique tow was conducted perpendicular to shore starting at approximately 25 m from shore. A 0.25 m diameter, 20-µm phytoplankton net was towed from 11 m depth to the surface. Tows were on average 10 min long, but ranged from 5 to 15 min depending on environmental conditions and phytoplankton biomass. After the tow, the contents of the codend were transferred to a clean sample jar through a 190-µm Nitex mesh pre-filter to remove zooplankton, except for August 2010 in which a 500-µm pre-filter was used. Generally, little material was left on the pre-filter. Surface temperature and salinity measurements were taken at the midpoint of the transect with a temperature/conductivity meter. When possible, water samples were collected at the surface (0.5–1 m depth) for spectrofluorometric chlorophyll a (Chl a) analysis (Jeffrey and Humphrey 1975). Sample jars were stored on ice in the dark until all three sites were sampled, then immediately returned to the Friday Harbor Laboratories (FHL) and filtered. Prior to filtration, a 3-ml aliquot from the concentrated POM sample was taken for visual analysis and preserved in 1.7% paraformaldehyde. The remainder of each sample was filtered onto a pre-fired (400°C for 4 h) Whatman GF/F or Millipore Quartz fiber filter. GF/F filter material interfered with MSI analyses of sulfur in particular and made it difficult to determine exact POM mass used in FA extractions. Therefore, we filtered a large volume of concentrated sample in order to gather enough biomass to be removed from the filter. The POM was gently removed from the filter and frozen for stable isotope or fatty acid processing. Thus, MSI, FA and visual analyses reflected the same concentration of material from each sample.

In addition to the seasonal sampling, two cruises were conducted to investigate POM variability at different scales. Sampling in May 2012 investigated small-scale temporal variability related to tidal mixing and advection of water masses. The R/V Centennial was anchored north of Skipjack Island in Boundary Pass (48°44′39.8″N, 123°0′19.6″W; Fig. 1) during a spring tide with a 2.8 m predicted tidal exchange. The site was selected to maximize exposure to freshwater runoff from the Fraser River on the ebb tide and oceanic water from the Strait of Juan de Fuca on the flood tide, yet to still be comparable to seasonal sampling sites. Every two hours over a 24-hour period, a CTD (conductivity, temperature, density) cast was conducted from the surface to approximately 10 m above the bottom (~90–100 m). The CTD gathered continuous temperature, salinity and Chl a data. Surface Chl a measurements are presented to be consistent with previous sampling. Concurrent to the CTD cast, a phytoplankton tow was conducted from 11 m to the surface to collect material for MSI, FA and visual analyses. Material from the codend was subsampled for visual analyses and the remainder was filtered and frozen immediately after each tow.

In July 2011, an offshore transect was conducted aboard the R/V Centennial, with the goal of investigating differences in MSI, FA and phytoplankton/detrital community composition with distance from sites of nearshore macrophyte productivity. Samples were collected in the Strait of Juan de Fuca (Neah Bay 48°14′96.0″N, 123°14′77.4″W; western Strait 48°22′58.9″N, 124°34′14.1″W) and at increasing distances from a *Nereocystis luetkeana* bed at Cape Flattery, WA (Fig. 1 inset). A bilge pump was used to collect water within the kelp bed throughout the entire water column. Net tows were conducted at 1, 3, 18, 28 and 40 miles offshore of the kelp bed. Subsamples were collected for visual analysis before filtration and freezing as in seasonal samples. Salinity was measured at each site, but an equipment malfunction prevented measurement of temperature. Chl a samples were collected from the surface at all sites and analyzed as in seasonal samples.

Visual quantification of composition

Our initial questions involved the contribution of macroalgal detritus to the POM. While detritus particles were extremely abundant in all samples, the size and shape of particles varied considerably and it was impossible to visually identify their sources. We therefore developed a point count method to estimate the relative proportion of phytoplankton, zooplankton and detritus in a sample based on the assumption that surface area (herein bio-area) would more accurately approximate the relative biomass than simple counts. We used two methods to visually quantify POM composition: cell counts (CC) to quantify taxonomic composition of living organisms, and point counts to estimate the relative percent bio-area of living phytoplankton and detritus. Both CC and bio-area were conducted with a Palmer–Maloney counting chamber and compound microscope. For cell counts, 300 intact cells were identified in each of three replicate subsamples. All cells were identified to genus. For bio-area, phytoplankton were identified to genus, and detritus was divided into four general categories: organic (pigmented,

'fuzzy' material including fecal pellets), inorganic (sediment-like, crystalline), biogenic inorganic (diatom frustules, spicules, lacking organic material), and unidentifiable small detritus (any small particles that did not fit the other descriptions). In each of three subsamples, all particles beneath the intersections of a 10×10-square gradicule installed on a compound microscope were identified until at least 100 objects were counted. The total number of intersections counted was recorded and the relative area (objects per category divided by total objects) of each category calculated. The method was developed with samples from different seasons to account for variability in detrital material. All samples were analyzed by the same observer to ensure consistent identification of phytoplankton and detritus.

Stable isotope analysis

Material used for MSI and FA analyses was freeze-dried and ground into a homogeneous powder. Two and eight mg dry biomass was required for carbon/nitrogen and sulfur isotope analysis, respectively. Samples were analyzed by the Washington State University Isotope Core Lab. Carbon isotopic ratios were calculated relative to Vienna Peedee Belemnite (VPDB) standard. Nitrogen isotope ratios were calculated relative to air and sulfur isotope ratios relative to Vienna Cañon Diablo Troilite (VCDT). The ratios of heavy to light isotope are expressed as $\delta x = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where $\times$ is carbon, nitrogen or sulfur and R is $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$.

Fatty acid analysis

FA analysis was conducted on seasonal samples from spring, summer and winter 2011 and all samples from the 24-h sampling and the offshore transect. Total FA were extracted from 10 mg of dry material when possible, except for low biomass samples for which 6–10 mg of material was used using a modified-Folch method (Taipale et al. 2011). Two ml of chloroform were added, followed by 1 ml of methanol, 1 ml of 2:1 chloroform: methanol mix, and 0.5 ml of DI water. This mixture was sonicated for 10 min, vortexed for 30 s, and finally centrifuged for 3 min at 3000 rpm to separate the phases. The lower (organic) phase was removed to a separate culture tube. An additional 3 ml of chloroform was added to the original sample and the process of sonication, vortexing, centrifugation, and removal was repeated two more times. The extracted organic layers were pooled and evaporated under nitrogen. Once dry, 1 ml of toluene and 2 ml of a 1% solution of sulfuric acid in methanol were added to re-suspend the lipids. This mixture was vortexed to ensure uniformity, and then placed into a 50°C water bath for 16 h to allow for FA methylation. Once cooled, 2 ml of 2% $KHCO_3$ solution and 5 ml of 1:1 hexane: diethyl ether were added. The mixture was vortexed for 10 s and centrifuged for 2 min at 1500 rpm to separate the phases. The organic layer was transferred to a new tube. To ensure total removal of fatty acid methyl esters (FAME), we added a further 5 ml of hexane: diethyl ether, then vortexed, centrifuged, and removed the upper phase into the same tube. These organics were evaporated under nitrogen, and the FAME dissolved into hexane for GC analysis.

Chromatograms were generated from a GC-FID, with an Agilent DB-23 column, and an 85-min method designed to separate 16 and 18 monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Taipale et al. 2011). Fatty acid peaks were identified by comparing retention times and relative areas with several standards and samples run on GC-MS, under the same method and analytical conditions. To quantify FA concentration (mg FAME g POM^{-1}), dilutions of a 569B standard (Nu-chek Prep) were analyzed with the GC-FID. Areas from the chromatogram were correlated to FAME concentrations (0.0025, 0.05, 0.1, 0.25, 0.5, 1 and 2 mg ml^{-1}) to determine a regression from which the concentration of FAME could be found after accounting for hexane volume and dry weight extracted.

Statistical analysis

Variation of MSI, FA proportions and POM composition related to spatial and temporal scales were tested with PERMANOVA in PRIMER 6 (Anderson et al. 2008). Factors in the PERMANOVA analyses were season (fixed), date (random, nested within season) and site (random, nested within season). Seasonal and 24-h samples were combined for quantitative comparisons of regional SJA POM composition and biomarkers. Genera in community composition datasets and individual FA that were present in fewer than five samples ($<7.5\%$ of samples) and made up less than or equal to 0.5% were removed from multivariate analysis. MSI data were normalized for multivariate analyses. Proportional FA, CC and bio-area data were arcsine-square root transformed. FA concentration data were square root transformed. Chl a was log-transformed. Correlations between the full multivariate composition (cell count: 35 taxa, bio-area: 26 taxa) and biomarker ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ and 35 FA) datasets were tested with the RELATE function in PRIMER. RELATE compares resemblance matrices to determine correlations among sample variation.

We investigated the relationships among POM composition and MSI and FA variables using distance-based linear models (DISTLM) (multivariate multiple regressions) in PRIMER ver. 6 (McArdle and Anderson 2001). A matrix was created with bio-area for diatoms (summed across genera), dinoflagellates (summed), and the four POM detritus categories. The DISTLM routine determined which MSI and FA explained the greatest amount of variation in POM community composition. Separate analyses were conducted for MSI and FA as these metrics co-vary. The individual MSI and FA identified in the DISTLM were then used to evaluate biomarkers for specific components of POM. In addition, biomarkers commonly associated in the literature with phytoplankton, bacterial, terrestrial or macroalgal components that were not identified in the DISTLM analyses were also compared to POM composition. FA were grouped into the categories of saturated fatty acids (SAFA), MUFA and PUFA to investigate dynamics in these FA groups with distinct physiological functions. We also summarized concentrations of long chain 'essential' FA (EFA; $\Sigma \geq C_{20}$ ω-3 and ω-6 FA), which are physiologically important FA which heterotrophs cannot synthesize de novo (Dalsgaard et al. 2003). Regression analyses then tested the relationships between biomarkers and diatoms, dinoflagellates or detritus. We separated dia-

toms and dinoflagellates for this analysis as these groups produce different FA and are often investigated separately in food web studies. The best-fit, positive relationships were reported under the assumption that an effective biomarker will increase proportionally to its source. Because detrital particles could not be assigned to a specific source, organic, inorganic and unidentified small detritus were summed for regressions. Biogenic inorganic detritus was excluded because of its known origin and positive correlation to diatom bio-area. Univariate comparisons were made with Welch's t-tests to allow for unequal variances. The relationship between $\delta^{13}C$ and Chl a was compared to data from another study of POM from the west coast of North America (Miller et al. 2013).

Results

Variation of nearshore POM

We included 35 taxa in the seasonal POM cell counts representing 25 diatom genera, 8 dinoflagellate genera, zooplankton and *Dictyocha* (Actinochrysophyceae). Diatoms numerically dominated cell counts in all seasons. Total phytoplankton bio-area exhibited a highly significant relationship to Chl a ($r^2 = 0.85$, $F = 185.82$, $p < 0.001$), indicating the point count method successfully approximated phytoplankton biomass. Diatoms represented a small fraction of POM in winter, but were seasonally abundant in spring and summer ranging from 17–39% of bio-area (Table 2, Fig. 2). The most common diatom taxa were *Skeletonema* spp., *Chaetoceros* spp. and *Thalassiosira* spp. Dinoflagellates represented < 1% of cells in spring 2011, spring 2012 and summer 2010, < 5% in winter 2010 and 2011 and approximately 20% in summer 2011 (Table 2). Detritus was extremely abundant in all samples ranging from 32 to 99% of bio-area and varied in composition among seasons (Fig. 2). Overall, POM community composition clearly varied among seasons using cell count (CC) or bio-area (PERMANOVA, $p < 0.001$ for both). There was considerable small-scale temporal and spatial variation in POM community composition as well. Within season, POM community composition varied significantly with date and site for both CC ($p < 0.002$ and $p < 0.003$, respectively) and bio-area ($p < 0.008$ and $p = 0.048$, respectively).

The biomarkers showed similar patterns of variation to community composition. POM MSI differed significantly

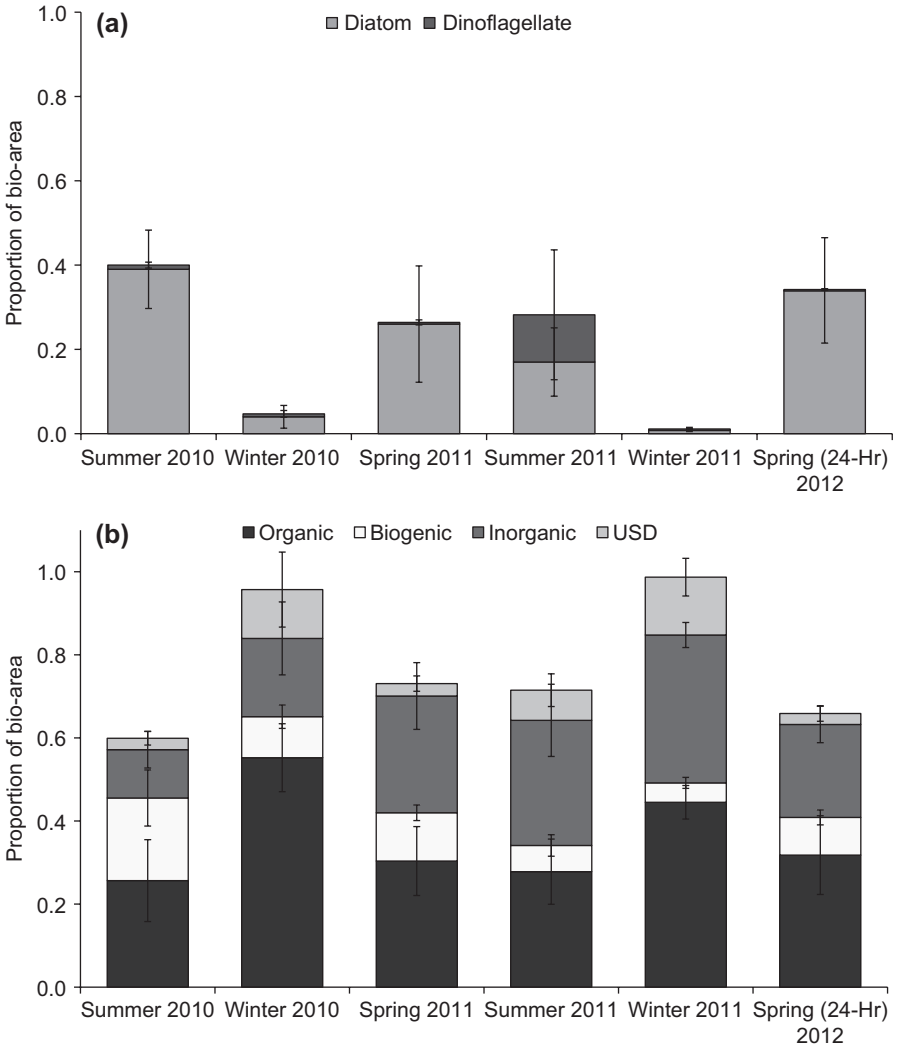

Figure 2. Seasonal mean (± SD) bio-area of (a) diatoms and dinoflagellates and (b) detrital categories from visual analysis of POM composition. See methods for definitions of detrital categories. USD = unidentified small detritus.

among seasons (PERMANOVA, $p < 0.001$, averages in Table 1). Within season, there was a highly significant effect of date ($p = 0.001$), however there was no effect of site ($p = 0.572$). Fatty acid signatures also differed significantly among seasons (PERMANOVA, $p < 0.001$). Within season, there was a significant effect of date ($p < 0.001$), but no effect of site ($p = 0.799$). Total FA concentrations in the samples differed among seasons (ANOVA, $F = 62.32$, $p < 0.001$), ranging from a low of 1.53 mg g^{-1} POM in winter to 16.22 mg g^{-1} POM in summer. The proportions of FA classes also varied among seasons (Fig. 2): PUFA and ω-3 FA were highest in summer, SAFA increased in winter as PUFA decreased. ω-6 FA generally represented a small proportion of total FA.

Comparisons among biomarkers and POM composition

Comparisons of the different methods of analyzing POM composition (cell count, bio-area, FA and MSI) showed strong correlations among these datasets. The cell count and bio-area methods of quantifying POM were strongly correlated (RELATE, rho = 0.75, $p = 0.001$). Both cell count and bio-area were significantly correlated to FA (rho = 0.74, $p = 0.001$ and rho = 0.71, $p = 0.001$, respectively) and MSI (rho = 0.44, $p = 0.001$ and rho = 0.50, $p = 0.001$, respectively). MSI and FA were also significantly correlated (rho = 0.59, $p = 0.001$). Since the resolution of MSI and FA is not generally expected to distinguish among fine taxonomic groups (e.g. genera of diatoms), further analyses were focused on summaries of the community components. A substantial portion (59.6%) of the variation in POM community composition could be explained by values of the three stable isotopes, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (DISTLM). A greater proportion of POM community variation was explained by FA variables (89.7%, using the 20 FA listed in Table 4).

To gain a more detailed understanding of the taxa and biomarkers that drive these relationships, we sought correlations between individual MSI and particular phytoplankton groups (diatoms, dinoflagellates, total phytoplankton). $\delta^{13}C$ was positively correlated to diatom bio-area ($r^2 = 0.53$, $F_{1,65} = 74.29$, $p < 0.001$; Table 4, Fig. 4a) but not to dinoflagellate bio-area ($r^2 = 0.0003$, $F_{1,65} = 0.02$, $p = 0.89$; Fig. 4a). Thus, the total phytoplankton bio-area explained less of the variation of $\delta^{13}C$ in the POM ($r^2 = 0.46$, $F_{1,65} = 54.67$, $p < 0.001$) than diatom bio-area alone. $\delta^{15}N$ was negatively correlated to diatom bio-area ($r^2 = 0.30$, $F_{1,65} = 27.53$, $p < 0.001$; Table 4, Fig. 4b) and showed a marginally significant positive correlation to dinoflagellate bio-area ($r^2 = 0.05$, $F_{1,65} = 3.73$, $p = 0.058$; Fig. 4b). $\delta^{34}S$ was positively correlated to both diatom and dinoflagellate bio-area ($r^2 = 0.57$, $F_{1,65} = 85.41$, $p < 0.001$ and $r^2 = 0.19$, $F_{1,65} = 14.82$, $p < 0.001$, respectively; Table 4, Fig. 4c).

Table 1. Seasonal averages (± SD) of stable isotope ratios.

Season	Year	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Summer	2010	−19.01 ± 0.25	4.99 ± 0.57	20.24 ± 1.05
Winter	2010	−21.06 ± 0.58	6.89 ± 1.61	13.40 ± 5.95
Spring	2011	−18.43 ± 0.48	5.12 ± 0.51	15.59 ± 3.60
Summer	2011	−19.89 ± 0.52	6.53 ± 0.98	18.35 ± 2.33
Winter	2011	−20.89 ± 0.24	7.28 ± 0.46	−0.29 ± 2.06
Spring (24-Hr)	2012	−19.61 ± 0.43	5.82 ± 0.25	15.15 ± 5.03

For fatty acids, total FAME concentration was positively correlated to total phytoplankton bio-area ($r^2 = 0.79$, $F_{1,47} = 175.04$, $p < 0.001$). The samples with the highest total FAME concentration were from summer 2011, coinciding with maximum dinoflagellate abundance (Table 2). SAFA, MUFA and PUFA concentrations all increased with diatom and dinoflagellate bio-area (Table 3, Fig. 5). SAFA and PUFA concentrations showed similar positive relationships to diatom and dinoflagellate bio-area, while the MUFA concentration was more strongly correlated to diatom bio-area than dinoflagellates (Fig. 5). Proportions of FA groups showed different patterns than these total concentrations in the POM. The proportion of SAFA decreased significantly with increasing diatom bio-area (Table 3), but showed no relationship to dinoflagellate bio-area. The opposite was true for MUFA proportions; no relationship was observed to diatom bio-area, but the proportion of MUFA decreased significantly with increased dinoflagellate bio-area (Table 3). The proportion of PUFA in a sample significantly increased with increasing diatom and dinoflagellate bio-area (Table 3).

Individual FA or specific ratios, rather than general summary FA categories may ultimately prove to be better biomarkers for identifying components of POM. Table 4 lists positive correlations between POM community components and FA. This analysis confirmed the utility of Σ16:1 (sum of monounsaturated 16 carbon-chain FA identified in our dataset), 16:2n-4, 16:4n-1 and 18:5n-1 as indicators of diatoms in the POM. The EFA 22:6n-3 (DHA) was the best indicator of dinoflagellate abundance, while 18:5n-3, 18:3n-3 (ALA), c20:0 and 20:5n-3 (EPA) also correlated significantly with dinoflagellate abundance. Ratios of 16:1/16:0 and 18:4/18:1 were correlated with diatom bio-area, while many showed a non-linear relationship to dinoflagellate bio-area. Ratios of 16:1/16:0 and EPA/DHA were consistently < 0.8 and < 1, respectively, when dinoflagellates were abundant (Table 4). The sum of long-chain EFA and the sum of all ω-3 FA were positively correlated to dinoflagellate bio-area. Proportions of bacterial marker FA were positively correlated to detrital bio-area ($r^2 = 0.64$, $F_{1,47} = 81.95$, $p < 0.001$; Table 4). The increase in the proportion of bacterial marker FA was associated with depletion of $\delta^{13}C$ ($r^2 = 0.36$, $F_{1,47} = 26.35$, $p < 0.001$) and $\delta^{34}S$ ($r^2 = 0.75$, $F_{1,47} = 144.48$, $p < 0.001$) and enrichment of $\delta^{15}N$ ($r^2 = 0.22$, $F_{1,47} = 12.96$, $p = 0.001$). The proportion of bacterial marker FA was also negatively correlated to ω-3 FA concentration (mg g POM^{-1}) ($r^2 = 0.50$, $F_{1,47} = 47.33$, $p < 0.001$).

A comparison of MSI values to FA concentrations revealed significant relationships among $\delta^{13}C$ and $\delta^{34}S$ and SAFA ($\delta^{13}C$: $r^2 = 0.22$, $F_{1,47} = 13.48$, $p < 0.001$; $\delta^{34}S$: $r^2 = 0.82$, $F_{1,47} = 208.49$, $p < 0.001$), MUFA ($\delta^{13}C$: $r^2 = 0.0.43$, $F_{1,47} = 35.03$, $p < 0.001$; $\delta^{34}S$: $r^2 = 0.79$, $F_{1,47} = 173.78$, $p < 0.001$) and PUFA ($\delta^{13}C$: $r^2 = 0.17$, $F_{1,47} = 9.42$, $p = 0.003$; $\delta^{34}S$: $r^2 = 0.71$, $F_{1,47} = 114.92$, $p < 0.001$). Both of these isotopes were depleted at very low FAME concentrations and rapidly enriched with increasing FA concentration. $\delta^{15}N$ was not significantly correlated to

Table 2. Seasonal averages (± SD) of phytoplankton from visual analysis of community composition. Proportion of cells represents results from cell count analysis, whereas proportion bio-area represents results from point count analysis.

		Diatoms		Dinoflagellates	
Season	Year	Proportion cells	Proportion area	Proportion cells	Proportion area
Summer	2010	1.00 ± 0.004	0.39 ± 0.093	0.003 ± 0.003	0.010 ± 0.007
Winter	2010	0.94 ± 0.021	0.04 ± 0.027	0.033 ± 0.015	0.007 ± 0.008
Spring	2011	0.99 ± 0.003	0.26 ± 0.138	0.004 ± 0.003	0.004 ± 0.006
Summer	2011	0.77 ± 0.203	0.17 ± 0.081	0.217 ± 0.208	0.112 ± 0.154
Winter	2011	0.95 ± 0.014	0.01 ± 0.005	0.027 ± 0.008	0.001 ± 0.001
Spring (24-Hr)	2012	1.00 ± 0.003	0.34 ± 0.125	0.002 ± 0.001	0.002 ± 0.002

concentrations of SAFA or PUFA, but showed a negative relationship to MUFA ($r^2 = 0.16$, $F_{1,47} = 9.06$, $p = 0.004$).

In addition to MSI and FA, we compared C:N of POM and Chl a, two general metrics of living biomass, to POM community composition. C:N of the POM overall differed among seasons (ANOVA, $F_{4,53} = 37.19$, $p < 0.001$) averaging 5.9 ± 0.53 in spring and summer and increasing to 7.9 ± 0.90 in winter. C:N was negatively correlated to total phytoplankton bio-area (log C:N: $r^2 = 0.74$, $F = 183.94$, $p < 0.001$) and positively correlated to total detritus (log C:N: $r^2 = 0.74$, $F = 182.15$, $p < 0.001$). $\delta^{13}C$ and $\delta^{34}S$ were negatively correlated to C:N (log C:N: $r^2 = 0.47$, $F_{1,65} = 53.52$, $p < 0.001$ and $r^2 = 0.39$, $F_{1,65} = 37.93$, $p < 0.001$, respectively). The relationship between $\delta^{15}N$ and C:N was best explained by a 2nd order polynomial, driven largely by high values of C:N from winter 2011 ($r^2 = 0.38$, $F_{1,65} = 20.14$, $p < 0.001$). Concentration of total FAME ($r^2 = 0.47$, $F_{1,47} = 42.45$, $p < 0.001$) was negatively correlated to C:N. $\delta^{13}C$ and $\delta^{34}S$ were positively correlated to Chl a ($r^2 = 0.79$, $F_{1,32} = 123.95$, $p < 0.001$ and $r^2 = 0.77$, $F_{1,32} = 108.13$, $p < 0.001$, respectively). $\delta^{15}N$ was negatively correlated to Chl a ($r^2 = 0.43$, $F_{1,32} = 24.64$, $p = 0.017$). Total FAME concentration was positively correlated to Chl a ($r^2 = 0.69$, $F_{1,32} = 72.31$, $p < 0.001$).

Offshore sampling

In the July 2011 samples taken along a 40 mile transect running offshore, diatoms dominated CC, ranging from 72.2–92.2% of cells. They represented 22.4-37.8% of bio-area at 1, 3, 18 and 28-mile stations; minimum values of 4.2 and 2.6% were observed at the most nearshore (kelp forest) and most offshore (40-mile) stations. Dinoflagellates were less abundant, and only represented > 5% of CC at the 40-mile station. Similar to seasonal samples in the San Juans, *Skeletonema*, *Thalassiosira* and *Chaetoceros* were abundant. *Corethron*, a genus not present in SJA samples, was abundant at 1, 3 and 18-mile stations. Unlike the seasonal samples, phytoplankton bio-area was not correlated to Chl a across a range similar to SJA samples ($r^2 = 0.34$, $F_{1,6} = 3.07$, $p = 0.130$), suggesting that some small phytoplankton are not efficiently quantified by our visual sampling methods but contribute to chlorophyll concentrations. $\delta^{13}C$ followed the expected relationship of depletion with distance offshore, decreasing from –19.1‰ at Neah Bay to –24.1‰ at the 18-mile station, until the 28-mile station where a value of –18.9‰ coincided with increased phytoplankton biomass. $\delta^{34}S$ varied from 18.75 to 22.2‰ along the transect, except for a value of 14.8‰ at Neah Bay. The relationship between Chl a and $\delta^{13}C$ (Fig. 6) and $\delta^{15}N$ was similar to that observed in the seasonal sampling. In contrast, POM FA signatures of the offshore transect were significantly different from all seasonal sampling periods in the SJA ($p < 0.001$ for all comparisons). Total FA concentration was marginally significantly correlated to Chl a ($r^2 = 0.45$, $F_{1,6} = 4.96$, $p = 0.067$) and had a similar range of concentrations seen in the SJA (4.63 to 17.20 mg g^{-1} POM). Minimum FA concentrations were observed in the kelp bed and at Neah Bay, while the maximum FA concentration was observed in the phytoplankton bloom at the 28-mile station.

Discussion

Variation of POM composition and biomarkers

We observed considerable variability in POM composition across hourly to interannual timescales, and regional spatial scales (Table 2, Fig. 2). Patterns of spatial and temporal variability were similar among community composition,

Table 3. Results of linear regressions between bio-area and FA categories. *indicates negative correlation. Based on 49 samples from seasonal and 24-h sampling in the SJA.

	Total phytoplankton			Diatoms			Dinoflagellates		
	r^2	F-ratio	p	r^2	F-ratio	p	r^2	F-ratio	p
FAME (mg g POM^{-1})	0.79	181.43	< 0.0001	0.54	54.06	< 0.0001	0.34	23.73	< 0.0001
SAFA (mg g POM^{-1})	0.73	127.75	< 0.0001	0.48	42.87	< 0.0001	0.36	26.25	< 0.0001
MUFA (mg g POM^{-1})	0.86	299.13	< 0.0001	0.83	236.72	< 0.0001	0.06	3.15	0.082
PUFA (mg g POM^{-1})	0.70	107.81	< 0.0001	0.40	31.38	< 0.0001	0.43	35.70	< 0.0001
Proportion SAFA	0.49*	45.57	< 0.0001	0.45*	38.55	< 0.0001	0.05*	2.24	0.141
Proportion MUFA	0.07	3.78	0.058	0.00	0.01	0.922	0.62*	75.81	< 0.0001
Proportion PUFA	0.44	36.14	< 0.0001	0.22	12.21	0.001	0.40	30.62	< 0.0001

Table 4. Empirical evaluation of putative biomarkers for POM components. FA and MSI biomarkers were selected by DISTLM best solution (see text) and from literature. The relationship shown represents the best-fit regression between the selected biomarker and diatoms, dinoflagellates or detrital bio-area from our dataset. Diatoms and dinoflagellates represent the sum of all genera identified in a sample. Detrital category includes organic, inorganic and unidentified small detritus. All bio-area data were arcsine-square root transformed. Equation for best-fit regression represents arcsine-square root transformed[1], square root transformed[2] or untransformed[3] biomarker data. Bacterial FA include c15:0, i-17:0, c17:0 and 18:1n-7. FA regressions: n = 49 samples, MSI regressions: n = 67 samples.

Biomarker	Putative source	Reference	Best fit	r^2	F	p	Relationship	
MSI from DISTLM								
$\delta^{13}C$	Multiple		Diatom	0.53	74.29	<0.0001	$y = -21.14 + 3.18$* Diatom	3
$\delta^{15}N$	Trophic level		Diatom	0.30	27.53	<0.0001	$y = 7.25 - 2.68$* Diatom	3
$\delta^{34}S$	Multiple		Diatom	0.57	85.41	<0.0001	$y = 3.01 + 24.71$* Diatom	3
FA from DISTLM								
∑16:1	Diatom	[1, 2]	Diatom	0.47	42.32	<0.0001	$y = 0.31 + 0.30$* Diatom	1
c17:0	Bacteria	[3]	Detritus	0.57	63.53	<0.0001	$y = -0.07 + 0.12$* Detritus	1
16:3n-4	Radiophytes	[4]	Diatom	0.08	3.85	0.06	–	1
16:4n-1	Diatom	[2, 5]	Diatom	0.40	31.84	<0.0001	$y = 0.10 + 0.18$* Diatom	1
c18:0	Detritus	[6]	Detritus	0.63	81.40	<0.0001	$y = -0.07 + 0.26$* Detritus	1
18:1n-7	Bacteria	[3, 6, 7]	Detritus	0.62	78.32	<0.0001	$y = 0.01 + 0.12$* Detritus	1
18:3n-3	Terrestrial plant, seagrass, green/brown macroalgae	[1, 7–10]	Dinoflagellate	0.34	23.87	<0.0001	$y = 0.05 + 0.14$* Dinoflagellate	1
18:5n-1	–	No reference	Diatom	0.54	54.96	<0.0001	$y = 0.03 + 1.24$* Diatom	1
c20:0	–	–	Dinoflagellate	0.24	14.74	0.0004	$y = 0.03 + 0.10$* Detritus	1
20:1n-9	Zooplankton	[1, 3, 11]	Detritus	0.48	43.77	<0.0001	$y = -0.08 + 0.11$* Detritus	1
20:1n-7	Zooplankton	[3]	Detritus	0.45	38.77	<0.0001	$y = -0\ 05 + 0.09$* Detritus	1
20:4n-6	Macroalgae	[8, 9, 12, 13]	Detritus	0.32	22.65	<0.0001	$y = 0.01 + 0.04$* Detritus	1
20:5n-3	Diatom, red/brown	[1, 6, 8]	Diatom	0.24	14.67	0.0004	$y = 0.26 + 0.12$* Diatom	1
	macroalgae		Dinoflagellate	0.23	14.10	0.0005	$y = 0.30 + 0.17$* Dinoflagellate	1
c22:0	Terrestrial plants	[2]	Detritus	0.28	18.69	<0.0001	$y = 0.00 + 0.04$* Detritus	1
22:1n-9	Zooplankton	[11]	Detritus	0.34	24.49	<0.0001	$y = -0.02 + 0.08$* Detritus	1
22:3n-3	–	–	N/A	–	–	–	–	1
22:5n-3	–	–	N/A	–	–	–	–	1
22:6n-3	Dinoflagellate	[3, 9]	Dinoflagellate	0.70	107.26	<0.0001	$y = 0.19 + 0.48$* Dinoflagellate	1
c24:0	Terrestrial plants, seagrass	[1, 2]	Detritus	0.33	22.79	<0.0001	$y = 0.00 + 0.06$* Detritus	1
24:1n-9	–	–	N/A	–	–		–	1
From literature:								1
16:2n-4	Diatom	[4]	Diatom	0.57	61.42	<0.0001	$y = 0.08 + 0.15$* Diatom	1
18:1n-9	Brown/green macroalgae, terrestrial plants, zooplankton	[3, 9, 14, 15]	Detritus	0.68	98.25	<0.0001	$y = -0.04 + 0.25$* Detritus	1
18:4n-3	Brown macroalgae	[1, 8, 12]	Dinoflagellate	0.39	20.38	<0.0001	$y = 0.14 + 0.15$* Dinoflagellate	1
18:5n-3	Dinoflagellate	[2, 16]	Dinoflagellate	0.29	18.99	<0.001	$y = 0.03 + 0.11$* Dinoflagellate	2
16:1/16:0	Diatom, dinoflagellate	[1, 2]	Diatom Dinoflagellate	0.62 –	76.31 –	<0.0001 –	$y = 0.53 + 0.94$* Diatom Dinoflagellate < 0.8	2
18:4/18:1	Diatom, dinoflagellate	[9]	Diatom	0.45	38.65	<0.0001	$y = 0.30 + 1.00$* Diatom	2
EPA/DHA	Diatom, dinoflagellate	[10]	Dinoflagellate	–	–		Diatoms > 1, Dinoflagellate < 1	2
>20-C EFA	Dinoflagellate	[9]	Dinoflagellate	0.64	82.06	<0.0001	$y = 0.36 + 0.48$* Dinoflagellate	1
∑ω-3	Dinoflagellate	[9]	Dinoflagellate	0.63	78.63	<0.0001	$y = 0.40 + 0.62$* Dinoflagellate	1
Bacterial FA	Bacteria	[2, 10]	Detritus	0.64	81.95	<0.0001	$y = 0.00 + 0.19$* Detritus	1

References: [1] Kelly, J. R. and Scheibling, R. E. 2012. Mar. Ecol. Prog. Ser. 446: 1–22. [2] Budge, S. M. et al. 2001. Mar. Chem 76: 285–303. [3] Alfaro, A. C. et al. 2006. Estuar. Coast. Shelf Sci. 70: 271–286. [4] Taipale, S. et al. 2013. Aquat. Microbiol. Ecol. 71: 165–178. [5] Nichols, P. D. et al. 1986. Phytochemistry 25: 1649–1653. [6] Kharlamenko, V. I. et al. 1995. Mar. Ecol. Prog. Ser. 120:231–241. [7] Kharlamenko, V. I. et al. 2001. Mar. Ecol. Prog. Ser. 220: 103–117. [8] Galloway, A. W. E. et al. 2012. J. Phycol. 48: 956–965. [9] Dalsgaard, J. et al. 2003. Adv. Mar. Biol. 46: 225–340. [10] Budge, S. M. and Parrish, C.C. 1998. Org. Geochem. 29: 1547–1559. [11] Graeve, M. et al. 1994. Deep-Sea Res. I 41: 915–924. [12] Hanson, C. E. et al. 2010. Aquat. Bot. 93: 114–122. [13] Takagi, T. et al. 1985. J. Jpn Oil Chem. Soc. 34: 1008–1012. [14] Richoux, N. B. and Froneman, P. W. 2008. Mar. Ecol. Prog. Ser. 357: 121–137. [15] El-Saabawi, R. W. et al. 2010. Estuar. Coast. Shelf Sci. 33: 738–752. [16] Nichols, P. D. et al. 1984. Phytochemistry 23: 1043–1047.

MSI and FA. The composition of taxon-specific living and general detrital components was strongly correlated to variation in MSI and FA, supporting the use of multivariate MSI and FA datasets to describe variation in the availability of POM sources. Much of this predictive power was driven by the strong correlations between phytoplankton abundance (rather than other POM components) and biomarkers, which were similar across seasonal, 24-h and offshore samples (Fig. 4–6). The concentrations of all FA categories, proportion of ecologically important PUFAs, $\delta^{13}C$ and $\delta^{34}S$ increased with increasing phytoplankton abundance. Conversely, when phytoplankton were less abundant, the relative proportion of SAFA was approximately twice that of PUFA (winter).

The correlation between abundances of FA and phytoplankton indicates that in situ phytoplankton production is the dominant source of FA to POM consumers in this nearshore ecosystem, with dinoflagellates representing a particularly important source of EFA (Fig. 5). The seasonal change of the most abundant diatom species and the proportional increase in dinoflagellate abundance explained a large percentage of the seasonal variation in MSI and FA profiles. These community changes may influence the transfer of energy and essential FA to higher trophic levels owing to variable preference and assimilation efficiencies of different diet types (Kreeger and Newell 2001). While the abundance of phytoplankton drove the large-scale patterns in MSI and FA signatures, detritus was extremely abundant in these samples (Fig. 2). The abundance of detritus was particularly high in winter. It may therefore be an important source of carbon, albeit of poorer quality than PUFA-rich phytoplankton, for nearshore consumer metabolism (Kreeger and Newell 2001, Kankaala et al. 2010).

Phytoplankton biomarkers

The wide range of POM composition and concentration in our dataset enabled quantification of the relationship between individual biomarkers and potential sources in mixed POM samples (Table 4). Had our POM samples been drawn from only one site or season, the limited range in most variables would have made it impossible to draw these connections between biomarkers and sources. The slope and intercept of the regressions presented in Table 4 are useful in evaluating POM biomarkers. These relationships are correlative and may be confounded by unknown factors, as is the nature with biomarkers, and therefore need to be considered in the context of the study system. Diatoms represented the greatest bio-area during spring and early-summer plankton blooms in the region. $\delta^{13}C$ and $\delta^{34}S$ were positively correlated to diatom abundance (Fig. 5), and in general, FA biomarkers commonly used for diatoms (Claustre et al. 1989, Dalsgaard et al. 2003) varied in proportion to diatom abundance (Table 4). As anticipated from the literature, the C_{16} FA (Σ16:1, 16:2n-4, 16:4n-1) were particularly useful for explaining variation in diatom abundance. EPA (20:5n-3) is commonly referred to in the biomarker literature as a diatom marker, but we found that it also varied in proportion to dinoflagellate abundance. We also caution that many macroalgae contain EPA in similar proportions (~10% in Laminariales) or variable but potentially high proportions in the Rhodophyta (Galloway et al. 2012). Dinoflagellates, which were abundant in late-summer blooms, were best predicted by DHA and abundance of long-chain EFA (Table 4). The ratio of 16:1/16:0 and EPA/DHA were particularly useful for distinguishing between diatoms and dinoflagellates in our samples. Other FA correlated to dinoflagellates were ALA, a marker often used for terrestrial plants (Kelly and Scheibling 2012), and the SAFA 20:0. ALA is also present in low amounts in cultured dinoflagellates (Mansour et al 1999); while comprising a small proportion of total dinoflagellate FA, the overall high concentrations of FA produced by dinoflagellates may explain the correlation between dinoflagellates and ALA and EPA proportions.

Detritus biomarkers

One of our initial goals was to use biomarkers to ascertain the sources of the abundant detritus in the POM. The FA groups SAFA and MUFA were positively correlated to the summed proportion of detritus, excluding biogenic inorganic material (Table 4). These FA include many bacterial biomarkers that were strongly correlated to detrital bio-area, showing that the proportional contribution of bacteria increases with detritus. Total FA concentration (mg g POM^{-1}) was low in detritus-dominated samples and predominately composed of SAFA (Fig. 3, 5), suggesting this detritus may contribute relatively little to overall ecosystem FA requirements (Litzow et al. 2006). The exception is 20:4n-6 (ARA), an EFA commonly reported as a macroalgal biomarker (Kelly and Scheibling 2012) that was proportionally more abundant in detritus dominated samples. While proportionally depleted in important PUFA and EFA, the abundance of detritus throughout the year may provide a critical carbon subsidy to seasonal phytoplankton productivity to meet metabolic needs of POM-consuming heterotrophs (Kankaala et al. 2010).

POM sources

$\delta^{13}C$ and $\delta^{34}S$ were depleted during the winter and in samples with low phytoplankton abundance when detritus was dominant (Table 2, Fig. 6). The reason for this pattern is unclear, but we propose two potential, non-exclusive hypotheses. First, the isotopic signature of POM reflects phytoplankton biomass, but the phytoplankton signature changes in response to variable carbon and sulfur substrates and growth rate-dependent fractionation (phytoplankton hypothesis). Second, the isotopic values are related to the heterogeneous composition of the POM and changes in the proportional contribution of different sources (detritus hypothesis). In consideration of the phytoplankton hypothesis, analysis of literature values of cultured and natural (mixed) samples of diatoms and dinoflagellates indicate that all of the $\delta^{13}C$ and $\delta^{15}N$ values observed are within the range of phytoplankton (Fry 1996, Burkhardt et al. 1999, Chanton and Lewis 1999). Depletion of $\delta^{13}C$ and $\delta^{34}S$ has been linked to admixture of riverine dissolved inorganic carbon and sulfate into marine systems (Chanton and Lewis 1999) and could explain the depleted $\delta^{13}C$ and $\delta^{34}S$ observed seasonally. Aside from the highly depleted $\delta^{34}S$ values in winter 2011, the range of observed $\delta^{34}S$ values were indicative of marine phytoplankton (17–20‰; Peterson and Fry 1987).

Other factors have been observed to alter phytoplankton isotopic signatures as well. Increased temperature during spring and summer blooms will lead to enriched $\delta^{13}C$ in phytoplankton (Degens et al. 1968, Savoye et al. 2003). There are considerable data showing that $\delta^{13}C$ values become enriched as phytoplankton productivity increases (Ramos et al. 2003, Savoye et al. 2003, Miller et al. 2013). The observations of enrichment with productivity changes are consistent with the patterns of isotopic variation relative to seasonal changes of phytoplankton abundance in the SJA, as well as the unexpected enrichment of offshore POM at the 28-mile station. Experimental degradation of phytoplankton

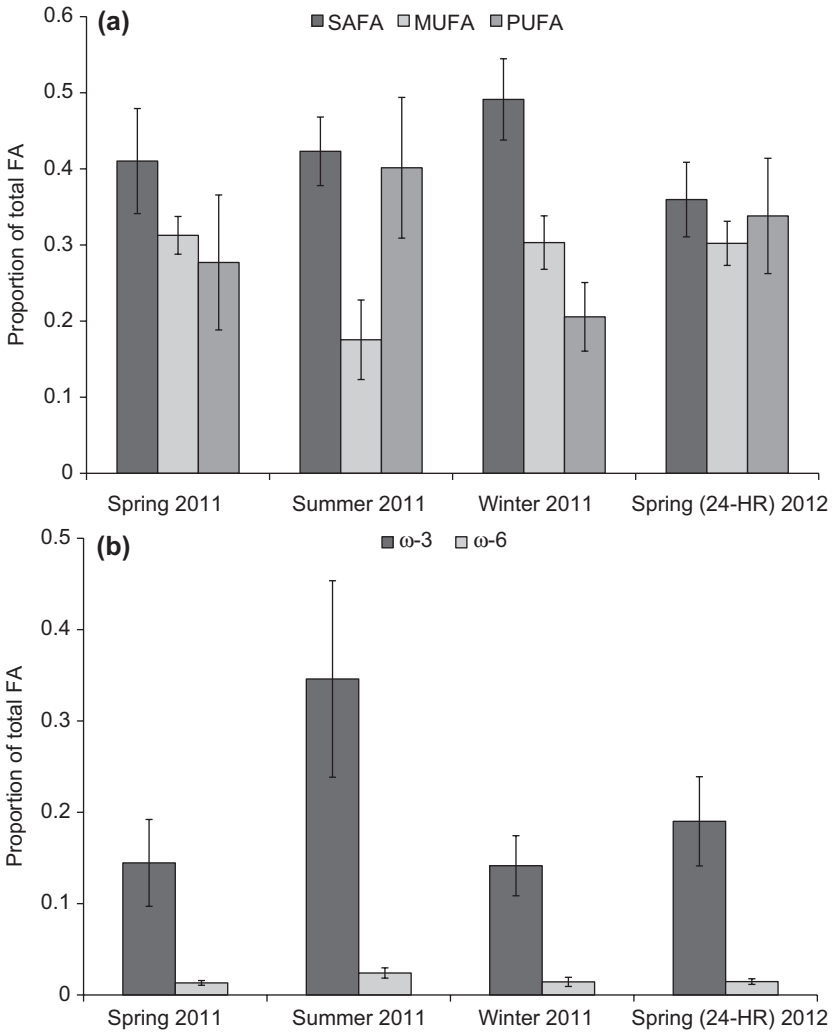

Figure 3. Seasonal comparison of FA composition (proportion) by (a) SAFA, MUFA, PUFA and (b) ω-3, ω-6 summary categories. All comparisons within FA categories significantly different among seasons (ANOVA, $p < 0.005$).

led to $\delta^{13}C$ depletion and increased C:N with loss of the labile fraction (Degens et al. 1968, Fujii et al. 2002), consistent with the $\delta^{13}C$ depletion and increase of C:N with decreased phytoplankton abundance. Total FA concentration was also positively correlated to phytoplankton abundance. MUFA concentration was significantly correlated to diatom bio-area (Fig. 5b), yet there was no variation in the proportion of MUFAs across the range of diatom abundance (0.3–54% of bio-area; Table 3) as would be expected if other sources with distinct FA were contributing significantly to the POM. The sum of these observations suggests that changes in the phytoplankton component alone could largely explain the variation of the biomarkers observed.

The variation of $\delta^{13}C$ in relation to Chl a (Fig. 6) was similar to patterns observed in the Santa Barbara Channel (Miller et al. 2013). The two study regions are characterized by very different oceanographic conditions, rates of potential terrestrial input and macroalgal growth dynamics. The similar correlation of $\delta^{13}C$ to phytoplankton biomass between regions highlights the importance of phytoplankton to POM dynamics, even in areas with highly productive nearshore kelp forest habitats. However, the range of $\delta^{13}C$ at low phytoplankton abundance was different between these systems, likely related to the abundance of detritus in the SJA. While the range was contracted in the SJA, the response of $\delta^{13}C$ to changes in Chl a was consistent, suggesting Chl a could be used to estimate $\delta^{13}C$ signatures of the POM over a wide area, as has been done for EFA (Budge et al. 2014). The overall positive correlation among MSI, FA and phytoplankton abundance, combined with the consistency of patterns of biomarker variation related to growth rate and substrate variation, provide evidence to support the phytoplankton hypothesis describing POM variation.

The detritus hypothesis predicts biomarker variation is driven by variation in the proportional contribution of disparate POM sources including macrophyte and terrestrial detritus. Increased contributions of macrophyte or terrestrial POM versus phytoplankton would lead to proportional changes in the biomarker signatures, which were observed in this study (Table 4). In the SJA, laminarian kelps dominate the benthic substrate from the shallow subtidal to approximately 20 m (Britton-Simmons et al. 2009). Previous estimates suggest little direct grazing on nearshore kelps, resulting in the production of considerable particulate

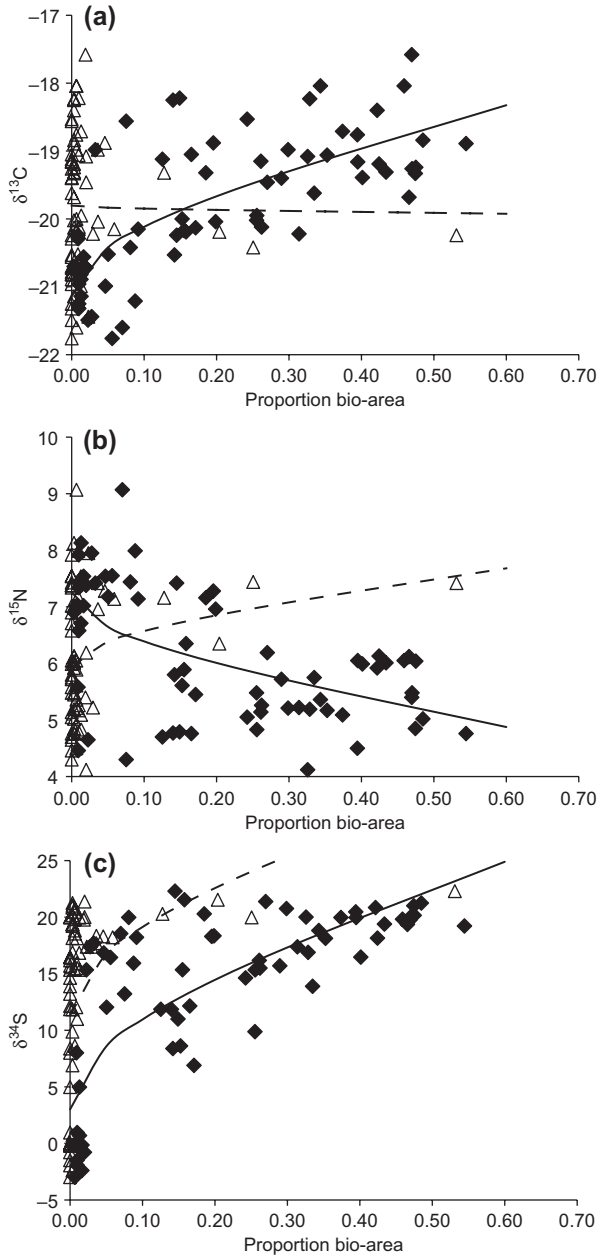

Figure 4. Relationship between (a) $\delta^{13}C$, (b) $\delta^{15}N$, (c) $\delta^{34}S$ and phytoplankton bio-area during seasonal and 24-h sampling in the SJA. Open triangles and dashed line show dinoflagellate bio-area. Diamonds and solid line show diatom bio-area. Lines are linear regressions fit to arcsin square root transformed bio-area data. Untransformed data shown. Note proportion bio-area is the explanatory variable in these plots, this differs from the equations presented in Table 4 that focus on predicting POM composition from biomarker measurements.

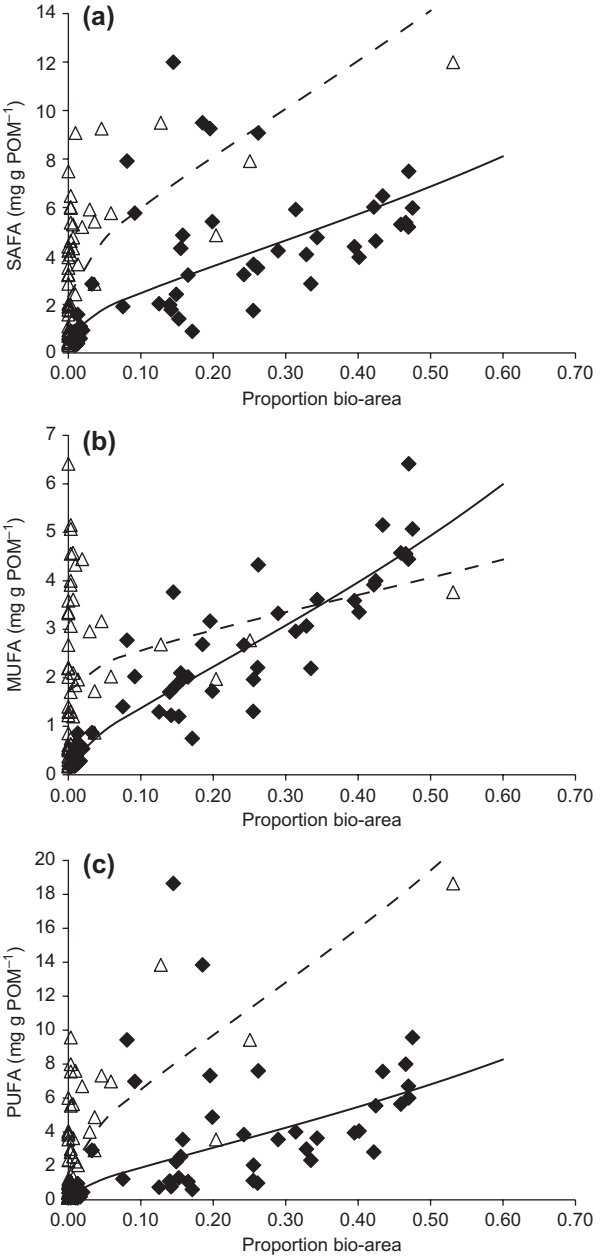

Figure 5. Relationship between fatty acid concentration (mg g POM^{-1}) of (a) SAFA (b) MUFA and (c) PUFA versus phytoplankton bio-area. Open triangles and dashed line show dinoflagellate bio-area. Diamonds and solid line show diatom bio-area. Lines are linear regressions fit to arcsin square root transformed bio-area and square root transformed FA concentration data. Untransformed data shown.

detrital biomass (Mann 1988). In general, the kelps that could contribute significant biomass to the POM in this region have enriched MSI relative to the POM (*Saccharina subsimplex* and *Nereocystis luetkeana*; Dethier et al. 2013), except for *Agarum fimbriatum*, the most abundant brown alga in the SJA (Carter et al. 2007), which exhibits a similar range of MSI as marine phytoplankton (Dethier et al. 2013). From the literature, we identified 18:1n-9, 18:4n-3 (SDA), EPA, and ARA as potential kelp biomarkers (Galloway et al. 2012, Kelly and Scheibling 2012). The FA 18:1n-9 was proportionally increased in the kelp bed, 1- and 3-mile offshore samples and correlated to detritus in the SJA (Table 4). However, this biomarker is also common in zooplankton in this region (El-Sabaawi et al. 2010), and was similarly enriched at the 40-mile offshore station. Stearidonic acid (SDA) is commonly found in dinoflagellates as well as kelps, and was positively correlated to dinoflagellate bio-area in the SJA (Table 4). While the possibility that

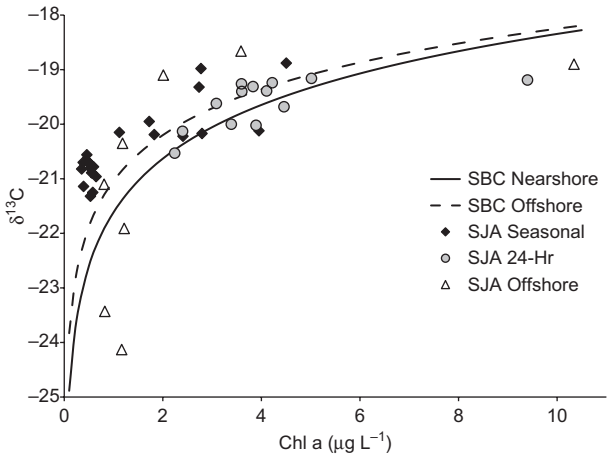

Figure 6. Comparison of $\delta^{13}C$ and Chl a data collected during the current study (SJA Seasonal, SJA 24-h and SJA Offshore) with equation fit to nearshore and offshore data collected in the Santa Barbara Channel (SBC; Miller et al. 2013).

macroalgal detritus increased concurrently to dinoflagellate bio-area cannot be ruled out, this observation weakens the argument for using SDA as a kelp biomarker when dinoflagellates are present. Similar trends were observed for EPA in its relationship to phytoplankton. The commonly used brown algal marker ARA was negatively correlated to diatom and dinoflagellate bio-area and positively correlated to detrital bio-area (Table 4); ARA did increase in winter, but generally represented a small proportion of total FA.

Depleted $\delta^{13}C$ and $\delta^{34}S$ values can reflect terrestrial inputs (Fry and Sherr 1984, Fontugne and Jouanneau 1987, Maier et al. 2011; average of POM from Nooksack, Fraser and Elwha Rivers: $\delta^{13}C = -26.6$, $\delta^{34}S = 7.18$) as well as the resuspension of sedimented detritus and bacteria (Chanton and Lewis 1999, Yamanaka et al. 2013). In high detritus samples, depletion of $\delta^{13}C$ was correlated with depletion of $\delta^{34}S$ indicating a greater contribution of terrestrial or bacterially-degraded material than macroalgae. In relation to FA, depleted $\delta^{34}S$ values corresponded to total FA concentrations of < 5 mg g POM^{-1}, a concentration observed at very low phytoplankton abundance ($< 15\%$ bio-area). The strong positive correlation between $\delta^{34}S$ and FA concentration suggests that the majority of FA production comes from marine sources. Two common terrestrial biomarker FA, ALA and 18:2n-6 (LIN; Budge and Parrish 1998), were not correlated to detrital bio-area as would be expected with terrestrial inputs, instead they were positively correlated to dinoflagellate bio-area (Table 4), a pattern observed in other systems (Ramos et al. 2003). This is potentially confounded by environmental factors, as dinoflagellates were most abundant in warmer, lower salinity water. Overall, our data suggest that while freshwater inputs influence POM composition and the energy available to benthic suspension feeders, this is not mediated through delivery of terrestrial detritus. Rather, we suggest the main influence of riverine inputs involve freshwater-driven stratification and promotion of marine phytoplankton production.

The increase in the proportion of bacterial FA biomarkers in the low phytoplankton samples and the corresponding dominance of inorganic and organic detritus provide evidence for the resuspension of bacterially-degraded organic matter from the sediments. Such resuspension is probable owing to the hydrodynamic forces resulting from large tidal exchanges in this region (Eckman et al. 2003) and supported by the consistently high abundance of detrital particles in all samples. Sediment resuspension would contribute depleted $\delta^{34}S$ (Yamanaka et al. 2013), which could explain the extremely depleted $\delta^{34}S$ in winter 2011, however experimental data confirming this observation are lacking. The increase of bacterial FA biomarkers in winter and spring samples corresponds to a significant increase in C:N and slight enrichment of $\delta^{15}N$, consistent with patterns of depleted $\delta^{13}C$ and enriched $\delta^{15}N$ in experimentally degraded material (Degens et al. 1968). Other studies have attributed depletion of ω-3 to bacterial degradation as evidenced by increases of bacterial FA and enrichment of $\delta^{15}N$ in consumer tissue (Galloway et al. 2013). While potentially confounded by seasonal fluctuations of ω-3 sources, proportional bacterial FA were observed to increase seasonally with decreased ω-3 FA. The increase in the proportion of bacterial FA corresponds to depletion of $\delta^{13}C$ and $\delta^{34}S$ and enrichment of $\delta^{15}N$, as would be expected if bacteria-colonized particles from the sediment were resuspended. This observation supports the detritus hypothesis that POM biomarker variation is influenced by mixing of multiple sources, however these results suggest a greater importance of the resuspension of local marine detrital sources rather than large amounts of terrestrial detritus similar to the observations of Fontugne and Jouanneau (1987) and Karageorgis et al. (2014).

Conclusions

Our seasonal sampling captured considerable variation in POM composition, allowing evaluation of the sources of carbon and FA available through the POM resource pool. Our study shows that phytoplankton dynamics drive nearshore marine POM composition in the San Juan Archipelago, yet this is buffered by the abundance of detritus. The data indicate that FA in the POM were primarily of marine (phytoplankton) origin, and that terrestrial inputs, if present, represented FA-poor carbon sources. This conclusion is consistent with studies in other coastal regions (Rothlisberg et al. 1994, Savoye et al 2003, Volkmann et al. 2008, Miller et al. 2013). Biomarkers reflected the total amount of phytoplankton (Table 4) but were also sensitive to the changes in community composition when phytoplankton were abundant (spring and summer). Our analyses confirm the utility of MSI and some FA, and suggest caution when using other FA commonly reported in the literature as biomarkers. We demonstrated that certain POM components can be independently estimated given MSI and FA signatures, or vice versa (Table 4). Many of these relationships can be used to determine phytoplankton growth dynamics in estuarine systems and are important to consider when estimating availability of various energy sources (Savoye et al. 2013, Budge et al. 2014).

Although seasonal POM variation was driven by phytoplankton blooms in the spring and summer and low phytoplankton abundance in winter, our results also suggest that the abundance of detrital material and bacterial degradation

of local marine production is seasonally important in the SJA. Individual biomarkers provided evidence of terrestrial ($\delta^{34}S$ ~ 0–10‰ in winter) and macroalgal inputs (increased proportion of ARA in samples with low phytoplankton abundance). Yet integration of multiple biomarkers instead indicated the importance of in situ marine productivity by phytoplankton as a source of living and detrital organic matter. These results highlight the importance of integrating multiple methods in biomarker studies, rather than using an individual biomarker, to improve food web resource tracking (Volkmann et al. 2008). Using FA concentrations allowed estimation of not only the relative proportions of disparate sources, but also the total contribution of available FA from that source. This information could be used to validate estimates of source contribution from biomarker mixing models. The strong correlation of all biomarkers to Chl a suggests this may be a simpler estimate of phytoplankton biomass for comparison to biomarkers. Additional techniques, such as bio-optical measurements, may enhance identification of detrital abundance (Karageorgis et al. 2014) for quantitative biomarker comparisons in coastal environments. Analysis of composition and biomarkers using the methods presented here can provide a greater understanding of energy sources available in a given ecosystem and allows comparison across aquatic environments (Fig. 6) that will improve studies of connectivity in aquatic and marine ecosystems.

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