The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems

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ARTICLE INFO

ABSTRACT

The processing of terrigenous dissolved organic matter (DOM) by aquatic food webs modifies its biochemical composition from riverine to coastal ecosystems. We used parallel factor analysis (PARAFAC) of fluorescence excitation–emission matrices (EEMs) and biodegradable dissolved organic carbon (BDOC) incubations to investigate changes in the biochemical composition and lability of terrigenous DOM in three estuaries of coastal southeastern Alaska: 1) a watershed with high glacial coverage, 2) low glacial coverage, and 3) low glacial coverage and high wetland coverage. Laboratory BDOC incubations were conducted for each site by inoculating filtered river water with microbial inocula collected from four different salinities (0, 2, 10 and 25) along the estuarine transect. The percent BDOC for all three sites ranged from 22 to 44% for the 28-day incubations and was greatest in the estuary draining the highly glaciated watershed. Moreover, percent BDOC was greatest for river water samples inoculated with marine compared to freshwater bacteria suggesting marine bacterioplankton were able to utilize a larger fraction of the terrigenous DOM pool than riverine microbes. PARAFAC modeling of fluorescence EEMs showed non-conservative estuarine mixing behavior for DOM including removal at low salinities and addition at mid-high salinities for all three sites. For example, tyrosine-like fluorescence decreased dramatically between salinity values 0 and 0.5 and was undetectable by salinity 2 for all three estuaries. However, humic-like C4 (correlated with aliphatic carbon content) and tryptophan-like fluorescence increased non-conservatively during estuarine mixing, likely associated with an increase in bacterioplankton growth. These results indicate that terrigenous DOM, particularly from glacial runoff, is an important source of carbon and nutrients to near-shore coastal zones of southeast Alaska.

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1. Introduction

The flux of terrigenous dissolved organic matter (DOM) from continent to ocean is a major source of reduced carbon to marine environments. Thus, estuaries are critical links in the transfer of DOM and nutrients between terrestrial and coastal marine ecosystems because these areas have some of the highest areal rates of heterotrophic bacterial production in aquatic ecosystems (Smith and Hollibaugh, 1993). The mixing behavior of terrigenous DOM in estuarine environments is quite variable and can change dramatically with riverine discharge, seasonality, and as a result of its biochemical composition (Spencer et al., 2007). This suggests that terrestrial ecosystem processes that alter the timing, magnitude, and lability of DOM delivery to estuaries have the potential to influence biogeochemical cycling in near-shore marine ecosystems.

Terrigenous DOM can mix conservatively in some estuaries, which indicates relatively little modification of the DOM precursor material (Abril et al., 2002). This DOM can be exported to coastal marine ecosystems where it can serve as a source of carbon and nutrients for aquatic heterotrophs (Raymond and Bauer, 2000). In contrast, terrigenous DOM also exhibits non-conservative mixing behavior in estuaries. For example, a transect study in two U.K. estuaries draining peatland watersheds showed estuarine dissolved organic carbon (DOC) removal as high as 58% (Spencer et al., 2007). Specific DOM removal mechanisms in estuarine ecosystems include: 1) physico-chemical transformations such as flocculation (Amon and Meon, 2004) and adsorption onto suspended sediments (Uher et al., 2001); 2) photochemical degradation (Hernes and Benner, 2003; Spencer

et al., 2009), which can directly degrade DOM into a range of compounds, some being readily available for bacterial uptake (Moran and Zepp, 1997); and 3) bacterial metabolism (Raymond and Bauer, 2000; Lønborg et al., 2009), although its biochemical composition can greatly impact on bacterial uptake in estuaries (Sondergaard et al., 2003). Because variability in removal processes makes understanding the potential fate of terrigenous DOM in estuaries challenging, studies have used parallel factor analysis (PARAFAC) of fluorescence excitation–emission matrices (EEMs) (Stedmon et al., 2003; Cory and McKnight, 2005) to help elucidate DOM dynamics in estuaries. Fluorescence characterization of DOM has shown both conservative and non-conservation mixing occurs for different fluorescent components in estuaries (Yamashita et al., 2008). Thus, there is great potential for PARAFAC analysis of EEMs to elucidate the production and removal of different DOM fractions during estuarine mixing.

Wetland soils strongly influence streamwater DOM concentrations in many regions of the world (Aitkenhead and McDowell, 2000) and thus, constitute a considerable source of organic matter to near-shore marine ecosystems in coastal regions draining landscapes with a high coverage of wetlands (Alkhatib et al., 2007; Baum et al., 2007). Wetland contributions of DOM to estuaries could be particularly important in southeast Alaska because wetlands occupy approximately 29% of the land area (USDA, 1997), and wetlands can export large concentrations of relatively labile DOM to coastal streams (Fellman et al., 2009a) that have short transit times to downstream marine ecosystems. Another potential source of terrigenous DOM and nutrients to estuaries of southeast Alaska are glacial ecosystems (Hood and Scott, 2008; Hood et al., 2009). Glacial runoff currently accounts for ~50% of the 870 km^{3}year^{-1} annual freshwater runoff in southeastern Alaska (Neal et al., 2010) and research from coastal watersheds has shown that the percentage of biodegradable dissolved organic carbon (BDOC) in coastal rivers along the Gulf of Alaska (GOA) is strongly correlated with watershed glacial coverage (Hood et al., 2009). Because thinning and recession of glaciers is particularly pronounced in southeastern Alaska (Arendt et al., 2002), future reductions in glacial coverage could substantially alter the timing and amount of labile DOM and nutrients delivered to near-shore marine ecosystems.

As terrigenous DOM moves through the watershed from its source in the terrestrial environment to the watershed outlet, the selective processing of certain DOM fractions (Kaplan and Bott, 1983) combined with high-internal biotic demand for labile DOM by stream communities in lower order streams generally results in a decrease in DOM lability with distance downstream (Leff and Meyer, 1991). Thus, it is possible that estuarine bacterioplankton are well adapted to utilize this relatively altered, terrigenous DOM typically delivered to coastal marine ecosystems. Previous DOM laboratory biodegradation studies have shown estuarine microbial communities are more efficient at removing allochthonous DOM (Stepanauskas et al., 1999a,b; Wikner et al., 1999) from the water column than riverine microbial communities; however, there is still conflicting evidence at this point (Langenheder et al., 2003; Sondergaard et al., 2003). Therefore, evaluating how the uptake of terrigenous DOM differs for marine compared to riverine microbes will shed new light on this topic and at the same time, allow us to evaluate how ongoing changes in the terrestrial landscape of southeast Alaska may impact near-shore coastal food webs.

In this study, we used PARAFAC modeling of fluorescence excitation–emission matrices (EEMs) and BDOC incubations to investigate changes in the biochemical composition and lability of terrigenous DOM along a salinity gradient in three estuaries draining watersheds with varying wetland and glacial coverage. We also performed BDOC incubations by inoculating filtered surface water collected from four additional rivers with varying watershed wetland and glacial coverage with microbial inocula collected from two different salinities (0 and 25). This sampling design allowed us to evaluate whether 1) wetland- and glacier-derived DOM is a source of labile carbon for bacterioplankton, and 2) if riverine and marine bacterial communities differ in their ability to utilize terrigenous DOM. Moreover, the evaluation of estuaries with quantitatively different riverine inputs from wetland soils and glacial ecosystems will enhance our understanding of how future reductions in watershed glacial coverage could affect the cycling of DOM and inorganic nutrients in coastal ecosystems along the GOA.

2. Methods

2.1. Study sites

River, estuarine and near-shore marine water samples were collected from coastal draining watersheds and adjacent waters near Juneau, southeast Alaska (Fig. 1). Southeast Alaska covers an area of approximately 85,000 km^2 consisting of a diverse range of ecosystems including water-logged peatlands mixed with heavily forested mountains that rise abruptly from a complex network of glacier carved fjords and inland marine waterways. More than 22,000 islands exist within this archipelago creating an intertwined network of fjords and inland waterways that provide a large source of water and nutrients to the coastal ocean. Juneau has a maritime climate characterized by mild winters, cool wet summers, persistent cloud cover and a mean annual precipitation of 1400 mm at sea level, most of which falls in the autumn as rain.

Estuarine transects were completed in June, 2008 in three different estuaries that drain into Lynn Canal (Fig. 1). Lynn Canal is a glacial-carved fjord approximately 145 km in length and over 600 m in depth, making it the deepest fjord in North America. Each of the three estuaries sampled drains a watershed that varies in its landscape coverage: 1) St. James, a watershed with high wetland coverage, 2) Endicott, a forested watershed with low glacial coverage and 3) Eagle, a watershed with high glacial coverage (Table 1). The three estuaries were generally shallow near the river inlets (<5 m), have diurnal tidal cycles with large tidal changes (4–8 m) and Eagle estuary had the greatest turbidity when sampled due to large contributions of glacial meltwater to surface water flow during the summer months. In addition to the three study estuaries, surface water was collected during June, 2008 from four additional rivers (Lemon Creek, Mendenhall River, Peterson Creek and Montana Creek; Fig. 1). Our goal for these four rivers was to further evaluate the lability of terrigenous DOM from wetland and glacial sources. The seven study watersheds represent different combinations of wetland coverage, glacial recession, dominant vegetation and watershed area (Table 1). The Mendenhall, Lemon and Herbert/Eagle all have watershed glacial coverage greater than 25% and contain extensive areas of high-elevation alpine tundra with exposed bedrock and shallow (<0.5 m) soils. Vegetation in the upper reaches of these watersheds is typically sparse and dominated by *Alnus* and *Salix* spp., and in the lower part of these watersheds, the landscape is older consisting of a mixture of coniferous forest dominated by *Tsuga heterophylla* and *Picea sitchensis*. Similarly, the Endicott watershed contains extensive high-elevation reaches of alpine tundra and has many successional attributes (e.g. shallow soils and vegetation dominated by *Alnus* and *Salix* spp.) typical of recently deglaciated terrain. In contrast, the Peterson, St. James and Montana watersheds have more gradual relief and greater wetland extent, particularly in the lower reaches of the watersheds.

2.2. Field methods

A four liter surface water grab sample was collected from the river mainstem in each of the seven watersheds (total of 7 riverine water samples) studied during one week in June, 2008. Estuarine transect cruises were conducted along a salinity gradient in the three estuaries (St.
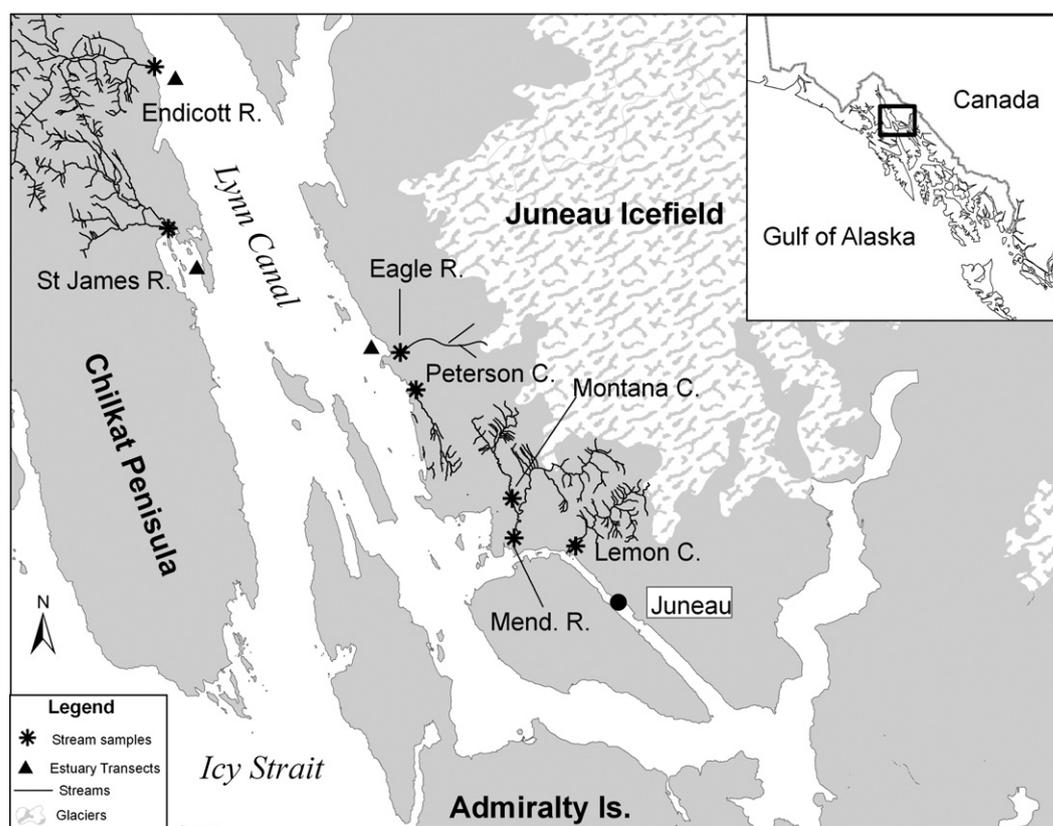
Fig. 1. Map of the three estuaries and seven watersheds studied near Juneau, Alaska.

James, Endicott and Eagle) during the same week riverine sampling occurred and on the same day as riverine grab samples were collected for the respective estuaries. Water samples were collected at 7–8 selected salinities (total of 22 estuarine water samples for all three sites) measured in situ using a portable multi-parameter probe (YSI model 556). Transects stretched from the riverine sampling point (e.g. freshwater endmember) to the mouth of each estuary (covering a salinity range of 0–25). A maximum surface salinity of ~25 is typical for many of the inland marine waterways of southeast Alaska due to the high freshwater yield from the terrestrial environment (Etherington et al., 2007). The estuarine sampling was timed to span high tide and was carried out ±2 h of peak high tide.

A two liter water sample was collected from a depth of <0.5 m (thus avoiding the surface microlayer) at all estuarine sampling sites and placed directly into acid-washed, high density polyethylene bottles (HDPE). All estuarine and riverine water samples were transported from the field to the laboratory in a cooler packed with ice. Upon return to the laboratory, water samples were immediately filtered through pre-combusted (450 °C for 4 h) Whatman GF/F filters (nominal pore size 0.7 μm). Water samples for analytical measurements were stored in either pre-combusted (450 °C for 4 h), amber glass bottles (DOM measurements) or acid-washed, HDPE bottles

(inorganic nutrients). Fluorescence characteristics and concentrations of DOM were determined within 48 h of collection and samples for δ^{13}C-DOC, lignin phenol and inorganic nutrient analysis were stored frozen after filtration and analyzed at a later date.

2.3. Laboratory analyses

Dissolved organic matter and inorganic nutrient concentration was determined for each of the seven riverine and 22 estuarine water samples. Concentrations of DOC (determined by non-purgeable organic carbon analysis) and total dissolved N (TDN) were measured via high temperature catalytic oxidation on a Shimadzu TOC/TN-V analyzer. All DOC and TDN data are reported here as the mean of three to five replicate injections, for which the coefficient of variance was always <2%. A spectrophotometric colorimetric detection method was used to measure NH_4–N and NO_3–N (Spencer et al., 2007) and dissolved organic N (DON) was calculated as the difference between TDN and dissolved inorganic N (DIN = NH_4–N + NO_3–N). Soluble reactive phosphorus (SRP) was measured using the ascorbic acid method (Murphy and Riley, 1962), total dissolved phosphorus (TDP) was determined with a persulfate digest (Valderrama, 1981), and dissolved organic phosphorus (DOP) was calculated as the difference between TDP and SRP. Three replicate analyses of individual samples for DON and DOP yielded concentrations that were always less than 10% of each other for DON and 5% for DOP.

Stable isotope analysis of DOC (δ^{13}C-DOC) was undertaken on the riverine endmembers (seven river samples in total) at the University of California, Davis stable isotope facility using an O.I. Analytical Model 1010 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Isotopic data are expressed with standard notation (δ^{13}C) in parts per thousand (‰) relative to the Pee Dee Belemnite standard, where δ^{13}C = [(Rsample/Rstandard) − 1] × 1000,

Table 1
Description of the seven study watersheds.

Watershed	Watershed size km²	Glacial cover %	Wetland cover %	Forest cover %
Eagle River	281	40	<5	19
Endicott River	408	5	7	22
Lemon Creek	59	25	<5	15
Mendenhall River	231	55	<5	6
Montana Creek	37	0	5	51
Peterson Creek	23	0	53	45
St. James River	130	0	14	27

and R is the ratio of ^{13}C to ^{12}C. Replicate analyses of individual samples for $\delta^{13}C$-DOC yielded $\delta^{13}C$-DOC values with standard deviations of ≤ 0.2‰ and the mean is reported.

Lignin phenol analysis was completed for the riverine endmembers (seven river samples in total) using 1–2 L of filtered water that was lyophilized using a Labconco freeze drier (FreezeZone 2.5) at -50 °C. The freeze-dried material then underwent alkaline CuO oxidation, followed by acidification and ethyl acetate extraction (Spencer et al., 2008). Lignin phenol quantification was carried out by GC–MS (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector and a DB5-MS capillary column) using cinnamic acid as an internal standard and a five point calibration scheme (Hernes and Benner, 2002). For all samples eight lignin phenols were quantified which included three vanillyl phenols (vanillin, acetovanillone, and vanillic acid), three syringyl phenols (syringaldehyde, acetosyringone, and syringic acid), and two cinnamyl phenols (p-coumaric acid and ferulic acid). Blank concentrations of lignin phenols were low (40–50 ng) and thus blanks never exceeded 3% of the total lignin phenols in an extract.

2.4. Fluorescence spectroscopy and PARAFAC modeling

Fluorescence excitation–emission matrices (EEMs) of DOM were measured on a Fluoromax-3 (Jobin Yvon Horiba) fluorometer with a xenon lamp following the procedures of Hood et al. (2007). Fluorescence EEMs were created on riverine and estuarine water samples by measuring fluorescence intensity across excitation wavelengths ranging from 240 to 450 nm (5 nm intervals) and emission wavelengths ranging from 300 to 600 nm (2 nm intervals). Excitation and emission slit widths were 5 nm and the instrument was configured to collect fluorescence measurements in ratio mode. If necessary, riverine DOM samples were diluted with Milli-Q water to an optical density of 0.02 at 300 nm to minimize inner filter effects (Green and Blough, 1994). Fluorescence EEMs of Milli-Q water run on the same day were subtracted from each sample EEM. All EEMs were Raman normalized using the area under the water Raman peak at excitation 350 nm and corrected for instrument bias using correction files provided by the instrument manufacturer. In addition, several sample EEMs were collected in triplicate and replicate EEMs were within 10% in terms of peak location and intensity.

We currently cannot relate the fluorescent properties of DOM to the exact biochemical composition of organic matter, but it is widely recognized that highly conjugated and aromatic compounds contribute largely to DOM fluorescence. Phenolic compounds have also been shown to contribute to the fluorescence signal and their fluorescence spectra can overlap with protein-like fluorophores (Hernes et al., 2009). Thus, fluorescence characterization of DOM does not provide definitive structural information like other discrete chemical measurements (e.g. analyses related to the molecular composition of DOM such as lignin phenols). However, spectrophotometric characterization provides unique source and process information rapidly, and relatively inexpensively, which allow for temporal and spatially extensive sampling programs necessary to trace DOM dynamics in aquatic ecosystems.

Fluorescence EEMs were analyzed using the multivariate modeling technique parallel factor analysis (PARAFAC), a three-way decomposition method that decomposes the fluorescence spectra of DOM into independent fluorescent components. PARAFAC modeling of EEMs was conducted with MATLAB using the PLS_toolbox version 3.7 (Eigenvector Research Inc., 2006) following the procedures of Stedmon et al. (2003). To obtain sufficient variation in the PARAFAC model, the data set for the model included 40 estuarine water samples and 80 riverine samples (data array consisted of 120 EEMs with 151 emission wavelengths and 43 excitation wavelengths) collected from glacial, forested and wetland streams in the Juneau area that drain into the Lynn Canal. Raman and Rayleigh scatter were removed from sample EEMs and a "triangle of

zeros" was added to the area of missing data. The appropriate number of modeled components was determined using core consistency diagnostics (Ohno and Bro, 2006) followed by a split-half validation (two random halves of 60 EEMs each, Stedmon et al., 2003). Our PARAFAC model identified a total of eight unique components within the EEMs (Table 2; Appendix A). We selected eight components as the best model fit since we found good agreement in the spectral loadings for the split-half validation (Appendix A) and the model had a core consistency score of 98.2%. All eight components identified by our model have been previously identified as either part of a PARAFAC model or through peak picking (visual inspection of EEMs to locate fluorophores) of EEMs (Table 2). Seven of the components (components 1–3 and 5–8) were previously identified in PARAFAC models for southeast Alaskan catchments (Fellman et al., 2009a,b) suggesting a tight linkage between catchment/riverine DOM sources and estuarine DOM. Humic-like C4 was the only component found in this study not identified in these previous fluorescence characterization studies of riverine DOM (Fellman et al., 2009a,b). Thus, it was particularly useful in elucidating changes in DOM biochemical composition during estuarine mixing.

2.5. DOC lability experiments

Laboratory incubations examining DOC lability were conducted for the three estuaries by inoculating filtered river water with microbial inocula collected from four different salinities (0, 2, 10 and 25). In addition, surface water collected from the four additional rivers (Lemon Creek, Mendenhall River, Peterson Creek and Montana Creek) was examined in 28 day BDOC incubations using both the riverine (salinity 0) and near-shore marine (salinity 25) inocula. Water samples were initially filtered through a 0.3 μm (pre-combusted 450 °C for 4 h ADVANTECH GF-75) glass fiber filter to remove the majority of microbial biomass. After filtration, 18 mL of the filtrate was placed into pre-combusted (450 °C for 4 h) 20 mL glass bottles, and a 10% by final volume microbial inocula was added. DOC concentration was measured at the start of the experiment and samples were incubated for 2, 7 and 28 days at 15 °C in the dark. At each time point of the incubation, the solution was re-filtered through a 0.3 μm filter, DOC was analyzed, and BDOC was calculated as the difference in sample DOC before and after the incubation. Three replicate BDOC incubations were completed for each time and salinity treatment. The four different microbial inocula were prepared by filtering riverine, estuarine and near-shore marine (salinities of 0, 2, 10 and 25) wholewater through a pre-combusted (450 °C for 4 h) Whatman GF/D filter (nominal pore size 2.7 μm).

Table 2
Characteristics of the eight individual components identified by the PARAFAC model in this study.

Comp.	Ex/Em maxima (nm)	Fluorophore name	Description
1	<250/450–470	A[1], 1[2], 2[3], 1[4], 1[5]	Widespread UVC humic-like fluorophore, but is most common in wetlands and forest streams
2	330/456–480	C[1], 4[2], 1[3], 2[4], 2[5]	High molecular weight and aromatic humic-like fluorophore
3	290/510	3[4], 5[3], 3[5]	Humic-like fluorophore, correlated with aromatic C content
4	240/384	12[3]	Humic-like fluorophore, correlated with aliphatic C content
5	240/414	3[2], 5[5]	Widespread UVC humic-like fluorophore
6	275/462	7[3]	Humic-like fluorophore
7	280/330–340	T[1], 7[2], 8[3], 4[4], 8[5]	Tryptophan-like, fluorescence resembles free tryptophan
8	275/304–306	B[1], 8[2], 13[3], 7[4], 9[5]	Tyrosine-like, fluorescence resembles free tyrosine

References: 1. Coble (1996); 2. Stedmon and Markager (2005); 3. Cory and McKnight (2005); 4. Yamashita et al. (2008); 5. Fellman et al. (2009b).

3. Results

3.1. Concentration and composition of riverine DOM

Riverine DOC concentrations ranged from 0.9 to 4 mg C L^{-1} while DON ranged from 16 to 131 µg N L^{-1}, with greatest concentrations in the wetland-dominated watersheds Peterson Creek and St. James River (Table 3). Concentrations of DOP ranged from 1 to 10 µg P L^{-1} and were higher in the glacial watersheds. Riverine NH$_4$–N concentrations were near or below detection for all sites (data not shown), thus NO$_3$–N was the dominant form of DIN present for all sites. Values for δ^{13}C-DOC ranged from −25.0 to −27.1‰ across the seven rivers and values were generally more enriched in the glacial compared to the wetland rivers (Table 3). The rather narrow range observed for our sites indicate predominantly organic carbon from C3 plants (Hobbes et al., 2000) with a more increased proportion of DOM from microbial biomass in the glacial-dominated watersheds (Hood et al., 2009).

Lignin phenol concentrations (Σ_8) ranged from 0.7 to 24.2 µg L^{-1} (mean = 5.7 µg L^{-1}) and were greater in the wetland-dominated watersheds than in rivers dominated by glacial meltwater (Table 3). The lignin phenol concentrations for the glacial rivers reported here were comparable to those reported in glacial tributaries of the Yukon River in Alaska (Spencer et al., 2008). In non-glacial watersheds such as Peterson Creek (high wetland coverage), Σ_8 values were similar to wetland concentrations (5.7–36.8 µg L^{-1}) on the Sacramento–San Joaquin Delta (Eckard et al., 2007). Carbon-normalized lignin concentrations (Λ_8), which can be used to estimate the contribution of vascular plant derived carbon to the bulk pool of DOC (Hernes and Benner, 2006; Spencer et al., 2008), ranged from 0.12 to 0.58 (mg (100 mg OC)$^{-1}$) with a mean of 0.21 (mg (100 mg OC)$^{-1}$) across the seven rivers. The presence of syringyl phenols (S) in angiosperms and cinnamyl phenols (C) in non-woody tissues allows us to compare their concentrations with the ubiquitous vanillyl phenols (V) to discriminate organic matter sources from angiosperm and gymnosperm plant types (S:V) and between non-woody and woody tissues (C:V) (Hedges and Mann, 1979). The C:V and S:V ratios ranged from 0.07 to 0.16 (mean = 0.11) and 0.19–0.47 (mean = 0.29), respectively. These ratios are consistent with a mixture of traditionally defined angiosperm non-woody tissues and gymnosperm woody sources, but could also be derived solely from degraded angiosperm non-woody tissues as S:V and C:V typically decrease with diagenetic processing.

3.2. Estuarine mixing behavior

Estuarine DOC concentrations for the St. James and Endicott estuaries exhibited slight non-conservative mixing at low salinities (between salinity values 0 and 0.5 for both estuaries), and concentrations decreased gradually with increasing salinity between 2 and 25 (Fig. 2a). Concentrations of DOC in Eagle estuary showed a dramatically different mixing pattern than that of St. James and Endicott, as concentrations increased from 0.9 to 1.4 mg C L^{-1} between salinity values 0.5 and 10, followed by a gradual decrease between salinity values 10 and 25. Although the absolute increase in DOC concentration in the Eagle estuary was relatively small (~0.5 mg C L^{-1}), this change corresponded to a concentration increase of ~60% between salinity values 0 and 10. Estuarine mixing patterns for DOP were similar for all three estuaries, as concentrations increased dramatically between salinity values 0.5 to 6 (Fig. 2b). The mixing behavior of DON was more variable than for DOC and DOP, as all three estuaries showed both removal followed by the slight production of DON from the inner to outer estuary (Fig. 2c). This removal/production pattern was most pronounced in the St. James estuary as DON concentration decreased ~40% between salinity 0 and 2. Concentrations of DOC and DON were always greatest in the St. James estuary, whereas the Eagle estuary showed the greatest DOP concentrations. Evaluating the inorganic nutrient concentrations showed that the production of NO$_3$–N at low salinities occurred concomitant with the observed removal of DON for all three estuaries (Fig. 3a). Estuarine SRP concentration decreased non-conservatively in all three estuaries, as concentrations dropped 1.8 µg P L^{-1} for Endicott, 2.2 µg P L^{-1} for St. James and 1.4 µg P L^{-1} for Eagle between salinity values 0 and 2 (Fig. 3b). Overall, the most dramatic changes in estuarine C, N and P concentrations were observed between salinity values 0 and 6.

A dramatic shift occurred in the biochemical composition of DOM during estuarine mixing at all three study sites, as evidenced by changes in the fluorescence intensity of the eight PARFAC components. Humic-like components 1–3 and 5–6 showed similar mixing behavior for all three estuaries, as fluorescence intensities decreased gradually around the theoretical dilution line between salinity values 2 and 25 (Fig. 4a–c, e and f). However, all three estuaries exhibited slight non-conservative mixing at low salinities, as fluorescence intensities decreased an average of 26% (average decrease for components 1–3 and 5–6) for Endicott, 17.8% for St. James, and 16.2% for Eagle between salinity values 0 and 2. In contrast, the mixing behavior of humic-like C4 was dramatically different compared to the other 5 humic-like components. The fluorescence intensity for C4 increased along the salinity gradient to reach a maximum at the middle salinity range (10–15) for all three estuaries, followed by sharp decrease between salinities 15 and 25 (Fig. 4d). There were also dramatic shifts in the protein-like components for all three estuaries, as tyrosine-like fluorescence (C8) decreased from a F_{max} of 0.17 in the Eagle riverine endmember to 0 by salinity 2 (Fig. 4h). Similarly, tyrosine-like fluorescence was undetectable for the St. James and Endicott estuaries by salinity 0.5. Shifts in tryptophan-like C7 were similar to that of DON, where both consumption at low salinities (0–2) and production in the middle salinity range (2–15) of tryptophan-like fluorescence were observed along the salinity gradient for all three estuaries (Fig. 4g).
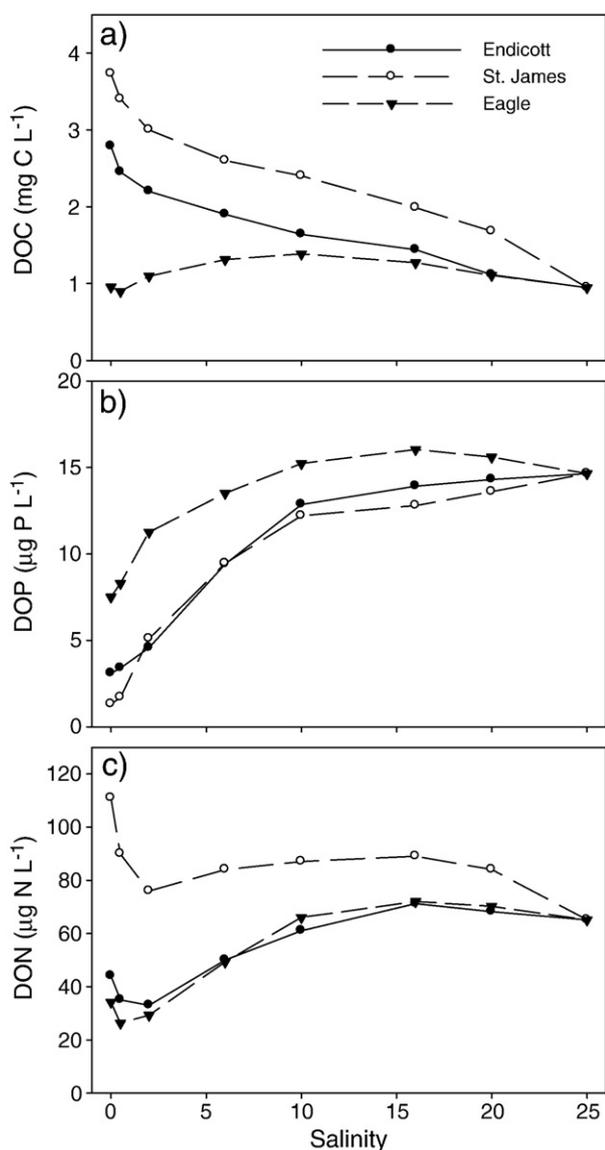
3.3. BDOC incubations

The percent BDOC ranged from 22 to 44% for the 28-day incubations using river water inoculated with microbial communities collected from a range of salinities (0, 2, 10 and 25) in the Eagle, St. James and Endicott estuaries (Fig. 5). Moreover, percent BDOC was

Table 3
Background riverine C, N, P and lignin phenol concentrations for the seven study watersheds.

	DOC mg C L^{-1}	DON µg N L^{-1}	DOP µg P L^{-1}	NO$_3$–N µg N L^{-1}	SRP µg P L^{-1}	δ^{13}C-DOC (‰)	Σ_8 (µg L^{-1})	Λ_8 mg	C:V	S:V
Eagle R.	0.9	44.0	7.5	18.4	3.0	25.8	1.3	0.13	0.13	0.29
Endicott R.	2.8	34.2	3.1	55.0	3.3	25.4	3.8	0.14	0.10	0.47
Lemon Cr.	1.1	30.6	9.8	29.0	1.1	25.2	1.1	0.12	0.15	0.37
Mendenhall R.	0.9	16.0	10.1	52.6	2.1	25.0	0.7	0.12	0.16	0.20
Montana Cr.	1.3	62.4	4.2	50.9	3.5	26.8	3.3	0.22	0.08	0.22
Peterson Cr.	4.0	131.4	6.4	39.7	3.1	27.1	24.2	0.58	0.07	0.19
St. James R.	3.7	110.9	1.3	30.8	4.2	25.3	5.6	0.15	0.11	0.30

Abbreviations: Σ_8, sum of 8 lignin phenols (µg L^{-1}); Λ_8, carbon-normalized lignin yield (mg (100 mg OC)$^{-1}$); C:V, cinnamyl:vanillyl phenol ratios; S:V, syringyl:vanillyl phenol ratios.

Fig. 2. Changes in the concentration of a) DOC, b) DOP and c) DON vs. salinity for the St. James (wetland), Eagle (glacial) and Endicott (forested) estuaries. The salinity of 25 is the near-shore marine surface endmember for all three estuaries.

Fig. 3. Changes in the concentration of a) NO₃–N and b) SRP vs. salinity for the St. James (wetland), Eagle (glacial) and Endicott River (forested) estuaries. The salinity 25 is the near-shore marine surface endmember for all three estuaries.

protein-like fluorescence (sum of tyrosine and tryptophan-like fluorescence components, $p<0.05$, Fig. 7b) and δ^{13}C-DOC ($p<0.05$, Fig. 7c) were all significantly correlated with percent BDOC. Interestingly, the riverine microbial inoculum was more strongly correlated with the percent contribution of protein-like fluorescence than the marine inoculum while the opposite pattern was observed for lignin phenol concentrations.

4. Discussion

4.1. Utilization of terrigenous DOM

Understanding the cycling of DOM in estuarine ecosystems is of considerable interest because these zones can have high areal rates of bacterial production (Smith and Hollibaugh, 1993) and terrigenous inputs of organic matter can support much of the heterotrophic carbon demand in estuarine ecosystems. We found DOC uptake during all incubations providing evidence that microbial degradation of terrigenous DOM is likely a considerable removal mechanism for DOM in near-shore marine environments of southeast Alaska. Furthermore, greater than half of the DOC consumed during incubations occurred during the initial few days of lability incubations suggesting that a substantial fraction of the terrigenous DOM pool is highly labile and turns over very rapidly in aquatic environments (Coffin et al., 1989; Sondergaard et al., 1995). The percent BDOC was also greatest in the estuary draining the highly glaciated watershed suggesting that glacial runoff from GOA watersheds, which is rapidly being altered by climatic warming, could have a major role in the metabolic stability of coastal food webs by supplying labile DOM and nutrients for marine bacterioplankton. Interestingly, percent BDOC was also relatively high (>15%) in the wetland-dominated rivers (Peterson Creek and St James River) which implies wetland
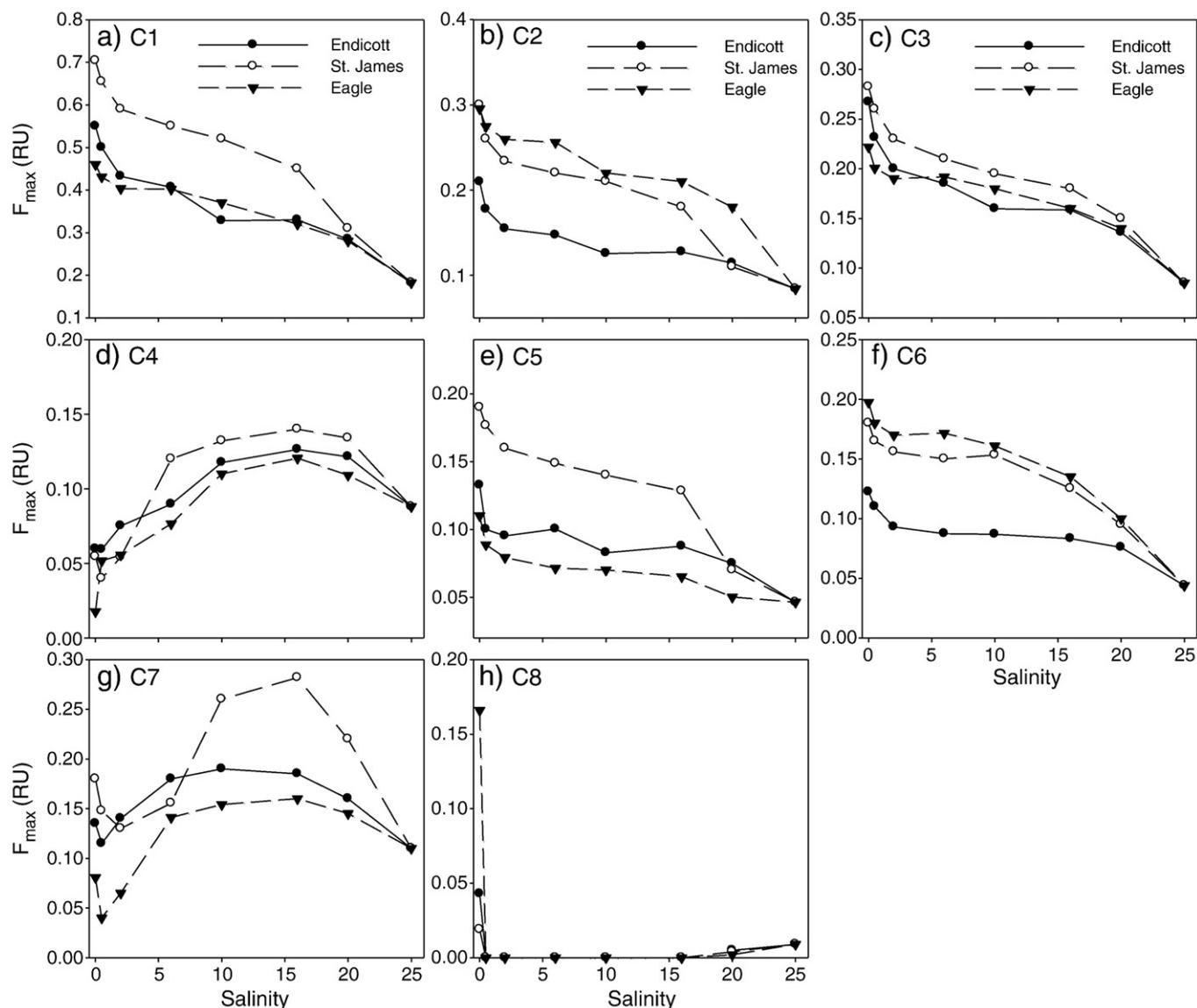
greatest with higher salinity inocula for all three estuaries, suggesting greater bacterial utilization of terrigenous DOC under estuarine/ marine than riverine conditions. We further evaluated this pattern by comparing all of the seven rivers sampled and we found that both percent BDOC and concentrations of BDOC for the salinity 25 microbial inoculum were significantly greater (t-test; $p<0.05$) than for the freshwater inoculum (Table 4). Microbial DOC utilization was greatest in rivers receiving glacial meltwater (Eagle, Lemon and Mendenhall) and lowest in the wetland-dominated watersheds (Peterson, St. James). In contrast to percent BDOC, concentrations of BDOC were generally greater in the higher DOC, wetland streams than in low DOC glacial streams (Table 4).

Temporal trends in the BDOC incubations showed that >58% of the total DOC consumed during incubations with the marine inoculum (salinity 25) occurred during the first week for the St. James, Eagle and Endicott Rivers (Fig. 6). This pattern of rapid utilization of DOC was most pronounced in the glacial watershed (Eagle River), where 80% of DOC consumption occurred during the first 2 days. The lability of DOM was also strongly correlated with its biochemical characteristics for both the marine and freshwater microbial inoculum, as carbon-normalized lignin yields (Λ_8) ($p<0.05$, Fig. 7a), percent contribution of

Fig. 4. Changes in the fluorescence intensity of PARAFAC components vs. salinity for the St. James (wetland), Eagle (glacial) and Endicott River (forested) estuaries. The salinity 25 is the near-shore marine surface endmember for all three estuaries and fluorescence intensities (F_{max} values) are in Raman Units (RU).
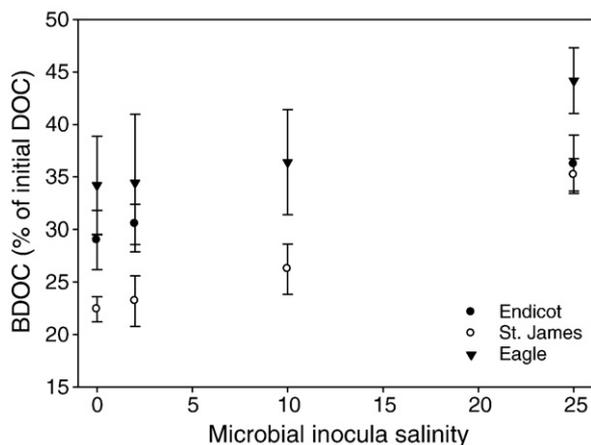

Fig. 5. The percent BDOC after the 28 day incubations for riverine samples (freshwater sample for each estuarine endmember) inoculated with water collected at different salinities for the St. James (wetland), Eagle (glacial) and Endicott River (forested) estuaries.

contributions of DOM could also satisfy a portion of the bacterial carbon demand in coastal food webs of southeast Alaska.

The observed relationship between DOM biochemical characteristics and percent BDOC supports previous research showing that the percent contribution of protein-like fluorescence is strongly correlated with DOM lability (Fig. 7b; Fellman et al., 2008). These findings indicate that phenolic compounds are not likely contributing to a large portion of protein-like fluorescence in this study. Percent BDOC was greatest in rivers receiving glacial runoff (Eagle, Lemon and Mendenhall), and is consistent with protein-rich, DOM derived from microbial populations that exist in both supra-glacial and sub-glacial ecosystems (Lafreniere and Sharp, 2004). Carbon-normalized lignin values (Λ_8) were also greatest in the wetland-dominated watershed (Peterson Creek) indicating a greater fraction of DOM derived from vascular plant material. Interestingly, the relationship between Λ_8 and percent BDOC was best fit with a single, exponential decay model likely indicating a component of the lignin pool that is not susceptible to microbial degradation, as previously reported with respect to the photochemical degradation of lignin phenols (Spencer et al., 2009). Thus, DOM inputs to estuaries from different landscapes (e.g. glacial vs. wetland) have the potential to impact coastal biogeochemical

Table 4
Mean (± 1 SE) concentration and percent BDOC during the 28 day incubations for the seven study watersheds.

	Salinity 0		Salinity 2		Salinity 10		Salinity 25	
	mg C L^{-1}	%	mg C L^{-1}	%	mg C L^{-1}	%	mg C L^{-1}	%
Eagle R.	0.32 (0.1)	34.2 (4.7)	0.33 (0.1)	34.4 (6.5)	0.37 (0.2)	39.4 (5.0)	0.42 (0.2)	44.2 (3.2)
Endicott R.	0.84 (0.1)	29.0 (2.8)	0.89 (0.1)	30.9 (1.9)	ND	ND	1.10 (0.1)	38.1 (2.8)
St. James R.	0.83 (0.1)	22.4 (1.2)	0.86 (0.1)	23.2 (2.4)	0.97 (0.1)	26.3 (2.4)	1.30 (0.2)	35.2 (1.6)
Mendenhall R.	0.38 (0.2)	43.2 (2.9)	ND	ND	ND	ND	0.41 (0.2)	46.4 (3.8)
Lemon Cr.	0.37 (0.1)	34.8 (4.4)	ND	ND	ND	ND	0.45 (0.2)	38.7 (3.4)
Montana Cr.	0.46 (0.1)	20.1 (2.9)	ND	ND	ND	ND	0.49 (0.1)	25.7 (2.3)
Peterson Cr.	0.91 (0.1)	14.7 (4.0)	ND	ND	ND	ND	1.22 (0.1)	22.9 (0.1)

ND indicates value not determined.

processes differently by influencing temporal patterns in heterotrophic productivity. Our findings taken together highlight the usefulness of characterizing DOM to predict the potential fate of terrigenous DOM in near-shore marine environments.

4.2. Lability of terrigenous DOM to freshwater and marine microbial inocula

At our study sites, marine microbial communities consumed a significantly larger fraction of terrestrially-derived DOC than the riverine microbes. This finding is consistent with previous DOM biodegradation studies showing estuarine microbial communities are more efficient at removing allochthonous DOC (Wikner et al., 1999) and DON (Stepanauskas et al., 1999a,b) from the water column than riverine microbial communities. One potential explanation given for this finding is that large mortality of the marine bacterial community could have occurred during the initial stages of the incubations, although we observed changes in BDOC throughout the entire incubation suggesting this did not occur. Moreover, at 20 fg C cell^{-1} (Lee and Fuhrman, 1987), and 1×10^5 cells mL^{-1} after the 1 in 10 dilutions, there would be 2 ng of bacterial carbon per liter. At a max growth efficiency of 50%, that would support 1 ng of bacterial carbon production per liter during the length of the incubations. These calculations suggest that mortality of freshwater intolerant microbes during the initial stages of the incubation do not likely explain the observed changes in BDOC. In addition, a freshwater lens, sometimes as deep as 1 m, can develop over the saline, marine water in the coastal waterways of southeast Alaska. We therefore hypothesize that marine bacterioplankton could be well adapted to tolerate the dramatic changes in salinity common to the inland coastal watersheds of southeast Alaska.
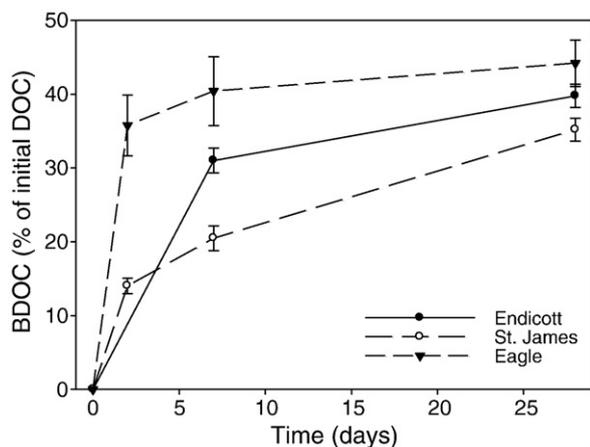
A shift in microbial community composition along the salinity gradient we sampled could result in an increased utilization efficiency by marine compared to freshwater bacteria, although research showing that site-specific bacteria are capable of obtaining a greater amount of carbon from the same source than another community are limited (Harvey et al., 2006). The increase in ionic strength in estuarine environments could also cause biochemical and conformational changes within DOM. This could result in the formation of aggregates and colloidal material (Kerner et al., 2003), and coiling of humic molecules (de Haan et al., 1987), both of which could allow for greater bacterial colonization and carbon utilization. However, results from a BDOC laboratory experiment found no significant difference in DOC utilization ($p < 0.05$) using a riverine microbial inoculum added to both river water and river water made up of different salinities with an artificial salt mixture (Fellman, unpublished data). Thus, chemical speciation changes in DOM associated with increasing salinity are not likely responsible for the observed difference in BDOC between the marine and riverine microbial inoculum in this study.

Another potential explanation given for increased utilization efficiency by marine microbes are their potential for more intensive synthesis of extracellular enzymes with greater diversity, which allows them to more effectively metabolize terrigenous DOM (Stepanauskas et al., 1999a). Previous research from a Danish river and estuary showed that riverine and estuarine bacterial communities differed in their preference to degrade different fractions of DOM (Sondergaard et al., 2003). In support, our finding that the percent contribution of protein-like fluorescence was more strongly correlated with percent BDOC for the riverine compared to the marine inoculum indicates that riverine microbes were more readily metabolizing the nitrogen-rich and presumably more labile fraction of DOM. We also found that carbon-normalized lignin yields were more strongly correlated with percent BDOC for the marine than the riverine inoculum suggesting differential uptake of certain DOM fractions by the two microbial communities. Therefore, we hypothesize that marine bacterioplankton are able to metabolize different and potentially certain fractions of the terrigenous DOM pool which are traditionally viewed as less labile than that utilized by riverine microbes.

Although we found greater DOM utilization by marine compared to freshwater bacteria, several additional estuarine studies evaluating DOM biodegradation showed no significant difference in DOC utilization between marine and freshwater microbial communities (Langenheder et al., 2003; Sondergaard et al., 2003). One possible reason for these divergent results is that different salinities were used in their laboratory incubations compared to ours, such as in Langenheder et al. (2003) where river water was compared to estuarine water with a salinity of only 4. In our incubations, we found the largest change in percent BDOC between salinities 10 and 25 and their associated microbial communities. Seasonal changes in the biochemical composition of terrigenous DOM (Spencer et al., 2008; Fellman et al., 2009b) could also shift the microbial community composition or deliver specific fractions of DOM that are more readily
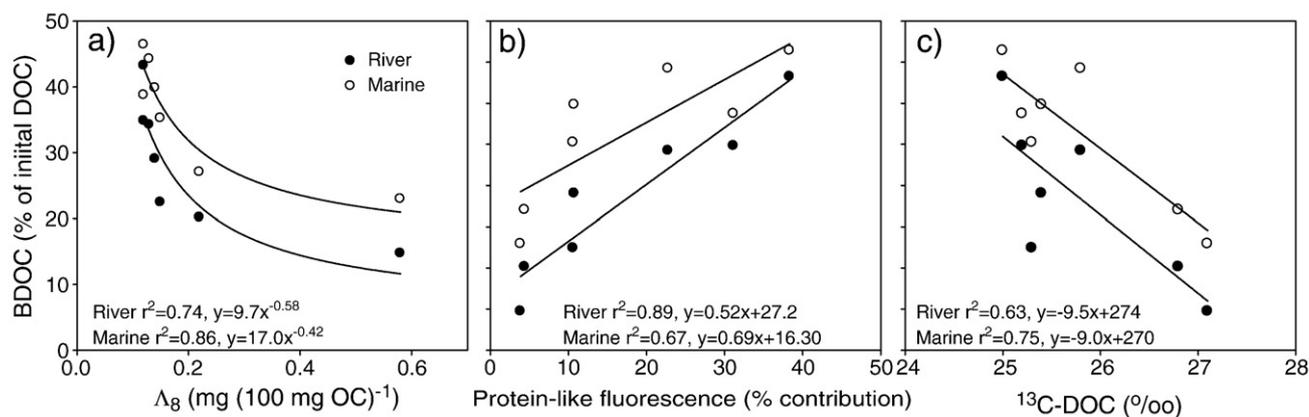

Fig. 6. The percent BDOC at time 2, 7 and 28 days for river water inoculated with the near-shore marine water (25 ppt) for the St. James (wetland), Eagle (glacial) and Endicott (forested) estuaries.

Fig. 7. Relationship between a) carbon-normalized lignin phenols (Λ_8), b) percent contribution of protein-like fluorescence (sum of tyrosine and tryptophan-like fluorescence components), c) δ^{13}C-DOC and percent BDOC using the riverine and marine microbial inocula for the seven study rivers. Protein-like components were reported as a percentage of total DOM fluorescence by quantifying the contribution for each of the eight components and dividing that by the total fluorescence of all the modeled components.

metabolized by either the riverine or marine microbes. A previous laboratory experiment using estuarine water and riverine DON amendments found that the composition of riverine DON can greatly affect its availability to estuarine planktonic communities (Seitzinger et al., 2002). Thus, shifts in DOM quality could be particularly important if near-shore marine microbial communities can metabolize DOM fractions that are commonly perceived to be more resistant to biodegradation by freshwater microbes.

4.3. Behavior of inorganic nutrients and DOM during estuarine mixing

Nitrogen and phosphorous speciation changed considerably with increasing salinity, indicating that in addition to conservative mixing of different water masses, seaward movement of N and P were modified by estuarine biotic transformations (El-Sayed et al., 2008), sorption/desorption processes (Uher et al., 2001) and photochemical degradation (Bushaw et al., 1996). In particular, the observed mixing behavior of NO_3–N and DON at low salinities is consistent with the ammonification of DON and nitrification of the released NH_4–N. Research from the Elbe Estuary showed as much as 85% of nitrification was from bacterial ammonification of DON (Kerner and Spitzy, 2001). However, NH_4–N can also be released during the photochemical degradation of DON that could stimulate rates of estuarine production (Bushaw et al., 1996). The observed changes in N species do not provide unequivocal evidence supporting either mechanism, although the high turbidity present at lower salinities (particularly in the glacial estuary) implies bacterial ammonification of DON is likely the dominant process.

There were dramatic shifts in the fluorescence properties of DOM during estuarine mixing, with observed changes in PARAFAC components consistent across all three estuaries. In particular, slight non-conservative mixing of humic-like components 1–3, 5 and 6 was observed at low salinity values (0–2), which was similar to that of bulk DOC. Bacterial degradation of DOM (Rochelle-Newall et al., 2004) is a possible removal mechanism, as are physicochemical transformations (e.g. flocculation; Amon and Meon, 2004) and adsorption onto suspended sediments (Uher et al., 2001). Similarly, both tyrosine and tryptophan-like fluorescence dropped considerably between salinity values 0 and 2, although the strong relationship between protein-like fluorescence and percent BDOC implies rapid bacterial metabolism is removing much of the protein-rich DOM in these estuaries. The fact that we found little evidence of nitrogen or phosphorous limitation in nutrient amended BDOC incubations (Fellman, unpublished data) is consistent with DOM as a source of nitrogen and carbon for riverine (Brookshire et al., 2005) and estuarine microbial communities (Kerner and Spitzy, 2001; El-Sayed et al., 2008). Overall, our findings provide evidence supporting the hypothesis (see Fellman et al.,

2009b) that tight biogeochemical linkages exist between terrestrial and downstream freshwater aquatic and marine ecosystems such that estuarine bacterioplankton are able to capitalize on the loss of biologically available DON from coastal temperate watersheds of southeast Alaska.

The differential mixing of fluorescent DOM fractions was observed between salinity values 2 and 20, as fluorescence intensities for humic-like components 1–3 and 5–6 gradually decreased around the theoretical dilution line while C4 increased from the inner to outer estuary. This gradual decrease in humic-like fluorescence (C1–3 and 5–6) indicates simple conservative mixing after the freshwater–seawater interface of DOM fractions derived primarily from catchment sources including soil organic matter and vascular plant material, which has been observed in other estuarine studies (Abril et al., 2002; Jaffe et al., 2004; Yamashita et al., 2008). Specifically, humic-like components 1 and 2 of this study have been shown to mix conservatively in two estuaries of Japan (Yamashita et al., 2008). In contrast, C4 increased non-conservatively in all three study estuaries suggesting autochthonous sources of DOM, potentially bacterioplankton production, are contributing to the pool of DOM. It is also possible that C4 could be produced photochemically (Murphy et al., 2006), as a laboratory photodegradation study of water collected from the Baltic Sea suggested that C4 could be a photoproduct of terrigenous DOM (Stedmon et al., 2007). These findings suggest that the decrease in certain DOM fractions is balanced by an increase in other fractions that could result in the apparent conservative mixing of DOC in the study estuaries. Thus, the differential mixing of DOM fractions can strongly influence its chemical quality without dramatically changing the concentration of the bulk DOM pool. Overall, our findings demonstrate that carbon dynamics in estuaries can be highly complex and using PARAFAC modeling of fluorescence EEMs to evaluate shifts in the biochemical composition of DOM during estuarine mixing can greatly improve our understanding of carbon source/sink relationships in estuaries.

The contribution of C4 has been shown to be correlated with aliphatic C content (Cory and McKnight, 2005), and DOM containing a high fraction of aliphatic C is likely derived from microbial precursor material (McKnight et al., 1994). This finding indicates that bacterioplankton production could be responsible for the large increase in fluorescence intensity of C4. In support, the tryptophan-like fluorescence, which has been related to fresh or less degraded peptide material (Mayer et al., 1999; Yamashita and Tanoue, 2004), also increased dramatically from low to mid salinities in the study estuaries. This result is similar to a previous estuarine transect study showing tryptophan-like fluorescence increased dramatically within the mid-salinity range of the estuary, which was attributed to biological production (Yamashita et al., 2008). Moreover, previous

research has also shown that planktonic communities can release labile DON compounds to the water column (Bronk et al., 1994; El-Sayed et al., 2008). The observed estuarine mixing behavior of PARAFAC components combined with the DOM utilization we found in our BDOC incubations provides strong evidence that terrigenous DOM and nutrients are being used to support bacterioplankton and/or phytoplankton growth in our study estuaries. Therefore, the processing of terrigenous DOM by estuarine food webs can strongly influence its incorporation into coastal food webs by utilizing more readily available fractions of DOM.

4.4. Implications of glacier loss on the productivity of near-shore marine ecosystems

Watersheds draining into the GOA are experiencing some of the highest rates of glacial erosion on Earth (Arendt et al., 2002) and this loss of glaciers has implications for the productivity of estuarine and near-shore marine ecosystems. Our finding that percent BDOC was greatest in the estuary draining the highly glaciated watershed combined with estimates that coastal glacial watersheds contribute ~0.10 Tg of highly labile DOC to the GOA (Hood et al., 2009) suggests that glacial runoff contributes greatly to the heterotrophic carbon demand of near-shore marine ecosystems of the GOA. Although glacial runoff into the GOA might increase in the short-term due to the loss of glaciers, our findings indicate that future reductions in glacial runoff and subsequent changes in riverine export of labile DOM and nutrients could affect the long-term ecosystem dynamics of near-shore coastal regions in southeast Alaska and the greater GOA. Furthermore, our findings show that wetland contributions of DOM to estuaries could also be an important source of carbon and energy for coastal food webs, although the specific water runoff and DOM yields are substantially higher in glacial watersheds during the summer ice-melt period and DOM yields from wetland/forested watersheds are more tightly linked to storms (Hood and Scott, 2008). This study highlights the need for future studies aimed at quantifying heterotrophic carbon demand in the GOA and how it changes throughout the primary runoff season that extends from April through October. This would greatly assist in predicting the impacts of ongoing changes in the terrestrial landscape of southeast Alaska toward near-shore coastal DOM dynamics and productivity in the GOA.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marchem.2010.03.009.

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