A multi-scale assessment of aquatic ecosystem processes across the land-ocean-atmosphere continuum: from headlands to the sea

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Abstract

A multi-scale assessment of aquatic ecosystem processes across the land-ocean-atmosphere continuum: from headlands to the sea

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Freshwater systems provide a unique cross-section of earth processes, linking terrestrial, aquatic, marine, and atmospheric reservoirs. Once thought to be passive pipes connecting land to sea, rivers are increasingly recognized as dynamic players in global carbon cycling. Rainfall mobilizes terrestrially-derived components from the landscape and as these components travel downstream they are greatly altered within the aquatic system. The transformation and remineralization of terrestrial organic matter (OM) fuels a state of net heterotrophy and CO_2 supersaturaton in the world's rivers, resulting in a globally-relevant flux of CO_2 to the atmosphere. I examined the complex sequence of processes resulting in fluvial carbon fluxes at various spatiotemporal scales in a range of latitudinal settings, from small streams in the Pacific Northwest and coastal Brazil to the mouth of the world's largest river, the Amazon.

High-resolution (hourly) measurements of dissolved and particulate geochemical parameters in the Pacific Northwest and the South Brazilian coast revealed a persistent positive correlation between river discharge and the concentration of carbon, nutrients, and terrestrial biomarkers. Discreet pools of soil carbon and nutrients with unique accumulation/mobilization

dynamics were identified with the analysis of lignin phenols and elemental stable isotopic composition. Compositional parameters in the Pacific Northwest revealed patterns of constituent mobilization from deep and shallow soil pools, whereas compositional variability in Espirito Santo revealed functionalization of unique landscape units throughout the course of a storm.

Seasonal measurements of particulate and dissolved organic matter made along the lower Amazon River revealed significant degradation of OM between the mouth and the historic upstream gauging station, Obidos. The relative abundance of both dissolved and particulate OM derived from the highland terrestrial environment, determined by isotopic and compound specific end member mixing models, significantly decreased between Obidos and each respective channel near the mouth. Highland-derived DOM was on average less than 10% of the DOC near the mouth, whereas highland POC was, on average, roughly 50% of total POC near the mouth. The concentration of POC decreased by roughly 70% between Obidos and the mouth, implying that the river contains both refractory and highly labile POC fractions. The flux of total organic carbon from the river mouth was 90% dissolved and 10% particulate. Through a series of incubation experiments it was estimated that, on average, the degradation of terrestrially derived macromolecules (e.g. lignin phenols) supports 30-50% of bulk respiration rates in the river. It is estimated that 40% of the vascular plant-derived organic carbon sequestered by the terrestrial biosphere is degraded within soils, 55% is degraded along the river continuum, and less than 5% is either stored in the basin or delivered to the ocean.

Table of Contents

Page

Table of Contents, continued

List of Figures

Page

List of Figures, continued

Page

List of Tables

List of Published Work and Co-Author Contributors

The below chapters are based on the following published manuscripts:

Chapter II: Ward, N.D.; Keil, R.G.; Richey, J.E. (2012). Temporal variation in river nutrient and dissolved lignin phenol concentrations and the impact of storm events on nutrient loading to Hood Canal, Washington, USA. Biogeochemistry. doi: 10.1007/s10533-012-9700-9

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Chapter VII: Ward, N.D. and Finley, R.J. (submitted). Hypoxia crime scene investigation: A model for integrating local research with high school science curriculum.

Chapter VIII: Ward, N.D.; Finley, R.J.; Keil, R.G.; Clay, T.G. (2013). Benefits and limitations of iPads in the high school science classroom and a trophic cascade lesson plan. Journal of Geoscience Education. Vol. 61, 3

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Chapter I: Introduction

The main goal of the collection of work described here is to explore the complex sequence of processes involved in transporting geochemical constituents from the terrestrial biosphere, along aquatic ecosystems, and into the ocean. In an effort to develop a conceptual and quantitative basis for fundamental aquatic ecosystem dynamics I performed studies at spatial scales ranging from small first order streams in temperate and subtropical latitudes (Chapters II and V) to the largest river in the world by volume, the Amazon River (Chapters III-IV), with sampling frequencies ranging from hourly to seasonal. Results and insights gained from these studies were used to quantify the respective terms of the evolving "active river" concept (Figure I.1). Further, the results of these studies were integrated into educational outreach activities to illustrate basic science principles to local high school students enrolled in Ecology and Environmental Science courses (Chapters VI-VII).

Early research in aquatic geochemistry suggested that the main role of river systems was to deliver geochemical constituents derived from the landscape to the ocean (e.g. Livingstone et al., 1963; Park et al., 1969a; Shurr et al., 1977; Richey et al., 1980). In a comprehensive review of geochemical data available at the time, Livingstone et al. (1963) described the mass export of inorganic geochemicals from the world's rivers and the factors influencing the composition of these fluxes. The solubility of the landscape was defined as the primary control on river chemistry; for example, water flowing over insoluble rocks was described to be nearly identical to rain water, whereas water flowing over/through permeable soils was described to attack the mineral constituents and leech soluble components into stream waters (Livingstone et al., 1963). Although the landscape was thought to be the primary control on river composition at the time,

the notion that organisms played a role in altering the balance of geochemicals in the aquatic environment was recognized by Lund et al. (1950) and others.

In Chapter II the sequence of processes leading up to the mobilization of geochemical components from the landscape into stream waters was explored in several temperate Pacific Northwest streams that drain into the Hood Canal estuary. High frequency measurements of dissolved organic carbon, nutrients, and lignin phenols revealed a tight correlation between the concentration of soluble carbon and nitrogen species and the river discharge rate during autumn storms. The response of a specific constituent to enhanced stream flow was mostly dependent on its inherent solubility, which has been shown to be affected by both sorptive and degradative processes (McKnight et al., 1992; Qualls and Haines, 1992; Day et al., 1994; Aufdenkampe et al., 2001; Hernes et al., 2007). For example, in Pacific Northwest soils, organic carbon was more readily mobilized than nitrogen and phosphorous, whereas silica, which is generally insoluble (Kennedy, 1971), was only mobilized by base flow and was diluted by rapid storm flow (Chapter II). In Chapter V a similar high frequency approach was taken in the tropical/sub-tropical SE coastal state of Espirito Santo, Brazil to determine the response of suspended sediments and organic matter to rapid storm flow and the effects of specific land use regimes on sediment transport processes. Measurements were made in the main channel of the Rio Santa Maria Vitoria downstream of two dams, which provides water to 30% of the population of the state's capital, Vitoria, and a small second-order tributary, the Rio Mangarai. Fluvial sediment transport plays an important role in the delivery of both sediments and sedimentary organic carbon to marine receiving waters (e.g. Hedges et al., 1976; Nittrouer et al., 1979). Abundant suspended sediments can also create significant difficulties in the sanitation of freshwater (Carr et al., 2008). This issue is of particular importance in the Rio Santa Maria da Vitoria studied in Chapter

V, as high sediment concentrations during heavy storm flow inhibits the production of clean freshwater.

The export of geochemical components from watersheds, and organic carbon in particular, was a topic of significant interest in the 1970's and 1980's. Degens et al. (1982) described three main sources for river organic carbon: allochthonous, or derived from terrestrial production; autochthonous, or derived from *in situ* aquatic production; and anthropogenic inputs derived from agricultural, industrial, and domestic activities. The export of total organic carbon from watersheds around the world was estimated to be roughly 1% of terrestrial net primary production (Whittaker and Likens, 1973). By compiling all available fluvial organic carbon export data collected during this time period Hope et al., (1994) noted interesting similarities and differences between watersheds around the world. For example, the per volume flux of OC was relatively similar across temperate forested watersheds studied in North America varying in size from small streams to large river networks such as the Columbia and St. Lawrence Rivers (e.g. Weber and Moore, 1967; Efford, 1972; Gross et al., 1972; Wetzel et al., 1972; Wetzel & Otsuki, 1974; Hobbie and Likens, 1973; Fisher, 1977; Richey et al., 1978; Moeller et al., 1979; Naiman and Sedell, 1979 Richey and Wissmar, 1979; Dahm et al., 1981; Schreiber and Duffy, 1982; Tate & Meyer, 1983). Likewise, the dynamics and magnitude of OC export were similar to measurements made in other temperate forested watersheds around the same time in New Zealand (e.g. Moore, 1989; Moore and Jackson, 1989) and Europe (e.g. Dueser, 1971; Pieczynska, 1972; Postma, 1973; Sholkovitz, 1976; Moeller et al., 1979; Cauwet and Martin, 1982; Stevens et al., 1989). In general, all of these temperate watersheds showed a positive correlation between river discharge and organic carbon concentrations that has been similarly measured on rapid timescales, here in Chapters II and V, and elsewhere (e.g. Boyer et al., 1997;

Hinton et al., 1998; Buffham et al., 2001; Sigleo and Frick, 2003; Hernes et al., 2008; Raymond and Saiers, 2010). Although discharge was the most commonly reported control on the concentration of river organic carbon, flow path and soil parameters were recognized to have a strong influence on the magnitude and composition of organic components (Edwards and Cresser, 1987; Billett and Cresser, 1992). For example, in NE Scotland a positive correlation was observed between the flux of OC and the relative predominance of hill peat land use regimes (Creasey, 1984; Edwards, 1984; Cresser and Edwards, 1987). Variability in the relationship between OC concentrations and discharge was similarly attributed to variability in flow paths and soil type in New Zealand watersheds (Moore and Jackson, 1989). Grieve et al. (1991) suggested that variability in the relationship between OC concentrations and discharge is related to the proportion of mineral versus peat-dominated soil coverage. The relationship between discharge and OC is further complicated by in stream retention mechanisms such as those described by Bilby and Likens (1980). Newbold et al. (1982) suggested that the overall ability for a watershed to retain, recycle, or export organic matter can be described as "carbon spiraling," or the balance between river flow and OC retention as opposed to a typical closed-loop system used for terrestrial ecosystems. This balance (i.e. how far downstream a spiral will persist) is influenced by climatic controls such as precipitation, river flow, and temperature (Minshall et al., 1983) and the inherent lability of the transported organic carbon (Lush et al., 1981).

Around the same time, Richey et al. (1980) made the first estimates of organic carbon fluxes from the world's largest river, the Amazon, which represented the first systematic assessment of organic carbon levels throughout the hydrograph in a large tropical river. OC levels in the Amazon were similarly highest at rising and peak river discharge, but the levels of

organic carbon relative to discharge were generally higher than the temperate systems described above (Richey et al., 1980). These Amazon River OC fluxes implied that, globally, freshwater systems contributed significantly more carbon to the ocean than previous estimates suggested (Richey et al., 1981; Schlesinger and Melack, 1981). The OC load at Obidos, roughly 900 km from the Amazon River mouth, was roughly 60% dissolved and 40% particulate load (Richey et al., 1980, 1990). Measurements made at the outflow of the Bonneville dam, roughly 200 km from the Columbia River mouth, on the other hand, showed that 90% of the OC load was in the dissolved phase (Dahm et al., 1981). The burial of sediments in Columbia River dam reservoirs is the most likely reason for this difference. Measurements made near the mouth of the Amazon River (Chapter IV), however, showed similarities to the observations by Dahm et al (1981) in the Columbia River, with roughly 90% of the OC load in the dissolved phase near the Amazon River mouth (Chapter IV). There are currently no dams along the Amazon River mainstem (Lewis et al., 1995; Richey et al., 1997), however, it is possible that ocean tides create conditions near river mouths that impose effects similar to that of a dam reservoir (Wright, 1977). Perhaps the most striking revelation from the Richey et al. (1980) organic carbon export estimates, however, was the presence of a mysterious "oxidation flux" that was required to balance the river carbon budget.

Delving into the inorganic carbon chemistry of freshwater, Park et al (1969a) constructed an alkalinity budget of the Columbia River, which indicated that the export of bicarbonate from rivers was much higher than previous estimates. Park et al. (1969a) further posited that the extensive damming of the Columbia River led to a homogenization of geochemical fluxes from the river compared to a naturally-flowing system that experiences high geochemical variability under varying flow conditions. Park et al. (1969b) made some of the first systematic

measurements of freshwater carbon dioxide levels in the Columbia River. These early measurements showed that the Columbia River was supersaturated with respect to atmospheric CO_2 by 200-870ppm throughout the year (Park et al., 1969b). Coyne and Kelley (1974) also observed a supersaturation of CO_2 in several Arctic Rivers, but levels were not as high as measured in the Columbia River. To understand the biotic mechanism driving the balance between aquatic O_2 and CO_2 levels, Schurr et al. (1977) measured rates of respiration and photosynthesis in several Swiss rivers. The rate of respiration measured by Schurr et al. (1977) was roughly double the rate of photosynthesis. These studies provided some of the early evidence that *in situ* metabolism plays an important role in the transformation of carbon along river systems. The river continuum concept was developed by Vannote et al. (1980) to describe the dynamic variability in river chemistry observed in the world's rivers. Rivers were described as a continuum from the headlands to the river mouth; inputs of organic matter varied along this continuum depending on the geological and biological makeup of specific terrestrial environments and the composition of biotic communities varied across the continuum to maintain equilibrium with the dynamic physical/chemical characteristics at any one location (Vannote et al., 1980).

As a result of the evidence described above, it became increasingly recognized that organic carbon in streamwaters plays an important role in numerous processes beyond export to the ocean (Schlesinger and Melack, 1981; Degens et al., 1982; Meybeck, 1982; Degens et al, 1991; Hope et al., 1994). For example, the presence of organic matter had been shown to alter the mobilization and transport of organic pollutants (Carter and Suffet et al., 1982) and metals such as iron, copper, aluminum, mercury, and zinc (Schnitzer and Khan, 1972; Reuter and Perdue, 1977; Baker and Schofield, 1982; Buffle, 1984). The influence of organic carbon on

particle surface and colloidal chemistry was observed by Tipping et al. (1986); this reversible exchange of organic matter from dissolved to particulate phases through particle sorption/desorption has been recognized to be an important control on organic matter composition, abundance, and mobilization (Easthouse et al., 1992; Aufdenkampe et al., 2001; Hernes et al., 2007). Zafiriou et al. (1984) noted the importance of organic carbon on the photochemistry of freshwater and marine environments, which has since been explored in freshwater systems worldwide (e.g. Hernes and Benner, 2003; Rodriguez and Zuniga, 2008; Spencer et al., 2009; Remington et al., 2011). Fisher and Likens (1973) constructed an energy budget for a New Hampshire stream ecosystem and estimated that 99% of the energy in the river was derived from the terrestrial biosphere. Fisher and Likens (1973) hypothesized that the leaf and branch compartments of terrestrial plant biomass turned over on the scale of 1 to 4.2 years, respectively, a pool of detritus of similar magnitude to the leaf and branch reservoir is stored in surface soils on an annual basis, and dissolved organic matter turns over very rapidly and does not accumulate. Results from Chapter II and V explore this phenomenon of accumulation/mobilization and indicated that in both tropical and temperate systems a shallow pool of organic matter accumulates during dry periods and is mobilized by rapid rainfall during rising and peak high water, similar to other studies performed in similar environments (e.g. Boyer et al., 1997; Hinton et al., 1998; Buffham et al., 2001; Sigleo and Frick, 2003; Hernes et al., 2008; Raymond and Saiers, 2010).

John Hedges developed novel tools and approaches to characterize terrestrially-derived organic compounds in aquatic and marine environments capable of assessing the potential role of riverine organic matter as food for aquatic organisms, a source of biogenic material to the marine biosphere, and an indicator of catchment climate and vegetation regimes (e.g. Hedges and

Parker, 1976; Hedges and Mann, 1979a; Hedges and Ertel, 1982; Ertel and Hedges, 1984, 1985). Leo and Barghoorn (1970) and Gardner and Menzel (1974) were the first to identify phenolic aldehydes in marine sediments, suggesting that these compounds were potential indicators of terrestrially-derived organic matter since phenolic structures were typically not present in planktonic organisms (Brauns and Brauns, 1960). Hedges and Parker (1976) made systematic measurements of a suite of lignin phenols and the stable isotopic composition of marine sediments in the Gulf of Mexico, determining that angiosperm-derived grass and leaf material was the primary source of terrestrially-derived organic matter to the inner continental shelf. These indicators were not found in high abundance beyond the inner shelf (Hedges and Parker, 1976). Keil et al. (1997) later made observations illustrating the disappearance of organic matter from particles in river deltas. Hedges and Mann (1979b) made similar measurements in coastal sediments along the southern Washington coast indicating that the terrestrial component of marine sediments was primarily derived from gymnosperm wood and non-wood angiosperm tissues. The higher abundance of gymnosperm material measured in marine sediments in the Pacific Northwest, which contains significant old growth conifer forests, relative to the Gulf of Mexico, which receives inputs from catchments dominated by grasses and flowering plants, illustrates the linkage between land use regime and the composition of river organic matter (Hedges and Parker 1976; Hedges and Mann, 1979b; Goni et al., 1997, 1998). Hedges and Mann (1979b) estimated that terrestrially-derived phenolic compounds remained largely unaltered for periods of over 400 years in marine sediments on the southern WA coast. Hedges et al. (1985) determined that 90-98 wt% of the polysaccharides and at least 15-25 wt% of the lignin in woods buried in marine sediments was degraded over a 2,500 year period. The preservation of terrestrially-derived organic matter was hypothesized to be facilitated primarily by the inherent

association of specific types of OM with mineral surfaces and the presence of coastal regions with low oxygen levels and high rates of sediment burial (Hedges and Keil, 1995; Keil et al., 1995; Cowie et al., 1997; Hedges et al., 1997; Keil et al., 1997; Thimsen and Keil, 1998; Keil and Cowie, 1999). Experiments performed in the laboratory by Hedges et al. (1988) indicated that white-rot fungi were able to degrade these phenolic compounds quite rapidly in soil environments and the signature of degradation was captured by the ratio of specific phenols to one another, similar to the species-dependent relationship between other specific phenols (Sarkanen and Ludwig, 1971). Benner et al. (1984a,b, 1986) also measured appreciable levels of degradation of lignocellulose material in salt marsh sediments and suggested that bacteria were the primary consumer of lignocellulose organic matter in these environments.

Cumulative evidence from this time period suggested that microbial metabolism of organic carbon was highly relevant to the overall flux of carbon from the basin. The balance between *in situ* primary production and microbial respiration was further explored by Cole et al. (1992), who measured phytoplankton production in the tidally-influenced portion of the Hudson River. Primary production was strongly light-limited as a product of river turbidity and mixing; for example, it was estimated that algal cells spent only 2-4 hours per day above the 1% light threshold, severely limiting the potential for photosynthetic activity (Cole et al., 1992). The idea that microbial respiration generally outweighs primary production in aquatic environments became widely accepted after observations by Cole et al. (1994) showing that lakes around the world, regardless of latitude, were generally net heterotrophic and, thus, supersaturated with respect to atmospheric CO_2. Prior to these global observations Kling et al. (1991) measured significant evasion of CO_2 and methane from Arctic lakes and peatland-draining streams. Systematic measurements at high latitudes indicated that aquatic environments in the Arctic were

generally net sources of CO_2 to the atmosphere, contrary to previous conceptions (Oechel et al., 1993). Continued work in the Arctic has revealed that the state of high latitude systems as either CO_2 sources or sinks can fluctuate depending on long term climate trends; for example, aquatic CO_2 evasion was enhanced by a warm period in the 1980's, but after continued warming, CO_2 evasion rates began to decrease and sometimes reverse to a net sink during the summer (Oechel et al., 2000). The presence of previously frozen organic carbon in permafrost further complicates the carbon balance of high latitude systems. Boreal and Arctic permafrost reservoirs store nearly twice as much CO_2 as is present in the atmosphere (Zimov et al., 2006; Schuur et al., 2008). This frozen OC has been shown to be highly biolabile once thawed and mobilized into the aquatic environment (Holmes et al., 2008; Schuur et al., 2009; Spencer et al., 2009). Thus, the thawing and release of high latitude permafrost reservoirs is suspected to be among the most significant potential positive feedbacks for global climate change (Davidson et al., 2006; Field et al., 2007; Guo et al., 2007; Walvoord et al., 2007; Heimann et al., 2008). Export of terrigenous organic matter from Arctic rivers behaves differently than temperate and tropical systems due to annual freeze/thaw cycles, which cause the majority of material flux to occur rapidly during spring thawing (Holmes et al., 2008; Spencer et al., 2009). In a sense, this rapid export during spring thawing is similar to the storm-driven export observed in temperate and tropical systems, but occurs over a much shorter time period (Drake and Cacchione, 1985; Ogston et al., 2004; Wheatcroft and Sommerfield, 2005; Dalzell et al., 2007; Hernes et al., 2008; Raymond and Saiers, 2010, Chapters II and V).

Shortly after the discovery of global CO_2 supersaturation in lakes by Cole et al. (1994), Raymond et al. (1997) determined that the partial pressure of carbon dioxide in the temperate Hudson River remained well above atmospheric saturation with respect to CO_2 throughout the

entire year. Even greater levels of CO_2 supersaturation were soon measured in the tropical Amazon River by Richey et al. (2002), who estimated that the Amazon River basin outgasses 0.5 Pg C yr^{-1}, which is roughly equivalent to the amount of carbon estimated to be annually sequestered by the Amazon rainforest (Malhi et al., 2008). Cole et al (2007) estimated that roughly 1.9 Pg C yr^{-1} moves through aquatic systems, of which 0.8 Pg C yr^{-1} is outgassed to the atmosphere, 0.2 Pg C yr^{-1} is stored, and 0.9 Pg C yr^{-1} is delivered to the ocean. Butman et al. (2011) measured significant rates of CO_2 evasion from rivers and streams around the continental United States implying even higher global rates than previously estimated. It is currently estimated that roughly 2.9 Pg C yr^{-1} is mobilized from the terrestrial biosphere into freshwater systems, globally, of which 2.1 Pg C yr^{-1} is remineralized and outgassed to the atmosphere before reaching the ocean and/or being buried in sediments (Raymond et al., 2013; Regnier et al., 2013). The global evasive CO_2 outgassing flux is roughly equivalent to the estimated terrestrial sink of 2.4 Pg C yr^{-1} of anthropogenically-produced CO_2 (Le Quere et al., 2009; Battin et al., 2009; Regnier et al., 2013). Tropical river systems represent hotspots for carbon dioxide outgassing, with approximately 70% of the global CO_2 flux evading from only 30% of the earth's landscape (Raymond et al., 2013). The transport of terrestrially-derived organic material through aquatic ecosystems is generally enhanced in the tropics relative to temperate and high-latitude counterparts, largely due to increased temperatures and precipitation (Meybeck, 1982; Aufdenkampe, 2011). This was evident when comparing high frequency data sets collected in the Pacific Northwest and SE coastal Brazil (Chapters II and V, respectively). For example, the positive relationship between particulate organic carbon concentrations with river discharge during storm flow was amplified in the tropical/sub-tropical coastal Brazilian watershed relative to the temperate Pacific Northwest.

Extensive research efforts have recently been applied to understanding the underlying dynamics and organic matter sources fueling the globally ubiquitous carbon dioxide evasion from inland waters and the terrestrial landscape. Schlesinger and Andrews (2000) suggested that soil respiration is the primary pathway for conversion of terrestrial carbon back to atmospheric CO_2. Degradation by fungi and bacteria has been shown to be the primary pathway for the breakdown of lignin in soils and aerated peat (Otto and Simpson, 2006; Kuzyakov et al., 2010; Thevenot et al., 2010). Feng et al. (2008) observed that the breakdown of lignin in soils was enhanced by increased temperatures. On a global scale, soil bulk respiration rates are also generally positively correlated with temperature and precipitation (Raich and Schlesinger, 1992; Kirschbaum, 1995; Raich and Potter, 1995). Because of this link, some suggest that soil respiration is another potential positive feedback for climate change (e.g. Woodwell et al., 1998; Cox et al., 2000; Lenton et al., 2000), whereas others have suggested that soil respiration rates may acclimatize to higher temperatures, weakening the feedback mechanism (Luo et al., 2001). Soil respiration can also be enhanced by perturbations such as soil tillage during agricultural cultivation and elevated temperatures, whereas processes such as increased nitrogen deposition can enhance soil/vegetation carbon sequestration (Schlesinger and Andrews, 2000). In addition to creating high levels of groundwater DIC, soil respiration plays an important role in the transformation and mobilization of organic matter into aquatic environments (Cole et al., 2013).

Direct CO_2 inputs from soil respiration, likely contribute significantly to CO_2 levels in small streams due to the tight land-water interface (Vannote et al., 1980; Peterson et al., 2001; McClain and Elsenbeer., 2001; Palmer et al., 2001; Johnson et al., 2008). On the other hand, observations in the main channel and tributaries of the Amazon River by Benner et al. (1995) and Devol et al. (1995) indicated that depth-integrated microbial respiration was the primary

source of elevated pCO_2 in large tropical river systems. Analyses of organic carbon and microbial communities made in the temperate Columbia River by Crump et al. (1998) suggested that the primary pathway for the breakdown of sedimentary organic matter was biological degradation by particle-bound bacteria. Stable and radiocarbon isotopic measurements of carbon dioxide and organic matter made by Mayorga et al. (2005) in the main channel and tributaries of the Amazon River revealed that young organic matter, derived from a mixture of C3 and C4 terrestrial vegetation, was the primary fuel for CO_2 outgassing from the Amazon River. Floodplain inputs of labile organic matter and aquatic plant respiration-derived CO_2 have also been suggested to contribute significantly to the Amazon River's evasive CO_2 gas flux (Quay et al., 1992; Abril et al., 2014). Whereas dissolved inorganic carbon (DIC) was ubiquitously young in the Amazon (Mayorga et al., 2005), Caraco et al. (2010) measured relatively old DIC ages in the temperate Hudson River. Although, aged DIC is typically a product of chemical weathering, microbes and plankton in the Hudson River were observed to have relatively old radiocarbon signatures, implying that a significant portion of bulk respiration was driven by the breakdown of old organic matter (Caraco et al., 2010). In the Amazon River, bulk organic carbon pools became younger downstream, implying that the bulk OC was reactive and overturned quite rapidly (e.g. less than 5 years); further, old carbon was generally degraded before it was able to escape the tropical river system (Mayorga et al., 2005). In the Hudson River, on the other hand, respiration was determined to be fueled by a small pool of OC that cycles on millennial time scales; the radiocarbon age of bulk OC did not decrease downriver, implying that the majority of bulk OC was not reactive and old OC could escape the aquatic system (Caraco et al., 2010). The observed differences between tropical and temperate systems are likely a result of a combination of

physical (e.g. soil, vegetation, land use, and topographical regimes) and climatic (e.g. temperature and precipitation) factors (Aufdenkampe et al., 2011; Cole et al., 2013).

Recent studies have explored the breakdown of specific compounds such as lignin phenols in the aquatic environment. For example, the radiocarbon age of specific particulate lignin phenols was determined to be ubiquitously young in the Mekong River, whereas the age of bulk OC varied throughout the hydrograph, implying that terrestrially-derived macromolecular compounds are turned over relatively quickly in the aquatic environment (Ingalls et al., 2010; Martin et al., 2013). Measurements made in the Mississippi River plume indicated that both photo-oxidative and microbial degradative processes played a role in the breakdown of terrigenous dissolved organic matter (Hernes and Benner, 2003). Some studies suggest that the breakdown of terrestrially-derived organic matter in aquatic environments can be stimulated by the addition of labile substrates such as algal biomass (Guenet et al., 2010; Bianchi et al., 2011). The chromophoric fraction of DOM (CDOM) generally correlates with terrestrially-derived compounds such as dissolved lignin phenols and black carbon (Spencer et al., 2009a; Stubbins et al., 2012). Spencer et al. (2009a) determined that dissolved lignin phenols, and their associated CDOM signatures, were highly photo-reactive in the tropical Congo River. Black carbon found in the deep ocean was similarly shown to be photo-reactive (Stubbins et al., 2012).

Chapter III explores the microbial breakdown of dissolved and particulate lignin phenols near the Amazon River mouth. The high rates of degradation measured suggest that the residence time of these terrestrially-derived macromolecules is roughly 2-3 weeks in the tropical Amazon basin. In Chapters II and IV variability in the concentration and composition of dissolved and particulate lignin was examined in the Pacific Northwest on hourly timescales and near the

mouth of the Amazon River on seasonal timescales, respectively. In the Lower Amazon River the abundance of lignin phenols was maximal at rising and high water and minimal at low water similar to measurements made further upstream in the Amazon (e.g. Hedges et al., 1986; Moreira-Turcq, 2013) and in other tropical rivers such as the Mekong (Ellis et al., 2011) and the Congo (Spencer et al., 2010), and temperate systems in the continental United States (Hernes et al., 2008; Spencer et al., 2012). High frequency measurements made in the Pacific Northwest showed a positive correlation between the concentration of dissolved lignin phenols and stream flow during periods of rapid rainfall (Chapter II), similar to measurements of episodic material export in similar environments (Drake and Cacchione, 1985; Ogston et al., 2004; Wheatcroft and Sommerfield, 2005; Dalzell et al., 2007; Hernes et al., 2008; Raymond and Saiers, 2010). Likewise, the export of sediment and particulate organic carbon in Espirito Santo, Brazil was greatly enhanced by short-term rainfall (Chapter V).

Beyond scientific discovery, a large goal of my work has been to bring the process and ideology of science to a broader audience. Chapters VI and VII describe my efforts to incorporate lessons learned from current research into curricular materials for educational outreach purposes. The goal was to create stimulating course materials about fundamental science concepts, provide students the opportunity to approach course materials (and life challenges) from a scientific perspective based on critical thinking, and illustrate the viability of science as a career choice to young students with no previous frame of reference.

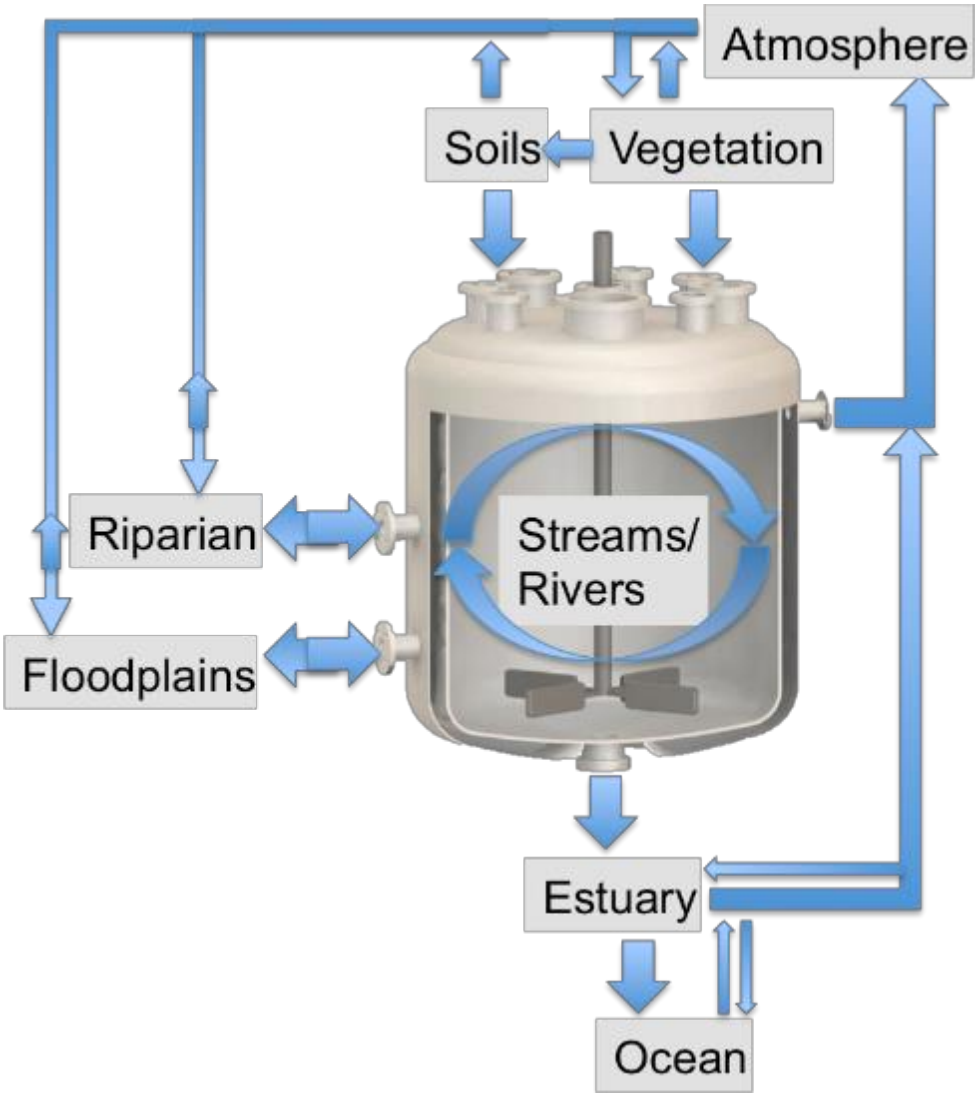


Figure I.1 Conceptual diagram of the "active river." The river system receives a continuous input of organic and inorganic components as water percolates through soil and vegetation layers (Livingstone, 1963). Carbon is constantly mobilized both in the form of organic compounds and CO_2 from organic material that has already been respired (Vannote et al., 1980; Peterson et al., 2001; McClain and Elsenbeer., 2001; Johnson et al., 2008). Lateral exchange with the riparian zone and floodplains is an additional source of energy and matter to the river (Sioli 1984; Junk and Howard-Williamson, 1984; Junk, 1997; Melack and Forsberg, 2001; Abril et al., 2014). As a

parcel of water travels downstream CO_2 is constantly outgassed and replenished by continuous remineralization of organic carbon (Cole et al., 1994; Raymond et al., 1997; Richey et al., 2002; Butman et al., 2011; Raymond et al., 2013)

Chapter II: Geochemical dynamics of soil-to-stream mobilization and the impact of storm events on nutrient loading to Hood Canal, WA

Foreword: *The following chapter is derived from the published work: Ward, N.D.; Keil, R.G.; Richey, J.E. (2012). Temporal variation in river nutrient and dissolved lignin phenol concentrations and the impact of storm events on nutrient loading to Hood Canal, Washington, USA. Biogeochemistry. doi: 10.1007/s10533-012-9700-9.*

II. 1 Introduction

The magnitude of nutrient export from a watershed has been demonstrated to be closely coupled with the rate of water discharge on seasonal time scales (Guillaud et al., 2007). Studies have also shown a tight coupling between river discharge rates and dissolved nitrogen and carbon concentrations on timescales from days to weeks (e.g. Boyer et al., 1997; Hinton et al., 1998; Buffham et al., 2001; Sigleo and Frick, 2003; Hernes et al., 2008; Raymond and Saiers, 2010). Rapid loading events have been estimated to contribute to as much as 86% of annual OC export in forested watersheds (Raymond and Saiers, 2010) and 71-85% of OC export in agricultural watersheds (Dalzell et al., 2007). Furthermore, it has been shown that OM biodegradability significantly increases with peak discharge in both temperate and high-latitude river systems, suggesting that storm events play a significant role in mobilization of potentially labile terrestrial carbon (Buffham et al., 2001; Holmes et al., 2008; Fellman et al., 2009). Small streams are the primary source of terrestrial material to river systems because of the tight land-water interface; rapidly changing conditions at this spatial scale leads to dynamic chemical conditions within the stream (Vannote et al., 1980; Peterson et al., 2001; McClain and Elsenbeer., 2001).

Our understanding of downstream N cycling has been greatly improved by various ^{15}N tracer studies, which allow the quantification of important processes such as nitrification and subsequent NO_3^- and NH_4^+ uptake (e.g. Mulholland et al., 2000; Peterson et al., 2001; Webster et al., 2003; Deegan et al., 2010). Observations of short term compositional changes in stream OC such as increases in aromaticity, detected by specific UV absorbance (SUVA), with peak discharge have allowed for a more detailed characterization of OC and hydrologic flowpaths (Hood et al., 2006; Vidon et al., 2008; van Verseveld et al., 2009). Spatial analysis suggests that distinct landscape units become functional during these hydrologic events, altering nutrient and DOC mobilization dynamics throughout the course of a storm (Hinton et al., 1998; McGlynn and McDonnell, 2003; Ocampo et al., 2006). However, the mechanistic mobilization dynamics of inorganic and organic constituents from the terrestrial biome are still not well constrained.

Input of nutrient-rich freshwater has been shown to cause eutrophication in nutrient-limited estuarine or coastal systems, which under certain conditions results in hypoxic events due to microbial respiration of sinking bloom-derived organic matter (OM) (Rabalais, et al., 2001; Howarth and Marino, 2006). Observations in Hood Canal, Washington—a branch of the Puget Sound estuary where this study is focused—have shown that low dissolved oxygen conditions in Hood Canal, especially in the southernmost Lynch Cove region (Figure II.1), have become more persistent and widespread in recent decades compared to the 1930's to 1960's, resulting in increased frequency of hypoxic and fish kill events (Newton et al., 1995; Newton et al., 2002). One possible driver for the observed increase in frequency of hypoxic events is an increase in watershed nutrient loading rates. Changes in land use can have profound effects on the chemical composition of a stream and greatly influence the potential for watershed nutrient delivery (Biggs et al., 2004; Thomas et al., 2004; Neill et al., 2006). Recent increases in urbanization and

N_2 fixing red alder tree prevalence has likely influenced the nutrient inventory of the Hood Canal watershed (Newton et al., 2002). Thus, understanding the timing and magnitude of river nutrient delivery is critical for predicting potential impacts of a watershed on the basin in which it drains. Furthermore, in order to assess the fate of terrestrially derived OM in the context of global biogeochemical cycling, it is crucial to first understand the mechanisms by which OM is mobilized from land into river systems.

In this study, variability in stream nutrient and OM concentration and composition were measured at a fine temporal resolution in an effort to enhance our understanding of the dynamical coupling of hydrologic and geochemical fluxes into streams. Storm events were sampled over a three to four day period on a three to four hour interval for an entire year in the Skokomish and Union Rivers, which drain into the Lynch Cove region of Hood Canal (Figure II.1). In addition to the measurement of dissolved nutrients and particulate OM, dissolved lignin phenol concentrations were measured to determine changes in the composition and source of dissolved organic carbon (DOC) throughout the course of a storm. Lignin is a terrestrial biomarker unique to vascular plants that can be used as an indicator of the OM source (e.g. Hedges and Ertel, 1982; Goni and Hedges, 1992); changes in phenolic ratios throughout a storm were used to determine variation in the composition of OM mobilized by base flow and rapid shallow flow. The goal of this study is two-fold: to assess the impact of short term storm events on nutrient loading rates and hypoxia in Hood Canal, Washington and to gain a better understanding of the mechanisms behind nutrient and OM mobilization in an effort to improve predictive capabilities concerning the fate of terrestrially derived material in the context of global biogeochemical cycling.

II. 2 Methods

II. 2.1 Study Area

The Hood Canal watershed (Figure II.1) has a surface area of ~3,050 km^2, of which Hood Canal itself comprises 12%. The watershed can be separated into three zones: 1) the large mountainous watersheds of the Olympic Mountains with hydrographs dominated by snowmelt, 2) the Skokomish River (the largest catchment in the watershed), and 3) the smaller, lower elevation watersheds that dominate the Kitsap Peninsula and the southern, southeastern, and northwestern parts of Hood Canal. More than 60% of the total annual precipitation occurs between November and January, and less than 10% occurs between June and August (Peterson et al., 1997). Similarly, TDN concentrations in the Skokomish and Union Rivers peak during autumn and winter months and reach a minimum during dry summer months (Steinberg et al., 2010). Hood Canal is characterized by slow marine flushing rates. Low DO conditions are most prevalent in the southernmost Lynch Cove region of Hood Canal, where DO concentrations less than 5 mg l^{-1} were observed below the pycnocline every month of the year from 1998-2000 (Figure II.1; Newton et al., 2002). For this reason, two rivers representative of those draining primarily into the Lynch Cove region, the Skokomish and Union Rivers were sampled.

The Skokomish River catchment, accounting for ~22% of the freshwater input into Hood Canal with an average annual runoff rate of 3.2 m/yr and discharge of 37.6 m^3/s, is situated in the southwest portion of the watershed, where annual precipitation rates are generally highest (Steinberg et al., 2010). The Skokomish catchment is sparsely populated with 0-4 people/km^2 and is primarily mature coniferous forest (46%) managed as national parks, and national or commercial forest (Steinberg et al., 2010).

The Union River drains into the southeast tip of Lynch Cove. The Union River and the numerous similar lowland draining streams account for ~24% of the freshwater input into Hood Canal with a runoff rate of 1.1 m/yr (Steinberg et al., 2010). Compared to the Skokomish catchment, these lowland catchments have experienced more disturbance by clearing, suburban development, and wetland draining (Ness and Fowler, 1960; McCreary, 1975; McMurphy, 1980). These watersheds have moderate population densities averaging 20 people/km^2. Dominant land use is primarily coniferous (~23% mature, ~15% young) and mixed deciduous (~30%) forests (Steinberg et al., 2010). Approximately 50% of the land covered by mixed deciduous forest consists of N_2-fixing red alder trees (L. McGeoch, unpubl. Data).

II. 2.2 Sampling and Analysis

Samples were collected at three to four hour intervals depending on forecasted storm duration using Teledyne ISCO autosamplers placed roughly 4km upstream of the river mouths of the Union (47º27'48.61" N, 122º49'55.92" W) and Skokomish Rivers (47º19'09.73" N, 123º08'22.47" W). Water was pumped through Teflon tubing placed near the center of the river and fitted with a coarse mesh to exclude large debris (7mm pore size). Twenty-four samples were collected for each storm in acid-washed 1 L polyethylene bottles, which were also pre-rinsed with river water prior to sampling. Upon completion of the autosampler sequence, samples were immediately placed on ice and transported to the lab for filtration and analysis. Significant storm events were sampled over a three or four day period from October 2008 through October 2009. Real-time river discharge data for the Skokomish River was obtained from USGS (http://waterdata.usgs.gov/nwis/rt). Union River discharge data was provided by the Hood Canal Salmon Enhancement Group (HCSEG).

Dissolved nutrient samples (NO_3^-, NO_2^-, NH_4^+, SiO_4^{4-}, and PO_4^{3-}) were filtered through Whatman® cellulose acetate filters (0.45 μm), and analyzed on an Alpkem RFA/2 autoanalyzer. TDN and DOC were measured on a Shimadzu DOC analyzer after filtering through pre-combusted Whatman® GF/F glass fiber filters (0.7 μm nominal pore size) into pre-cleaned glass vials. Dissolved Organic Nitrogen (DON) concentration was calculated by subtracting all inorganic nitrogen species from TDN. For particulates, between 100 and 600 mL of stream water (depending on turbidity) were filtered through pre-combusted Whatman® GF/F glass fiber filters. Particulates captured on the filters were then dried at 55 °C for 24 hours, acid-fumigated for 24 hours, dried at 55 °C for an additional 24 hours, and packed in tin capsules. The samples were analyzed for N and C concentration and stable isotope ratios at UC-Davis Stable Isotope Facility on a PDZ Europa ANCA-GSL elemental analyzer. Total Suspended Sediment (TSS) was assessed by filtering between 100-600 mL of water through pre-weighed/combusted Whatman® GF/F glass fiber filters. The filters were then dried 55 °C for 48 hours, and reweighed.

Solid phase extraction of dissolved lignin was performed as described by Keil and Neibauer (2009); water samples were prefiltered through combusted Whatman® GF/F glass fiber filters, acidified to pH 2 with HCl, and passed through 200 mg Waters Oasis HLB cartridges at a rate of 3mL min^{-1}. Solid phase extraction cartridges were pre-conditioned sequentially with 1 mL methanol, 1 mL ethyl acetate and 1 mL acidified (pH 2) MilliQ water. The solid phase was then eluted from the cartridge with 10 mL ethyl acetate at 3 mL/minute. Samples were analyzed via Gas Chromatography using an Agilent 6890N GC-FID following an alkaline CuO oxidation performed in a CEM Microwave Accelerated Reaction System as described by Goni and Montgomery, 2000. Particulate lignin samples were analyzed in the same fashion; the Whatman®

GF/F glass fiber filters used to measure TSS were placed directly into the Teflon microwave vessels to undergo CuO oxidation (Goni and Montgomery, 2000). Combusted filters were used as blanks to assess any lignin signature present on the GF/F filters, which was nominal.

II. 3 Results

II. 3.1 Dissolved Organic and Inorganic Constituents

During the first significant autumn storm (i.e. several days of consistent rainfall) after a dry summer, both TDN and DOC concentrations are tightly coupled with river discharge in both the Union and Skokomish Rivers (Figure II.2, Table II.2). For example, throughout the sampled 3 Oct 2008 storm, DOC and TDN concentrations increased from 42μmol l^{-1} and 4.2μmol l^{-1} to 193μmol l^{-1} and 12.6μmol l^{-1}, respectively, in the Skokomish River. The peak in both TDN and DOC concentrations occurred approximately 3-6 hours after the peak in river discharge. This strong coupling persisted through the November samplings in the Union River. Although DOC and TDN concentrations increased with the large peak in discharge at the end of the November Skokomish River sampling, there was less correlation between discharge and DOC and TDN concentrations during smaller peaks in discharge throughout the week long sampling (Table II.1).

After several months of consistent rainfall, the correlation between discharge and DOC and TDN concentrations began to diminish; for example, during the January 2009 Union River sampling, a 137% increase in river discharge yielded a 22% and 8% increase in TDN and DOC concentrations, respectively, whereas a 62% increase in river discharge in the October 2008 sampling yielded an approximately 52% and 132% increase in TDN and DOC concentrations,

respectively (Figure II.2). Furthermore, the correlation coefficients for the relationship between discharge and DOC and TDN concentrations decreases from October (DOC R^2 = 0.88) to January (DOC R^2 = 0.27) (Table II.2). March and June 2009 samplings showed even less response in TDN and DOC concentrations to increased river discharge (Table II.1); TDN and DOC concentrations did not move significantly from the average during these rainfall events and correlation constants, especially for TDN, remained low (Table II.2). However, TDN concentrations also remained fairly constant during the September 2009 sampling, with a small increase in DOC in both rivers (Table II.1). During this sampling, there was almost no correlation between river discharge and TDN and DOC concentrations in the Union River (DOC R^2 = 0.03), whereas the Skokomish River showed a slight correlation between discharge and DOC concentrations (DOC R^2 = 0.51) (Table II.2). DOC:TDN, DOC:DON, TDN:PO_4^{3-}, and DON:PO_4^{3-} ratios are all positively correlated with river discharge in autumn and winter storms. The correlation between C:N and N:P ratios and discharge is strongest when the correlation between nutrient concentrations and discharge is strongest (Figure II.3).

Throughout the sampled 2008-2009 water year, TDN was on average composed of approximately 80% NO_3^- and 17% DON in the Union River and 51% NO_3^- and 43% DON in the Skokomish River (Table II.1). Relative to inorganic N, DON contributes more to the TDN pool during wet autumn months than the rest of the year. For example, in the Skokomish River TDN is composed of 44% NO_3^- and 52% DON from October through November whereas TDN is composed of 65% NO_3^- and 27% DON from January through September (Table II.1). Short term dissolved NO_3^- concentrations followed the same pattern as TDN. DON concentrations also increased with increasing river discharge in autumn and winter storms; this relationship diminished during spring and summer samplings (Table II.1). Peaks in both NO_3^- and DON

concentrations exhibited similar timing to TDN—peak concentrations occurred 3-6 hours after peak river discharge. Phosphate concentrations increase only to a small extent prior to peak river flow, then level out, which is reflected in the N:P ratios discussed previously. In all storm events sampled throughout the year, SiO_4^{4-} concentrations decrease with increasing river discharge (Figure II.2, Table II.1).

II. 3.2 Total Suspended Sediments and Particulate Organic Nitrogen and Carbon

TSS concentrations were correlated with river discharge during all sampled storm events; R^2 values were above 0.80 for every sampled storm (Table II.2). TSS concentrations generally reached a maximum simultaneously with or slightly prior to peak river discharge, and began to drop several hours (~6-12 h) before river flow decreased. Similar to the observed trends in dissolved nutrient concentrations, particulate organic N and C (PON and POC) concentrations are strongly coupled with river discharge during autumn and winter storms (Figure II.4). As with dissolved nutrient concentrations, the coupling between particulate N and C concentrations and river discharge began to diminish after several major winter storms; for example, R^2 values for POC dropped from 0.94 in January to 0.01 in March (Table II.2). A key difference between dissolved and particulate nutrient concentrations is that POC and PON concentrations peaked simultaneously or prior to peak river discharge similar to TSS.

Stable isotope analysis of POC revealed several notable trends. ∂^{13}C-POC generally became more depleted with increasing river discharge in all sampled storms. For example, ∂^{13}C-POC varied from -25.3‰ to -27.0‰ throughout the week long November sampling of the Skokomish River with the most depleted values occurring at peak discharge (Table II.1). As with other measured parameters, this trend was most evident in autumn samplings. Throughout

the year, ∂^{13}C-POC values ranged from -25.3‰ to -29.2‰, indicating a primary input of C_3 terrestrial OM.

II. 3.3 Dissolved and Particulate Lignin Phenols

As with DOC, the total concentration of dissolved lignin was correlated with river discharge during autumn storms (Figure II.5); data from the 13-17 October 2009 sampling showed the clearest trends and tightest correlations between discharge and the measured parameters (R^2 =0.88), and will thus be considered to best represent autumn storm conditions. During this sampling, both DOC and dissolved lignin concentrations increased with discharge, however, the total mass of dissolved lignin per mass of carbon (mg lignin/100 mg DOC) decreases, indicating a dilution of the DOC pool with non-lignin organic carbon (Figure II.5). During autumn storms there is an apparent increase in the ratio of cinnamyl to vannilyl phenols (C/V ratio) with increasing discharge as well as a decrease in the ratio of syringal to vannilyl phenols (S/V ratio; Figure II.5). Furthermore, there is a systematic increase in the ratio of vannilyl acids to vannilyl aldehydes (Ad/Al (v)) throughout the autumn storm event (Table II.1). Similar results are seen during the November 2008 and January 2009 samplings in terms of lignin concentrations and the ratio of lignin to DOC, although trends in C/V, S/V, and Ad/Al ratios are less distinguishable (Table II.1). During the March 2009 sampling there appears to be a decrease in both total lignin concentrations and the lignin to DOC ratio and no distinguishable trends in Ad/Al (v), S/V, C/V, and (Table II.1).

Particulate lignin concentrations were measured during the September 2009 storm. As with POC, total particulate lignin concentrations increased with increasing discharge (Figure II.6). Λ, or mg lignin per 100mg POC, also increased with river discharge in contrast to

observations in the ratio of dissolved lignin to DOC (Figure II.5). Trends in the particulate lignin phenol ratios also showed opposite trends relative to dissolved lignin measurements during autumn storms (i.e. October 2009 sampling). With increasing discharge, S/V increased and both C/V and Ad/Al (v) decreased (Figure II.6).

II. 4 Discussion

II.4.1 Nutrient Accumulation and Mobilization

The cumulative data presented here suggests that nutrients accumulate in the upper soil layers in the Hood Canal watershed during long dry periods and are mobilized during autumn and winter rainfall events. Excess nutrients are exhausted by successive winter storms. By late winter/early spring, nutrient concentrations exhibit little response to increased river discharge suggesting that the watershed has two distinct nutrient pools; (i) a deep soil nutrient pool that supports base flow nutrient concentrations throughout the year and (ii) an additional nutrient pool that accumulates in the shallow subsurface soil layer during periods of low soil saturation (i.e. summer dry periods) (Figure II.7). Hornberger et al. (1994) described the N-flushing hypothesis, which suggests that when the soil saturation deficit is high, nutrients accumulate in upper soil layers, and as the soil saturation deficit decreases, a saturated subsurface layer flushes nutrients from the upper soil layers into the stream. The importance of hydrologic flowpaths through the uppermost OM rich soil layer in controlling soil OM concentrations has been demonstrated; during rapid rain events young OM has been shown to be flushed from upper soil layers, depleting soil OM concentrations and leaving behind older OM in deeper soil horizons (Schiff et al., 1998; van Verseveld et al., 2009). The 3-6h time lag observed between peak river discharge and peak dissolved nutrient concentrations suggests that the accumulated shallow

nutrient pool is mobilized by saturation of the shallow subsurface layer rather than by direct surface runoff. Based on endmember mixing models, Easthouse et al. (1992) estimated that base flow largely consists of OM from deep soil layers, whereas 50-65% and 35-50% of the OM mobilized by peak flow originates from the B and O soil horizons, respectively. The observed decrease in silica concentrations in storms sampled throughout the year is the result of a dilution of base flow conditions; silica is relatively insoluble and is not mobilized by rapid shallow flow (Kennedy, 1971; Hill et al., 1999).

As with dissolved nutrient concentrations, POC and PON are tightly correlated with river discharge during autumn and winter storms (Table II.2). The correlation between POC and PON concentrations and river discharge diminishes by spring and summer samplings; for example the R^2 value for POC drops from 0.96 in January 2009 to 0.01 in March 2009 (Table II.2). The persistent correlation between discharge and TSS throughout the year supports the hypothesis that accumulated nutrients are completely leached out of soils by frequent winter storms. Thus, as with dissolved nutrients, a shallow pool of particulates with high nutrient content accumulates during long dry periods. Whereas dissolved constituents are mobilized primarily via saturation/solubilization of the sub surface soil layer, the occurrence of peak POC and PON concentrations simultaneously or prior to peak river discharge indicates that surficial processes such as displacement of discrete storage pools, surface runoff, and bank erosion are likely the primary means for mobilization of accumulated particulate nutrients and carbon. For example, in the September 2009 sampling POC and PON are moderately correlated with river discharge (R^2 = 0.88 and 0.80 respectively), whereas dissolved nutrient concentrations are not (DOC R^2 = 0.51; TDN R^2 = 0.14; Table II.2). Relative to later autumn storms, the September sampling occurred during a light rain event, which was likely not sufficient enough to saturate the upper soil layer

and mobilize dissolved nutrients, whereas particulate material was mobilized by rapid surficial processes. For example, discharge in the Union River during the September 2009 sampling ranged from only 0.65 to 0.91 m^3/s, whereas discharge in the October 2009 sampling ranged from 0.59 to 2.24 m^3/s (Table II.1).

II.4.2 Organic Matter Characterization

Additional layers of complexity can be added onto the simple two nutrient pool model described above based on trends seen in carbon to nitrogen and lignin phenol ratios. Upon first inspection, the observed correlation between river discharge and DOC:DON ratios appears to indicate that the source of material to the shallow dissolved nutrient pool is of distinct origin from the source of the base flow pool; for example, material mobilized from the shallow soil pool has an elevated DOC:DON, which may indicate that the source of this pool is derived more from wood/vascular plant detritus than the base flow pool (Liaw and MacCrimmon, 1977; Naiman and Sedell, 1979). The increase in total dissolved lignin with discharge seems to support this, since lignin is unique to vascular plants (Sarkanenk and Ludwig, 1971). However, since the material in the base flow pool persists in soils longer than in the shallow pool it is also possible that the two pools are of similar origin and that the source signature has been altered by biogeochemical processes such as degradation and sorption (e.g Aufdenkampe et al., 2001; Hernes et al., 2007).

Throughout autumn storms, the ratio of lignin to DOC decreases with increasing discharge. One explanation for the apparent enrichment of dissolved lignin in the deep base flow pool is that dissolved lignin is relatively more difficult to mobilize from soils than other dissolved organic carbon compounds; thus, other carbon compounds are more readily mobilized

from soils into streams by rapid shallow flow. For example, Kaiser et al. (2004) observed that the hydrophobic fraction of lignin-rich DOM in soils is much less mobile relative to the hydrophilic fraction of DOM, composed primarily of carbohydrates, due to sorption of lignin-derived compounds by Al and Fe oxides-hydroxides. Easthouse et al. (1992) also observed a pronounced increase in the relative proportion of hydrophilic acids in stream water during an autumn storm event, followed by a lesser contribution in a subsequent storm. Another possible explanation is that the more rapidly cycled shallow pool of dissolved constituents has undergone less biodegradation than the deep base flow pool. Since few organisms are capable of directly consuming lignin, it is often considered relatively refractory compared to other carbon compounds (Trojanowski, 1969; Crawford, 1980). However, the observed increase in the ratio of dissolved vannilyl acids to aldehydes (Ad/Al (v)) with increasing river discharge is contradictory to the above explanation. Upon biodegradation Ad/Al typically increases (Ertel and Hedges, 1984; Hedges et al., 1988, Opsahl and Benner, 1995), implying that DOM that is mobilized by storm events is more highly degraded than the base flow of DOM. In fact, many recent studies have began to show that lignin in the dissolved phase is much more labile than once thought (Benner et al., 1991; Haddad et al., 1992; Opsahl and Benner, 1995; Dittmar et al., 2001; Otto and Simpson, 2006; Ward et al., in prep).

It is likely that both biodegradation and sorption, which can be a rapid process relative to degradation (McKnight et al., 1992; Qualls and Haines, 1992; Day et al., 1994; Aufdenkampe et al., 2001; Hernes et al., 2007), play an important role in the mobilization of dissolved carbon compounds. The observed data may imply that non-degraded dissolved lignin phenols (e.g. aldehydes) are relatively more difficult to mobilize from soils than their degraded counterpart (e.g. acids). The products of dissolved lignin degradation are mobilized by rapid shallow flow,

whereas non-degraded dissolved lignin phenols are much less soluble and persist in soils until further degradation. Prolonged exposure to water (i.e. base flow), on the other hand is capable of mobilizing the somewhat insoluble non-degraded dissolved lignin phenols similarly to silica mobilization. This hypothesis is in contrast to observations in high-latitude settings. Spencer et al (2008) suggest that relatively less degraded OM, including lignin phenols, is released into high latitude rivers during spring flushing events. Cold winter temperatures prior to a high-latitude spring flushing, however, would inhibit OM degradation within soils, while drastic increases in river discharge may mobilize substantial amounts of OM regardless of its relative mobility. This DOC, however, has been shown to be highly labile once mobilized into the aquatic setting (Holmes et al., 2008). In contrast, biodegradation within soils is promoted during warm summer and autumn months in the temperate system studied here, allowing a preferential mobilization of more soluble/degraded OM during smaller peaks in discharge relative to high latitude spring flushings. Alternatively, it is possible that the less-degraded Ad/Al(v) signature of base flow dissolved lignin is unrelated to sorption processes and is simply an indicator that the deep soil horizons contain old, recalcitrant OM, whereas upper soil layers contain more fresh, labile OM that has been recently degraded. Numerous studies have shown that peaks in discharge release young labile OM relative to base flow (Schiff et al., 1998; Sanderman et al., 2009; van Verseveld et al., 2009).

The ratio of cinnamyl (C), vannilyl (V), and syringyl (S) phenols is an indicator of OM source; an increased C/V indicates a more non-woody than woody source and an elevated S/V indicates an angiosperm source (Hedges and Mann, 1979; Hedges and Ertel, 1982). Thus, the observed increase in C/V and decrease in S/V ratios of dissolved lignin with increasing river discharge indicates that the mobilized material contains more leafy/gymnosperm-derived

compounds compared to the base flow of DOM. On the other hand, the systematic changes to C/V and S/V may indicate a preferential mobilization of cinnamyl phenols and retention of syringyl phenols relative to vanillyl phenols. Opposite trends in the ratio of lignin to OC, C/V, S/V, and Ad/Al (v) are observed in particulate lignin (Figure II.6). Perhaps compounds that are less likely to be mobilized by rapid flow are sorbed to the particulate phase, which is either retained in soils or mobilized into the stream by surficial processes. However, particulate lignin data was only collected for one storm and is insufficient to entirely support the above hypothesis. It is likely that dissolved C/V and S/V ratios are controlled by the same mechanism described previously—preferential degradation of vanillyl versus syringyl phenols produces relatively more vanillyl acids (which are more easily mobilized than the non-degraded counterpart) than syringyl acids, for example. Recent dissolved lignin degradation experiments in the Amazon River basin showed significant degradation of total dissolved lignin as well as preferential decay of vanillyl phenols in the aquatic setting (Ward et al, 2013). Likewise, it is possible that the shallow pool of young labile OM has simply undergone more degradation, and thus more phenolic fractionation, than the old refractory base flow pool.

II. 5 Conclusion

The cumulative results from this year-long data set indicate that a shallow nutrient-rich pool of particulate and dissolved organic matter accumulates in watersheds during periods of soil-saturation deficiency (summer). Autumn and winter storms mobilize this pool of accumulated nutrients from surface soils, which is exhausted with successive winter storms. Results from this study will allow for more accurate nutrient and carbon export estimates from rivers with the development of more sophisticated watershed chemical models, which can be

applied to river systems worldwide and used to tackle a diverse set of hydrological and biogeochemical issues. Furthermore, this unique high-resolution data set has contributed to a deeper understanding of the mechanisms involved in the mobilization of nutrients and OM from terrestrial biomes into river systems—a necessary step toward understanding the overall role of river systems in global biogeochemical cycling.

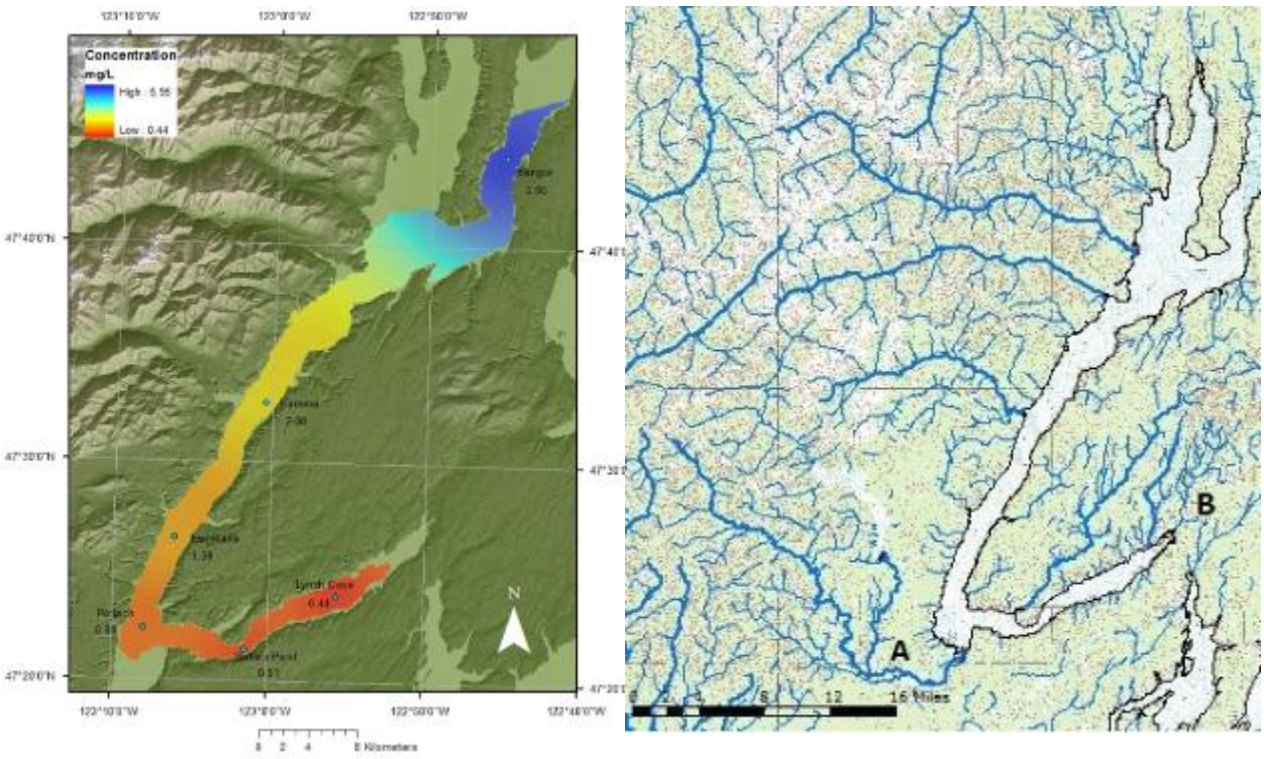


Figure II.1 Map of Hood Canal watershed and dissolved oxygen levels. (left) Modeled dissolved O_2 concentrations in the Hood Canal basin during the August 2006 hypoxic event (HCDOP Citizen Monitoring Data and UW Spatial Analysis Lab projection). (right) Map of the Hood Canal drainage basin. Sample sites for this study are (A) the Skokomish River, which is relatively large, and (B) the Union River, which is representative of the many small streams that compose the Kitsap Peninsula watershed PRISM Internet Map Server).

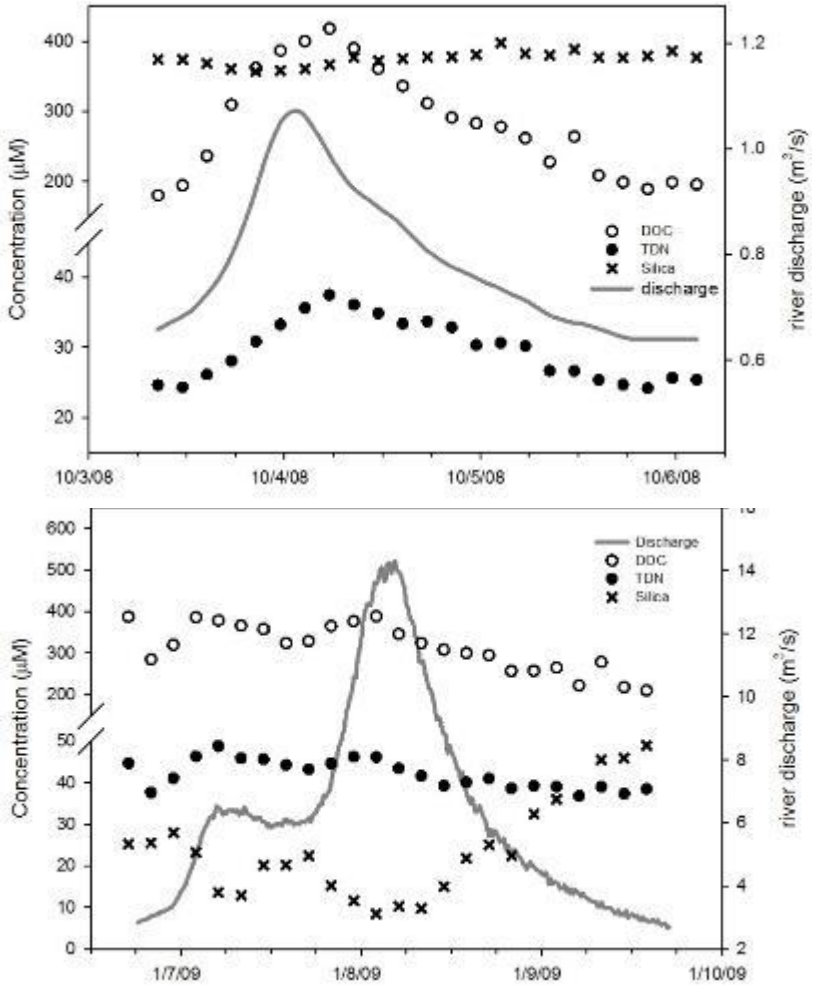


Figure II. 2 Dissolved nitrogen, organic carbon, and silica during an autumn storm. Total Dissolved Nitrogen, Dissolved Organic Carbon, and silica concentrations in the Union River during the first significant autumn storm of 2008 (top), and a subsequent winter 2009 storm (bottom).

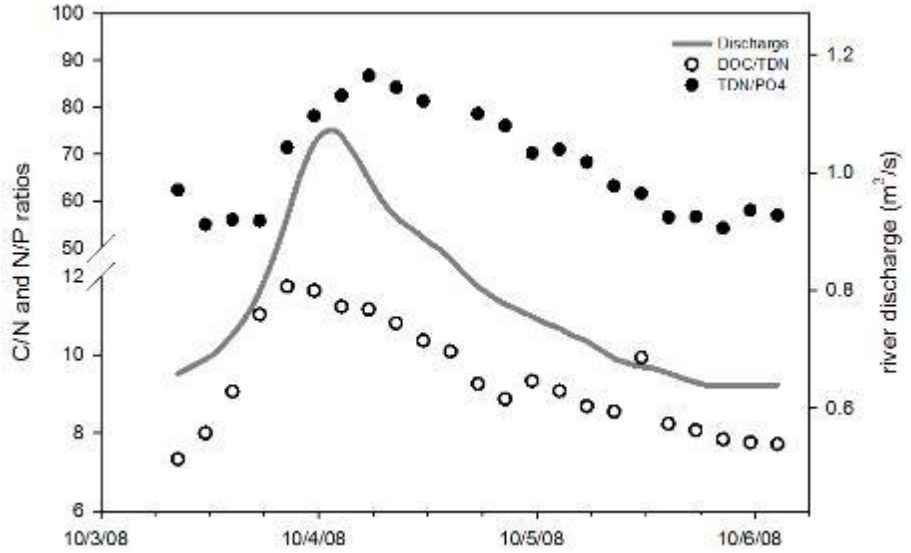


Figure II.3 Carbon to nitrogen and nitrogen to phosphorous ratios during an autumn storm. The ratio of Dissolved Carbon to Nitrogen (DOC:TDN) and the ratio of Dissolved Nitrogen to Phosphorus (TDN:PO_4^{3-}) in the Union River during the first autumn storm in 2008

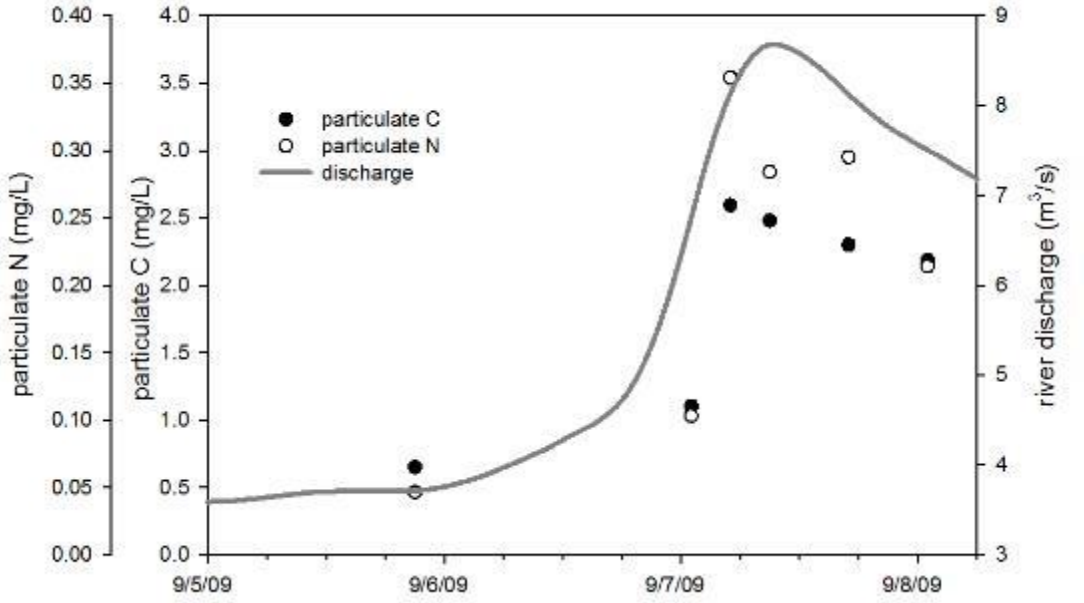


Figure II.4 Particulate organic carbon and nitrogen during an autumn storm. Particulate Organic Carbon and Particulate Organic Nitrogen concentrations in the Union River September 2009

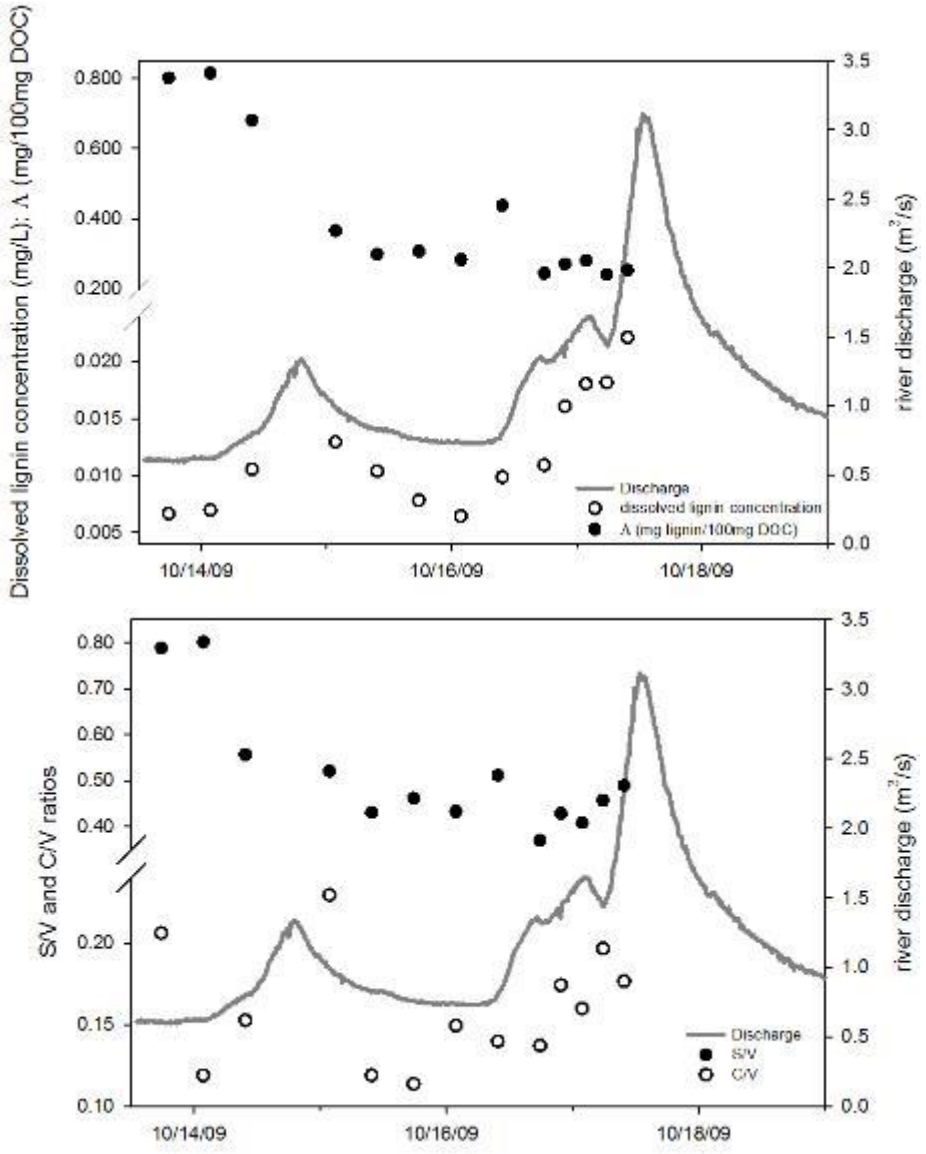


Figure II.5 Dissolved lignin concentrations and phenolic ratios during an autumn storm. Total dissolved lignin concentrations and the lignin to Dissolved Organic Carbon ratio (top) and the ratio of syringal to vannilyl dissolved lignin phenols (S/V) and the ratio of cinnamyl to vannilyl dissolved lignin phenols (C/V) (bottom) in the Union River during the first significant autumn storm of 2009. As C/V increases material is more woody vs. leafy; as S/V decreases material is more gymnosperm-derived vs. angiosperm derived.

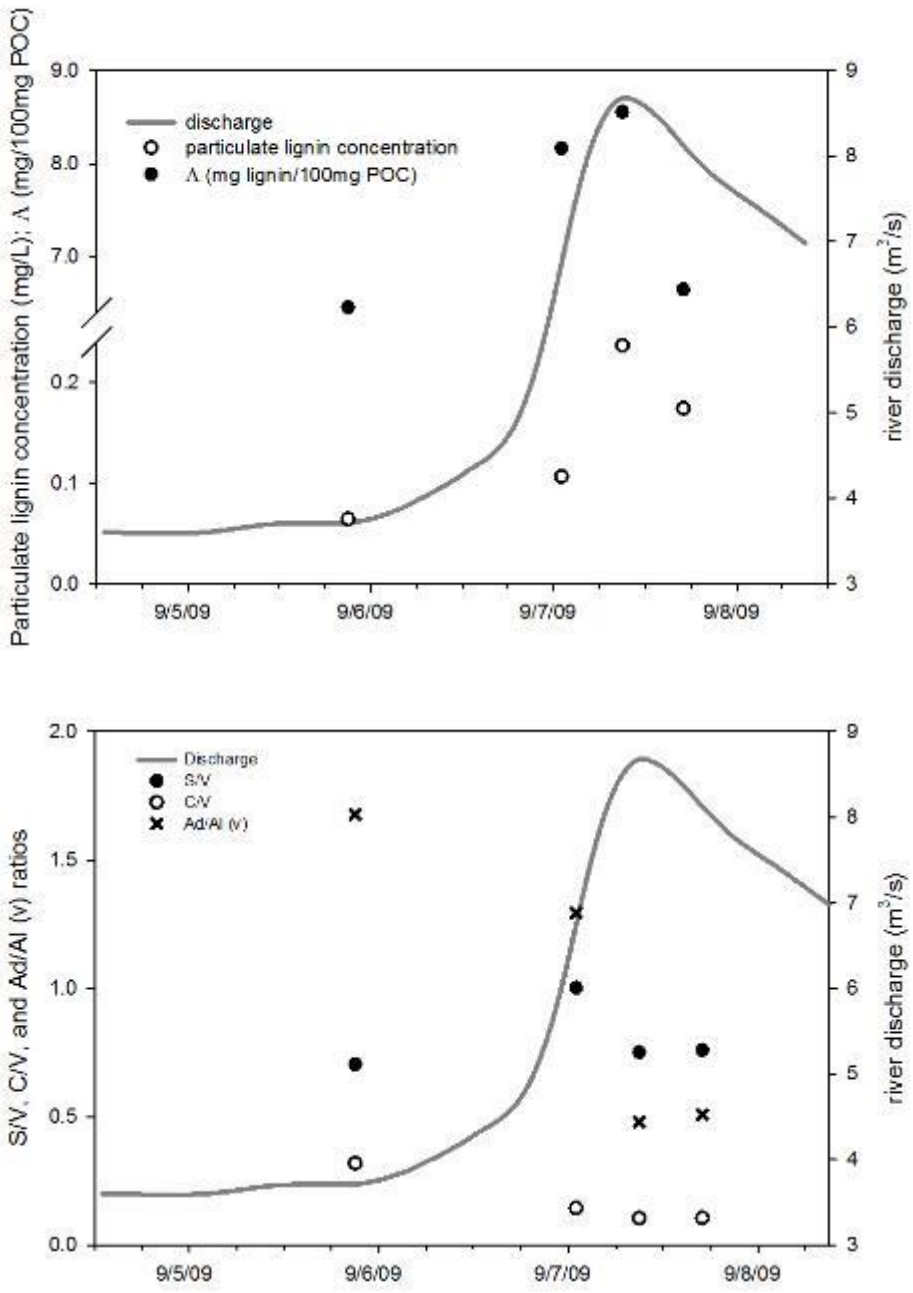


Figure II.6 Particulate lignin concentrations and phenolic ratios during an autumn storm. Total particulate lignin concentrations and Λ, or mg lignin / 100 mg POC (top) and the particulate S/V, C/V, and Ad/Al (v) ratios (bottom) in the Skokomish River during the September 2009 storm.

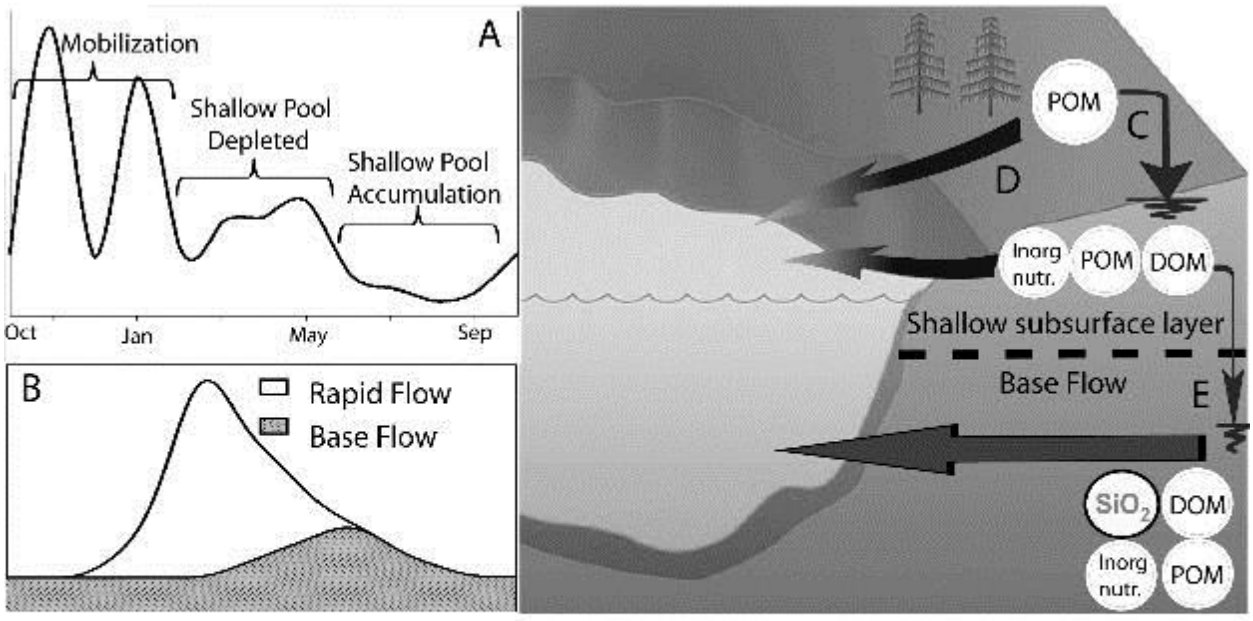


Figure II.7 Overview of accumulation and mobilization of shallow nutrient/OM pools.

(A) Nutrients accumulate in the shallow subsurface layer in dry summer months, are mobilized during autumn/winter storms, and are depleted from the accumulated shallow subsurface pool by late winter/spring. (B) Base flow is the sum of deep subsurface flow and delayed shallow subsurface flow (Ward and Robinson, 1990). Rapid flow consists of surface runoff and shallow subsurface flow. (C) Particulate organic matter (i.e. litterfall) degrades providing dissolved and particulate OM to the accumulated shallow subsurface nutrient/OM pool. (D) Surface and shallow subsurface material is mobilized by rapid flow leading to increased nutrient/OM concentrations during storm events. (E) Compounds that do not easily mobilize eventually fuel base flow chemical flux. Base flow of water provides a constant chemical flux to the river.

Table II.1 Storm sampling data for the Union and Skokomish Rivers

River	Date	Time	River flow (m^3/s)	DOC µmol l^{-1}	TDN µmol l^{-1}	SiO_4 µmol l^{-1}	NO_3^- µmol l^{-1}	PO_4^{3-} µmol l^{-1}	DON µmol l^{-1}	TSS mg l^{-1}	POC mg l^{-1}	PON mg l^{-1}	$\Delta^{13}C$ ‰	$\Delta^{15}N$ ‰	Total lignin mg l^{-1}	S/V	C/V	Ad/Al(v)
SKOK	10/31/08	16:30	14.24	70	6.7	165	1.2	0.02	5.34	4.6	0.60	0.04	-26.3	2.1	6.5E-03	0.58	0.16	1.20
SKOK	10/31/08	20:30	15.06	69	6.4	166	1.6	0.01	4.56	6.2					5.8E-03	0.63	0.20	0.97
SKOK	11/1/08	0:30	17.61	65	4.3	166	0.8	0.02	3.16	7.7	0.66	0.10	-25.8	2.9	7.0E-03	0.77	0.25	1.60
SKOK	11/1/08	4:30	18.63	68	7.9	166	2.0	0.01	5.49	6.0								
SKOK	11/1/08	8:30	17.95	59	4.7	166	1.2	0.01	3.34	9.3	0.98	0.07	-26.2	2.9	5.3E-03	0.61	0.18	1.35
SKOK	11/2/08	0:30	17.10	49	3.5	169	1.2	0.00	2.19	2.2	0.63	0.04	-25.3	3.4				
SKOK	11/2/08	4:30	17.27	52	4.9	169	1.4	0.00	3.26	2.2	0.71	0.04	-26.4	3.1	5.3E-03	0.70	0.21	1.44
SKOK	11/2/08	8:30	18.12	51	4.0	170	1.5	0.00	2.28	2.4					4.9E-03	0.75	0.22	1.43
SKOK	11/2/08	12:30	19.14	48	4.4	170	1.7	0.00	2.52	2.9					8.4E-03	0.58	0.19	1.40
SKOK	11/2/08	16:30	21.24	52	4.4	171	1.6	0.00	2.67	1.4					8.6E-03	0.70	0.28	1.14
SKOK	11/2/08	20:30	22.68	45	3.5	169	1.5	0.00	1.75	2.3	0.47	0.03	-26.2	3.8	9.4E-03	0.63	0.20	0.95
SKOK	11/3/08	0:30	23.98	72	4.0	169	1.3	0.00	2.61	3.6					6.3E-03	0.68	0.20	1.04
SKOK	11/3/08	4:30	24.95	51	3.8	171	0.9	0.00	2.65	2.8	0.62	0.04	-26.1	3.5	8.7E-03	0.69	0.22	1.28
SKOK	11/3/08	12:30	28.32	55	4.1	169	0.8	0.00	3.17	4.5	0.71	0.05	-26.4	2.6				
SKOK	11/4/08	0:30	30.30	64	3.8	160	1.1	0.01	2.41	9.5	0.60	0.03	-25.4	2.2	9.5E-03	0.63	0.32	1.64
SKOK	11/4/08	15:00	31.43	77	5.4	162	3.2	0.01	0.74	1.4					7.8E-03	0.39	0.15	1.13
SKOK	11/4/08	21:00	29.73	68	5.9	164	3.4	0.01	1.69	6.2					7.1E-03	0.46	0.17	1.11
SKOK	11/5/08	3:00	28.32	58	5.1	177	3.6	0.01	0.50	6.4	0.44	0.02	-26.0	3.5	1.1E-02	0.44	0.17	1.09
SKOK	11/5/08	6:00	27.69	61	4.3	164	3.7	0.01	0.00		0.57	0.03	-26.4	3.0	1.0E-02	0.54	0.17	1.96
SKOK	11/5/08	9:00	26.90	56	4.8	166	3.7	0.01	0.00	4.6					8.7E-03	0.40	0.16	1.31
SKOK	11/5/08	21:00	24.95	49	4.5	177	3.4	0.01	0.00	3.6	0.38	0.09	-26.7	2.5	1.1E-02	0.41	0.14	1.08
SKOK	11/6/08	3:00	25.15	50	4.8	165	3.5	0.01	0.00	3.7	0.34	0.02	-26.4	3.4	1.9E-02	0.69	0.12	1.64
SKOK	11/6/08	9:00	27.89	55	4.5	164	3.6	0.00	0.00	3.3	0.51	0.03	-26.6	2.9	1.4E-02	0.58	0.21	1.43
SKOK	11/6/08	15:00	44.46	72	5.1	159	3.9	0.03	0.38	4.7	0.31	0.07	-26.7	3.1	1.2E-02	0.62	0.15	1.52
SKOK	11/6/08	21:00	226.8			135	7.2	0.09	0.00	117.8	2.81	0.29	-27.4	2.8	5.7E-03	0.61	0.15	1.13
SKOK	11/7/08	3:00	387.9	214	14.3	135	6.9	0.12	6.51	943.4	5.32	0.65	-27.0	2.6	4.7E-03	0.44	0.15	0.98
SKOK	11/7/08	9:00	487.1	156	10.3	145	6.4	0.10	3.21						6.3E-03	0.45	0.18	1.03
UNIO	1/6/09	20:00	3.06	285	37.6	25	31.4	0.24	4.93	44.9	3.04	0.25	-27.1	3.8	3.6E-01	0.54	0.29	1.88
UNIO	1/7/09	5:00	6.48	378	48.7	14	39.4	0.24	7.95	239.6	7.08	0.56	-28.0	-3.0	3.6E-01	0.57	0.21	1.53
UNIO	1/7/09	17:00	6.14	329	43.1	22	35.4	0.22	6.65	83.1	9.38	0.81	-27.9	-4.4	6.7E-01	0.12	0.05	1.13
UNIO	1/8/09	2:00	13.56	389	46.1	8	38.6	0.19	6.14	434.6	19.05	1.49	-27.8	-2.1	2.6E-01	0.65	0.26	1.08
UNIO	1/8/09	11:00	8.81	308	39.2	15	34.1	0.16	4.29	379.5	8.90	0.79	-27.9	-5.9	2.6E-01	0.64	0.26	1.19
UNIO	1/9/09	14:00	2.92	210	38.4	49	33.1	0.20	4.24	32.5	2.56	0.16	-26.9	1.8	2.8E-01	0.89	0.20	0.99
SKOK	3/13/09	19:30	19.14	40	8.3	35	2.8	0.03	5.52	11.8	0.64	0.034	-25.3	2.8	1.2E-02	0.61	0.09	0.89
SKOK	3/14/09	11:30	22.14	36	4.5	66	3.6	0.06	0.31	4.1	0.45	0.040	-27.4	3.3	8.1E-03	1.54	0.84	0.37
SKOK	3/14/09	23:30	24.18	41	5.7	48	3.6	0.04	1.04	5.6	0.86	0.042	-28.1	3.3	9.8E-03	0.69	0.18	1.26
SKOK	3/15/09	23:30	23.79	40	5.4	53	4.1	0.04	0.39	8.0	0.47	0.061	-25.8	3.4	3.4E-02	0.04	0.01	0.13
SKOK	3/16/09	23:30	25.32	44	6.5	78	4.4	0.08	1.60	8.2	0.62	0.047	-28.3	2.0	5.9E-03	0.70	0.18	1.07

SKOK	3/17/09	15:30	26.31	44	6.1	47	3.9	0.05	2.08	10.4	0.61	0.041	-26.6	2.3	6.8E-03	0.83	0.25	2.20
River	Date	Time	River flow (m^3/s)	DOC µmol l^{-1}	TDN µmol l^{-1}	SiO_4 µmol l^{-1}	NO_3^- µmol l^{-1}	PO_4^{3-} µmol l^{-1}	DON µmol l^{-1}	TSS mg l^{-1}	POC mg l^{-1}	PON mg l^{-1}	$\Delta^{13}C$ ‰	$\Delta^{15}N$ ‰	Total lignin mg l^{-1}	S/V	C/V	Ad/Al(v)
UNIO	6/19/09	14:00	1.10	82	20.4	385	16.5	0.29	2.9	2.5					8.4E-03	0.54	0.14	0.86
UNIO	6/20/09	6:00	1.36	88	21.6	406	17.5	0.33	3.2	1.4					9.0E-03	0.66	0.23	0.71
UNIO	6/20/09	10:00	1.30	80	18.9	397	17.0	0.28	1.6	8.5					7.9E-03	0.69	0.16	0.73
UNIO	6/20/09	14:00	1.13	87	20.4	399	17.1	0.31	3.2	1.6					6.8E-03	0.50	0.13	0.76
UNIO	6/20/09	22:00	1.25	83	20.7	397	18.7	0.29	1.6	2.1					8.5E-03	0.55	0.18	0.51
UNIO	6/21/09	2:00	1.36	85	22.0	397	19.0	0.28	2.7	1.9					6.7E-03	0.54	0.14	0.91
SKOK	9/5/09	21:00	3.71	34	4.0	211	2.4	0.22	1.57	4.8	0.65	0.046	-26.2	2.9				
SKOK	9/6/09	1:00	3.71	34	3.9	211	2.6	0.21	1.28						4.34E-03	0.14	0.11	1.02
SKOK	9/7/09	1:00	6.31	50	4.6	202	3.0	0.14	1.54	15.5	1.10	0.103	-27.6	1.6	7.48E-03	0.23	0.11	1.31
SKOK	9/7/09	5:00	7.99	48	4.3	197	3.1	0.12	1.11	50.2	2.59	0.354	-28.9	0.5	6.97E-03	0.25	0.16	1.18
SKOK	9/7/09	9:00	8.72	54	5.1	195	3.8	0.11	0.68	65.3	2.48	0.284	-29.2	-4.3	6.86E-03	0.26	0.14	1.42
SKOK	9/7/09	17:00	8.30	53	4.0	196	3.6	0.13	0.22	42.0	2.30	0.295	-27.8	-2.3	6.21E-03	0.48	0.17	0.86
SKOK	9/8/09	1:00	7.56	58	5.4	200	3.9	0.15	0.82		2.18	0.214	-29.1	-3.5				
UNIO	9/5/09	19:00	0.65	97	18.7	399	17.4	0.51	0.64	3.9	0.67	0.056	-28.2	1.7	7.40E-03	0.70	0.12	1.04
UNIO	9/6/09	10:00	0.65	115	21.0	401	18.8	0.55	1.10	4.6	0.84	0.063	-26.8	2.1	6.19E-03	0.25	0.13	1.90
UNIO	9/6/09	13:00	0.79	125	19.2	393	18.0	0.51	0.97	4.0					6.98E-03	0.36	0.19	1.07
UNIO	9/6/09	16:00	0.91	131	18.5	392	17.5	0.50	0.90	4.2	0.65	0.098	-28.4	3.1	6.28E-03	0.34	0.14	0.81
UNIO	9/6/09	19:00	0.85	130	18.6	394	17.7	0.51	0.87	3.5					5.72E-03	0.36	0.16	1.11
UNIO	9/6/09	22:00	0.76	130	19.5	400	18.6	0.53	0.45	4.2	0.72	0.049	-26.6	2.7	5.94E-03	0.30	0.15	1.00
UNIO	9/7/09	1:00	0.74	153	22.1	402	19.4	0.56	1.42	2.3					6.19E-03	0.27	0.16	1.27
UNIO	9/7/09	4:00	0.71	126	20.7	402	20.0	0.48	0.61	3.4	0.85	0.058	-26.5	2.0				
UNIO	9/7/09	10:00	0.68	124	21.0	407	19.7	0.53	0.79	3.1	0.67	0.105	-27.1	2.7				
UNIO	9/7/09	16:00	0.65	137	20.5	402	18.4	0.55	1.49	2.7	0.58	0.047	-26.5	3.7				
UNIO	9/7/09	22:00	0.68	177	23.6	391	19.7	0.63	3.58	6.4	1.01	0.104	-28.0	3.8				
UNIO	10/13/09	17:45	0.59	69	19.1	397	12.5	0.31	6.3		0.84	0.119	-27.2	2.3	6.6E-03	0.79	0.21	0.99
UNIO	10/14/09	1:45	0.62	71	19.4	395	13.0	0.28	6.0		0.66	0.043	-26.8	3.3	7.0E-03	0.80	0.12	0.39
UNIO	10/14/09	9:45	0.79	129	20.4	384	11.9	0.40	8.2		0.88	0.065	-27.9	3.1	1.1E-02	0.56	0.15	0.72
UNIO	10/14/09	17:45	1.27	310	30.6	346	17.3	0.41	12.8		2.45	0.214	-28.1	3.1				
UNIO	10/15/09	1:45	0.99	295	37.2	367	25.4	0.25	10.7		1.85	0.144	-28.1	3.4	1.3E-02	0.52	0.23	0.72
UNIO	10/15/09	9:45	0.82	290	34.1	384	21.2	0.29	12.3		0.82	0.113	-28.4	3.1	1.0E-02	0.43	0.12	0.77
UNIO	10/15/09	17:45	0.76	213	30.7	405	19.9	0.22	9.6		0.90	0.066	-26.8	3.2	7.8E-03	0.46	0.11	0.73
UNIO	10/16/09	1:45	0.74	190	29.5	409	19.0	0.26	9.3		1.08	0.083	-27.5	3.5	6.5E-03	0.43	0.15	0.70
UNIO	10/16/09	9:45	0.79	188	26.4	399	16.8	0.30	8.4		0.68	0.080	-27.2	3.4	9.9E-03	0.51	0.14	0.95
UNIO	10/16/09	17:45	1.33	374	32.8	373	19.3	0.31	12.9		2.60	0.159	-28.3	2.7	1.1E-02	0.37	0.14	1.22
UNIO	10/16/09	21:45	1.36	496	43.0	349	26.5	0.40	15.8		2.67	0.196	-26.8	3.8	1.6E-02	0.43	0.17	1.06
UNIO	10/17/09	1:45	1.61	538	45.1	342	28.2	0.31	16.3		3.94	0.270	-28.1	3.1	1.8E-02	0.41	0.16	0.95
UNIO	10/17/09	5:45	1.44	630	63.8	348	42.5	0.27	20.4		12.35	0.954	-28.6	3.0	1.8E-02	0.46	0.20	1.03
UNIO	10/17/09	9:45	2.24	728	63.5	290	41.3	0.46	21.5		10.89	0.801	-28.5	2.6	2.2E-02	0.49	0.18	0.71

Table II.2 Correlation between river discharge and DOC, TDN, POC, PON, and TSS during sampled storms

River	Storm Start Date	DOC R^2	TDN R^2	POC R^2	PON R^2	TSS R^2
Union	3 Oct 2008	0.88	0.73			
Skokomish	2 Oct 2008	0.90	0.71			
Union	31 Oct 2008	0.87	0.92			
Skokomish	31 Oct 2008	0.11	0.11	0.96	0.96	0.84
Union	6 Jan 2009	0.27	0.33	0.94	0.94	0.86
Union	13 Mar 2009	0.73	0.38			
Skokomish	13 Mar 2009	0.50	0.28	0.01	0.19	0.80
Union	19 Jun 2009	0.11	0.13			
Union	4 Sep 2009	0.03	0.29			
Skokomish	4 Sep 2009	0.51	0.14	0.88	0.80	0.85
Union	13 Oct 2009	0.88	0.70	0.95	0.84	
Skokomish	13 Oct 2009	0.66	0.16			

Chapter III: The relative contribution of terrestrially-derived macromolecule degradation to bulk respiration at the mouth of the Amazon River

Foreword: The following chapter is derived from the publication: Ward, N.D.; Keil, R.G.; Medeiros, P.M.; Brito, D.C.; Cunha, A.C.; Dittmar, T.; Yager, P.L.; Krusche, A.V.; Richey, J.E. (2013). Degradation of terrestrially derived macromolecules in the Amazon River. Nature Geoscience. doi: 10.1038/ngeo1817 and additional unpublished data by Ward et al.

III.1 Introduction

Nearly all temperate and tropical rivers are overwhelmingly heterotrophic, providing a large net source of CO_2 to the atmosphere (Cole et al., 1994; Richey et al., 2002; Duarte and Prairie, 2005; Butman and Raymond, 2011), yet little is known about the organic matter (OM) that fuels these globally-relevant carbon fluxes. Lignin—one of the most abundant biochemicals in the terrestrial biosphere (Boerjan et al., 2003)—is often assumed to be refractory and hard to degrade once mobilized from soils into the aquatic setting. The data presented here shows that lignin and other terrestrially-derived macromolecules are, in fact, quite reactive in tropical river systems. For the first time, the heterotrophic consumption of a vast suite of specific terrestrial OM compounds was directly measured in the lower reach of the world's largest river, the Amazon River mainstem. On average, the degradation of lignin-like compounds supported 30-50% of bulk respiration rates, implying that lignin is a highly important OM fuel for CO_2 outgassing from the world's inland waters. These results overturn the long held paradigm that lignin and other terrestrial macromolecules are recalcitrant and persist for decades to centuries once delivered to aquatic systems (e.g. Hedges et al., 1984; Hedges and Cowie, 1988; Gough et al., 1993; Opsahl and Benner, 1997) and reveal a highly dynamic and essential pathway for terrestrial carbon remineralization.

The immense outgassing of CO_2 from inland waters is now part of the paradigm of the global carbon cycle (Richey et al., 2002; Butman and Raymond, 2011). Globally, inland waters process, transport, and bury 2.7 Pg C y^{-1} (Battin et al., 2008; tranvik et al., 2009), a flux nearly equivalent to the terrestrial sink for anthropogenic CO_2 of 2.8 Pg C y^{-1}(Battin et al., 2008; Canadell et al., 2007). The Amazon River accounts for roughly 20% of the freshwater discharged to the world's oceans, making it an ideal test bed for understanding large scale biogeochemical processes and fluxes. The Amazon rainforest is responsible for nearly 10% of global primary production, or the fixation of 8.5 Pg C y^{-1} (Field et al., 1998; Malhi et al., 2008). Respiration of contemporary OM originating on land and near rivers is the dominant source (Mayorga et al., 2005) sustaining a CO_2 supersaturation in the Amazon River that drives an outgassing of 0.5 Pg C y^{-1} (Richey et al., 2002), roughly equivalent to the amount of carbon actually sequestered by the forest (Field et al., 1998; Malhi et al., 2008). Our current understanding of the source, chemical composition, and microbial controls of the OM pool driving this vast CO_2 outgassing, however, is vague at best.

Richey et al (2002) estimates that approximately 75% of the CO_2 evasive gas flux from the Amazon River basin originates from *in situ* respiration of OM as it travels downstream through the river continuum. The OM respired in the Amazon has been shown to be relatively young, with a radiocarbon age of less than five years old (Mayorga et al., 2005). This pool of OM is thought to be rapidly overturned, whereas terrestrially-derived compounds with complex macromolecular structures, such as lignin, are assumed to be refractory and implicitly old (e.g. Hedges et al., 1984; Hedges and Cowie, 1988; Gough et al., 1993; Opsahl and Benner, 1997). However, new evidence suggests the contrary: for example, in the Mekong River, the radiocarbon age of particulate lignin phenols has been shown to be modern over the entire

hydrograph, in contrast to the aged pool of bulk particulate organic carbon (POC), implying that lignin is potentially a highly dynamic fraction of OM in the aquatic setting (Martin et al., 2013).

The most abundant biochemicals on land are cellulose, hemicellulose, and lignin (Boerjan et al., 2003). They represent as much as 80% of the biomass in a typical forest and as much as 60% of the biomass in a typical field (natural or crop; Bose et al., 2009; Martens et al., 2004). Lignin composes roughly 30% of the organic carbon in the terrestrial biosphere (Boerjan et al., 2003). Numerous studies have evaluated lignocellulose degradation in soils and marshes, and the conclusion is that lignin is reactive in the soil setting because of the presence of white-rot fungi, a lignin-consuming specialist (Paterson and Lundquist, 1985; Bergbauer and Newell, 1992; Kiem et al., 2003; Feng et al., 2008). Once lignin is mobilized into the aquatic setting, it is assumed to be refractory and hard to degrade by fluvial and marine microorganisms (Hedges et al., 1984; Hedges and Cowie, 1988; Gough et al., 1993; Opsahl and Benner, 1997). However, this assumption is at odds with numerous observations showing that marine sediments are not accumulating vast amounts of these compounds (Hedges and Oades, 1997). The implication is that lignin, and similar terrestrial macromolecules, must be remineralized along the river-ocean continuum, playing an important role in the net community respiration of the world's rivers and resulting CO_2 gas efflux.

III.2 Methods

The total contribution of lignin and lignin-like compound remineralization to net community respiration was determined at the Amazon River mouth by tracking the abundance of eight standard lignin phenols (L_8) and a suite of the most abundant 95 phenolic compounds (T_{phen}) detectable by ultra high resolution time-of-flight mass spectrometry (GC-ToF-MS). These

dark incubation experiments were performed simultaneously with measurements of bulk respiration and conventional chemical inventories. A second mass spectrometry approach, electrospray ionization Fourier transform ion cyclotron (ESI-FTICR-MS), was used to broadly characterize the chemical structure of the entire DOM pool (~2,800 compounds) throughout the same set of incubations.

Chemical measurements and dark incubations were performed in the Amazon River during four expeditions from September 2010 to September 2011 to the north channel of the mainstem near Macapá (S 00º05.400', W 51º 03.200'). Whole water was collected with a Shurflo submersible pump from 50% river depth (annual average river depth = 28m) at the center and margins of the river channel. Bulk respiration rates were determined by measuring the consumption of oxygen in 60mL BOD bottles via Winkler titration; triplicate samples were incubated for 26-39 hours in the dark at ambient river temperature (annual average river temperature = 30 °C). Compound specific incubations were performed in triplicate for 5-7 days in 4L polycarbonate bottles. Dissolved nutrients were consumed, but remained at measureable concentrations throughout the incubations. Killed control incubations showed no statistical change in phenol concentrations. Particulate OM samples were collected on pre-combusted 142mm diameter Whatman GF/F glass fiber filters (0.7μm) and frozen. Dissolved OM samples were pre-filtered through 142mm diameter Pall 0.2um Supor filters, acidified to pH=2, and extracted at 3 mL/min on Waters Oasis HLB solid phase extraction cartridges for lignin analysis (Keil and Neibauer, 2009) and PPL cartridges for ESI-FTICR-MS analysis (Dittmar et al., 2008). ESI-FTICR-MS analysis of dissolved OM was performed with a Bruker Daltonics 15 Tesla FT-ICR mass spectrometer at Max Planck Institute, Oldenburg, Germany. Particulate and dissolved lignin samples underwent CuO oxidation performed in a CEM Microwave Accelerated Reaction

System (Goni and Montgomery, 2000), and were analyzed using a GC-ToF-MS (Agilent 7890A GC, Leco TruToF HT). Free lignin monomers were measured by direct injection of the DOM extract onto the GC-ToF-MS. Bacterial abundance samples were fixed with 4% buffered formaldehyde and kept cold and dark until they could be stored frozen (within one month). Upon return to the lab, they were thawed, sonicated, stained with DAPI (Porter and Feig, 1980), and counted by epifluorescence microscopy.

Triplicate POC and $PO^{13}C$ samples were filtered on combusted Whatman GFF filters, packed in tin capsules after drying and acid-fumigation, and analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. The filtrate was analyzed for DOC in triplicate on a Shimadzu TOC-VCPH analyzer. River discharge was measured using a Teledyne Workhorse Rio Grande 600 khz Acoustic Doppler Current Profiler (ADCP). ADCP profiles were made across the north and south mainstem channels and Belem (e.g. Tocantins) at approximately 1 hr intervals for 13 hr in order to assess river velocity over the span of a tidal cycle and calculate the total Amazon River discharge to the ocean. Total daily discharge from the Amazon River was calculated by ADCP measurements at the North and South channels of Macapá and Agência Nacional de Águas (ANA) stage/discharge records at Óbidos and the Tapajós, Xingu, and Tocantins tributaries.

Basin-wide estimates of the fate of lignin in soils and the river were made by first calculating the total amount of standing lignin biomass in the Amazon basin. Here, conservative values of 70 Pg C were used for for total carbon biomass (Hedges et al., 1988), and multiply by 16.5%, or the fraction of total C biomass that is lignin (Schmidt et al., 2011). Basin-wide annual lignin remineralization rates in soils were estiated using the above value for total lignin and the

empirically determined half-life of lignin phenols in the soil setting (Goni and Montgomery, 2000). Annual river remineralization rates were determined by scaling the average phenolic degradation rates measured here to total average annual Amazon River discharge. These two rates were then compared to the annual sequestration of lignin by the terrestrial biosphere to determine the relative proportion of sequestered lignin that is respired in soils versus in the river. A value of 0.5 Pg C y^{-1} was used for annual C sequestration (Malhi et al., 2008; Mayorga et al., 2005) and multiplied by 16.5% to normalize to lignin (Schmidt et al., 2011). Respiration in soils and the river accounted for 95% of the annually sequestered lignin; the remaining 5% was assumed to be delivered to the ocean.

III.3 Results and Discussion

ESI-FTICR-MS results showed a complete loss of specific dissolved compounds, approximately 60% of which had a 'lignin-like' structure (i.e. fall within van Krevelen ratios characteristic of lignin (Figure III.1) implying that some compounds similar in structure to lignin are highly degradable. Direct measurements via GC-ToF-MS revealed an annual average degradation rate of 0.67±0.08 and 0.57±0.07 mg C m^{-3} d^{-1} for dissolved and particulate L_8 lignin, respectively. The average degradation rate of total phenolic compounds (T_{phen}) in the dissolved and particulate phases were 17.9±3.8 and 4.3±0.9 mg C m^{-3} d^{-1}, respectively. Bulk respiration was, on average, 58.0±5.8 mg C m^{-3} d^{-1}. Thus, T_{phen} respiration accounts for approximately 30-50% of bulk respiration rates in the river. Furthermore, these degradation rates relative to measured concentrations imply that the entire pool of lignin and related phenolic compounds in the Amazon River completely overturns in only 2-3 weeks, orders of magnitude faster than the

notion that lignin persists for decades to centuries in aquatic systems (e.g. Hedges et al., 1984; Hedges and Cowie, 1988; Gough et al., 1993; Opsahl and Benner, 1997).

Although OM concentrations and bacterial abundances peaked during high water condition in May (Table III.2), bulk respiration rates were lowest in May and highest in December (Figure III.2), in agreement with previous respiration measurements made in the Amazon showing minimal respiration rates and maximal bacterial growth efficiency during high water (Benner et al., 1995). On the contrary, L_8 and T_{phen} respiration rates were loosely correlated with abundance and OM inventories, reaching their maximum at high water (Figure II.2). Thus, the rates of L_8 and T_{phen} degradation correspond with substrate availability, whereas bulk OC respiration does not. In May, for example, total phenolic DOC accounts for less than 7% of DOC, yet T_{phen} respiration accounts for 73 ± 21% of bulk respiration rates (Figure II.2), implying that much of the bulk OC mobilized by high discharge is not biologically available relative to the lignin/phenolic fraction. Since lignin concentrations correlate with discharge both on seasonal (Ellis et al., 2012) and hourly to daily (Ward et al., 2012) timescales in tropical and temperate systems alike, I hypothesize that other river systems behave similarly in regards to lignin remineralization. The remaining balance of OM sources that contribute to bulk respiration rates is likely a combination of autochthonous OM and terrestrially-derived celluloses and hemicelluloses. Their relative contributions are likely seasonally dependent; for example, floodplain drainage during falling water delivers a large source of labile algal-derived OM to the mainstem.

Based on measurements made here and those made by others (Field et al., 1998; Malhi et al., 2008; Bose et al., 2009; Hedges et al., 1988; Houghton et al., 2001), the fate of lignin in the

Amazon watershed as it travels from soils, through the river, and into the ocean is described (Figure III.3). It is estimated that roughly 80 Tg C of lignin is sequestered in Amazonian soils annually. Roughly 40% of this lignin is respired in soils, 55% is respired within the river continuum, and less than 5% is delivered to the ocean. The collective results from this study present strong evidence of the biodegradability of terrestrially derived macromolecules in the aquatic setting and provide a significant step in integrating the emerging paradigm regarding chemical recalcitrance (Schmidt et al., 2008) with global carbon budgets.

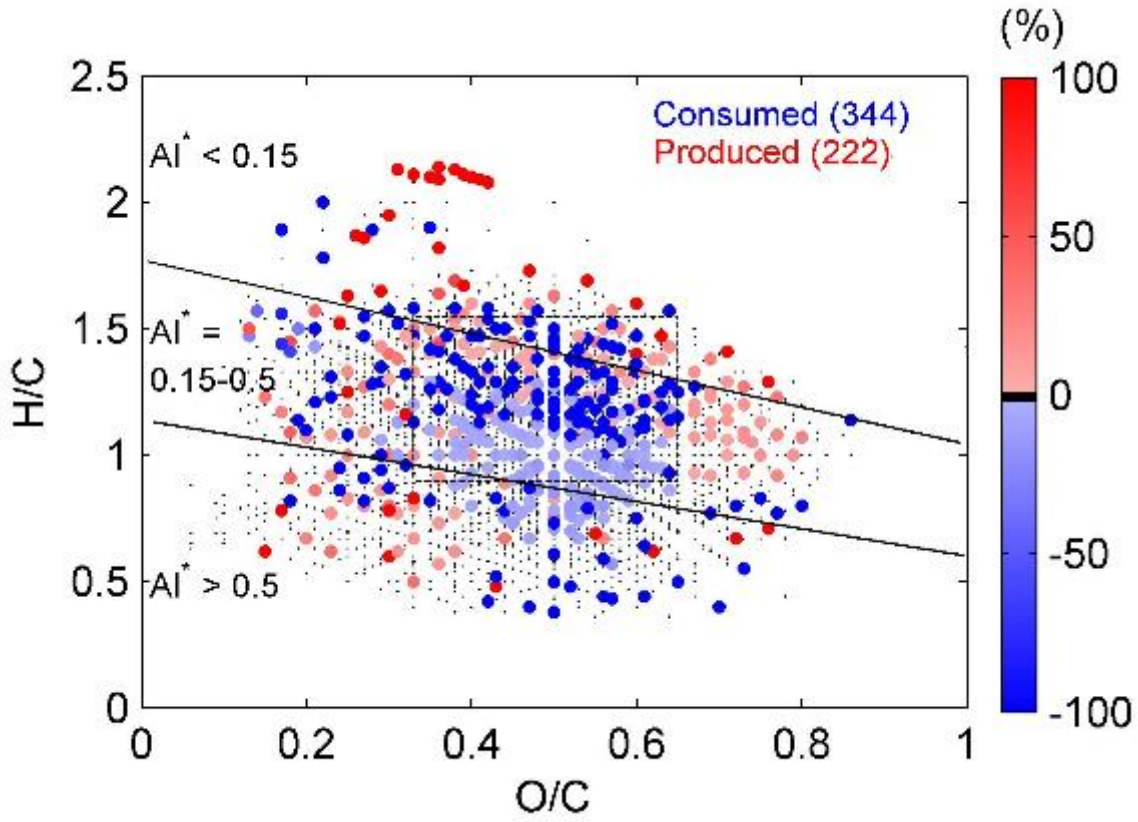


Figure III.1 Van Krevelen diagrams of incubated Amazon River DOM. The relative consumption/production of DOM compounds (based on hydrogen-to-carbon and oxygen-to-carbon ratios) after a 10-day incubation period. Of the 2,804 compounds detected (black), 344 were consumed (blue) and 222 were produced (red). The majority of consumed DOM compounds have vK ratios denoting lignin-like compounds (centered box) and Aromaticity Index (AI) values from 0.15-0.5 (black lines).

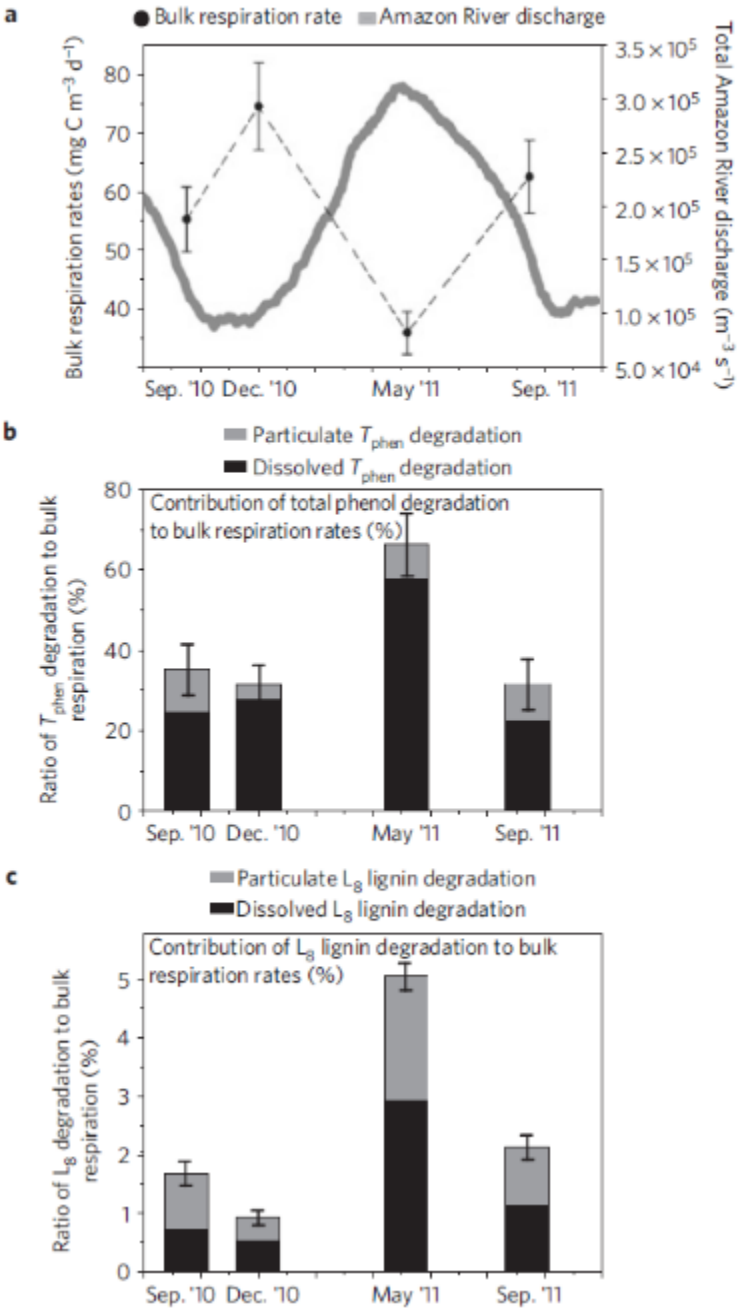


Figure III.2 Bulk and specific respiration rates in the Amazon River. (Top) Bulk respiration rates (black) and total Amazon River discharge (grey). (Center) The proportion of bulk respiration that is dissolved (black) and particulate (grey) total aromatic compounds (T_{phen}). (Bottom) The proportion of bulk respiration that is dissolved (black) and particulate (grey) standard lignin phenols (L_8).

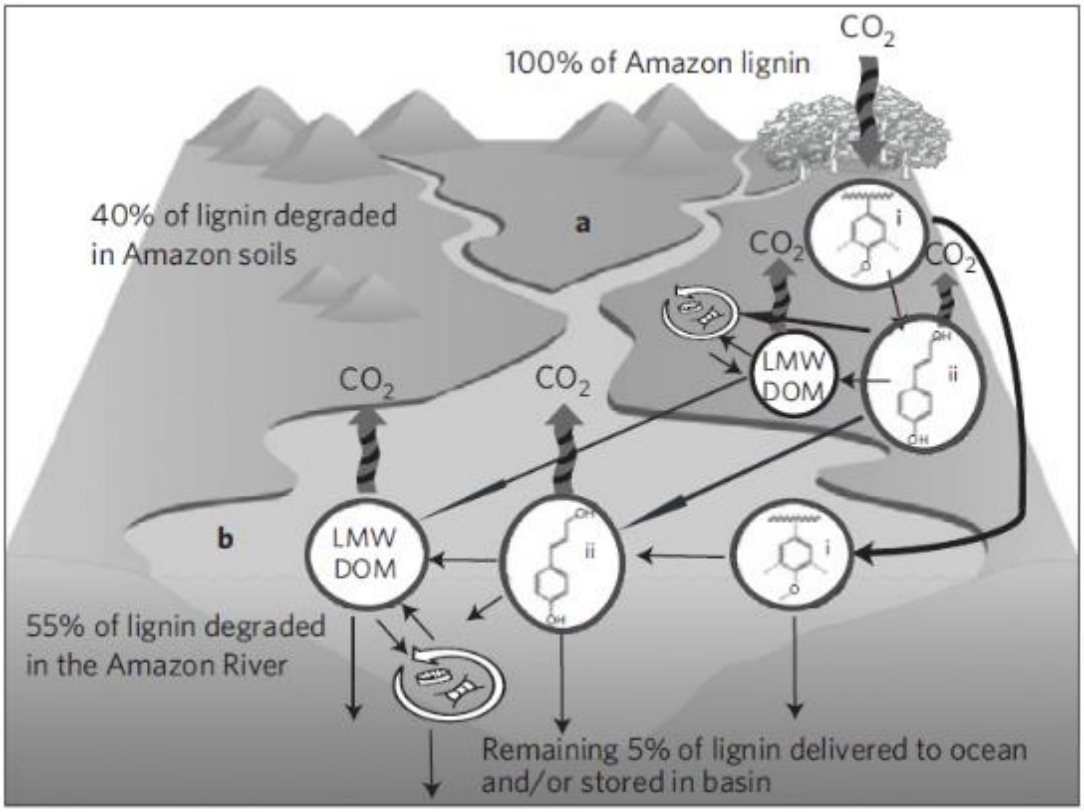


Figure III.3 Fate of lignin in the Amazon River watershed. (A) Terrestrial primary production converts atmospheric CO_2 into biomass dominated primarily by lignin, cellulose, and hemicellulose compounds. Within soils (i) large lignin macromolecules are broken down into (ii) free lignin monomers. Respiration of lignin in soils remineralizes roughly 40% of the lignin sequestered in the terrestrial biosphere. Lignin monomers and macromolecules are both mobilized into the river mainstem through stream networks (in addition to celluloses and hemicelluloses).

(B) Within the river-ocean continuum, terrestrial macromolecules are continuously broken down into monomers, which are either respired directly to CO_2, broken down into low-molecular weight DOM intermediates, or cycled within the microbial food web, supporting micro and macrofaunal heterotrophic production. 55% of the lignin sequestered on land is respired in the river. The remaining <5% is delivered to the ocean, where it is either buried or respired.

III.4 Supplemental Methodological Details

The main mass spectrometry approach used to measure the net consumption/production of phenolic compounds was two-tiered. First, the natural abundance of the eight standard lignin phenols (L_8) in particulate (>0.7μm), dissolved (<0.2 μm), and free monomer phases was monitored in order to directly measure their degradation rate next to known standards (Table III.3). Second, a vast suite of the most abundant 95 compounds containing an phenolic ring structure, similar to lignin, was tracked on the GC-ToF-MS allowing the measurement of the relative abundance of all detectable phenolic compounds before and after incubation (Table III.1). This list of compounds is composed of each phenolic compound that was identifiable in at least one sample (natural abundance or incubated), and represents the most abundant phenolic compounds in the DOM and POM extracts. Any known contaminants were removed from this list. Annually averaged, the suite of measured phenoliic compounds compose 7.4 ± 1.2% and 11.6 ± 1.2 % of bulk DOC and POC, respectively (Table III.2).

The relative abundance of this large suite of phenolic compounds (based on peak areas) was compared to the relative proportion of L_8 standard phenols to calculate a normalized degradation rate for the entire pool of aromatic compounds (Table III.3). The normalized degradation rate (d^{-1}) was multiplied by the initial concentration of total aromatic compounds (mg C L^{-1} d^{-1}), which was calculated by comparing the relative peak abundance of the total pool of phenolic compounds to the eight standard lignin phenols with standard-calibrated concentrations. This approach measures both the consumption and production of a vast suite of phenolic compounds, enabling the calculation of a net respiration rate of specific compounds that is directly comparable to ΔO_2 measurements of bulk community respiration. Since only

compounds with a ring structure were targeted, a phenolic compound was considered to be remineralized/respired once the ring structure was cleaved.

The second approach taken was a broad characterization of the chemical structure of the entire DOM pool with electrospray ionization Fourier transform-ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). ESI-FTICR-MS is a powerful non-targeted molecular trace approach that provides the molecular formula of thousands of individual compounds within the polar fraction of DOM (e.g., Tremblay et al., 2007; Sleighter and Hatcher, 2008; Kujawinski et al., 2009). The ultrahigh resolution and mass accuracy of ESI-FTICR-MS generates molecular masses that are accurate to within 1 ppm, and thus, it can be used effectively to detect mass changes within a suite of DOM molecules and subsequently to resolve the molecular-level impact of different biogeochemical processes on DOM composition. Combination of ESI-FTICR-MS with GC-ToF-MS and conventional biomarker and isotope analyses gives a picture of unprecedented detail of the microbial degradation of terrestrially-derived compounds in this major river.

Table III.1 List of the 95 phenolic BSTFA derivatives identified via GC-ToF-MS.

Compound Name	CAS #	Molecular Weight	Quant Mass
(2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone	78417-24-0	316	147
(4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	103077-68-5	265	137
[1,1'-Biphenyl]-4,4'-diamine, 3,3',5,5'-tetramethyl-	54827-17-7	240	240
1-(2,2-Dimethylpropyl)-1,2,3,4-tetrahydroisoquinoline	87443-62-7	203	132
1-(4-Anisidino)-2-phenyl-2-(1,2,4-thiadiazol-5-yl)ethane	153333-55-2	309	309
1,2,4-Benzenetricarboxylic acid, trimethyl ester	204-44-6	252	221
1,2,4-Trioxolane, 3,3,5-triphenyl-	23246-12-0	304	105
1,2-Benzisothiazole, 3-butoxy-	40991-40-0	207	151
1,2-Benzisothiazole-3-propanoic acid	50565-45-2	207	135
1,3-Benzenedicarboxylic acid	106450-31-1	310	295
1,3-Benzenediol, 5-iodo-	64339-43-1	236	236
1,3-Benzodioxole-5-carboxylic acid	613-95-6	280	223
1,3-Bis(trimethylsiloxy)benzene	4520-29-0	254	239
1,3-Dithiolane, 2-benzyl-2-methyl-	20137-72-8	210	119
1,4-Benzenedicarboxylic acid	4147-84-6	310	295
1,4-Butanedione, 1,4-diphenyl-	495-71-6	238	105
1-[(Pyrazinylcarbonyl)oxy]-2,5-pyrrolidinedione	342612-63-9	221	52
1-Allyldimethylsilyloxy-3-methylbenzene	532-66-2	206	165
1-Butyl(dimethyl)silyloxy-2-phenylethane	259862-61-8	236	179
1-Heptene, 1,3-diphenyl	5378-12-0	338	281
1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	3910-35-8	236	221
1H-Indole-2-carboxylic acid, 5-ethyl	74367-45-6	333	320
1-Naphthalenesulfonic acid, 5-(dimethylamino)-, phenyl ester	55837-12-2	327	327
2,2,4-Trimethyl-4-(4'-trimethylsilyloxyphenyl)chromane	155726-87-7	340	325
2,2'-Bibenzothiazole	866-23-8	268	268
2,2-Bis[4'-cyanooxyphenyl]propane	80-05-7	278	263
2,5-Dihydroxyacetophenone	490-78-8	296	281
2,6-Dihydroxyacetophenone	699-83-2	296	281
2-Butenoic acid, 4-oxo-4-[(1-phenylethyl)amino]-	108088-06-8	219	54
2-Ethyl-acridone	65753-77-7	223	208
2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl	55299-24-6	342	342
2-Iodo-3-pyridinol	40263-57-8	221	221
2-Phenyl-1,2-bispropane	294847-15-7	296	193
3,4 dihydroxybenzoic acid TMS	99-50-3	154	281
3,4-Diethoxybenzaldehyde	2029-94-9	194	166
3,4-Dimethoxyphenol	2033-89-8	226	226

3,5-Bis(4-methoxyphenyl)-1,2,4-selenadiazole	68723-61-5	346	221
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	234	219
3-Methoxyphenylpyruvic acid	27750-66-9	338	485
3-Pyridinecarboxylic acid	25436-37-7	195	180
4-(Benzyloxy)-N,N-dimethyl-1-butanamine	71126-70-0	207	58
4-Benzaldehyde	1012-12-0	194	179
4H-Pyran-4-one, 5-2-methyl	55557-21-6	286	271
4-hydroxy Benzoic acid	2078-13-9	282	224
4-hydroxyacetophenone	18803-29-7	208	151
5á-Podocarpa-8,11,13-trien-16-oic acid, methyl ester	3745-36-6	272	197
7-Oxodehydroabietic acid, methyl ester	110936-78-2	328	253
Acethydrazide, N2-[4-(thiitan-3-yloxy)benzylideno]-	9601-81-2	250	250
Acetic acid, (2-benzothiazolylthio)-	6295-57-4	225	181
Acetic acid, (3,4-dimethoxyphenyl)- methyl ester	2911-70-8	298	239
Acetic acid, phenyl(trimethylsiloxy)-, methyl ester	29233-93-0	238	179
Acetosyringone	55045-03-9	253	238
Acetovanillone	498-02-2	238	208
Anthra[1,9-cd]pyrazol-6(2H)-one	129-56-6	220	220
Anthraquinone, 7-methoxy-2-methyl-1,4,5-trihydroxy-	476-57-3	300	300
As-Indacene, 1,2,3,6,7,8-hexahydro-1,1,6,6-tetramethyl-4-(1-methylethyl)-	17465-47-3	256	241
Benzaldehyde, 2,4-bis	33617-38-8	282	267
Benzaldehyde, 3-ethoxy-4-propoxy-	350988-41-9	208	137
Benzene	18544-92-8	268	253
Benzene, 1-acetyl-3-ethyl-2-(2-ethenyl-6-ethylphenylazo)-	183-99-5	306	291
Benzeneacetic acid, 4	27750-57-8	296	73
Benzeneacetic acid, à-oxo-	55517-36-7	222	105
Benzeneacetic acid	2078-18-4	208	164
Benzenesulfonamide, N-butyl-	3622-84-2	213	141
Benzo[1,2-b:5,4-b']difuran-4,8-dione, 2-[1-(hydroxymethyl)vinyl]-5-methyl-	26962-41-4	258	258
Benzoic acid	65-35-7	194	179
Benzoic acid, 2-oxy	3789-85-3	282	439
Benzoic acid, 2-methyl	78324-00-2	250	119
Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	1421-49-4	250	235
Benzoic acid, 4-chloro-	25436-27-5	228	213
Benzoic acid, 4-methoxy-	2078-14-0	224	135
Benzoic acid, 4-methyl-, tert-butyl	345647-19-0	250	119
Benzoic acid, 5-methyl	624-53-7	296	237
Butylphosphonic acid, 3-chlorophenyl heptyl ester	6425-38-6	346	249

Carbocaine	96-88-8	246	98
Cinnamic acid, à-phenyl	63938-16-9	296	178
Cinnamic acid, m-methoxy	27750-64-7	250	235
Cinnamic acid, p-(trimethylsiloxy)-, trimethylsilyl ester	10517-30-3	308	293
Dibenzofuran-3ol, 2-methoxy-9a-(morpholin-4-yl)-5a,6,7,8,9,9a-hexahydro-	94420-49-2	305	226
Ethene, 1,2-diphenyl-1,2-bis	78375-55-0	324	309
Ferulic acid	10517-09-6	338	308
Hydrocinnamic acid, p-	27750-62-5	310	179
N(N'-Methyl-N'-nitroso(aminomethyl))benzamide	59665-02-0	193	105
p-Coumaric acid	10517-30-3	308	249
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	166273-38-7	306	191
Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	206	206
Propiophenone, 2,2',4',6'-tetramethyl-	2040-22-4	190	147
Pyrocatechol, bis	67-946-8	338	115
Pyrrolidine, 1-[2-(4-bromophenoxy)ethyl]-	1081-73-8	269	84
Syringaldehyde	6651-62-3	254	239
Syringic acid	10517-29-0	342	297
trans-Cinnamic acid	140-10-3	220	131
Valerophenone	33342-91-5	250	193
Vanillic acid	2078-15-1	312	282
Vanillin	6689-43-6	224	209

III.5 Supplemental Results and Discussion

Near the mouth of the Amazon River DOC concentrations were nearly an order of magnitude greater than POC concentrations (Table III.2). DOC and POC concentrations both appear to follow the hydrograph seasonally. The concentration of both dissolved L_8 lignin phenols and total dissolved phenolic compounds peaked in May 2011 and were lowest in September 2010 and 2011 (Table III.2). Contrary to bulk DOC, dissolved lignin concentrations are elevated at low rising water (December 2010) relative to low water in September (Table III.2). The proportion of free monomers to dissolved L_8 lignin phenols is anomalously high in December, suggesting that during the early stages of rising water dissolved phenolic compounds, rich in monomeric subunits from partial degradation, are mobilized from subsurface soils into the river through stream networks (e.g. Ward et al., 2012). Free monomers were completely degraded (e.g. undetectable post-incubation) in every incubation experiment. In both dissolved and particulate phases, the ratio of vanillyl acids to aldehydes (Ad/Al(v)) increased with river discharge (Table III.2), indicating that both the dissolved and particulate components mobilized into the river by rising and high discharge are more highly degraded than at low or decreasing flow. This trend is persistent not only in the tropics on a seasonal timescale, but has been observed on hourly timescales in temperate streams and rivers (Ward et al., 2012). Both particulate and dissolved L_8 lignin showed an elevated ratio of cinnamyl to vanillyl (C/V) phenols in September, indicating an elevated proportion of grass/leaf material (as opposed to woody material), and the lowest values in December at low-rising water. The ratio of syringyl to vanillyl (S/V) phenols was highest in May at high water and lowest in December, although the seasonal S/V variability does not appear to be statistically significant.

The absolute rates of degradation of both L_8 lignin and total phenolic compounds vary seasonally, following the hydrograph. However, when these rates are normalized to the initial L_8 and T_{phen} concentrations there is no statistically significant seasonal variability. This implies that lignin and aromatic respiration rates are likely primarily controlled by substrate availability in the river (e.g. Michaelis Menton). Bulk respiration rates, on the other hand, appear to be controlled by substrate quality since rates are inversely proportional to the hydrograph and OC concentrations. The composition of L_8 lignin consistently changed over the course of each incubation. For example, S/V, C/V, and Ad/Al (v) all consistently increased, indicating a preferential consumption of vanillyl phenols and higher degradation state after incubation. However, these small compositional changes were statistically insignificant.

Date	DOC (mg C L^{-1})	Λ_8 Lignin (μg C L^{-1})	Fraction of free Λ_8 lignin monomers (%)	Total phenolic DOC (mg C L^{-1})		S/V	C/V	Ad/Al (v)	Ad/Al (s)
16-Sep-10	4.8 ± 0.5	3.6 ± 0.4	0.98 ± 0.14	0.33 ± 0.05		0.37 ± 0.05	0.48 ± 0.07	0.52 ± 0.07	0.34 ± 0.05
1-Dec-10	3.5 ± 0.3	5.2 ± 0.5	6.40 ± 0.91	0.31 ± 0.04		0.21 ± 0.03	0.09 ± 0.01	0.58 ± 0.08	0.49 ± 0.07
7-May-11	5.7 ± 0.2	9.9 ± 1.0	0.04 ± 0.01	0.38 ± 0.05		0.33 ± 0.05	0.19 ± 0.03	1.25 ± 0.18	0.45 ± 0.45
13-Sep-11	3.8 ± 0.01	3.4 ± 0.3	0.73 ± 0.10	0.27 ± 0.04		0.27 ± 0.03	0.49 ± 0.06	0.42 ± 0.15	0.26 ± 0.04
Date	**POC** (mg C L^{-1})	Λ_8 Lignin (μg C L^{-1})		Total phenolic POC (mg C L^{-1})	δ13C-POC (‰)	S/V	C/V	Ad/Al (v)	Ad/Al (s)
16-Sep-10	0.45 ± 0.002	5.5 ± 0.6		0.076 ± 0.011	-26.8 ± 0.2	0.79 ± 0.11	0.15 ± 0.02	0.44 ± 0.06	0.44 ± 0.06
1-Dec-10	0.44 ± 0.002	3.4 ± 0.3		0.038 ± 0.005	-26.6 ± 0.2	0.71 ± 0.10	0.02 ± 0.00	0.46 ± 0.06	0.83 ± 0.12
7-May-11	0.56 ± 0.003	13.8 ± 1.4		0.061 ± 0.009	-27.7 ± 0.2	0.89 ± 0.13	0.09 ± 0.01	0.59 ± 0.08	0.42 ± 0.06
13-Sep-11	0.63 ± 0.003	6.8 ± 0.7		0.063 ± 0.009	-30.9 ± 0.2	0.74 ± 0.10	0.13 ± 0.02	0.40 ± 0.06	0.68 ± 0.10

Table III.2 Carbon and lignin concentrations and compositions at the mouth of the Amazon. Initial concentrations of dissolved (top, white) and particulate (bottom, grey) total organic carbon, Λ_8 lignin phenols, free Λ_8 monomers, and total phenolic compounds prior to incubation and the initial composition determined by the stable isotopic signature of organic carbon (δ^{13}C-POC), the ratio of syringyl to vanillyl phenols (S/V), the ratio of cinnamyl to vanillyl phenols (C/V), the ratio of vanillic acids to aldehydes (Ad/Al(v)), and the ratio of syringyl acids to aldehydes (Ad/Al (s)). Free Λ_8 monomers were completely consumed (i.e. undetectable) in all incubated samples.

Date	Bulk OC respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Λ_8 Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Λ_8 respiration ($\mu g\ C\ \mu g\ C^{-1}\ L^{-1}\ d^{-1}$)	Total Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Total Lignin respiration ($pA^2\ pA^{-2}\ L^{-1}\ d^{-1}$)
16-Sep-10	55.4 ± 5.5	0.40 ± 0.05	0.11 ± 0.02	13.7 ± 2.9	0.042 ± 0.006
1-Dec-10	74.7 ± 7.5	0.40 ± 0.05	0.08 ± 0.01	21.0 ± 4.4	0.067 ± 0.010
7-May-11	39.4 ± 3.9	1.16 ± 0.14	0.12 ± 0.02	22.8 ± 4.8	0.060 ± 0.009
13-Sep-11	62.7 ± 6.3	0.72 ± 0.09	0.21 ± 0.03	14.2 ± 3.0	0.053 ± 0.008
Date	Bacterial abundance (10^9 cells L^{-1})	Λ_8 Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Λ_8 respiration ($\mu g\ C\ \mu g\ C^{-1}\ L^{-1}\ d^{-1}$)	Total Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Total Lignin respiration ($pA^2\ pA^{-2}\ L^{-1}\ d^{-1}$)
16-Sep-10		0.53 ± 0.07	0.096 ± 0.015	5.9 ± 1.2	0.077 ± 0.012
1-Dec-10	2.37 ± 0.41	0.29 ± 0.04	0.085 ± 0.014	2.4 ± 0.5	0.064 ± 0.010
7-May-11	4.00 ± 0.7	0.83 ± 0.10	0.061 ± 0.010	3.3 ± 0.7	0.055 ± 0.008
13-Sep-11	3.90 ± 0.6	0.61 ± 0.08	0.090 ± 0.014	5.5 ± 1.2	0.087 ± 0.013

Table III.3 Bulk carbon and lignin respiration rates at the mouth of the Amazon River. Measured respiration rates of bulk organic carbon, dissolved (top, white) and particulate (bottom, grey) Λ_8 lignin phenols, and dissolved and particulate total phenolic compounds (T_{phen}). The normalized L_8 respiration rate is the ratio of the measured respiration rate to the initial L_8 concentration. The normalized T_{phen} respiration rate is the ratio of the rate of total phenolic respiration, based on relative GC-MS-TOF peak areas ($pA^2\ L^{-1}\ d^{-1}$), to the initial sum of peak area for total lignin-like compounds (95 identified compounds). The absolute rate of T_{phen} respiration ($\mu g\ C\ L^{-1}\ d^{-1}$) is determined by multiplying the normalized respiration rate by the ratio of total phenolic compounds to standardized L_8 lignin phenols and their initial concentration.

Chapter IV: The compositional evolution of organic matter along the lower Amazon River

Foreword: Data presented in this chapter is the result of a large collaboration between US and Brazilian Institutions as part of the River Ocean Continuum of the Amazon (ROCA) project. Drs. Daimio Brito, Alan Cunha, Rodrigo da Silva, Jose Mauro Sousa Moura, Troy Beldini, Patricia Yager, Richard Keil, Alex Krusche, and Jeffrey Richey all contributed significantly to the collection of the data presented in this chapter. Lignin analyses were performed in the UW Aquatic Organic Geochemistry Lab with assistance by Jaqui Neibauer, Ariel Townsend, and Rebekka Gould. Sampling in Macapá and Santarem was assisted by Henrique Sawakuchi, Joel Estevão Melo Diniz, Keila dos Santos, Eldo Santos, Brian Zelinsky, Christa Smith, Brandon Satinsky, Caroline Fortunato, and others.

IV.1 Introduction

A significant fraction of the carbon dioxide (CO_2) removed from the atmosphere by terrestrial primary production is mobilized into freshwater systems and remineralized along the river-to-ocean continuum, resulting in a flux of CO_2 from inland waters to the atmosphere that is an order of magnitude greater than the flux of carbon from rivers to the ocean (Cole et al., 1994; Benner et al., 1995; Richey et al., 2002; Duarte and Prairie, 2005; Tranvik et al., 2009; Aufdenkampe et al., 2011; Raymond et al., 2013; Ward et al., 2013). However, our understanding of rivers as a continuum, connecting headlands to the ocean and atmosphere, is fundamentally limited by a lack of measurements along the lower reaches of large river systems (Schlesinger and Melack, 1981). For example, most systematic measurements of the concentration and composition of organic material in the world's largest river by volume, the Amazon, have only been made as far downstream as Obidos, which is toughly 900 km from the

river mouth (Hedges et al., 1986; Quay et al., 1992; Devol et al., 1995; Moreira-Turcq et al., 2003; Battin et al., 2008), with some recent measurements downstream of Obidos (e.g. Mortillaro et al., 2011; Mortillaro et al., 2012; Moreira-Turcq, 2013). These relatively understudied reaches near the mouths of large rivers represent essential conduits in the delivery of organic and inorganic components from rivers to the ocean (Wehrli, 2013). Biogeochemical and physical processing mechanisms that occur within these reaches have a direct influence on the composition and magnitude of the material flux from a river.

The influence of rivers on regional and global carbon budgets extends well into the coastal ocean from the effect of fluvial nutrient delivery on marine primary production (Mayer et al., 1998). For example, the oligotrophic tropical North Atlantic Ocean is generally considered to be a net source of 30 Tg C y^{-1} to the atmosphere (Takahashi et al., 2002). However, a nearly equivalent CO_2 sink of 28 Tg C y^{-1} has been observed in the Amazon plume, as a result of nutrient inputs from the Amazon River (Subramaniam et al., 2008). This effectively reverses the normal tropical surface ocean condition and leads to CO_2 uptake in areas that would otherwise be outgassing. Accurately estimating the magnitude of these globally-relevant CO_2 fluxes is dependent on a well-constrained description of the nutrients, DIC and alkalinity exiting the river mouth, but there are currently little to no high-quality measurements of river chemistry or discharge at the mouth of large tropical rivers such as the Amazon (Cooley and Yager 2006; Cooley et al. 2007). Representing approximately 16% of the global freshwater discharge to the ocean, the Amazon is significant in its own right, and likely well-representative of large tropical rivers worldwide. Further, tropical regions are of unique importance as they provide the majority of both the global flux of riverine OC to the ocean and the evasive gas flux of CO_2 from inland waters to the atmosphere (Meybeck, 1982; Aufdenkampe et al., 2011).

Terrestrial and marine ecosystems are currently estimated to offset about half of the 7.9 Pg C yr^{-1} emitted to the atmosphere via fossil fuel emissions, however, it is unclear what proportion of the 2.4 Pg C yr^{-1} absorbed by terrestrial ecosystems is actually sequestered versus remineralized back into the atmosphere over modern time scales (Le Quere et al., 2009; Battin et al., 2009; Regnier et al., 2013). It is currently estimated that roughly 2.9 Pg C yr^{-1} is mobilized from the terrestrial biosphere into freshwater systems, globally, of which 2.1 Pg C yr^{-1} is remineralized and outgassed to the atmosphere before reaching the ocean and/or being buried in sediments (Raymond et al., 2013; Regnier et al., 2013). Tropical river systems represent hotspots for carbon outgassing, with approximately 70% of the global CO_2 flux evading from only 30% of the earth's landscape (Raymond et al., 2013). Draining an area of more than six million km^2, the Amazon basin represents 15% of terrestrial gross primary production (GPP) and ~25% of the evasive CO_2 gas flux from inland waters, globally (Richey et al., 2002; Raymond et al., 2013; Field et al., 1998; Malhi et al., 2008).

The region between Obidos and the mouth represents ~13% of the basin's surface area (Figure IV.1). Large lowland "mouth-bay" tributaries, the Rios Tapajos, Xingu, and Tocantins, in particular, contribute an additional ~20% to the Amazon River's total discharge to the ocean downstream of Obidos (Figure IV.2). At the river mouth the force of ocean tides reverses the flow of the river at semi-diurnal frequency (Ferraz, 1975). The influence of tidal forcing can be felt nearly 1,000 km upstream from the river mouth (Kosuth et al., 2009). The journey between Óbidos and the ocean takes a parcel of water three to five days to complete, during which in situ processing transforms organic and inorganic river constituents, fueling microbial respiration and CO_2 outgassing (Richey et al., 2002; Mayorga et al., 2005; Ward et al., 2013). The composition of OM is further altered in transit by processes such as sorption, photo-oxidation, and lateral

exchange with floodplains (e.g. Amon and Benner, 1996; Aufdenkampe et al., 2001; Hernes et al., 2007; Eckard et al., 2007; Spencer et al., 2010; Galy et al., 2011). This transit time is quite significant considering recent estimates that the entire pool of vascular plant derived macromolecular OC (e.g. lignin phenols) in the Amazon mainstem turns over in only 2-3 weeks (Ward et al., 2013). The counteracting forces from tides and river outflow create unique conditions for sediment transport and deposition at the mouth (Wright, 1977), which results in immense cross-channel variability in the concentration of suspended sediments near the mouth of the river. The dynamic variability of sediment loads at the mouth greatly complicates estimation of the flux of particulate organic carbon to the ocean. Further, the effects of tides on the residence time of water and dissolved material at the river mouth remains unclear.

The lower river receives organic carbon inputs from the terrestrial biosphere derived from both upstream sources (e.g. headlands) and lateral inputs from the surrounding lowland landscape (via direct input and stream/tributary networks). This terrestrial carbon load is augmented by autochthonous production in floodplains and productive lowland tributaries. Extensive floodplains, or varzea, are dispersed along the lower reaches of the Amazon River, which represent regions of significant *in situ* primary production, dominated by phytoplankton, periphyton, aquatic herbaceous plants, and flooded forests (Sioli 1984; Junk and Howard-Williamson, 1984; Junk, 1997; Melack and Forsberg, 2001). Covering a region of over 350,000 km^2 (Hess et al., 2003; Melack et al., 2010), these floodplains are thought to contribute to a significant fraction of the labile organic carbon in the mainstem. For example, Quay et al. (1992) estimated that roughly 40% of the organic carbon respired in the mainstem was derived from C4 plants, which are representative of grasses typically found in varzea. It is estimated that over 30% of the water in the Amazon River mainstem is derived from water that has passed through a

floodplain network (Richey et al., 1989). Varzea floodplains along with floodplain lakes such as the Lagoa Grande de Curuai and the Lagoa Grande de Monte Alegre provide a seasonal source of water to the mainstem, likely bringing with it locally produced OM (Bourgoin et al., 2007; Bonnet et al., 2008). Recent observations suggest that both the respiration of floodplain-derived OM and the direct input of CO_2 to the water from aquatic plant metabolism provides a significant fraction of the CO_2 to the river that is outgassed from the basin (e.g. Engle et al., 2008; Abril et al., 2014). The contribution of large floating macrophytes, produced primarily in floodplains, may be significant relative to the basin's overall carbon budget, but the magnitude of their production is relatively understudied (Melack and Engle, 2009). In addition to exporting labile OM to the mainstem, Moreira-Turcq et al. (2004) suggest that productive floodplains also represent important sinks for sedimentary OC.

In this chapter the composition and flux of particulate and dissolved organic material is examined at Obidos, the three main channels near the Amazon River mouth (Macapa North/South and Belem channels), and the Rio Tapajos tributary based on measurements made during a series of five cruises from September 2010-July 2012. The concentration and stable isotopic composition of bulk organic carbon was measured along with the concentration and composition of specific lignin phenols, a biomarker that has been used extensively to trace the origin, abundance, and alteration/preservation of OM derived from the terrestrial biosphere (Goni and Hedges, 1995; Tarq et al., 2004; Rezende et al., 2010; Ellis et al., 2012; Ward et al., 2012; Ward et al., 2013). Bulk isotopic signatures, specific lignin phenol ratios, and measurements of chlorophyll concentrations were used to construct a four-endmember mixing model to determine the relative contribution of highland terrestrial, lowland terrestrial,

varzae/floodplain, and algae-derived organic matter to dissolved and particulate organic carbon pools at Obidos and across each respective channel near the mouth.

IV. 2 Methods Summary

IV.2.1 Study area and chemical measurements

Chemical measurements were performed in the Amazon River mainstem at Obidos (S 01º55.141', W 55º 31.543'), the Rio Tapajos tributary near Santarem (S 02º29.063', W 55º 00.450'), and at the river mouth in the north channel of the mainstem near Macapá (S 00º05.400', W 51º 03.200'), the south channel near Macapá (S 00º09.415', W 50º 37.353'), and near Belem (S 01º31.162', W 48º 55.077') during five expeditions from September 2010 to July 2012. Whole water was collected at three cross-channel sites with a Shurflo submersible pump from the surface and 50% river depth at the center and margins of the river channel.

POC and particulate lignin samples were collected on pre-combusted 25mm and 142mm diameter, respectively, Whatman GF/F glass fiber filters (0.7μm) and frozen. Triplicate POC samples were filtered on combusted Whatman GFF filters, packed in tin capsules after drying and acid-fumigation, and analyzed for $PO^{13}C$ on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. DOC samples were pre-filtered through 25mm Whatman GF/F glass fiber filters, preserved with HCl, and analyzed in triplicate on a Shimadzu TOC-VCPH analyzer for DOC and an O.I. Analytical Model 1030 TOC Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer with a GD-100 Gas Trap Interface for $DO^{13}C$. Dissolved lignin samples were pre-filtered through 142mm diameter Whatman GF/F glass fiber filters and Pall 0.2um Supor filters, acidified to pH=2, extracted at 3

mL/min on Waters Oasis HLB solid phase extraction cartridges, and frozen for dissolved lignin analysis (Keil and Neibauer, 2009). Particulate and dissolved lignin samples underwent CuO oxidation performed in a CEM Microwave Accelerated Reaction System (Goni and Montgomery, 2000) and were analyzed using a GC-ToF-MS after derivatizing with BSTFA (Agilent 7890A GC, Leco TruToF HT).

River discharge was measured using a Teledyne Workhorse Rio Grande 600 khz Acoustic Doppler Current Profiler (ADCP). ADCP profiles were made across the north and south mainstem channels and Belem (e.g. Tocantins) at approximately 1 hr intervals for 13 hr in order to assess river velocity over the span of a tidal cycle and calculate the total Amazon River discharge to the ocean. Total daily discharge from the Amazon River was calculated by ADCP measurements at the North and South channels of Macapá and Agência Nacional de Águas (ANA) stage/discharge records at Óbidos and the Tapajós, Xingu, and Tocantins tributaries.

IV.2.2 End member mixing model equations

The composition of dissolved and particulate OM was classified based on four endmembers: algae/phytoplankton (OM_A), varzea grasses and macrophytes (OM_V), lowland terrestrial ($OM_{T\text{-}Lo}$; e.g. wood, bark and leaves), and highland terrestrial ($OM_{T\text{-}Hi}$; Equation 1). The relative contribution of algae was determined using chlorophyll a measurements made here and in the literature (Moreira-Turcq et al., 2013) assuming a C:Chl ratio of 40 (Equation 2; Meybeck, 1982; Saliot et al., 2001). An average contribution of algae to total OC of 1.5%, 1.6%, 1.7%, and 1.8% at North Macapa, South Macapa, Belem, and Obidos respectively was measured during September 2011. The ratio of cinnamyl to vannilyl lignin phenols (C:V) was used to determine the proportion of OM that was terrestrially-derived (e.g., $OM_{T\text{-}Hi}$ + $OM_{T\text{-}Lo}$) versus

OM that was varzea-derived (Equation 3). Grass and macrophyte species representative of the lowland varzea floodplains have a higher C:V ratio compared to forest-derived wood, bark, and leaves, while phytoplankton does not produce appreciable amounts of lignin phenols (Hedges et al., 1986). Plant endmember values of 1.15 and 0.01 were used for OM_V and ($OM_{T\text{-}Hi}$ + $OM_{T\text{-}Lo}$), respectively; values were derived from values determined by Ellis et al. (2012) in the Mekong River and Hedges et al. (1986) in the Amazon River. With OM_A, OM_V, and ($OM_{T\text{-}Hi}$ + $OM_{T\text{-}Lo}$) determined, a four endmember mixing model based on $\delta^{13}C$ stable isotopic signatures was used to determine the relative proportion of $OM_{T\text{-}Hi}$ and $OM_{T\text{-}Lo}$ individually (Equation 4). The endmember $\delta^{13}C_A$ was determined by the average $\delta^{13}C$-POC of phytoplankton measured in the Rio Tapajos, $\delta^{13}C_V$ was determined by the average $\delta^{13}C$ of grasses (-23‰) and macrophytes (-25.3‰; Ellis et al., 2012), and $\delta^{13}C_{THi}$ and $\delta^{13}C_{T\text{-}Lo}$ were determined by the average $\delta^{13}C$ of forest-derived OM in Amazonian forests above and below an altitude of 2500m (Korner et al, 1988; Quay et al., 1992).

Equation (1) $1 = OM_A + OM_V + OM_{T\text{-}Hi} + OM_{T\text{-}Lo}$

Equation (2) $OM_A = [40 \text{ g C g chl}^{-1} \text{ x } [chl]] / [OC]$

Equation (3) $(C{:}V)_{sample} = [(C{:}V)_V \text{ x } OM_V] + [(C{:}V)_{THi+TLo} \text{ x } OM_{THi+TLo})]$

Equation (4) $^{13/12}R_{sample} = (^{13/12}R_A \text{ x } OM_A) + (^{13/12}R_V \text{ x } OM_V) + (^{13/12}R_{T\text{-}Hi} \text{ x } OM_{T\text{-}Hi}) + (^{13/12}R_{T\text{-}Lo} \text{ x } OM_{T\text{-}Lo})$

$\delta^{13}C_{sample} = [(^{13/12}R_{sample} / {}^{3/12}R_{V\text{-}PDB}) - 1] \text{ x } 1000$

$\delta^{13}C_A = -35.1‰$

$\delta^{13}C_V = -24.25‰$

$\delta^{13}C_{T\text{-}Hi} = -26.5‰$

$\delta^{13}C_{T\text{-}Lo} = -28.8‰$

$(C:V)_V = 1.15$

$(C:V)_{THi+TLo} = 0.01$

IV.3 Results and Discussion

A combination of isotopic and compound specific endmember mixing models were used to determine the relative abundance of OC from the following categories: macophytes and submerged grasses from varzea floodplains; wood, bark, and leaves from highland and lowland terrestrial forests; and phytoplankton/algae produced in lowland tributaries and floodplains. The relative abundance of algal OM, determined by measurements of chlorophyll made during the September 2012 sampling, was less than 2 ± 0.5% of the particulate organic carbon at Obidos and each respective channel at the mouth. Turbid conditions greatly limit the potential for *in situ* algal production in the Amazon mainstem, which is typically net heterotrophic (e.g. Melack and Fisher, 1990; Benner et al., 1995; Devol et al., 1996; Ellis et al., 2012; Moreira-Turcq et al., 2013). Particulate organic carbon in the Rio Tapajos, on the other hand, was composed of roughly one-third algal-derived OM, on average. The values measured in the Rio Tapajos are similar to isotopic measurements that suggest roughly 36% of Amazon floodplain sediments are composed of algal-derived OM (Martinelli et al., 2003). Unlike the mainstem, the Rio Tapajos tributary contains very low sediment levels due to its shallow incline and slow moving waters (Goulding et al., 1993). For example, during the September 2010 collection at low water, the

concentration of fine suspended sediment was 3.3 ± 0.3mgL^{-1} in the Rio Tapajos, compared to 23.9 ± 0.4 mg L^{-1}, 19.8 ± 0.5 mg L^{-1}, and 29.4 ± 1.9 mg L^{-1} across the North Macapa, South Macapa, and Obidos channels, respectively (Table IV.3).

Lignin phenols compose a portion of the structural backbone for vascular plants; the ratio of specific lignin phenols to one another varies depending on species and tissue type (Ertle and Hedges, 1982). The ratio of cinnamyl to vannilyl phenols (C:V) of plant biomass, for example, varies between forest-derived and typically floodplain-derived species; the C:V ratio of forest-derived barks, woods, and leaves generally ranges from 0.01 to 0.35, whereas the C:V ratio of floddplain-derived grass and macrophyte species ranges from roughly 1.0 to 2.0 (Hedges et al., 1986; Ellis et al., 2012). The C:V ratio of lignin phenols was used to estimate the proportion of dissolved and particulate organic carbon that was derived from the macrophyte and C4 grass species representative of varzea floodplain production (C:V = 1.15) and forest-derived biomass (C:V = 0.01; Hedges et al., 1986; Ellis et al., 2013). The C:V ratio of particulate lignin at Obidos ranged from 0.08 ± 0.01 to 0.17 ± 0.02, with an average value of 0.12 ± 0.04 (Table IV.2). The C:V ratio was maximal at peak and falling water, indicative of an increased contribution of macrophyte and varzea derived POC during high water. Based on the C:V endmember mixing model, varzea-derived OM composed roughly 10 ± 4% of the POC at Obidos.

Once the relative abundance of algal and varzea-derived OM was known, the remaining contribution of lowland and highland terrestrially-derived OM could be calculated based on the stable isotopic value of organic carbon (δ^{13}C-OC). The stable isotopic signature of POC at Obidos ranged from -31.1 ± 0.7‰ to -26.2 ± 0.3‰ during the sample period, with a mean value (± one SD) of -27.4 ± 2.1‰ (Table IV.2). The mean δ^{13}C-POC measured here is similar to

previous measurements made at and upstream of Obidos (e.g. Quay et al., 1992; Mayorga et al., 2005), but a wider range of values was observed here. Based on these δ^{13}C-POC values, POC at Obidos was, on average (± one SD), composed of 65 ± 37% and 23 ± 39% of highland terrestrially-derived OM and lowland terrestrially-derived OM, respectively (Figure IV.3).

The C:V ratio of particulate lignin at the three channels near the mouth ranged from 0.02 ± 0.01 to 0.28 ± 0.04, with average values of 0.11 ± 0.05, 0.15 ± 0.05, and 0.21 ± 0.11 across the North Macapa, South Macapa, and Belem channels, respectively (Table IV.2). Belem had the largest proportion of macrophyte and varzea-derived POM, with 17 ± 9% on average, compared to 8 ± 5% and 12 ± 4% at the north and south Macapa channels, respectively. The δ^{13}C of POC across both Macapa channels ranged from -26.3 ± 0.7‰ to -30.7 ± 0.3‰, with a mean value of -27.8 ± 0.5‰ and -27.6 ± 0.7‰ in the North and South Macapa channels, respectively. In Belem, δ^{13}C-POC ranged from -22.4 ± 0.4‰ to -30.2 ± 1.1‰, with a mean value of -26.5 ± 1.1‰. Based on these isotopic values, POC near the river mouth was composed of relatively less highland terrestrial-derived OM compared to Obidos, with an average highland contribution of 49 ± 31%, 50 ± 32%, and 53 ± 42% across the North Macapa, South Macapa, and Belem channels, respectively, compared to 66 ± 37% at Obidos (Figure IV.3). Lowland terrestrially-derived OM composed 41 ± 31%, 36 ± 35%, and 28 ± 36% of bulk POM across the North Macapa, South Macapa, and Belem channels, respectively.

The high standard deviation of these annually averaged estimates of POM composition illustrates the significant seasonal variability in POM composition (Figure IV.3). This variability was differentially expressed in each channel, with the South Macapa channel and Obidos behaving somewhat similarly in contrast to the North Macapa and Belem channels. The presence

of lowland terrestrially-derived at Obidos and the South Macapa channel was minimal during rising and high water and highest during falling and low water (Figure IV.3). Highland terrestrially-derived POM was most abundant at rising and high water at Obidos and the South Macapa channel. Observations of relatively old (i.e. radiocarbon-depleted) POC present during rising and peak high water have been made in the Amazon and other large tropical rivers, similarly implying an export of aged headland-derived POC during periods of increasing discharge (Mayorga et al., 2005; Martin et al., 2013; Moreira-Turcq et al., 2013). The endmember mixing model calculated an anomalously high contribution of lowland relative to highland terrestrial POM at all sample sites for the September 2011 low water collections. The most likely explanation is that the contribution of algae to POM in the mainstem was underestimated. During this low water sampling, δ^{13}C-POC values ranged from -30.2 ± 1.1‰ to -31.1 ± 0.7‰ at Obidos, Belem, and both channels near Macapa, indicating higher presence of algal-derived POM than chlorophyll measurements suggest; however, similarities in isotopic signature makes it difficult to distinguish between algae and lowland terrestrial OM endmembers. The relative abundance of macrophyte and varzea-derived POM was highest during rising and high water at Obidos. The north channel of Macapa, on the other hand, had the highest relative abundance of varzea-derived POM at low and falling water, while the South Macapa channel had the largest varzea POM contribution at both peak and falling water. The Belem channel had a relatively consistent contribution of varzea POM, except for during the falling water cruise in July 2012. This differential expression of seasonal variability in POM composition observed along each channel of the mouth is likely to due to a combination of the presence of localized environments/inputs and varying effects of the interaction between river flow and tidal forcing.

Sediment transport mechanisms at the mouth of a river are influenced by the balance between river outflow and tidal forcing (Wright, 1977); import/export dynamics of water and POM from tidally inundated floodplains are similarly influenced by this balance. The magnitude and upstream extent of tidal fluctuations generally increases as river velocity slows down relative to tidal forcing (Wright, 1977). Total Amazon River discharge during the study period was roughly 203,759 m^3 s^{-1}. Based on ADCP measurements made across each respective channel of the mouth, roughly 50% of the total annual flow is through the South Macapa channel, 30% through the North Macapa channel, and 20% through the Belém channel. These differences in discharge through each channel, which vary seasonally, may influence the relative effects of tidal forcing on the movement of water and sediments through each channel and the patterns/timing of floodplain inundation. For example, it is possible that the enhanced flow through the South Macapa channel dampens the influence of tidal forcing relative its North Macapa and Belem counterparts. The profiles of the North and South Macapa channels are bathymetrically unique, possibly as a result of the balance between flow and tidal forces. For example, the North Macapa river profile is characterized by three deep channels with shallow sand bars interspersed, whereas South Macapa is characterized by one consistent channel. Additional work needs to be done exploring the interactions between river flow and tidal forcing in the numerous unique environments near the mouth in order to further understand the mechanisms driving the transport of sediment and sedimentary organic carbon. The compositional signals of POM observed at each channel are likely affected by tidally-influenced sediment transport processes and localized POM sources/sinks.

The tidal dynamics discussed above influence the abundance and composition of DOC by transporting it to and from unique environments over a short time period (e.g.

floodplains/flooded forests versus open channel) where OM remineralization and import/export dynamics can greatly differ (e.g. Twilley et al., Junk et al., 1989; Goulding et al., 1993; Amon and Benner, 1996). For example, the presence of highly labile algal and macrophyte-derived OM and unique microbial communities in floodplains potentially results in enhanced breakdown of mainstem-DOM in these environments (e.g. Bianchi et al., 2010; Ellis et al., 2012). The C:V ratio of dissolved lignin at Obidos ranged from 0.09 ± 0.01 to 0.39 ± 0.05, with an average value of 0.25 ± 0.11. Based on the C:V endmember mixing model, macrophyte/varzea-derived OM composed roughly 21 ± 10% of the DOC at Obidos on average, and was maximal at low and falling water (Table IV.1). The C:V ratio of dissolved lignin at the three channels near the mouth ranged from 0.07 ± 0.01 to 0.49 ± 0.05, with average values of 0.29 ± 0.19, 0.18 ± 0.10, and 0.19 ± 0.10 across the North Macapa, South Macapa, and Belem channels, respectively (Table IV.1). North Macapa had the highest average contribution of macrophyte and varzea-derived DOM, with 24 ± 16% on average, compared to 15 ± 9% at both the South Macapa and Belem channels. As with POM, the contribution of macrophyte and varzea-derived DOM at Obidos was highest at low and falling water. This trend was also evident in DOM across each channel at the mouth, whereas the seasonality of varzea contribution to POM was different in North Macapa and Belem relative to Obidos and the South Macapa channel. This difference is most likely driven by tidal influences on sediment transport mechanisms discussed previously that do not affect dissolved constituents in the same manner.

The stable isotopic signature of DOC (δ^{13}C-DOC) at Obidos ranged from -25.5 ± 0.2‰ to -29.4 ± 0.2‰ during the sample period, with a mean value of -27.4 ± 0.7‰. The annual average δ^{13}C-DOC at Obidos was roughly equal to the δ^{13}C-POC, with a slightly lower range of values for δ^{13}C-DOC (Table IV.1). Based on these δ^{13}C-DOC values, DOC at Obidos was, on

average, composed of 33 ± 46% and 44 ± 41% of highland terrestrially-derived OM and lowland terrestrially-derived OM, respectively (Figure IV.4). POC at Obidos was composed of nearly twice the proportion of highland OM compared to DOC, whereas DOC was relatively more enriched in lowland OM. The abundance of highland versus lowland terrestrial DOM was highly seasonally variable. Highland DOM was completely absent at Obidos during low and rising water and its contribution was maximal at high water, composing 91 ± 8% and 76 ± 9% of DOC during peak high water and falling water, respectively (Figure IV.4). δ^{13}C-DOC across the three channels near the mouth ranged from -27.4 ± 0.2‰ to -30.4 ± 0.2‰, with a mean value of -29.1 ± 1.2‰, -29.0 ± 1.1‰, and -28.4 ± 0.5‰ in the North Macapa, South Macapa, and Belem channels, respectively. There is a systematic decrease in δ^{13}C-DOC from Obidos to the river mouth, which indicates either an increase in lowland versus highland terrestrially-derived OM or a relative increase in algal DOM. Based on the endmember mixing model, the contribution of highland OM to DOC near mouth was on average 10 ± 22% at both the North and South channels of Macapa and 11 ± 13% across the Belem channel. As with Obidos, highland DOM was absent from most samplings. For example, Highland DOM composed roughly 20 ± 3% and 25 ± 5% during rising and high water, respectively, whereas highland DOM was only present at rising water in the North and South Macapa channels, with a relative abundance of 51 ± 7% and 48 ± 5%, respectively. Lowland terrestrial OM composed on average 64 ± 19%, 73 ± 20%, and 72 ± 13% of the DOM across the North Macapa, South Macapa, and Belem channels.

The differences observed in the composition of particulate and dissolved organic carbon between Obidos and the river mouth yield insight into the biodegradability of organic matter derived from highland, lowland, and floodplain sources. For example, floodplain-derived OM composed 10-20% of DOC and POC throughout the lower river based on compound specific

measurements, whereas isotopic measurements indicate that the respiration of floodplain-derived OM contributes to roughly 40% of the river's CO_2 supersaturation (Quay et al., 1992). This implies that floodplain-derived OM is rapidly remineralized in the river, but constantly resupplied at a variable rate depending on inundation frequency and magnitude. Varzea-derived OM was found in similar proportions at Obidos and the mouth because lateral transport provides a constant supply that balances in transit remineralization. Each channel of the mouth had different proportions of varzea-derived OM due to localized inputs and import/export dynamics. Abril et al. (2014) estimated that roughly 0.21 Pg C yr^{-1} is outgassed from central Amazonian floodplains, roughly 40% of the total CO_2 evasive gas flux from the Amazon basin (Richey et al., 2002).

Both highland and lowland terrestrially-derived DOM also appeared to be highly bio-labile along the river continuum. The relative contribution of highland DOM, for example, decreased by roughly 20% from Obidos to the river mouth. The ratio of vanillic acids to vanillic aldehydes (Ad/Al_v), is a useful proxy for determining the relative degradation state of vascular plant-derived OM (Ertel and Hedges, 1982). The mean Ad/Al_v ratio of dissolved lignin (± one SD) increased from 0.67 ± 0.43 to 1.00 ± 0.45 from Obidos to the mouth (flow-weighted average of each channel), indicating that the terrestrial macromolecular DOM present at the mouth was relatively more degraded. The South Macapa channel had the highest average dissolved Ad/Al_v ratio of 1.11 ± 0.51, followed by Belem and the North Macapa channel with an average Ad/Al_v of 0.92 ± 0.38 and 0.86 ± 0.51, respectively (Table VI.1). The difference in dissolved Ad/Al_v between Obidos and each channel across the mouth was generally highest at peak high water, implying the highest relative amount of vascular plant derived OM degradation occurred during this time period. Ward et al. (2013) observed that the degradation rate of specific lignin phenols

was also highest during this same high water sampling period, accounting for roughly 70% of bulk respiration rates at high water. Both dissolved and particulate Ad/Al_v ratios increased during these dark incubations conducted near the river mouth (Ward et al., 2013). As opposed to the dissolved phase, the Ad/Al_v ratio of particulate lignin, was only elevated at Belem relative to Obidos, whereas the particulate Ad/Al_v ratio at Obidos and the Macapa channels were nearly equivalent (Table IV.2). The mean particulate Ad/Al_v ratio at Obidos was 0.69 ± 0.12, compared to 0.50 ± 0.10, 0.70 ± 0.11, across 1.07 ± 0.42 at the North Macapa, South Macapa, and Belem channels.

The concentration of fine particulate organic carbon (FPOC) significantly decreased between Obidos and the mouth. The average concentration of FPOC at Obidos was 2.60 ± 0.94 mg L^{-1}, reaching its maximum at high water. Across the mouth, the concentration of POC averaged 0.64 ± 0.27 mg L^{-1}, 0.56 ± 0.25 mg L^{-1}, and 0.65 ± 0.19 mg L^{-1} at the North Macapa, South Macapa, and Belem channels, respectively (Table IV.2). The Rio Tapajos tributary had an average POC concentration of 0.47 ± 0.17 mg L^{-1}. Annually averaged fine sediment concentrations at Obidos typically range from 60-80 mg L^{-1} (Dunne et al., 1998; Filizola, 2003; Gibbs, 1967; Meade et al., 1979; Meade et al., 1985; Richey et al., 1990; Moreira-Turcq et al., 2013). Fine suspended sediment levels measured near the mouth were generally lower than these historic measurements made at and upstream of Obidos, with average concentrations (± one SD) of 52 ± 31 mg L^{-1}, 38 ± 19 mg L^{-1}, and 29 ± 13 mg L^{-1} across the North Macapa, South Macapa, and Belem channels, respectively. The percentage of sedimentary organic carbon (%OC) varied from 1.2 to 3.0% across each respective channel near the mouth, with the highest values generally occurring at low water; mean %OC values were 1.5 ± 0.43, 1.9 ± 0.77, and 2.1 ± 0.79 across the North Macapa, South Macapa, and Belem channels, respectively (Table IV.3).

Previous studies observed a slightly higher mean %OC and greater variation in %OC values at and upstream of Obidos, with a mean of roughly 2.5% and a range from 1 to 6% (Hedges et al., 1986; Hedges et al., 2000; Moreira-Turcq, 2013). The concentration of particulate lignin phenols (Σ_8) also decreased between Obidos and the mouth. The mean concentration (± one SD) of particulate Σ_8 lignin at Obidos was 28.6 ± 22.6 μg L^{-1}, reaching its highest value during rising water (Table IV.2). Near the mouth the mean concentration of particulate Σ_8 lignin was 11.6 ± 6.4 μg L^{-1}, 12.2 ± 4.3 μg L^{-1}, and 8.7 ± 1.7 μg L^{-1} across the North Macapa, South Macapa, and Belem channels, respectively. As with bulk POC, a large portion of the flux of vascular plant-derived POM through Obidos was lost along the river, due to a combination of remineralization and potential sedimentation processes.

Unlike POC levels, the concentration of dissolved organic carbon generally increased between Obidos and the mouth. The average concentration of DOC was 3.9 ± 0.6 mg L^{-1} at Obidos, compared to 4.2 ± 1.0 mg L^{-1} at both the North and South Macapa channels and 4.3 ± 1.5 mg L^{-1} at Belem. The average concentration of DOC in the Rio Tapajos was 2.0 ± 0.5 mg L^{-1}. Similarly the average concentration of dissolved Σ_8 lignin increased from 7.2 ± 5.7 μg L^{-1} at Obidos to 9.0 ± 4.2 μg L^{-1}, 8.6 ± 3.6 μg L^{-1}, and 8.8 ± 4.8 μg L^{-1} across the North Macapa, South Macapa, and Belem channels, respectively. The observed enrichment of dissolved organic material at the mouth compared to Obidos is likely due to a combination of the breakdown of large particulate compounds into low molecular weight dissolved compounds and lateral inputs of DOC from floodplain exchange (Abril et al., 2014) and lowland blackwater tributaries. The relative proportion of total organic carbon comprised of DOC increased from 60% at Obidos to roughly 90% across the mouth, on average, indicating that the OC flux to the ocean is primarily dissolved load (Figure IV.5). Flux estimates from the mouth were not made here due to large

uncertainty concerning the influence of tidal fluctuations on constituent concentrations. Large variability was measured in the particulate phase, in particular, throughout a tidal cycle. Systematic measurement of the underlying dynamics of this variability is required to calculate flux measurements with confidence.

IV.4 Conclusion

The culmination of these observations indicate that very little dissolved organic matter persisted from the headlands to the river mouth; highland DOM was only present at the mouth in low amounts during rising/high water. A significant fraction of particulate organic matter, on the other hand, persisted from the headlands to the mouth. Roughly 50% of the POC remaining at the mouth was highland-derived, implying that a portion of the river's sedimentary organic carbon load was relatively refractory and aged as suggested by others (Richey et al., 1980; Richey et al., 1990; Moreira-Turcq et al., 2003; Mayorga et al., 2005; Moreira-Turcq et al., 2013). Observations of ubiquitously modern compound specific radiocarbon ages of particulate lignin phenols in the Mekong River and high degradation rates of particulate and dissolved lignin in the Amazon, however, imply that the macromolecular components of terrestrial vascular plants are highly bio-labile (Martin et al., 2013; Ward et al., 2013). The compilation of these results suggest that there are two distinct pools of POM in the river: (i) a pool of labile POM from modern terrestrial and floodplain primary production that overturns on rapid to moderate timescales, and (ii) a pool of aged refractory POM that is buried along the lower river and coastal shelf.

The annual flux of organic matter from the river basin to the ocean represents less than 5% of the total carbon that moves through the system (Richey et al., 2002; Raymond et al.,

2013). Our quantitative understanding of this phenomenon can be greatly improved upon by a more detailed evaluation of the respective processes controlling both the input and outgassing of carbon to/from the system. For example, constraining gas transfer coefficients (k_{600}) for physically unique reaches of the basin and enhancing spatial coverage of pCO_2 measurements is essential for accurately quantifying CO_2 evasion. I hypothesize that gas exchange is enhanced in the lower river by the rough water surface conditions caused by strong winds and tidal flow reversals. Further, the ecosystem processes driving this evasive CO_2 flux are poorly constrained. For example, although we assume depth-integrated respiration is the primary source of CO_2 in the Amazon, little is known about the potential role of photo-oxidative biological "priming" processes in enhancing the biodegradability of OM as it travels along the continuum (Bianchi et al., 2011; Remington et al., 2011; Rodriguez-Zuniga et al., 2008; Spencer et al., 2009).

In order to construct basin-wide watershed carbon budgets that are accurate enough to predict the sensitivity of ecosystems to future change, understanding of each respective process that affects the exchange of carbon to and through aquatic ecosystem continuums must be refined (Devol et al., 1995; Lovett et al., 2006; Buttman and Raymond, 2011). Key dynamics remain quantitatively elusive and require systematic evaluation along the entire river-to-ocean continuum in order to accurately quantify the amount of carbon moving through and staying within a particular system. Reconciling global energy budgets requires defining a global carbon budget that is informed by detailed studies of basin-scale watershed dynamics across latitudes and biomes.

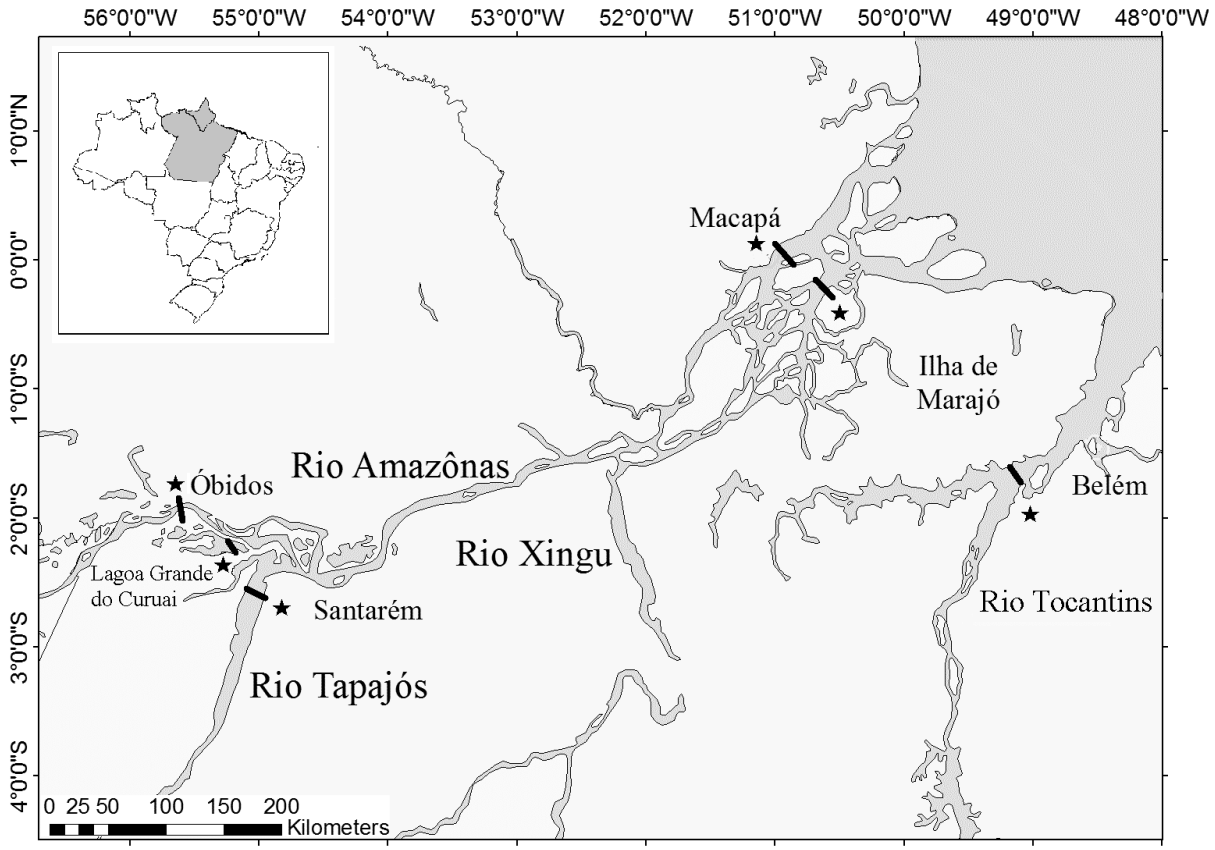


Figure IV.1 Study boundaries. The lower Amazon River, from Obidos to the mouth near Macapa (north/south channels) and Belem, including the Rio Tapajos tributary and Lagoa Grande de Curuai floodplain lake, were chemically characterized here.

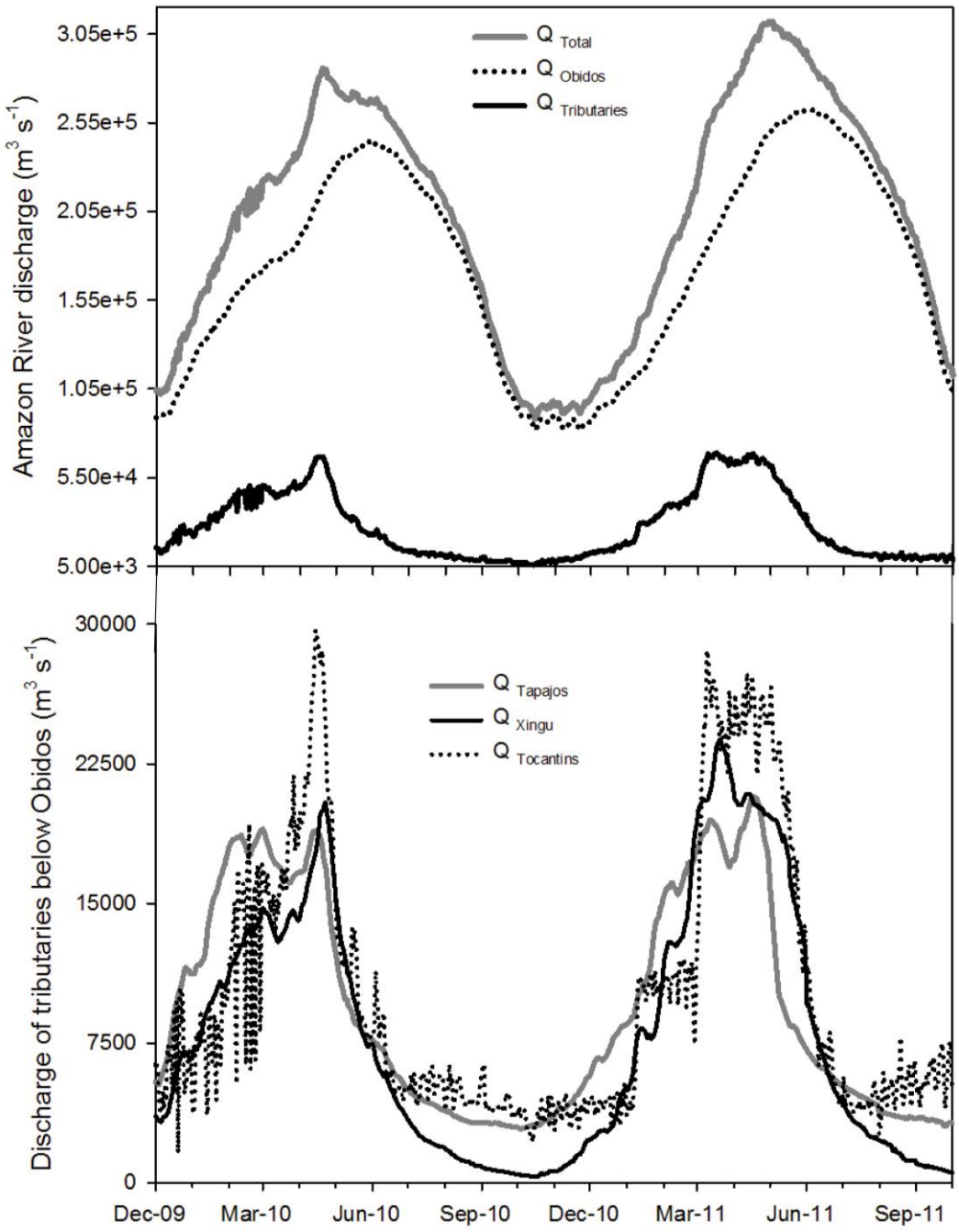


Figure IV.2 Amazon River discharge. Total discharge from the mouth of the Amazon River (sum of discharge at Belém and Macapá north/south channels), discharge at Óbidos, and discharge from the three major tributaries downstream of Óbidos—Rios Tapajós, Xingu, and Tocantins—from December 2009 through July 2012.

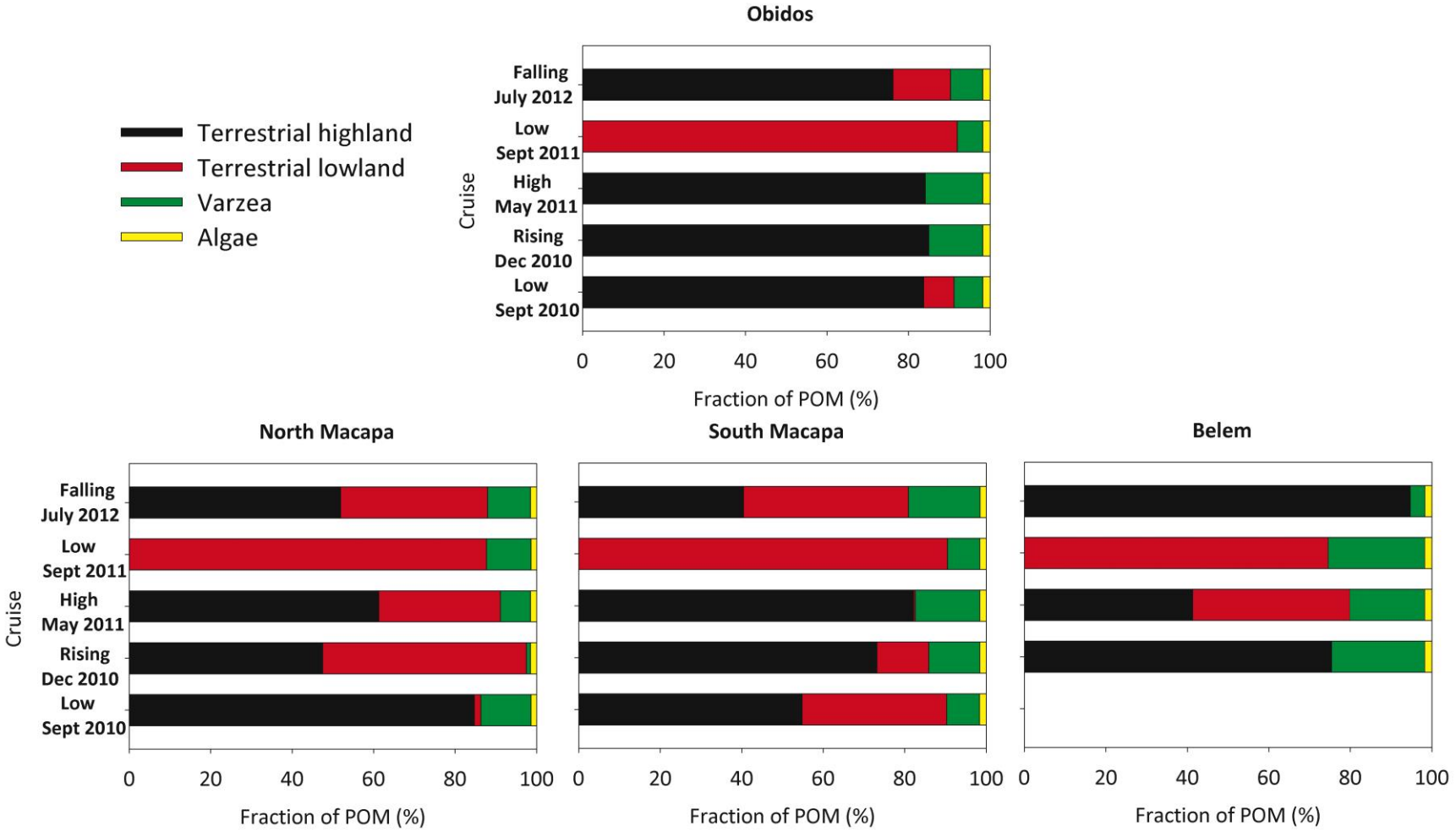


Figure IV.3 Particulate organic matter composition. The relative fraction of POM composed of highland terrestrial, lowland terrestrial, algal, and varzea-derived DOM at Óbidos (top) and each respective channel near the Amazon River mouth (bottom).

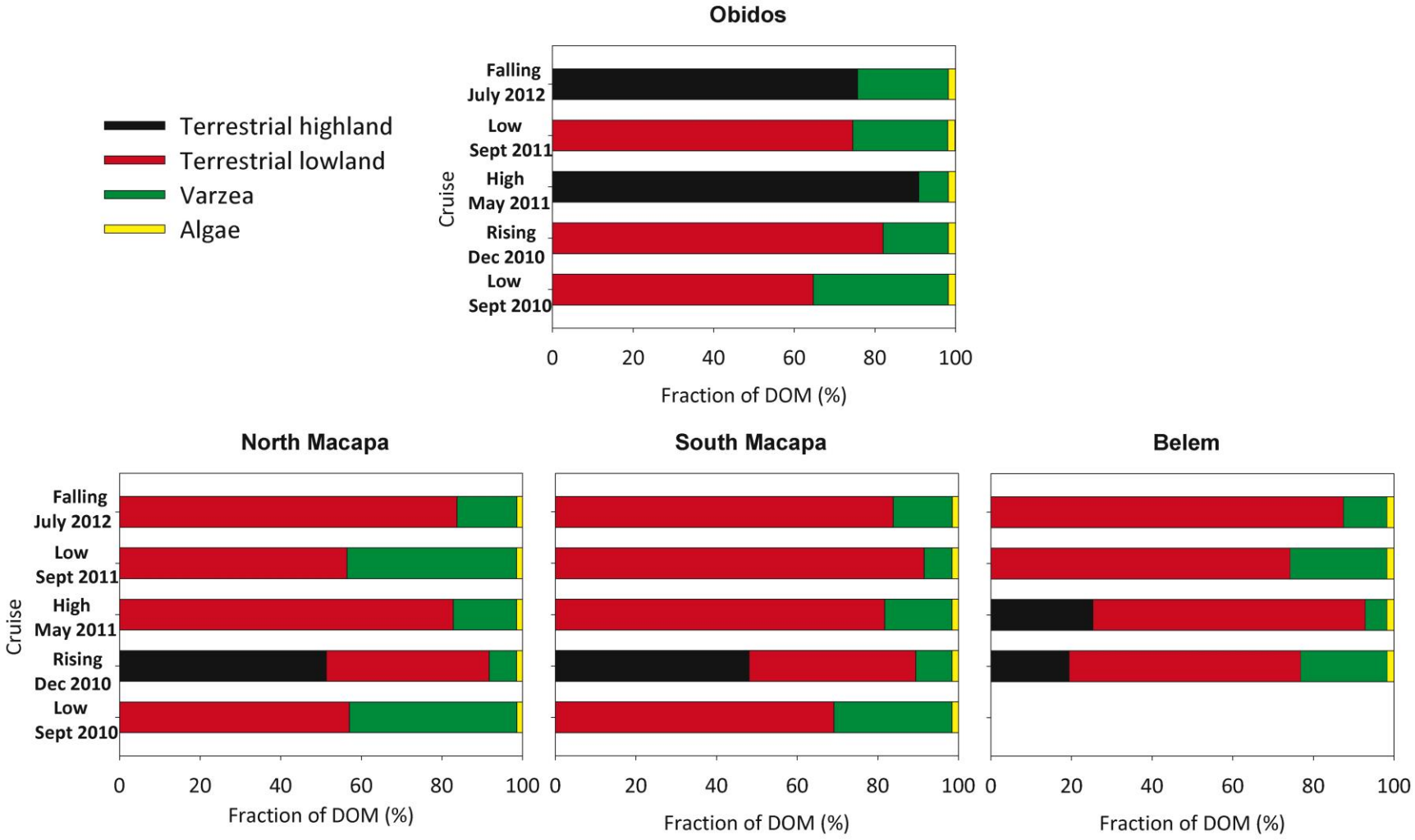


Figure IV.4 Dissolved organic matter composition. The relative fraction of DOM composed of highland terrestrial, lowland terrestrial, algal, and varzea-derived DOM at Óbidos (top) and each respective channel near the Amazon River mouth (bottom).

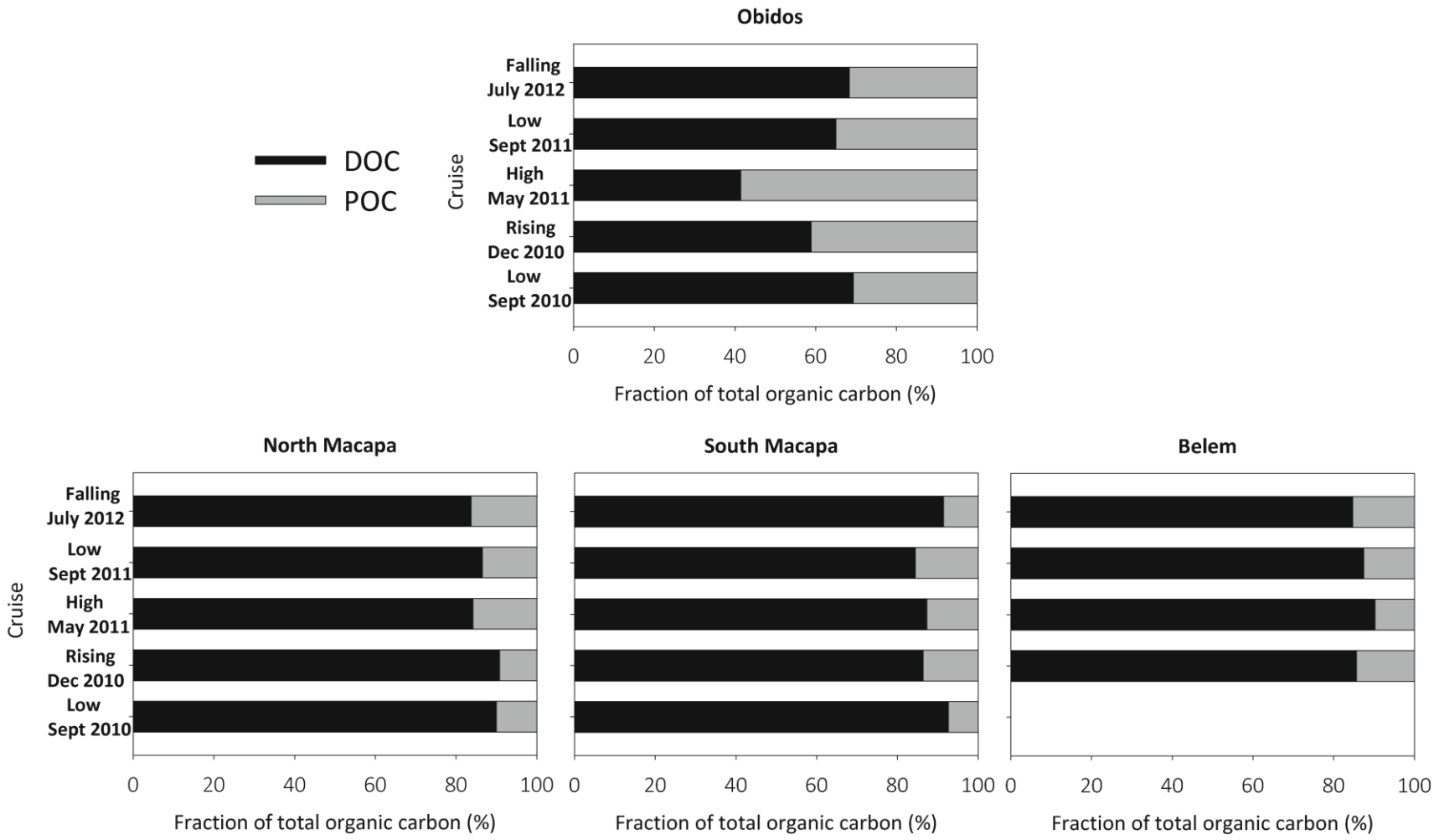


Figure IV.5 Dissolved versus particulate fraction of total organic carbon. The relative fraction of total organic carbon that is in the dissolved phase (black) versus the particulate phase (grey) at Óbidos (top) and each respective channel near the Amazon River mouth (bottom).

Station/Date	DOC	$DO^{13}C$	Dissolved Lignin	Dissolved λ_8	S/V	C/V	Ad/Al (v)	Ad/Al (s)
North Macapa	(mg C L^{-1})	(‰ VPDB)	(ug lignin L^{-1})	(ug lignin 100 mg DOC^{-1})				
16-Sep-10	4.4 ± 0.5	-30.2 ± 0.2	5.8 ± 0.6	0.013 ± 0.002	0.37 ± 0.08	0.48 ± 0.06	0.52 ± 0.06	0.34 ± 0.28
1-Dec-10	3.5 ± 0.3	-27.4 ± 0.2	8.2 ± 0.8	0.023 ± 0.003	0.21 ± 0.03	0.09 ± 0.01	0.58 ± 0.07	0.49 ± 0.06
7-May-11	5.7 ± 0.2	-28.5 ± 0.2	15.8 ± 1.6	0.028 ± 0.003	0.33 ± 0.04	0.19 ± 0.02	1.25 ± 0.16	0.45 ± 0.06
13-Sep-11	3.8 ± 0.1	-30.4 ± 0.2	5.5 ± 0.5	0.014 ± 0.002	0.27 ± 0.03	0.49 ± 0.05	0.42 ± 0.13	0.26 ± 0.03
23-Jul-12	3.6 ± 0.1	-29 ± 0.2	9.6 ± 1.0	0.027 ± 0.003	0.64 ± 0.08	0.18 ± 0.02	1.55 ± 0.22	1.20 ± 0.22
South Macapa								
18-Sep-10	4.3 ± 0.3	-30.1 ± 0.2	5.3 ± 0.5	0.012 ± 0.001	0.44 ± 0.11	0.34 ± 0.04	0.65 ± 0.08	0.44 ± 0.18
3-Dec-10	2.8 ± 0.2	-27.4 ± 0.2	6.2 ± 0.6	0.022 ± 0.003	0.22 ± 0.03	0.11 ± 0.01	0.76 ± 0.09	0.25 ± 0.03
9-May-11	5.6 ± 0.3	-28.5 0.2	14.5 1.5	0.026 ± 0.003	0.34 ± 0.04	0.20 ± 0.02	1.59 ± 0.21	0.70 ± 0.23
15-Sep-11	4.7 ± 0.4	-29.7 ± 0.2	8.3 ± 0.8	0.018 ± 0.002	0.97 ± 0.12	0.09 ± 0.01	0.89 ± 0.11	1.16 ± 0.15
25-Jul-12	3.9 ± 0.3	-29.2 ± 0.2	8.6 ± 0.9	0.022 ± 0.003	1.01 ± 0.13	0.18 ± 0.02	1.65 ± 0.27	1.35 ± 0.24
Belem								
7-Dec-10	5.3 ± 0.4	-27.5 ± 0.2	2.8 ± 0.3	0.005 ± 0.001	0.79 ± 0.10	0.25 ± 0.03	0.68 ± 0.08	1.20 ± 0.15
13-May-11	5.9 ± 0.4	-28.1 ± 0.2	13.5 ± 1.4	0.023 ± 0.003	1.51 ± 0.19	0.07 ± 0.01	1.45 ± 0.18	0.37 ± 0.05
21-Sep-11	3.0 ± 0.2	-29.4 ± 0.2	7.2 ± 0.7	0.024 ± 0.003	0.85 ± 0.11	0.28 ± 0.04	0.93 ± 0.12	0.44 ± 0.05
1-Aug-12	3.2 ± 0.2	-28.4 ± 0.2	11.5 ± 1.1	0.036 ± 0.004	0.77 ± 0.10	0.13 ± 0.02	0.62 ± 0.08	1.38 ± 0.17
Tapajos								
25-Sep-10	1.5 ± 0.1	-28.2 ± 0.2	8.9 ± 0.9	0.060 ± 0.007	0.43 ± 0.05	0.06 ± 0.01	1.08 ± 0.14	4.30 ± 0.54
14-Dec-10	2.4 ± 0.2	-28.7 ± 0.2	7.8 ± 0.8	0.032 ± 0.004	0.20 ± 0.02	0.09 ± 0.01	0.90 ± 0.11	0.66 ± 0.08
20-May-11	2.5 ± 0.6	-28.8 ± 0.2	9.0 ± 0.9	0.036 ± 0.004	1.89 ± 0.24	0.11 ± 0.01	0.71 ± 0.09	0.58 ± 0.07
26-Sep-11	1.4 ± 0.1	-28 ± 0.2	9.6 ± 1.0	0.069 ± 0.008	0.74 ± 0.09	0.10 ± 0.01	1.05 ± 0.13	1.17 ± 0.15
6-Aug-12	1.9 ± 0.1	-29.3 ± 0.2	13.4 ± 1.3	0.069 ± 0.008	0.72 ± 0.09	0.20 ± 0.03	0.46 ± 0.06	1.18 ± 0.15
Obidos								
23-Sep-10	4.1 ± 0.3	-28.2 ± 0.2	4.0 ± 0.4	0.010 ± 0.001	0.64 ± 0.08	0.39 ± 0.05	0.61 ± 0.08	1.30 ± 0.16
13-Dec-10	4.2 ± 0.3	-29.4 ± 0.2	2.8 ± 0.3	0.007 ± 0.001	0.72 ± 0.09	0.19 ± 0.02	0.41 ± 0.05	0.15 ± 0.02
19-May-11	2.9 ± 0.6	-25.5 ± 0.2	17.1 ± 1.7	0.059 ± 0.007	0.64 ± 0.08	0.09 ± 0.01	1.23 ± 0.15	0.97 ± 0.12
25-Sep-11	3.9 ± 0.3	-27.9 ± 0.2	5.5 ± 0.5	0.014 ± 0.002	1.67 ± 0.21	0.28 ± 0.03	0.14 ± 0.02	0.37 ± 0.05
5-Aug-12	4.5 ± 0.3	-26.1 ± 0.2	6.6 ± 0.7	0.015 ± 0.002	0.67 ± 0.08	0.27 ± 0.03	0.95 ± 0.12	1.55 ± 0.24

Table IV.1 Dissolved organic carbon and lignin concentrations and compositions. The concentration and ^{13}C stable isotopic composition of DOC and the concentration of dissolved lignin phenols and the ratio of syringyl to vanillyl phenols (S/V), cinnamyl to vanillyl phenols (C/V), vanillyl aldehydes to vanillyl acids (Ad/Al_v), and syringyl acids to aldehydes (Ad/Al_s) along the lower Amazon River.

Station/Date	POC	PO^{13}C	Particulate Lignin	Particulate λ_8	S/V	C/V	Ad/Al (v)	Ad/Al (s)
North Macapa	(mg C L^{-1})	(‰ VPDB)	(ug lignin L^{-1})	(ug lignin 100 mg POC^{-1})				
16-Sep-10	0.49 ± 0.04	-26.4 ± 0.5	9.0 ± 0.9	0.18 ± 0.022	0.79 ± 0.1	0.15 ± 0.02	0.44 ± 0.1	0.44 ± 0.1
1-Dec-10	0.36 ± 0.15	-27.8 ± 0.8	5.6 ± 0.56	0.16 ± 0.019	0.71 ± 0.1	0.02 ± 0.00	0.46 ± 0.1	0.83 ± 0.1
7-May-11	1.07 ± 0.77	-27.1 ± 0.3	22.6 ± 2.26	0.21 ± 0.025	0.89 ± 0.1	0.09 ± 0.01	0.59 ± 0.1	0.42 ± 0.1
13-Sep-11	0.59 ± 0.05	-30.7 ± 0.3	11.1 ± 1.11	0.19 ± 0.022	0.74 ± 0.1	0.13 ± 0.02	0.40 ± 0.1	0.68 ± 0.1
23-Jul-12	0.70 ± 0.28	-27.2 ± 0.8	10.1 ± 1.01	0.14 ± 0.017	0.92 ± 0.1	0.13 ± 0.02	0.62 ± 0.1	0.55 ± 0.1
South Macapa								
18-Sep-10	0.34 ± 0.04	-27.3 ± 0.4	7.2 ± 0.72	0.21 ± 0.025	0.74 ± 0.1	0.10 ± 0.01	0.66 ± 0.1	0.50 ± 0.1
3-Dec-10	0.44 ± 0.02	-26.6 ± 0.2	9.5 ± 0.95	0.22 ± 0.026	0.75 ± 0.1	0.15 ± 0.02	0.74 ± 0.1	0.63 ± 0.1
9-May-11	0.81 ± 0.02	-26.3 ± 0.7	17.7 ± 1.77	0.22 ± 0.026	0.91 ± 0.1	0.19 ± 0.02	0.78 ± 0.1	0.69 ± 0.1
15-Sep-11	0.86 ± 0.14	-30.7 ± 0.3	15.4 ± 1.54	0.18 ± 0.021	0.71 ± 0.1	0.10 ± 0.01	0.52 ± 0.1	0.85 ± 0.1
25-Jul-12	0.36 ± 0.06	-27.2 ± 0.5	10.9 ± 1.09	0.30 ± 0.036	0.87 ± 0.1	0.21 ± 0.03	0.81 ± 0.1	0.64 ± 0.1
Belem								
7-Dec-10	0.88 ± 0.13	-22.4 ± 0.4	9.1 ± 0.91	0.10 ± 0.012	0.59 ± 0.1	0.27 ± 0.03	0.97 ± 0.1	1.33 ± 0.2
13-May-11	0.63 ± 0.12	-27.1 ± 1.3	7.9 ± 0.79	0.13 ± 0.015	0.65 ± 0.1	0.22 ± 0.03	1.24 ± 0.2	1.41 ± 0.2
21-Sep-11	0.43 ± 0.07	-30.2 ± 1.1	6.9 ± 0.69	0.16 ± 0.019	0.77 ± 0.1	0.28 ± 0.04	1.53 ± 0.2	1.22 ± 0.2
1-Aug-12	0.56 ± 0.31	-26.1 ± 0.3	10.9 ± 1.09	0.19 ± 0.023	0.63 ± 0.1	0.05 ± 0.01	0.54 ± 0.1	0.44 ± 0.1
Tapajos								
25-Sep-10	0.25 ± 0.01	-34.2 0.2	4.0 ± 0.4	0.16 ± 0.019	0.77 ± 0.1	0.28 ± 0.04	1.12 ± 0.1	2.23 ± 0.3
14-Dec-10	0.64 ± 0.01	-22.4 ± 0.2	5.2 ± 0.52	0.08 ± 0.010	0.61 ± 0.1	0.23 ± 0.03	1.30 ± 0.2	2.10 ± 0.3
20-May-11	0.55 ± 0.01	-23.9 ± 0.2	3.0 ± 0.3	0.05 ± 0.007	0.64 ± 0.1	0.24 ± 0.03	1.42 ± 0.2	2.33 ± 0.3
26-Sep-11	0.32 ± 0.01	-36 ± 0.2	4.6 ± 0.46	0.14 ± 0.017	0.77 ± 0.1	0.31 ± 0.04	1.44 ± 0.2	2.42 ± 0.3
6-Aug-12	0.57 ± 0.01	-25.6 ± 0.2	3.6 ± 0.36	0.06 ± 0.008	0.65 ± 0.1	0.26 ± 0.03	1.40 ± 0.2	2.25 ± 0.3
Obidos								
23-Sep-10	1.80 ± 0.21	-26.7 ± 1.3	10.3 ± 1.03	0.06 ± 0.007	0.77 ± 0.1	0.09 ± 0.01	0.74 ± 0.1	0.53 ± 0.1
13-Dec-10	2.90 ± 0.22	-26.2 ± 0.3	66.4 ± 6.64	0.23 ± 0.027	0.84 ± 0.1	0.16 ± 0.02	0.81 ± 0.1	0.86 ± 0.1
19-May-11	4.10 ± 0.42	-26.2 ± 0.7	28.5 ± 2.85	0.07 ± 0.008	0.81 ± 0.1	0.17 ± 0.02	0.65 ± 0.1	0.55 ± 0.1
25-Sep-11	2.10 ± 0.09	-31.1 ± 0.7	12.2 ± 1.22	0.06 ± 0.007	0.75 ± 0.1	0.08 ± 0.01	0.49 ± 0.1	0.77 ± 0.1
5-Aug-12	2.08 ± 0.95	-26.8 ± 1.5	25.7 ± 2.57	0.12 ± 0.015	0.81 ± 0.1	0.10 ± 0.01	0.74 ± 0.1	0.68 ± 0.1

Table IV.2 Particulate organic carbon and lignin concentrations and compositions. The concentration and ^{13}C stable isotopic composition of DOC and the concentration of dissolved lignin phenols and the ratio of syringyl to vanillyl phenols (S/V), cinnamyl to vanillyl phenols (C/V), vanillyl aldehydes to vanillyl acids (Ad/Al_v), and syringyl acids to aldehydes (Ad/Al_s) along the lower Amazon.

Station/Date	FSS ($mg\ L^{-1}$)	%OC
North Macapa		
16-Sep-10	23.9 ± 0.4	0.2 ± 0.03
1-Dec-10	30.2 ± 0.6	0.3 ± 0.04
7-May-11	92.7 ± 3.6	0.1 ± 0.03
13-Sep-11	35.6 ± 2.7	0.2 ± 0.05
23-Jul-12	77.0 ± 3.3	0.1 0.03
South Macapa		
18-Sep-10	19.8 ± 0.5	0.3 ± 0.04
3-Dec-10	27.4 ± 0.4	0.3 ± 0.03
9-May-11	65.1 ± 1.6	0.1 ± 0.03
15-Sep-11	28.5 ± 0.4	0.4 ± 0.04
25-Jul-12	49.7 ± 1.4	0.2 0.03
Belem		
7-Dec-10	33.0 ± 0.3	0.5 ± 0.03
13-May-11	40.5 ± 0.8	0.4 ± 0.04
1-Aug-12	14.4 ± 1.1	0.4 ± 0.06

Table IV.3 Lower river suspended sediment concentration and OC content. The concentration of suspended sediments and %OC carbon content at each respective channel near the Amazon River mouth. Sediment data from Obidos and the Rio Tapajos is currently unavailable.

Chapter V: The response of sediments and particulate organic matter to rapid rainfall in the Santa Maria da Vitoria watershed, Espírito Santo, BR

Foreword: The work described in this chapter was a collaboration between numerous US and Brazilian researchers and agencies. The overall project, "Floresta para vida," in Espirito Santo was funded by a contract between the World Bank and the government of the state of Espirito Santo. The main goal of this project was to enhance our understanding and predictive capabilities of the hydrologic dynamics of the state's watershed and determine the effects of specific land use regimes on hydrologic and geochemical processes. A large part of this project is an effort to model the hydrology of the state on a coarse scale using the Variable Infiltration Capacity Model (VIC) and of specific basins on a finer scale using the Distributed Hydrology Soil Vegetation Model (DHSVM). The geochemical measurements made here were collected with the eventual goal of integrating geochemical parameters with the DHSVM modeling framework. Model development efforts are currently underway, led primarily by primarily by UW doctoral candidate Amanda Tan, with input and guidance from the entire project team, led by Jeffrey Richey. Harvey Greenberg and Amir Sheikh have spearheaded the preparation of model dataframe layers with assistance from numerous employees of Brazilian government agencies (e.g. IEMA, INCAPER, ANA, and CESAN). Historic monthly geocehmical measurements of geochemical parameters presented here were collected by IEMA employees. I performed the additional field collections made in 2013 and compiled the various sources of historic. As a direct result of the field collections made here using the ISCO autosamplers, CESAN, the sanitation agency of Espirito Santo, has recently signed a contract with the Federal University of Espirito Santo (UFES) with additional support from INCAPER and IEMA to continue monitoring the Rio Mangarai and Santa Maria da Vitoria in high resolution throughout an

entire water year (similar to the study described in Chapter II). UW researchers will aid in the training of a M.S. student at UFES who will conduct water quality monitoring and deployment of the ISCO autosamplers during significant storm sequences throughout the next year. The purpose of this foreword is to provide insight into the scope and goals of the overall project.

V.1 Introduction

A strong link has been observed in temperate and tropical watersheds between short-term increases in river discharge and enhanced concentrations of geochemical components such as suspended sediments, particulate organic matter (POM) and dissolved organic matter (DOM) has been (Boyer et al., 1997; Hinton et al., 1998; Buffham et al., 2001; Sigleo and Frick, 2003; Ward et al., 2012). A significant fraction of the annual flux of sediments from Pacific Northwest catchments, for example, occurs during episodic storm events (Drake and Cacchione, 1985; Ogston et al., 2004; Wheatcroft and Sommerfield, 2005). Likewise, it is estimated that nearly 70% of the total nitrogen flux from several catchments in the Pacific Northwest occurs during winter and autumn storms (Sigleo and Frick, 2003; Steinberg et al., 2010; Ward et al., 2012). These types of rapid loading events have been estimated to contribute to as much as 86% of annual organic carbon (OC) export in forested watersheds (Raymond and Saiers, 2010) and 71-85% of OC export in agricultural watersheds (Dalzell et al., 2007).

As rainfall flows over and through the terrestrial landscape, organic compounds are mobilized from vegetation and soils into stream and groundwater networks. Small streams are the primary source of terrestrially-derived material to river systems because of the tight land-water interface. Quickly changing conditions at this spatial scale leads to dynamic chemical conditions within a stream (Vannote et al., 1980; Peterson et al., 2001; McClain and Elsenbeer.,

2001). Spatial analyses suggests that distinct landscape units become functional during rapid hydrologic events, which can greatly alter the dynamics of particulate and dissolved organic matter mobilization throughout the course of a storm (Hinton et al., 1998; McGlynn and McDonnell, 2003; Ocampo et al., 2006). The composition of organic material mobilized from a natural landscape depends largely on the type of vegetation present, soil characteristics, and latitude-dependent seasonal accumulation/depletion dynamics (Liaw et al., 1977; Easthouse et al., 1992; Hornberger et al., 1994; Boyer et al., 1997; Hinton et al., 1998; Buffam et al., 2001; McGlynn and McDonnell, 2003; Sigleo and Frick, 2003; Thomas et al., 2004; Hood et al., 2006; Neill et al., 2006; Dalzell et al., 2007; Guillaud et al., 2008; Spencer et al., 2008; Vidon et al., 2008; Fellman et al., 2009; Sanderman et al., 2009; Raymond and Saiers, 2010; Ward et al., 2012). Most of the organic material that is mobilized from the landscape was originally derived from terrestrial primary production. The majority of this plant-derived carbon has been shown to be photo-reactive in rivers (e.g. Rodriguez-Zuniga et al., 2008; Spencer et al., 2009) and highly biodegradable in both soil and aquatic environments (Trojanowski, 1969; Crawford and Crawford, 1980; Paterson and Lundquist, 1985; Hedges et al., 1988; Benner et al., 1991; Haddad et al., 1992; Qualls and Haines, 1992; Opsahl and Benner, 1995; Dittmar and Lara, 2001; Feng et al., 2008; Holmes et al., 2008; Ward et al., 2013). Observations made in both temperate and high-latitude river systems have shown that OM biodegradability significantly increases with peak discharge in both temperate and high-latitude river systems, suggesting that storm events play a significant role in mobilization of potentially labile terrestrial carbon (Buffham et al., 2001; Holmes et al., 2008; Fellman et al., 2009). A significant fraction of the carbon dioxide (CO_2) removed from the atmosphere by terrestrial primary production is mobilized into freshwater systems as organic matter and CO_2 from soil respiration; the majority of this

mobilized carbon is remineralized in the river and outgassed to the atmosphere, resulting in a flux of CO_2 from inland waters to the atmosphere that is an order of magnitude greater than the flux of carbon from rivers to the ocean (Cole et al., 1994; Benner et al., 1995; Richey et al., 2002; Duarte and Prairie, 2005; Tranvik et al., 2009; Aufdenkampe et al., 2011; Raymond et al., 2013; Regnier et al., 2013; Wehrli et al., 2013).

Here the influence of natural and anthropogenic landscapes on the mobilization and transport of particulate organic carbon and nitrogen during rapid storm events was examined in the Santa Maria da Vitoria basin in the southern coastal state of Espirito Santo, Brazil. The Rio Santa Maria da Vitoria is one of the primary sources of freshwater for the greater Vitoria metropolitan area, the capital city of Espirito Santo. The headwaters of the Santa Maria da Vitoria are characterized by relatively pristine forested environments, transitioning into primarily agricultural and rural land uses, and finally reaching the large urban center of Vitoria near its marine receiving waters. The flow of sediments through the Rio Santa Maria da Vitoria is a large concern to local governing bodies because (i) the capital city's water sanitation facilities become overwhelmed by sediments during periods of high rainfall, and (ii) the Bay of Vitoria, a bustling commercial port, may eventually become inaccessible to container ships without extensive dredging.

Seasonal patterns of suspended sediments, dissolved nitrogen, pH, dissolved oxygen, and dissolved nitrogen were assessed based on quasi-monthly measurements made at five stations along the Rio Santa Maria da Vitoria mainstem from 2006-2012 by the Brazilian agency, IEMA. This dataset well represented base flow conditions, but lacked many measurements made during peak river discharge. The concentration of suspended sediments, particulate organic carbon (POC), and particulate organic nitrogen (PON) was measured during a week-long period with

high rainfall in November 2013 to determine constituent concentrations during peak discharge and assess the response of particulate components to rapid rainfall. The isotopic composition of POC and PON was measured throughout the week-long period to assess variability in the sources of organic matter inputs and determine whether specific landscape features were functionalized differently throughout storms and dry periods. Samples were collected on a 3-6h interval in the Rio Santa Maria da Vitoria mainstem and one of its tributaries, the Rio Mangarai.

V.2 Methods

V.2.1 Study Site

The southeastern Brazilian coastal state of Espirito Santo covers an area of 46,180 km^2. The topography of the state is categorized by 40% lowland coastal landscape and 60% "Serra" highlands. The state's watershed is divided into twelve sub-basins. Its largest river, the Rio Doce, extends 853 km, originating at the confluence of the Piranga and Carmo near the foothills of the Mantiqueira and Espinhaco mountain ranges in the neighboring state of Minas Gerais. A significant amount of the region's agricultural (coffee and sugar cane in particular), industrial, and mining production occurs within the Rio Doce basin, which contains a population of roughly 3.1 million people.

The Rio Santa Maria da Vitoria and the Rio Jucu are the main river basins that supply water to Vitoria, the capital of Espirito Santo (Figure V.1). The Rio Santa Maria da Vitoria watershed covers a region of 1,660 km^2, with a river length of 122km. The upper region of the Santa Maria da Vitoria basins is characterized by mountainous landscapes and intense whitewater rapids. The landscape of the Santa Maria da Vitoria watersheds is composed of

roughly 40% natural forested land cover, which is mostly concentrated in the upper regions of the basins (Luciano de Firme, personal communication). The central SMV basin is primarily influenced by rural development, pasture land, and agricultural uses such as banana, coffee, eucalyptus, and sugar cane. There is little to no treatment of wastewater in the upper and central regions of the basin. The SMV passes through five municipalities of varying size: Santa Maria de Jetiba, Santa Leopoldina, Cariacica, Serra, and Vitoria. The SMV river mouth empties into the Bay of Vitoria, an important commercial port for the region, and forms the island of Vitoria, the capital city of Espirito Santo. The Rio Jucu enters the Atlantic Ocean south of the Bay of Vitoria near Vila Velha. The greater Vitoria metropolitan area is the 14th largest city in Brazil, with a population of roughly 1.6 million people. The region receives most of its rain during the October through January time period and receives an average rainfall of roughly 918mm annually. The Rio Santa Maria da Vitoria provides water for roughly 30% of the greater Vitoria population.

There are two dams on the SMV upstream of Vitoria, the Rio Bonito and Suico dams, which produce ten and thirty megawatts of electric energy per year, respectively. These dams significantly alter the sediment transport and storage dynamics of the basin, storing large amounts of sediments in their reservoirs. A significant amount of sediments from the riparian zone and tribuataries enters the river downstream of these dams. Here the Rio Mangarai is examined as an example of one of the tributaries entering the Rio Santa Maria da Vitoria downstream of the two major dams.

V2.2 Analytical Methods

Monthly collections were analyzed by IEMA for determination of total suspended sediments (TSS) from 2006-2012. Stream samples were collected from October 30 through

November 6, 2013 on 3-6h frequency in the Rio Santa Maria da Vitoria at latitude/longitude -20.11687, -40.44339 and the Rio Mangarai at latitude/longitude -20.15879, -40.45773. Samples collected during the November 2013 field campaign were analyzed for determination of the concentration of TSS, particulate organic carbon (POC), particulate organic nitrogen (PON), and the stable isotopic composition of POC and PON. Each ISCO sequence collected 24 samples at 3-6h intervals in acid-washed/sample-rinsed 1L HDPE bottles. The ISCO autosampler was filled with ice to minimize degradation of samples throughout the sequence. Samples were collected at approximately mid depth in the center of the stream channels.

TSS was determined by filtering whole water through pre-weighed 0.45μm pore-size, 47mm diameter cellulose acetate filters (Pall). Samples were dried for 48h at 50°C and weighed in triplicate. PO^{13}C/^{15}N samples were filtered on combusted 47mm Whatman GFF filters (0.7 μm nominal pore size) and packed in pre-cleaned tin capsules after drying for 24h at 50°C, acid-fumigation for 24h, and drying for 24h at 50°C. Samples were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer.

V.3 Results and Discussion

During the 2006-2012 time period monthly total suspended sediment concentrations varied from 10-180 mg L^{-1} at five stations along the Rio Santa Maria da Vitoria (Figure V.3). Sediment concentrations were maximal at rising and peak high water and minimal at low water. It should be noted, however, that this type of sampling regime largely underestimates the total flux of sediments through the river. Even during high water, samples were typically not taken

during periods of extreme rainfall due to logistical difficulties, meaning that peak concentrations were typically not observed in the monthly dataset.

In November 2013 sediment measurements were made at a 3-6h interval during a week long period with moderate rainfall to assess sediment concentrations during peak storm flow. It should be noted that river discharge values for this time period are currently unavailbale from the Brazilian monitoring agencies (e.g. ANA), but will be made available once properly quality controlled. Further, once calibrated/validated outputs from the modeling efforts described here can be used to estimate discharge during this sampling period. From October 30-November 5, 2013 there was periodic light rainfall. Sediment concentrations in both the Santa Maria da Vitoria and Rio Mangarai reflected rainfall/river discharge, ranging from roughly 20-60 mg L^{-1} (Figure V.4). During this period, sediment concentrations were more variable (i.e. peaked and dropped more often) in the Rio Mangarai than the Santa Maria da Vitoria main channel. The Rio Mangarai is a small second-order stream where river conditions closely reflect a direct connection with the surrounding landscape. The Rio Santa Maria da Vitoria, on the other hand, recieves tributary and direct inputs from a much larger area. Material concentrations in the Santa Maria da Vitoria are, thus, a blend of signals coming from a large landscape with variable weather and landscape conditions. For example, during the October 30-November 5, 2013 time period the Rio Mangarai received localized rainfall that was not consistent across the entire Santa Maria da Vitoria basin. Sediment concentrations in the Santa Maria da Vitoria main channel, thus, reflected both the inputs of tributaries receiving localized rainfall such as the Rio Mangarai and base flow inputs from tributaries across the basin experiencing dry conditions at the time. From November 5-7, 2013 the entire region experienced significant rainfall. During this period, sediment concentrations in the Rio Mangarai increased from roughly 35 to 600 mg L^{-1} at peak

discharge (Figure V.4). Sediment concentrations in the Santa Maria da Vitoria increased from roughly 25 to 325 mg L^{-1}. Similar to the period of light localized rainfall the week before, sediment concentrations increased proportionally less in the Santa Maria da Vitoria compared to the Rio Mangarai, illustrating the direct connection between small streams and their surrounding landscape and the integration of varying signals in larger river channels (Vannote et al., 1980).

The concentration of particulate organic carbon (POC) and nitrogen (PON) showed a similar positive correlation with river discharge during the periods of both light and heavy rainfall during the November 2013 campaign (Figures V.4 and V.8). The percentage of organic carbon relative to total suspended sediments (%OC) slightly increased with increasing river discharge (Figure V.6). This likely indicates that storm flow mobilizes sediments that are rich in organic material relative to base flow. The importance of hydrologic flowpaths through the uppermost OM rich soil layer in controlling soil OM concentrations has been previously demonstrated; during rapid rain events young OM has been shown to be flushed from upper soil layers, depleting soil OM concentrations and leaving behind older OM in deeper soil horizons (Schiff et al., 1998; Sanderman et al., 2009; van Verseveld et al., 2009). Easthouse et al. (1992) estimated that base flow largely consists of OM from deep soil layers, whereas 50-65% and 35-50% of the OM mobilized by peak flow originates from the B and O soil horizons, respectively. The molar ratio of POC to PON generally increased with river discharge (Figure V.9), but was not well correlated. The ratio of dissolved organic carbon to nitrogen, on the other hand, displayed a strong correlation with river discharge in the Pacific Northwest (Ward et al., 2012). The elemental composition of dissolved constituents that are mobilized into streams via storm flow are controlled primarily by solubility (Kennedy, 1971; Hornberger et al., 1994; Hill et al., 1999). That is to say, specific constituents are more readily mobilized from soils into

streamwater depending on their inherent solubility even though these constituents may have been initially derived from the same source material. In the Pacific Northwest, carbon-rich compounds were relatively easier to mobilize than nitrogen or phosphorous rich compounds (Ward et al., 2012). The elemental composition of particulate organic material in the Rio Santa Maria da Vitoria, on the other hand, appears to be controlled primarily by variability in source materials.

The isotopic signature of both particulate organic carbon (∂^{13}C-POC) and particulate organic nitrogen (∂^{15}C-PON) generally became more enriched with increasing river discharge at both locations (Figures V.7 and V.10). For example, ∂^{15}N-PON ranged from roughly 2.0‰ to 10.0‰, becoming relatively more enriched in ^{15}N with increasing discharge during the November, 2013 storm. Organic nitrogen derived from fertilizers and wastewater is generally enriched in ^{15}N relative to natural vegetation (McClelland et al., 1997). Storm runoff in the Mangarai and Santa Maria da Vitoria mobilizes a mixture of nitrogen sources derived from fertilizers, untreated sewage, and natural landscapes into the river network based on the observed enrichment of ∂^{15}N-PON with storm flow. This enrichment in ∂^{15}N-PON is significantly higher than observations made in the Pacific Northwest during a similar sampling regime (Ward et al., 2012). In the Pacific Northwest, ∂^{15}N-PON was not directly correlated to river discharge and only reached a maximum value of 3.8‰. In the Rio Mangarai and Santa Maria da Vitoria, however, ∂^{15}N-PON was much more variable and reached much higher values at peak runoff. This is most likely due to stark differences in land use and wastewater treatment practices rather than latitudinal differences. For example, in the Pacific Northwest study region, N_2-fixing red alder trees were the primary source of nitrogen to the system and the isotopic signal of red alder inputs dominated the isotopic composition of particulate organic matter throughout the sampled storm sequenes (Steinberg et al., 2010). Fertilizer inputs from residential neighborhoods were

present, but insignificant relative to the red alder inputs. Sewage treatment in the region was primarily via septic tanks. Septic tank outfall would provide the river with enriched organic nitrogen values, however, these inputs primarily occur near the river-marine interface, and were, thus, not observed by the stream sampling regime. In stark contrast, there is very little actual wastewater treatment along many parts of the Santa Maria da Vitoria basin refer to an appropriate figure here. Thus, the river receives direct input of raw sewage, which has a enriched $\partial^{15}N$ signature. Likewise, the region, especially in the lowlands, has a large abundance of agricultural activities. Whereas the Pacific Northwest streams studied by Steinberg et al. (2010) and Ward et al. (2012) received minimal fertilizer inputs from residential developments, the Rio Santa Maria da Vitoria receives significant fertilizer inputs from widespread agricultural activities.

The stable isotopic composition of particulate organic carbon also became relatively more enriched with increasing storm runoff. For example, $\partial^{13}C$-POC varied from -27.1‰ to -24.0‰ throughout the week-long November sampling with the most enriched values occurring at peak discharge (Figure V.7). The range of values measured indicates a primary input of C_3 terrestrial organic matter and an enhanced input of C_4 organic matter during peak discharge. This likely indicates that POC export from pasture and some agricultural land uses is enhanced relative to forest inputs during heavy rainfall reference to another work that also shows this?., the trend in $\partial^{13}C$-POC values observed here was the opposite of what was previously observed in the Pacific Northwest during a similar sampling regime (Ward et al., 2012). In the Pacific Northwest $\partial^{13}C$-POC became more depleted in ^{13}C with increased discharge. This observed decrease in $\partial^{13}C$-POC likely indicated that the particulate material mobilized by rapid surface runoff in the Pacific Northwest was relatively “more degraded” than that which was mobilized by base flow.

The majority of particulate organic matter mobilized by early autumn storms in the Pacific Northwest was the product of litterfall accumulation during a long dry summer period. The region was primarily characterized by coniferous and deciduous forest land cover, whereas the Rio Santa Maria da Vitoria study contains a much higher abundance of agricultural and pasture land uses. In the Pacific Northwest, prior to mobilization by surface runoff, the accumulated litterfall experienced enhanced degradation relative to the particulate organic matter buried deep in soil layers. The particulate fractions present in deep soil layers also experience an exchange of matter between the dissolved phase via physical sorption, which can greatly alter its composition (e.g Aufdenkampe et al., 2001; Hernes et al., 2007). It is likely that both biodegradation and sorption play an important role in the composition of both dissolved and particulate organic carbon compounds that are mobilized by base flow and rapid storm flow (McKnight et al., 1992; Qualls and Haines, 1992; Day et al., 1994; Aufdenkampe et al., 2001; Hernes et al., 2007).

The trend of relatively more degraded particulate organic matter being mobilized by surface runoff compared to baseflow that was observed in the Pacific Northwest was not clearly evident in the tropical/sub-tropical Rio Santa Maria da Vitoria basin. Instead, the systematic enrichment of ∂^{13}C-POC revealed the mobilization of a unique source of organic material. Interestingly, the base flow values of ∂^{13}C-POC are very similar in both Espirito Santo and the Pacific Northwest, but opposite trends occur during rapid runoff. It is likely that old forest-derived organic carbon is the primary contributor to deep soil carbon that is mobilized by baseflow. Since forested land cover dominated the Pacific Northwest study region, the carbon that accumulates in shallow surface layers is, in a sense, of the same origin as the carbon that is buried in deep soil layers. However, these two pools experience different degradation and sorption regimes based on their lifespan (e.g. deep soil carbon was at one point present in the

shallow surface pool and was subsequently altered by a series of physical and biological processes). The Rio Santa Maria da Vitoria basin, on the other hand, is much less uniform and contains significant landscapes that are characterized by agricultural or open pasture land uses. The observed variability in ∂^{13}C-POC is most likely related to the mobilization of carbon from unique landscapes that become functionalized during periods of rapid rainfall (e.g. Hinton et al., 1998; McGlynn and McDonnell, 2003; Ocampo et al., 2006). Depending on the exact vegetation type, agricultural and pasture land use regimes typically express a more enriched ^{13}C signature (Cambardella et al., 1992; Cloern et al., 2002). Thus, the observed increase in ∂^{13}C-POC during storm flow in the Santa Maria da Vitoria basin indicates enhanced carbon inputs from pasture and agricultural land use regimes during periods of rapid rainfall. Whereas deep baseflow was primarily comprised of inputs from forested lands, rapid surface runoff mobilized relatively more particulate organic matter from pasture and agricultural lands.

V.5 Conclusion

Water and land resource management decisions have a direct effect on the environmental and economical sustainability of a watershed system. Major concerns focus on consequences of land use change for water supply and demand, for local and downstream hydrological hazards, and for biodiversity conservation (Vorosmarty et al., 2000; Tong et al., 2002; Schroter et al., 2005; Bates et al., 2008). There is a growing need for a comprehensive set of tools, capable of accurately predicting the impact of land use changes on small to large spatial scales, as global development continues to steadily increase (Defries et al., 2004). Accurate estimation of mass and chemical export from a watershed is needed to predict the biogeochemical response of a system to changes in both land use and climate (Hope et al., 1994; Hope et al., 1997; Tranvik et

al., 2002). The primary motivation for the government of Espirito Santo to explore sediment fluxes and dynamics in the Santa Maria da Vitoria watershed is to inform improved freshwater resource and sanitation management practices. For example, sediment concentrations reach such high levels during heavy rainfall that water sanitation facilities become inoperable (Figure V.4). When water is most abundant, local citizens have little to no access to clean freshwater because of the effect of enhanced sediment levels on water sanitation facilities. The Rio Santa Maria da Vitoria provides roughly one-third of the freshwater supply to the capital city, Vitoria.

In this chapter both long-term, coarse frequency and short-term, high-frequency suspended sediment datasets were examined in an effort to understand the complex processes governing the mobilization of biogeochemical components from a watershed's landscape into streams and rivers. Both types of datasets have their merits and drawbacks. For example, making monthly measurements over the span of years to decades is invaluable for describing seasonal patterns and identifying the sensitivity of a watershed to climatic variabiity (Richey et al., 1980; Schlesinger and Melack, 1981; Meybeck, 1982; Hope et al., 1994; Tranvik et al., 2002). However, a low frequency collection regime typically underrepresents the most extreme hydrologic events (Boyer et al., 1997; Hinton et al., 1998; Buffham et al., 2001; Sigleo and Frick, 2003; Hernes et al., 2008; Raymond and Saiers, 2010; Ward et al., 2012). Sample collections during extreme rainfall can be logistically difficult to achieve, and, further, river conditions during these time periods are highly variable, greatly decreasing the likelihood of collecting one sample that can adequately represent the river's dynamic composition. For example, if one were to attempt to collect a representative monthly sample from the Rio Mangarai during the November 5-November 7, 2013 time period, which was measured here in high frequency, the sediment concentration determined from one sample could vary anywhere

from 20-582 mg L^{-1} depending on the time of day that the sample was collected. As a result, coarse sampling regimes often largely underestimate the total flux of material through a river. In most cases large storm events, such as the one examined here, result in the majority of material flux through a system annually since both the amount of water flow and constituent concentrations increase greatly (e.g. Drake and Cacchione, 1985; Ogston et al., 2004; Wheatcroft and Sommerfield, 2005; Steinberg et al., 2010).

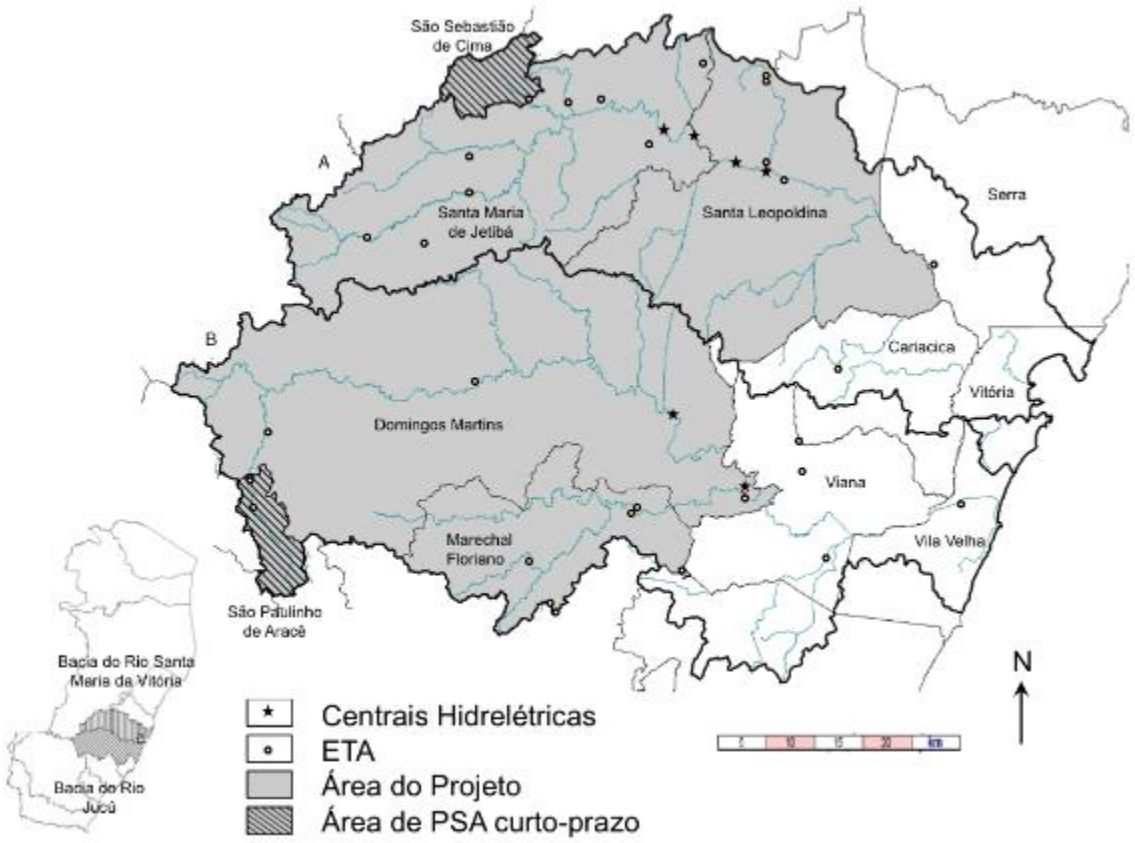


Figure V.1 Santa Maria da Vitoria and Jucu basins. Map of the (a) Rio Santa Maria da Vitoria (a) and (b) Rio Jucu basins in the state of Espirito Santo, Brazil (map courtesy of IEMA).

Table V.1 Sample sites along the Jucu and Santa Maria da Vitoria basins. The Rio Mangarai is a tributary of the Rio Santa Maria da Vitoria. ISCO autosamplers were deployed at SMV-ISCO and MAN-ISCO stations from October 30-November 6, 2013. Historic suspended sediment samples were collected by IEMA at stations SMV A-E from 2006-2012.

River	Station	Latitude	Longitude
Rio Santa Maria do Vitoria	SMV ISCO	-20.11687	-40.44339
	SMV-A	-20.051917	-40.764222
	SMV-B	-20.038167	-40.729694
	SMV-C	-20.081594	-40.584047
	SMV-D	-20.101333	-40.517778
	SMV-E	-20.171375	-40.394264
Rio Mangarai	MAN ISCO	-20.15879	-40.45773

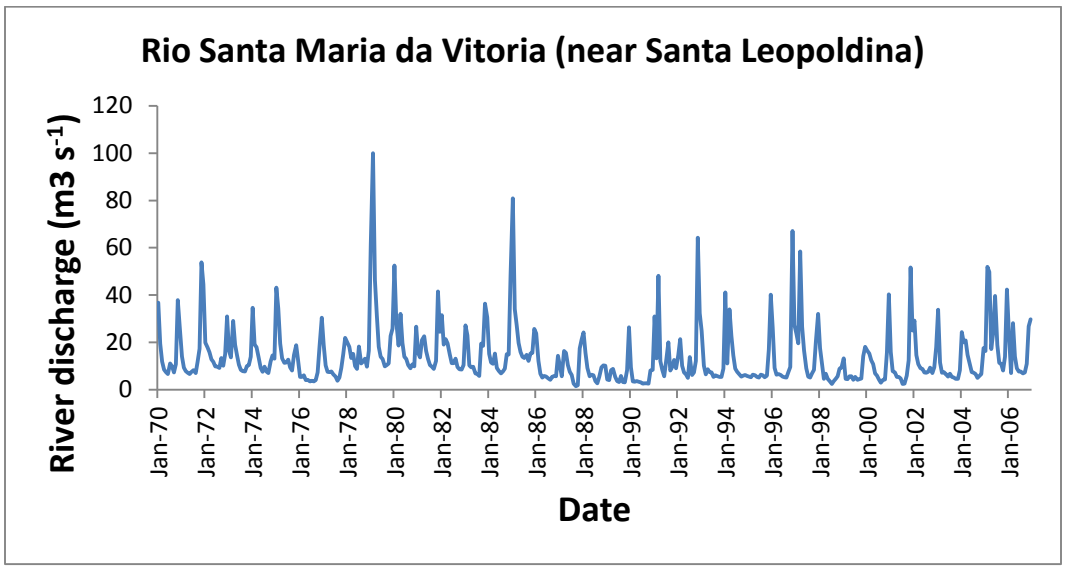


Figure V.2 Historic discharge in the Rio Santa Maria da Vitoria. ANA records of discharge in the Rio Santa Maria da Vitoria near Santa Leoploldina from 1970-2006.

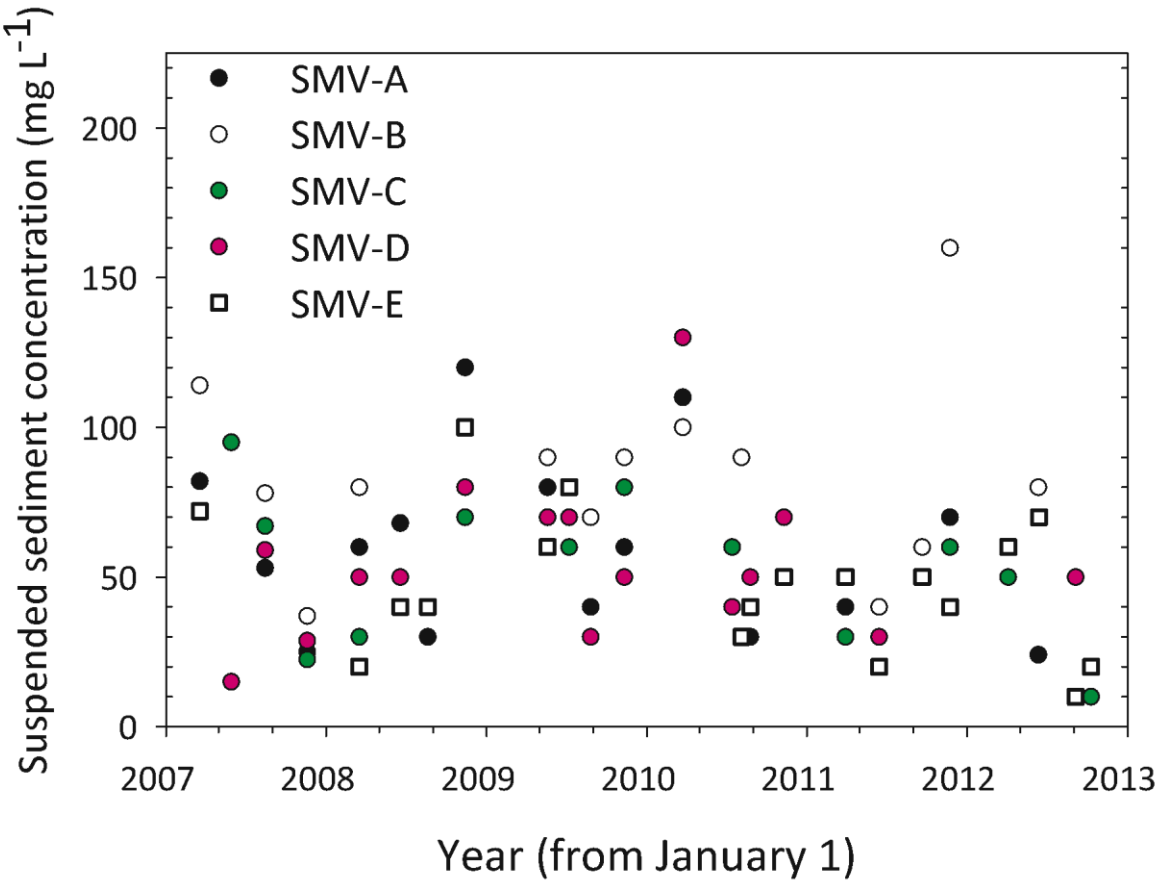


Figure V.3 Historic measurements of suspended sediment concentrations. Seasonal measurements of total suspended sediment concentrations in the Rio Santa Maria da Vitoria from April 2007 to October 2012. Station coordinates are described in Table V.1 (data courtesy of IEMA)

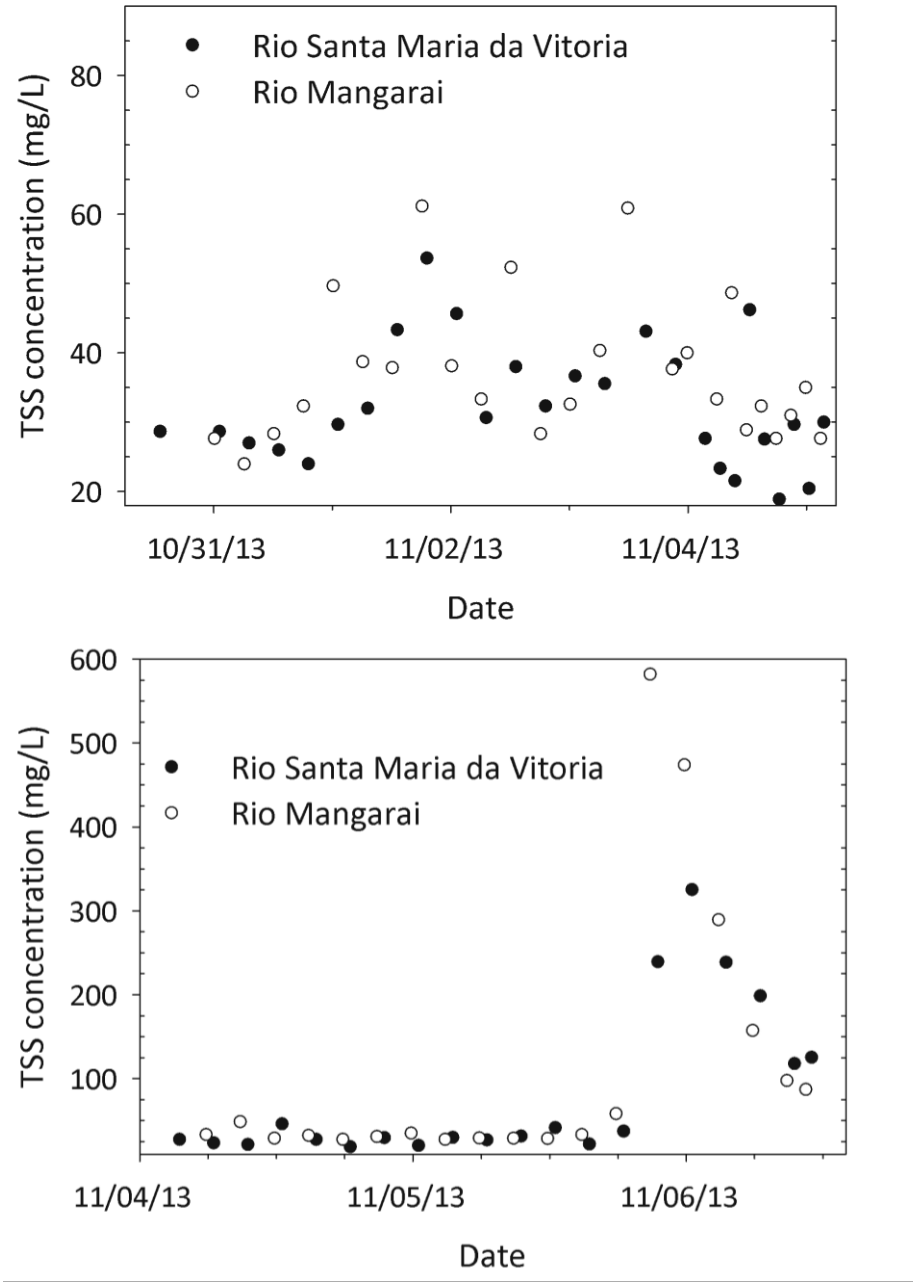


Figure V.4 Suspended sediment concentrations during storm flow. Measurements of total suspended sediment concentrations during light rainfall (top) and heavy storm flow (bottom) in the Rio Santa Maria da Vitoria and its tributary, Rio Mangarai, from October 30, 2013 to November 6, 2013. Samples were collected on a 3-6h interval.

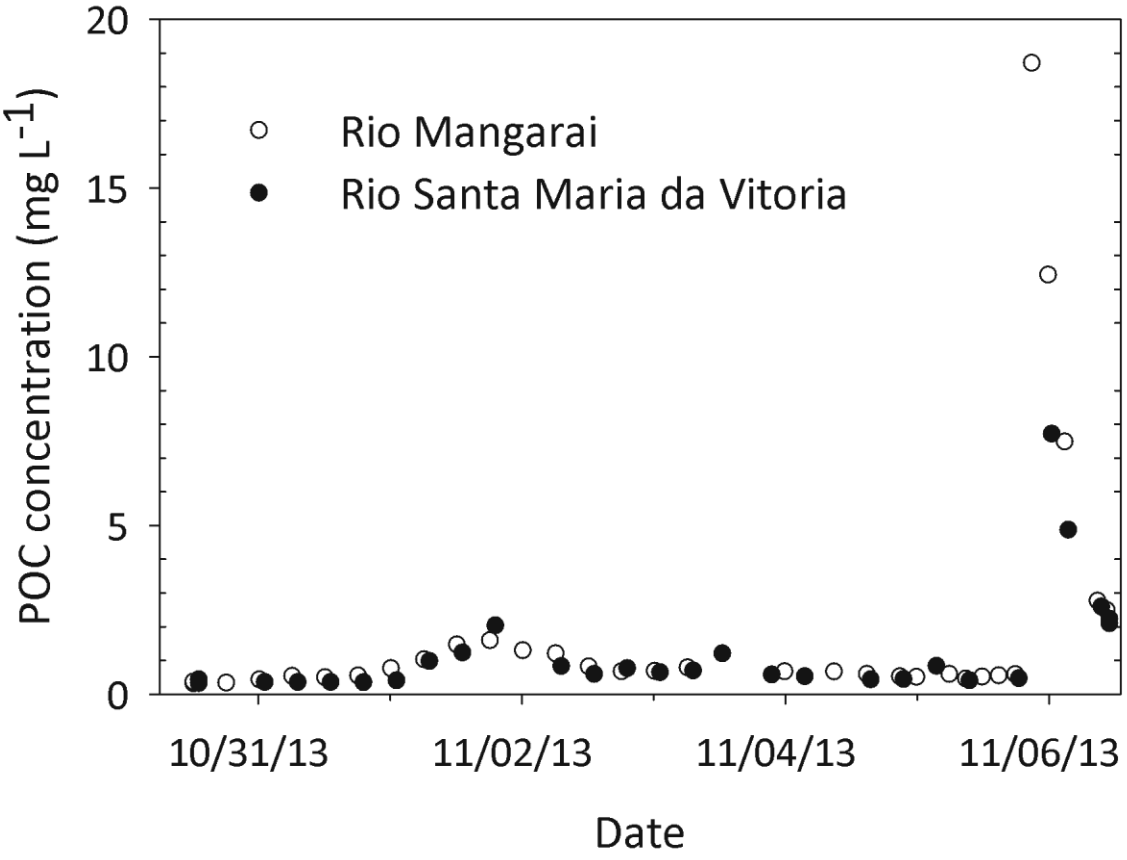


Figure V.5 Storm flow particulate organic carbon concentrations. 3-6 hour frequency measurements of the concentration of particulate organic carbon in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

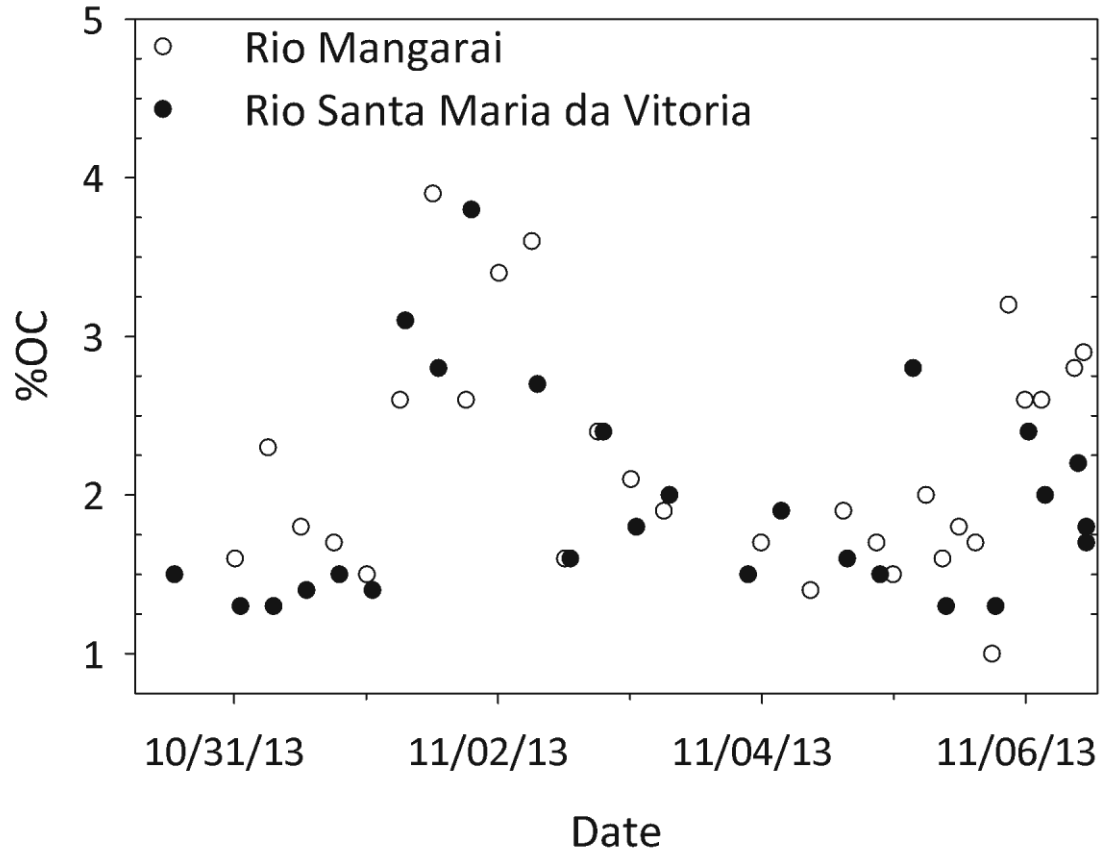


Figure V.6 Storm flow percentage particulate organic carbon. 3-6 hour frequency measurements of the percentage of particulate organic carbon relative to total suspended sediments in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

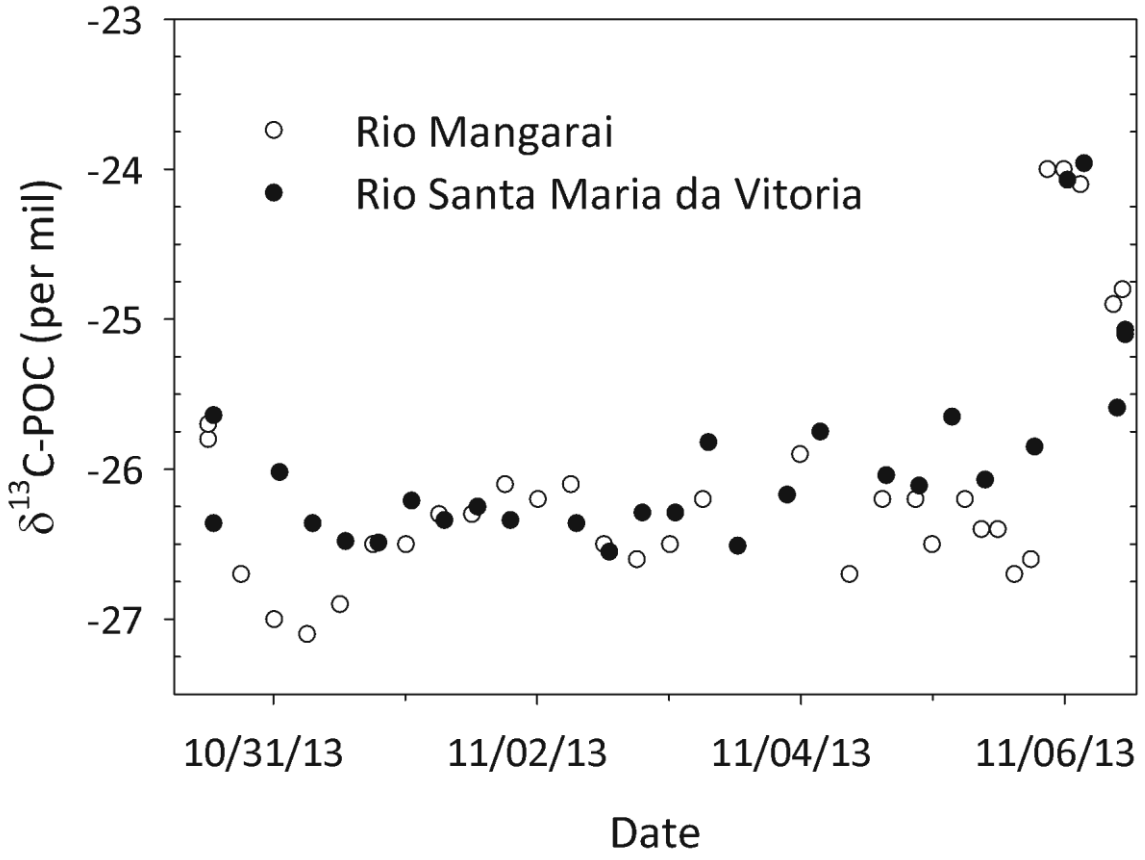


Figure V.7 Storm flow isotopic composition of particulate organic carbon. 3-6 hour frequency measurements of the stable isotopic composition of particulate organic carbon in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

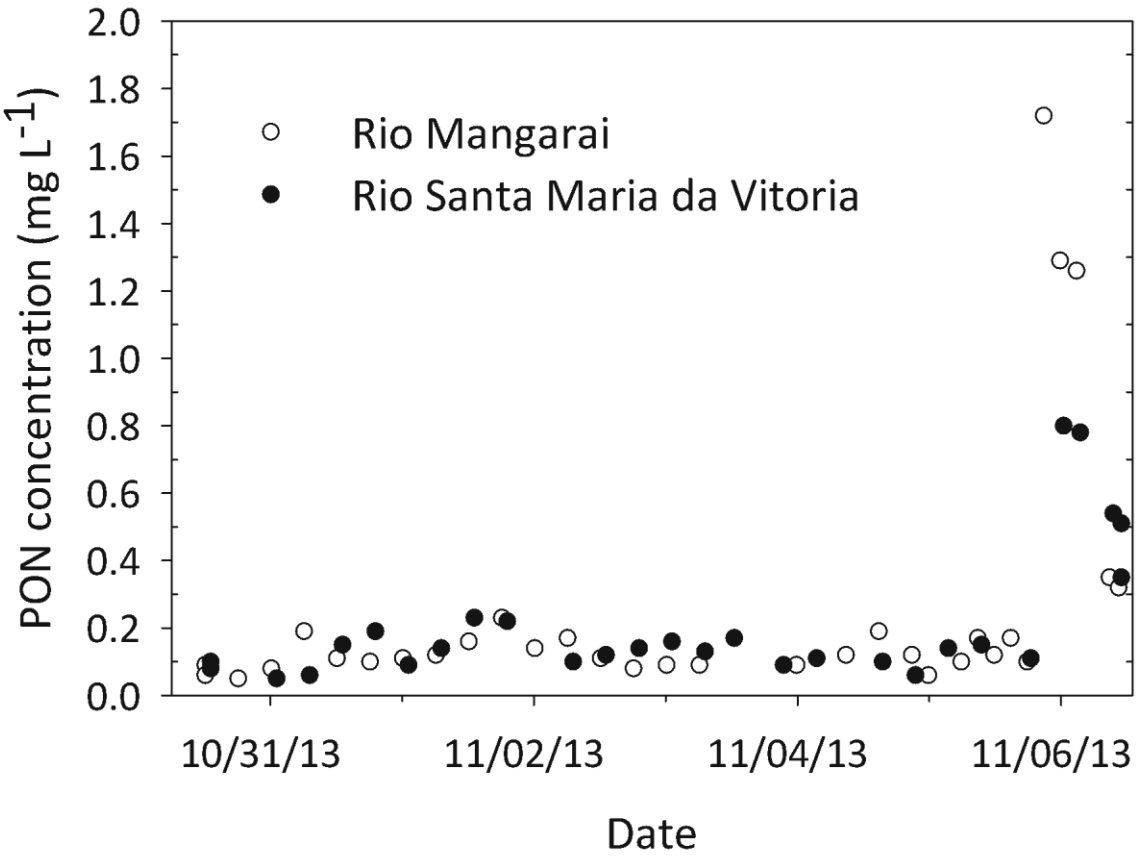


Figure V.8 Storm flow particulate organic nitrogen concentrations. 3-6 hour frequency measurements of the concentration of particulate organic nitrogen in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

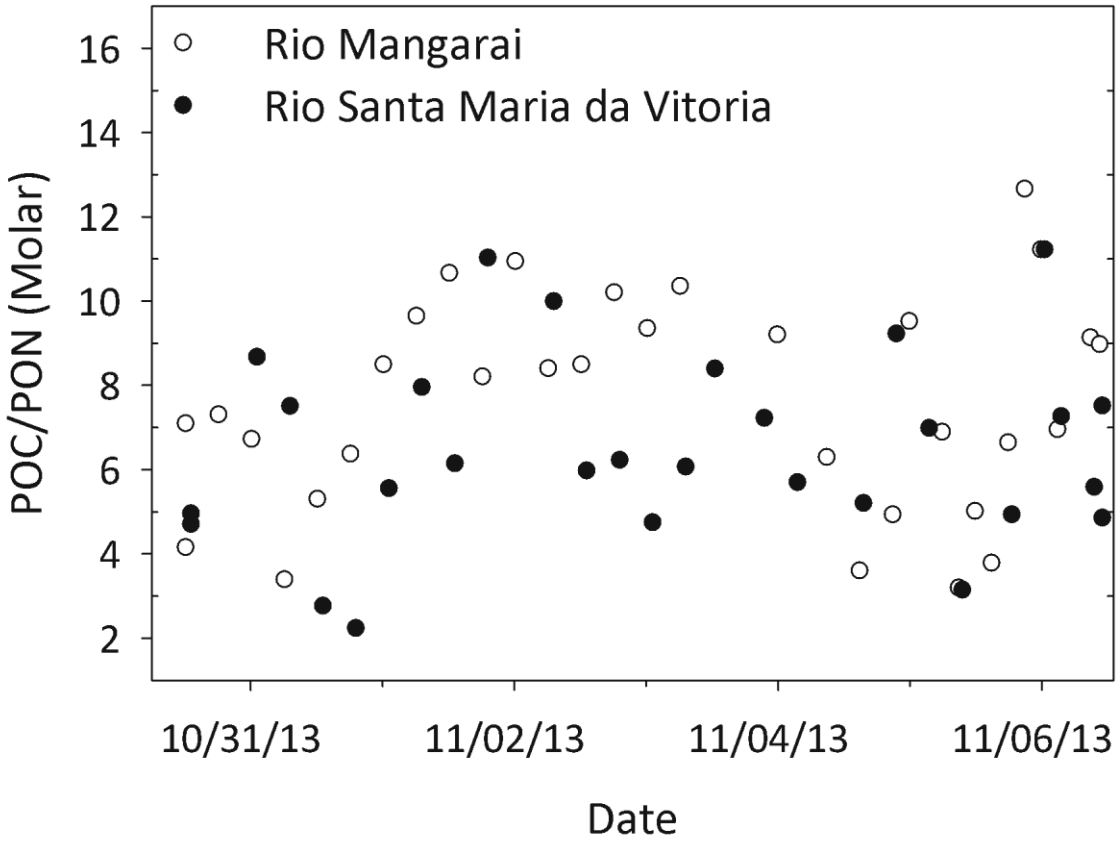


Figure V.9 Storm flow particulate organic carbon to nitrogen ratios. 3-6 hour frequency measurements of the molar ratio of particulate organic carbon to nitrogen in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

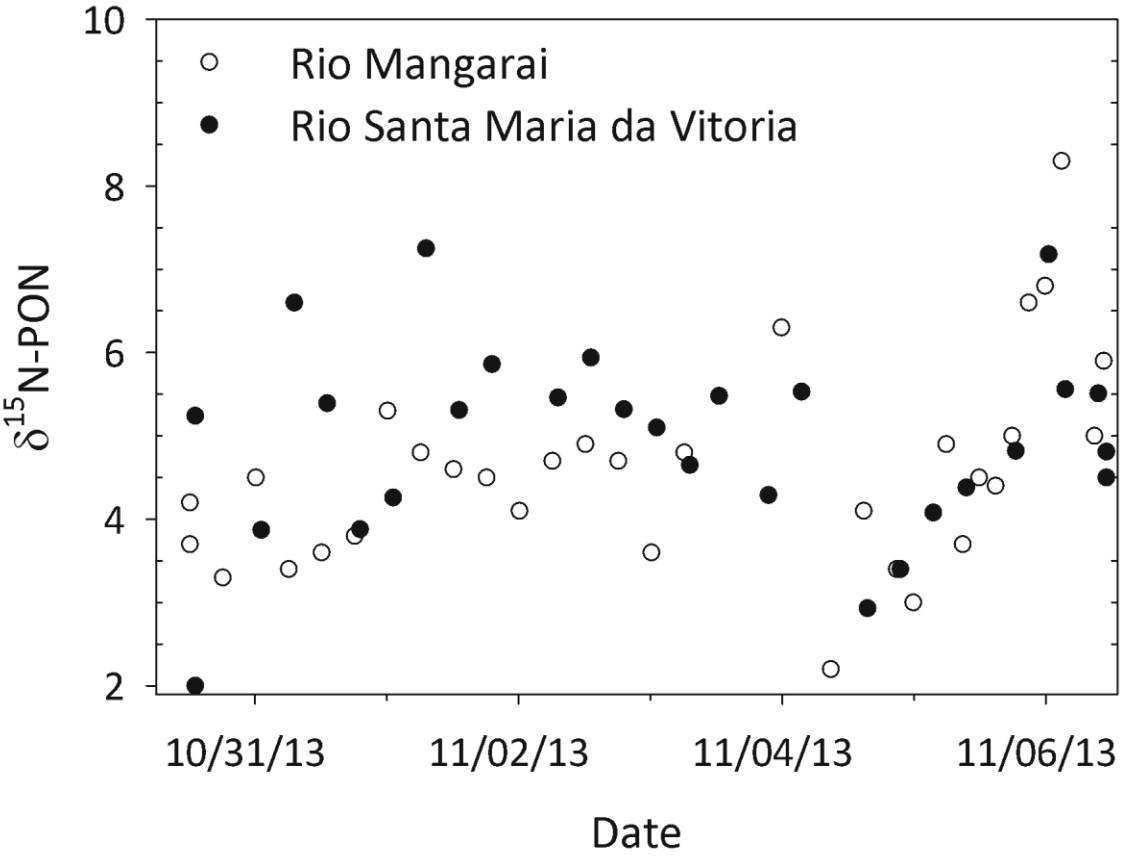


Figure V.10 Storm flow isotopic composition of particulate organic nitrogen. 3-6 hour frequency measurements of the stable isotopic composition of particulate organic nitrogen in the Rio Santa Maria da Vitoria (black) and its tributary, the Rio Mangarai (white), during a period of variable rainfall from October 30, 2013 through November 6, 2013.

Chapter VI: A successful framework for integrating local environmental research topics in a K-12 science classroom

VI.1 Introduction

Collaboration between university researchers and high school educators can provide an invaluable exchange of teaching philosophies and educational tools. Here, we detail a three to four week long inquiry-based unit on biogeochemical processes based on results from local scientific research. This curriculum was developed through the partnership of a high school science educator and a doctoral student in Oceanography. The goal of this curriculum was to both actively engage high school students in local environmental issues and increase their understanding of core biogeochemical principles.

We hoped to maximize student interest by making a tangible connection between course content and their local community and by basing the course materials on real data generated by real people in their community. Inquiry-based science activities generally have positive effects on acquisition of scientific knowledge, development of skills, and motivation (Purser and Renner, 1983; Ertepinar and Geban, 1996; Mao and Chang, 1998). We framed the entire unit as a crime scene investigation of a recent fish kill event in Hood Canal, Washington, in which students were given additional pieces of evidence to solve the mystery as they satisfied checkpoints in their understanding of key concepts. Positive student engagement can be enhanced by providing students with personalized course materials (Deci and Ryan, 1985). This investigation-style unit allowed students to learn the material at their individual pace, with the same end goal of solving the mystery. In this framework the instructor could readily provide differentiated materials such as simplified background readings or visual learning aids for

struggling students or more detailed news articles and primary literature for more advanced students.

The majority of this unit consisted of group (three to four students) and individual work. Students were given pieces of evidence to examine such as data plots, maps, laboratory exercises, or computer simulations. Each piece of evidence explored several key biogeochemical or oceanographic processes; students were given a worksheet with guiding questions and some background reading along with the evidence. The main role of the instructor (in this case a team of two instructors) was to circulate the classroom to answer clarifying questions and gauge student progression, allowing students to move on to the next step once they demonstrated satisfactory understanding of the content. This strategy could be difficult for an individual teacher to achieve with a large class size, but proved to be very effective with the graduate student-teacher tandem (or, likewise, a teacher-student teacher pair).

The material presented here is readily adaptable for use with middle school to undergraduate age groups. For example, this content could be incorporated into an undergraduate oceanography or environmental science curriculum as a one to two week unit. Here we provide generated course content for a detailed biogeochemistry unit and a replicable framework for integrating geoscience research into the high school science classroom.

VI.2 Class Demographics

This unit was included in the curriculum for six Ecology classes at an urban 9-12 high school in the Seattle, WA area. Students who are enrolled in this Ecology course are typically in their second year of high school and have taken an introductory Biology course during their first

year. Class size ranged from fifteen to thirty students, with an even gender distribution. The high school is made up of a diverse student population, with a school-wide distribution of 37.6% White, 30.2% Black, 22.1% Asian/Pacific Islander, 21.9% Asian, 7.7% Hispanic, and 0.8% American Indian/Alaskan Native students. 41.3% of the student body qualifies for free or reduced-cost meals. 6.2% are enrolled in special education programs and 5.6% of the students are transitional bilingual.

VI.3 The Unit

This unit was given over a three week period, with an additional week of review and synthesis work. Students compiled sequential pieces of evidence (e.g. scientific plots, lab exercises, and simulations) to unravel the mystery of the fish kill in Hood Canal (Figure VII.1). Students received worksheets with background reading and guiding questions for each evidence piece. Class periods typically began with a warm up question linking the concepts currently being explored to the overall mystery, followed by a brief five to ten minute lesson on key concepts or areas of confusion. The majority of class time was spent working through the evidence in groups or individually working on projects. We present this unit in the 5E format—engage, explore, explain, elaborate, evaluation (Bybee et al, 1997).

VI.3.1 Engage

We began the unit by viewing a video of diver observations in Hood Canal during the early stages of a hypoxic event (WA Dept. of Fish & Wildlife, 2006). The divers noted irregular fish behavior, including slow movement, shortness of breath, irregularly timed spawning, and migration of deep water fish to the surface. The video was paused and students were asked to

ponder why the fish were behaving strangely and what might be causing the odd behavior. Some ideas from the students were that perhaps the fish were tired, didn't have enough food, were sick, or maybe were poisoned by some toxin in the water. Some students noted the shortness of breath and related it to their dog panting after a long run.

The remainder of the video shows groups of dead and dying fish near the surface. Most students reacted enthusiastically and seemed genuinely concerned about what was happening to the poor fish. Students were surprised to hear that this was happening in their own backyard. When asked why the fish might be dying, many students stuck with their hypothesis that the fish were probably sick or poisoned by some toxin in the water. Some students made the connection between the behavior patterns and hypothesized that the fish had "run out of air." This set the stage for the investigation of the mysterious fish kill.

VI.3.2 Explore and Explain

Students were given additional pieces of evidence as their understanding of the key concepts behind each exhibit progressed (Figure VII.1). Students were given worksheets for each exhibit with background information on new vocabulary/concepts and questions to help guide the students towards connecting the evidence to the overall mystery. An "evidence organizer" was used by students to record the significance of each piece of evidence and its significance to unraveling the mystery. Students summarized what the data/map/activity "showed," what it "means for the ecosystem," and how the evidence helps us understand the fish kill. Students also worked in groups of three to four to organize their evidence and revelations on large 4'x4' whiteboards.

Students who progressed quickly or showed high interest in certain topics were given links to additional resources to further explore the concepts for extra credit. Students who struggled were given readings with simplified explanations in an easier to read format or additional visual aids. The unit was guided by a progression of seven driving questions, described below:

Exhibit 1: Why did the fish die? The beginning of the class period began with a warm up discussion of why sea-life needs oxygen and how they get it from water. We then transitioned into a brief lesson on what it means for oxygen gas to be dissolved in water, and how much oxygen different species of sea-life need. Students were given a map of Hood Canal showing dissolved O_2 concentrations in the surface waters during the August 2006 hypoxic event and a handout asking them to explain what they observed and its relevance to the overall mystery. Students realized fairly quickly that O_2 levels in the southern reach of Hood Canal were well below the 2 ppm threshold for fish survival and determined that the fish likely died due to a lack of oxygen. Students were also then given readings and a brief lesson on the natural history of Hood Canal to better understand the physical makeup of the watershed. One of the key points is that due to its bathymetry, the water in Hood Canal flushes with the ocean very slowly. Now that the students knew that the fish died due to a lack of O_2, rather than from some toxin, they went on to learn about the sequence of processes leading up to hypoxic conditions in Hood Canal.

Exhibit 2: How/why do O_2 levels vary in Hood Canal? The next piece of evidence was a series of *in situ* data collected by the University of Washington Oceanic Remote Chemical Analyzer (ORCA) program. Students examined the change in O_2 levels, salinity, and temperature with depth before, during, and after the fish kill. Prior to the fish kill, depth profiles showed a classic

"stratified" water column, with salty/cold/low-O_2 waters at depth and fresh/warm/high-O_2 water at the surface. During the fish kill, the water column became "well-mixed," with low O_2 levels and consistent salinity/temperature throughout the entire water column. After the fish kill, the water column returned to its typical stratified conditions. Depth profiles of rockfish populations revealed a migration of fish towards the surface to escape low O_2 levels at depth. Along with these pieces of evidence, students were given worksheets with readings and questions to guide their thinking. These concepts were solidified with formal lessons on estuarine circulation patterns and basic oceanographic principles.

Exhibit 3: Why is O_2 depleted in deep waters during "stratified" conditions? To understand the underlying mechanism leading to depleted O_2 levels at depth, students explored the SimBio Nutrient Pollution simulation. This simulation models the response of plankton/fish populations and O_2 levels to variable nutrient inputs. Students were able to visualize and quantify the process by which O_2 is depleted from bottom waters as a result of enhanced nutrient input: increased nutrient input promotes algal growth in surface waters (providing photosynthetically-produced O_2), once the algae dies it sinks to bottom waters where it is respired by bacteria (consuming O_2). Through guided calculations, graphing, questioning, and formal lessons students gained a key understanding of the reversible relationship between photosynthesis and respiration and the nutrient cofactors required for these processes. Once this understanding was reached, students were given a depth profile of chlorophyll concentrations in Hood Canal (a proxy for algae/phytoplankton population size), which revealed high levels of algae growth in surface waters prior to the fish kill. Students were taught the concept of using biomarkers or proxies for measuring ecosystem processes in order to understand the link between chlorophyll concentrations and algae population size.

Exhibit 4: How does O_2-poor water move to the surface? Students began by performing a food color salinity lab exercise to understand the relationship between salinity and water density. They observed that, when unperturbed, high salinity water generally sits on the bottom, creating a layered, or stratified, water column. When disturbed, the waters mix together, creating a uniform distribution of salinity. Through guided questions and brief mini-lessons, students reached an understanding that other dissolved chemical constituents (such as O_2 and nutrients) also move with the water particles similarly. The students were then given a map showing the changes in surface salinity (a proxy for wind-driven upwelling of deep/salty water) during the fish kill, which revealed very high upwelling rates in where fish deaths were most prominent. Students were asked to ponder what may have caused the large upwelling event, and were able to come to the conclusion that a large southerly windstorm must have pushed the surface water out of Hood Canal. Students were also given additional formal lessons on estuarine circulation describing the role of wind and tides in moving water masses.

Exhibit 5: What was the limiting factor for algae growth prior to the fish kill? Students ready to move on to this step now understood that the fish died because (1) there was a large algae bloom, which died, sank, and was consumed by bacteria, depleting the O_2 in deep waters; (2) this O_2-poor deep water was moved to the surface via upwelling caused by a strong southerly wind storm. The next step was to determine what factor allowed the large algae bloom to occur. Students had learned from Exhibit 3 that photosynthetic organisms typically need two main nutrients to grow—nitrogen and phosphorous. Students examined a plot of N and P levels in Hood Canal, observing a large spike in both N and P prior to the fish kill, a rapid drawdown of both nutrients, and a second smaller spike in concentrations. Students determined that there was some initial input of N and P, which got rapidly consumed by algae. After lessons and readings

on nutrient cycling, they were able to recognize that the second spike in nutrient levels possibly occurred because the dead algae had released, or "remineralized," the nutrients they had originally taken up. Students determined that N was the main limiting factor for algae growth because N levels dropped to zero, whereas some P remained. Students were given a very cursory lesson on the nitrogen cycle, with the main goal being to express the concept that nutrients are used by primary producers and "remineralized" by heterotrophic metabolism.

Exhibit 6: Where did the nitrogen come from that fueled the large algae bloom? Now that students had a key suspect (nitrogen) for their investigation, they went on to determine where that additional nitrogen came from. Although in reality both marine and terrestrial sources are important to the system, we decided to focus the rest of the unit on land-derived nitrogen to tie in to previous units exploring the connection between humans and watersheds. Students were given a plot of nitrogen fluxes to Hood Canal from marine and terrestrial sources, which revealed a constant N supply from the ocean and a large spikey input from land. In previous units, students had learned that rainfall carries with it elevated levels of nutrients and contaminants (e.g. Ward et al, 2012), so they suspected that a large rainstorm likely flushed these nutrients into the canal.

Exhibit 7: What are the different terrestrial sources of Nitrogen to Hood Canal? The final step in determining the fish kill culprit was to determine what land-derived sources contributed to nitrogen loading. Students assessed several land use maps of the Hood Canal watershed (Figure VII.3). They quickly determined that the southern corner of the watershed had the most urban influence. Similarly, southern and eastern shores had a large amount of deciduous forest, relative to the old growth coniferous forest-dominated western shore. Additional maps showed that red alder trees constituted roughly 50% of the deciduous forest landcover. Red alder trees are unique

in that they have symbiotic bacteria allowing them to fix N_2 gas from the atmosphere. This means that alder trees are a significant source of additional nitrogen to soils. In previous units we had discussed the role of alder trees in succession after a volcanic eruption. Students recognized that nitrogen fixation is an evolutionary advantage that allows alder trees to replace dead or removed old growth forest. After mini-lessons on the various human-derived nitrogen sources such as septic tanks, pet excrement, and fertilizer students hypothesized that human and alder-derived nitrogen from the southernmost region was the main culprit for depleted O_2 levels in Hood Canal. To close the case, students were given real data from stable isotopic analyses (similar to how DNA evidence always comes in to save the day on television), which revealed that 25% of the terrestrial nitrogen flux into Hood Canal was from old growth conifer forests, 50% was from deciduous/alder forests, and 25% was from anthropogenic sources.

VI.3.3 Elaborate and Evaluation

After closing the case, students were assigned a final project to synthesize their evidence and findings in the form of a Public Service Announcement (Table VII.1). They were given the option to make a poster, video, comic book, or presentation (Figures VII.4-5). Students were required to describe how hypoxia can occur naturally, how humans can influence this cycle, and make recommendations on how to limit anthropogenic inputs to the system. Students were evaluated based on both their final project and an open-note test in which they were asked to report their findings based on the evidence given throughout the unit (Table VII.2).

VI.4 Discussion and Conclusion

This unit received fantastic feedback from the students. On an anonymous survey, students noted that they thought this course content was more difficult than their average science lesson, but that they had more fun and were more interested. Most responded that they would not have been as interested in studying this if it was based on a non-local fish kill. However, interestingly, less than 75% of the students had even known where or what Hood Canal was prior to this unit. Only two students responded that they knew about this anoxia issue prior to the unit. Similarly, at the beginning of the school year many of these urban high school students were not familiar with Puget Sound, a huge body of water literally in their backyard. This and other units based around Puget Sound explored throughout the year markedly enhanced both student awareness of local environmental issues and student engagement in class activities. Further, this approach made a personal connection between students and science.

The teacher-graduate student tandem proved to be an effective way to both develop this type of curricular material and execute the evidence-based unit framework. Lab activities and time when students were primarily working in groups exploring the evidence were scheduled to be done when the teaching pair was present since the graduate student was only in the classroom half of the week. The role of the instructors during these activities was to circulate the classroom to gauge student understanding, provide guiding questions, and determine which groups were ready to move on to the next step. This role was difficult for a teacher to achieve on his/her own because of the sheer reality of classroom size, thus formal lessons and assessments were scheduled for days when the graduate student was not present. This type of inquiry-based unit framework could be similarly successful with a student teacher-teacher tandem. However,

bringing research science-based graduate students into the K-12 classroom is a unique opportunity to blend modern science with STEM education.

Further, we found great success in terms of student engagement by merging locally-relevant issues with course content. As our global community becomes increasingly interconnected, popular culture has trended towards an emphasis on the importance of local communities. The current generation of students is growing up in an era in which it is becomingly increasingly "cool" to support locally produced goods and services such as clothing, food, music, and sports. Thus, creating a local connection with classroom content is likely a powerful tool for engaging a student's interest. Here we provide a framework for integrating local research into the K-12 classroom, a fully adaptable unit on core biogeochemical principles, and illustrate the potential outgrowths of geoscience graduate student involvement in K-12 STEM education.

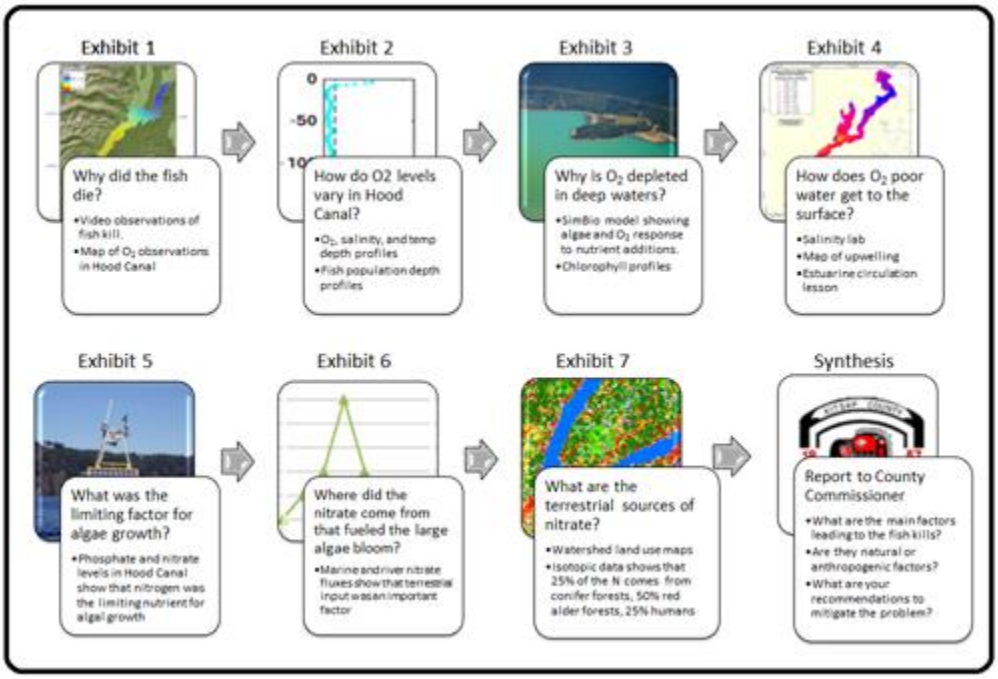


Figure VI.1 Progression of evidence pieces used to unravel the cause of the Hood Canal fish kill. Students completed worksheets for each exhibit, and were able to move through the unit at their own pace. Faster students were given additional resources to go into greater detail on certain concepts.

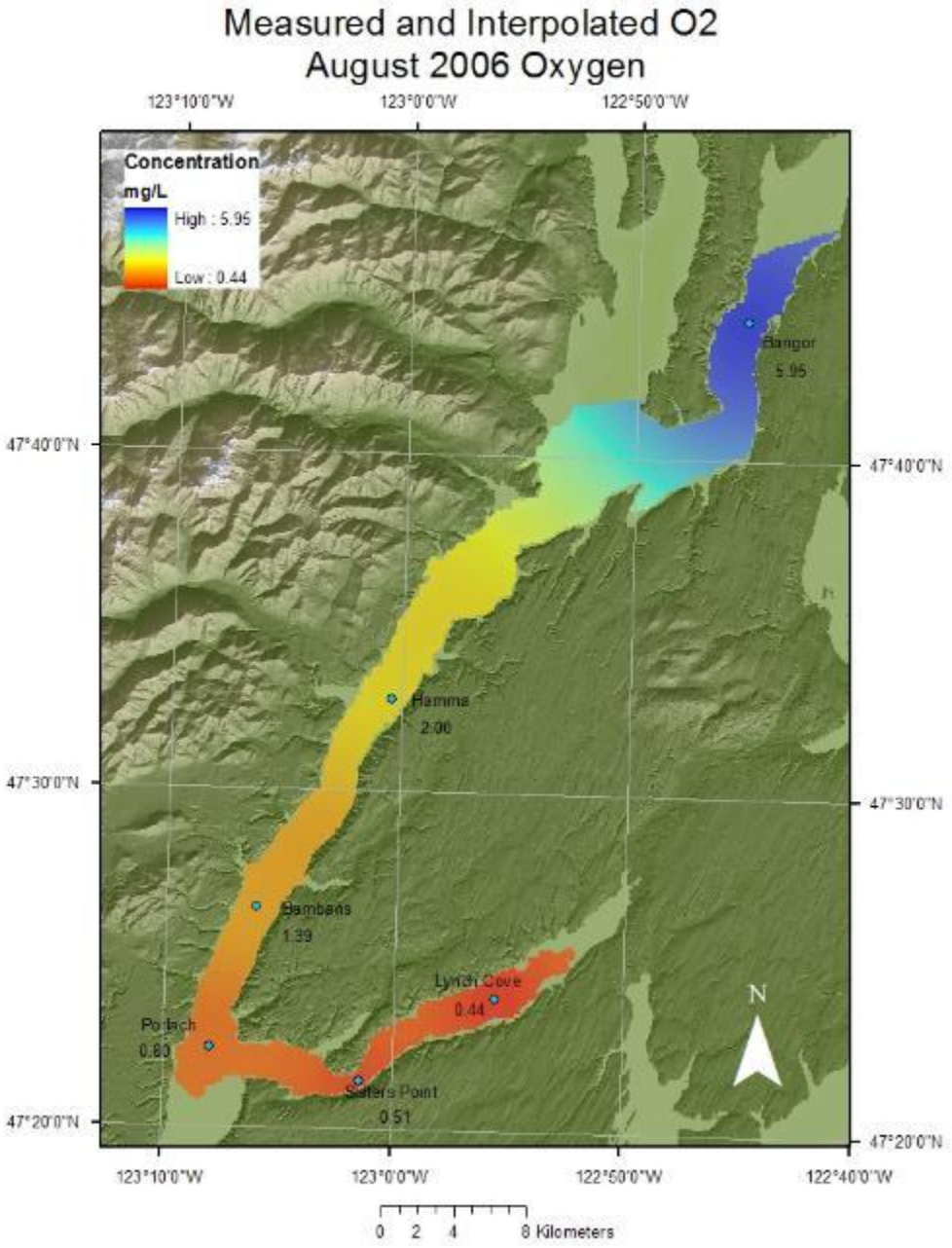


Figure VI.2 Example of evidence pieces used: a map of dissolved oxygen concentrations in Hood Canal surface waters during the August 2006 fish kill event (Ward et al, 2012).

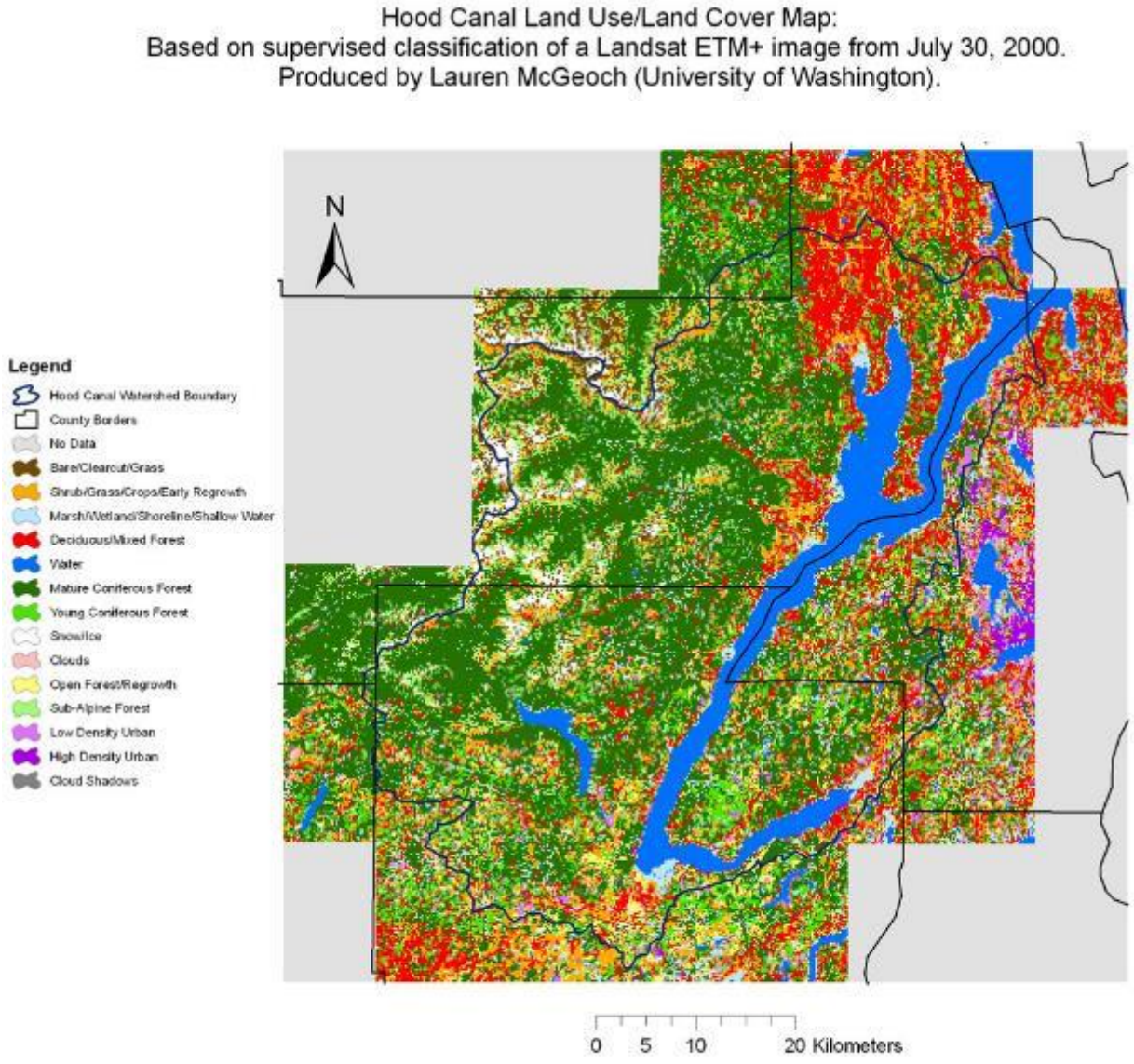


Figure VI.3 Example of evidence pieces used: a map of Hood Canal watershed land use coverage (L. McGeoch, unpublished).

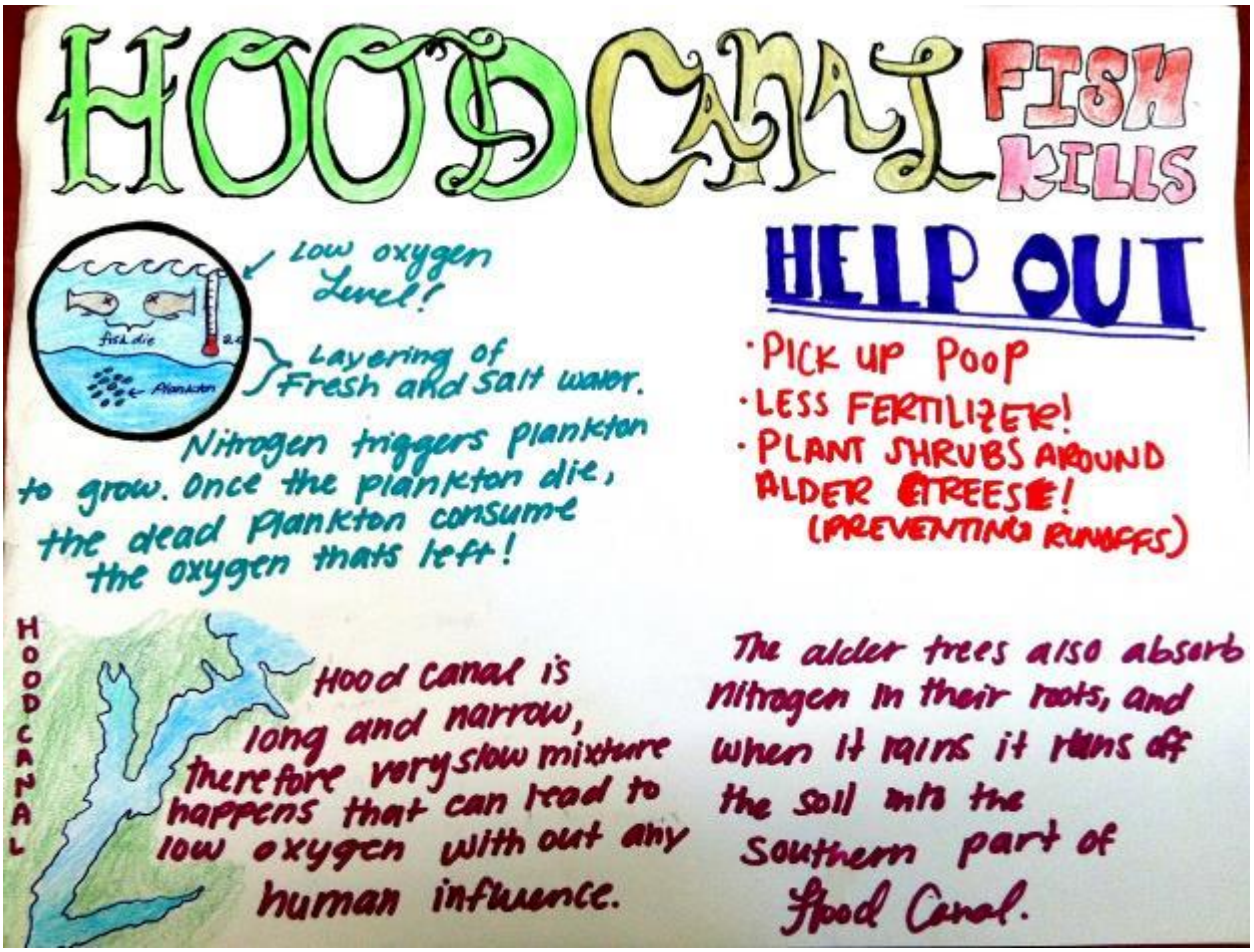


Figure VI.4 Example of student work on the final Public Service Announcement project

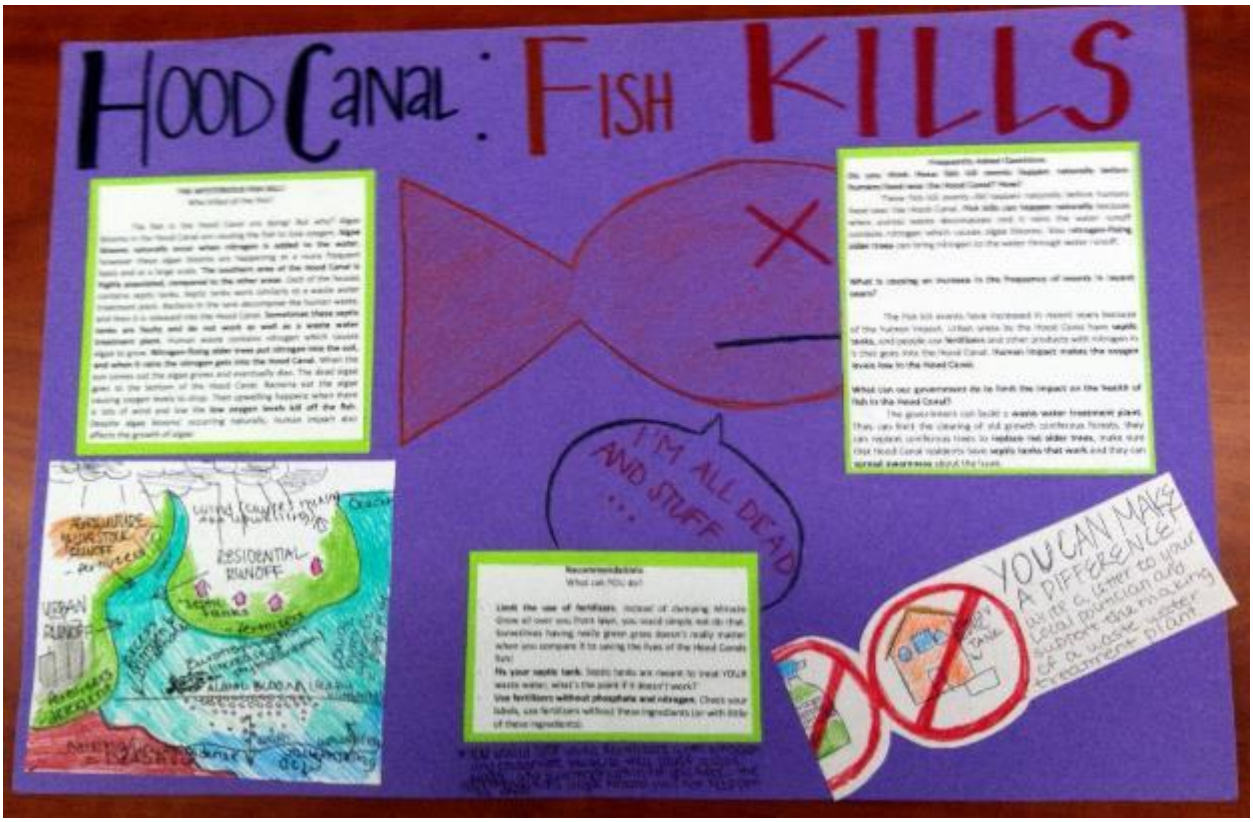


Figure VI.5 Example of student work on the final Public Service Announcement project

Table VI.1 Rubric for Public Service Announcement project (poster, video, comic book, or presentation).

Category	Excellent (5 pts)	Good (4 pts)	Satisfactory (3 pts)	Poor (0-2 pts)
Explanation of event	PSA recognizes that fish kills can happen naturally and provides a short summary of what causes low oxygen/fish kills under pristine conditions (no humans) and during recent events.	PSA provides thorough explanation of recent events, leaves out naturally occurring events that happened in the past.	PSA does an adequate job of explaining recent fish kill events.	PSA does not reflect an understanding of the events that cause fish kills.
Human Impact	PSA **both illustrates AND describes** that the impact of humans can increase the frequency of low O_2 events, and push us over the tipping point from an event with somewhat low O_2 to an event with so little O_2 that all the fish die.	PSA **illustrates OR describes** does not discuss the tipping points created by humans.	PSA shows **minor errors** in the illustration OR description of the tipping points created by humans.	PSA shows **major errors** in the illustration OR description of the tipping points created by humans.
Recommendations	The PSA makes **at least 3 recommendations** for how to reduce human impacts. Details are given on HOW each recommendation will decrease events. See description below.	The PSA only details **2 recommendations**, or does not give adequate details on how the recommendations will decrease events.	PSA does insufficient job explaining recommendations.	Recommendations missing or major errors in explanations
Project Appearance	We are wowed by the professional appearance, and aesthetics of the PSA No errors or edits	Professional appearance but nothing above and beyond	Project complete, but without aesthetics or any enhancements	Not final draft quality, unfinished appearance
Overall	Appropriate use of vocabulary with correct spelling Correct scientific values used to support answers	Appropriate use of vocabulary with only minor errors Scientific values used, but with errors in units or use	Some errors in vocabulary use, notations, and spelling	Major errors in vocabulary use, notations, and spelling

The PSA makes at least 3 of the following recommendations for how to reduce human impacts:

1) People can limit their use of fertilizers in their home gardens.
2) People can scoop and properly dispose of their pet waste in compost bins.
3) People can ensure that their septic systems work efficiently and don't leak.
4) The County government can build a sewer/waste water treatment system to reduce Nitrogen seapage from septic tanks.
5) The County government can limit the clearing of old growth conifer forests (N_2-fixing red alder trees dominate cleared land)
6) The County government can replant coniferous trees where red alders have taken over

Table VI.2 Final assessment: Official Crime Scene Report

1. Describe the crime in as much detail as possible. This should **only be observations**, not ideas about what caused the initial incident.
2. Where did the crime take place? What is significant about the location in terms of the crime?
3. Describe the timeline of events leading up to the crime? This should **only be a timeline** and **not** have details into HOW it happened.
4. Describe **5** key pieces of evidence that helped you formulate your conclusion. If helpful, use the sentence starter to construct your answer.
5. What is your conclusion as to the cause of death? This needs to be based on the appropriate evidence with an explanation on the correct sequence of events.

VI.5 Student Worksheet Examples

Exhibit 1: This is a map of dissolved O_2 concentrations in Hood Canal. Red colors indicate very low O_2 levels and blue indicates high (e.g. "normal") O_2 levels. The scale goes from 0.4-6.0 mg O_2/L. Remember, fish cannot survive long with **less than 2 mg O_2/L**.

Things to think about:

1) Where do you see the lowest O_2 levels in Hood Canal?

2) Is there anything unique about the area where O_2 levels are lowest?

3) What correlations/patterns do you see between this map and your other lines of evidence?

Exhibit 2: These graphs show **depth profiles** of O_2 levels, salinity, and temperature over a 3-day period. A **depth profile** shows you how these parameters (x-axis) vary from the surface of Hood Canal (top of y-axis) to the bottom of Hood Canal (bottom of y-axis). We are looking at a place where the water column is 100m deep (1 football field).

Looking at the salinity tells us the relative amount of fresh water (rivers) vs. salt water (ocean). If you see **different salinities at different depths**, this **indicates unique water masses/layers in the water column**. If a water column is **layered**, we say it is "**stratified"**

These different water masses will have different chemistries (e.g. O_2, NO_3, etc).

If you see a **constant salinity throughout the water column**, this **indicates a well-mixed water column**

In this case, water chemistry will be the same throughout the entire water column.

Things to think about:

Just look at the 19-Sept-2010 plots for these first questions – this is "normal" conditions for Hood Canal

1) At what depth do you see the lowest O_2 levels in Hood Canal?

2) Is there anywhere in the water column with enough O_2 for fish to survive? Hint–the 2mg/L limit is denoted by the dotted line.

3) At what depth(s) do you see the highest/lowest salinities?

4) Why do you think you see this pattern with salinity (i.e. where does the water come from)?

Now start looking at how these plots change over the 3-day period

5) How does the depth profile of O_2 change over the 3-day period?

6) Is there ever a time when O_2 levels are below the 2 mg/L threshold in the *entire* water column?

7) How would fish respond to these conditions?

8) How does the depth profile of salinity change throughout the 3-day period?

9) Look at the salinity on 21-Sept-2010 06:17:16, is the water column **stratified** or **well-mixed**?

10) What are two physical factors that cause a water column to be mixed?

11) Summarize what you think these salinity and O_2 profile are showing us and how does it relate to dying fish in Hood Canal?

Exhibit 2b: We will be using a computer simulation called *SimBio* to help us understand what factors affect O_2 levels throughout the water column. This simulation models levels of different chemicals (e.g. NO_3, O_2, pH, etc) and the population size of phytoplankton (aquatic plants), zooplankton (eat the phytoplankton), fish (eat the zooplankton), and bacteria (eat dead sinking plankton).

Things to think about:

1) Which organisms produce O_2 in the water? Where are they located in the water column (deep or shallow)?

2) Which organisms consume O_2 in the water? Where are they located in the water column (deep or shallow)?

3) Why is O_2 highest at the surface and lowest at the bottom (what biological processes are occurring where)?

As long as there's enough O_2 somewhere in the water column, fish can move vertically to find enough to live. Fish kills occur when there is literally nowhere for the fish to go.

Tying to other evidence

1) How do low O_2 waters move from the deep to the surface? Do you think it is caused by a biological, chemical, or physical process?

Evidence 2c: This plot shows the depth profile of chlorophyll on 19-Sept-2010 (the first day shown on the exhibit 2 plots). Any primary producer, or organism that photosynthesizes, uses chlorophyll, so we measure chlorophyll to determine the abundance of primary producers (e.g. algae and phytoplankton).

1) Where are chlorophyll concentrations highest in the water column?

2) How does the chl depth profile relate to the O_2 profiles in exhibit 2?

3) How does this information help us figure what is causing low O_2 in Hood Canal?

Exhibit 2d: These plots show the distribution of rockfish populations at 2 locations: Sund Rock (further south) and Pulali Point (further north). These measurements were made by divers who counted fish populations. We already have **qualitative** evidence that fish moved towards the surface during the fish kill event based on our observations (the video we watched).

However, these plots provide **quantitative** evidence (real numbers!)

The y-axis denotes the water depth where fish were found, the bubbles represent how many fish were observed at each depth (larger bubble = larger population). The x-axis represents time (1 year).

1) What is the difference between **qualitative** and **quantitative** evidence?

2) At the Sund Rock location, at what depth do rockfish typically live? (hint, only look at the data prior to Sept 19th)

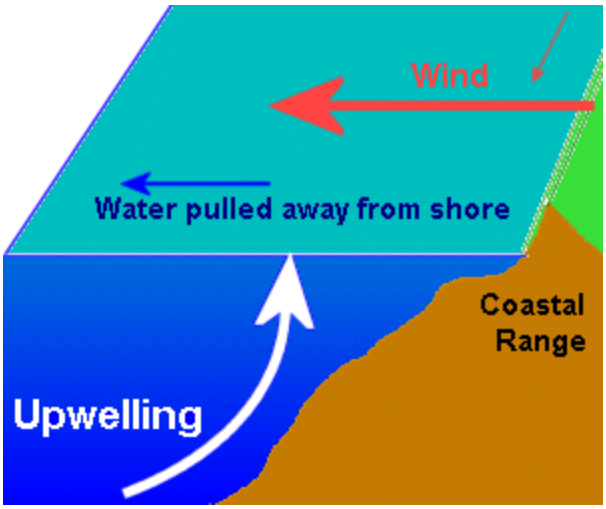
3) At the Sund Rock location, at what depth are most of the fish located on Sept 19th and the several days prior?

4) Why do you think the fish at Sund Rock swam closer to the surface around Sept 19th and how can you relate this observation to your other pieces of evidence?

Exhibit 3: This map shows the change in salinity at the surface of Hood Canal during the fish kill event. The **change in surface salinity** tells us approximately how much **deep water has upwelled** to the surface.

Normally, the surface of Hood Canal is less salty than the bottom. This is because salty water is denser. Thus, fresh water from rivers sits on top of salty ocean water.

However, when there is strong wind, it blows the surface water away from shore (see picture below). Because the fresh surface water is moving somewhere else, the deep salty water **upwells to the surface.**



When the deep water upwells we can measure how much the salinity has changed (or, in other words, how much deep salty water has risen to the surface).

When the water upwells, it also brings with it any dissolved chemicals or gasses or lack thereof (e.g. O_2, NO_3, etc)

1) Where in Hood Canal is upwelling (or the change in salinity) the greatest?

2) What direction do you think the wind would need to blow to push water **out of Hood Canal**?
(hint: from north to south or from south to north?)

3) Based on your other evidence, how much dissolved O_2 is found in the deep water of Hood Canal?
4) What impact does upwelling of this deep water have on the fish?

Exhibit 4: At this point you should have figured out the following:

- A large algae bloom occurred several days prior to the fish kill
- The algae died and began sinking to the bottom.
- Bacteria consumed the dead algae, also consuming O_2
- This lead to very low O_2 levels deep in the water column
- The deep water (with low O_2) was upwelled to the surface by a Southerly windstorm, causing O_2 levels to be uniformly low in the water column, leaving nowhere for the fish to go.

Now we that we know generally what happened, we need to dig into more detail to figure out the true "culprit." We still do not know what caused the unusually large algae bloom to occur.

Exhibit 4 shows the surface concentration of two essential nutrients for algae to thrive (nitrate and phosphate)…remember most plants can't fix N_2 gas directly, so they need to use NO_3.

Typically there are different amounts of these nutrients. A **nutrient** becomes **limiting** when it is completely **depleted** (and the other necessary nutrients are still abundant).

1) Based on this plot, which nutrient do you think is limiting to algae in Hood Canal?

2) If we added more phosphate would more algae grow?

3) If we added more nitrate would more algae grow?

4) Now we know the concentration of nutrients, but we still don't know *where* they came from. List 4 possible sources of nitrate to Hood Canal.

Exhibit 5: Both the ocean and land (through rivers) provide nitrate to Hood Canal. Deep ocean water is typically high in nitrate because of bacterial decomposition of primary producers (algae).

Our Crime Scene Investigation Team has informed us that the **marine** flux of nitrate to Hood Canal remained constant both before and after the fish kill event. **Flux** means the total amount of chemical moving from one reservoir to another. **Marine** means that it came from the Ocean.

Since the marine flux of nitrate remained constant, we suspect that the **terrestrial** nitrate flux provided much of the extra nutrients that fueled the large algae bloom. **Terrestrial** means that it came from land. Nutrients from land are carried by rain water, through rivers/streams, and into Hood Canal.

Exhibit 5 is a plot showing the terrestrial nitrate flux over a week-long period.

1) Why do you think the terrestrial nitrate flux increases so much from Day 1 to Day 2?

2) How do you think the timing of this plot lines up with the timing of your other pieces of evidence?

3) List 3-4 ideas of where you think the nitrate is coming from (hint: it's a combination of many sources)

Exhibit 6: This map shows the **land use** of the region surrounding Hood Canal. **Land use** means the type surface that is present at a specific location (for example, forest, concrete, open field, etc). Land use is often measured by satellite imagery and/or aerial photographs. This field of study is called **GIS, or Geographic Information Systems.**

Looking at this map can help us figure out some potential sources of nitrate because different types of land cover provide different amounts and types of Nitrogen. For example, all types of forest provide a natural source of Nitrogen to rivers. On the other hand, places populated by humans may provide **anthropogenic**, or man-made, sources of Nitrogen (such as fertilizers, dog poop, human poop, etc).

1) Where in the region do you see the most Mature Coniferous Forest?
2) Where in the region do you see the most Deciduous Forest?
3) What's the main difference between a deciduous forest and coniferous forest?

4) Where in the region do you see the most urban land use (where do the most people live)?
5) How does this map help us to explain the Hood Canal fish kill?

Exhibit 6b: This land use map gives us more quantitative data than Exhibit 6. The map on the left shows the distribution of mixed deciduous forest throughout the region (red dots). The map on the right shows the distribution of human populations (red and black dots).

The reason we want to look at the distribution of deciduous forests, is because many of the deciduous forests in the region contain **red alder trees**. Roughly 50% of the deciduous forests in the region are composed of red alder trees.

Red alder trees are unique because they live symbiotically with **nitrogen fixing bacteria**, which allows the red alder tree to use N_2 gas from the atmosphere for its source of Nitrogen. Because of this evolutionary advantage, alder trees usually are the first trees to grow back after an area has been cleared.

This is the final piece of evidence we will have to determine where the excess nutrients in Hood Canal are coming from.

If we can figure out what the different terrestrial Nitrogen sources are qualitatively, then the CSI team will be able to do a fancy isotopic analysis to reach a quantitative conclusion. But first we need your investigative help to narrow it down!

1) Where do you observe the most deciduous forests?
2) Do you think more Nitrogen would come out of a deciduous forest or coniferous forest? Why?
3) Would you consider the Nitrogen coming out of a forest to be **natural** or **anthropogenic?**

4) Where do you observe the most humans?

5) List 3 ways that humans provide Nitrogen to Hood Canal.

6) Are these **natural** or **anthropogenic** sources?
7) Based on all of your evidence, what do you think the 3 main sources of terrestrial Nitrogen to Hood Canal are? (hint: make generalizations)

8) Explain why a fish kill could have happened in Hood Canal 5,000 years ago when few if any humans inhabited the region surrounding Hood Canal.
9) Why do you think these fish kills are happening more often in the present?
10) Write a complete summary/timeline of the events leading up to the Hood Canal fish kill event.

Chapter VII: Benefits and limitations of tablet-pcs in the K-12 science classroom

VII.1 Introduction

Innovations in personal electronics technology are often not driven by the needs of educators, but by the desires of retail consumers. However, there is great potential to connect with learners on a more personal level as mobile devices become increasingly interconnected with our daily lives. Technological innovations provide novel tools to educators to connect and communicate with students. Implementation of these tools may allow for major positive transformations in our perception of the traditional lecture-based pedagogy (Bransford et al., 2000), but it is important that educators consider both the benefits and inherent limitations of a platform before adopting it institution-wide. Further, in some cases the utility of a platform are limited by school policy, highlighting the need for educators and administrators to work together when adopting new technology in the classroom.

The most obvious benefit of utilizing innovative technology in the classroom is the ability to spark student interest and provide a seemingly personalized educational experience. Student motivation, an important factor in deep learning, is thought to be enhanced by educational programs crafted for individual student needs (Deci and Ryan, 1985). For example, incorporation of computer-mediated communication has been successfully used to foster constructivist and individualized learning programs (Abrami and Bures, 1996; Muir-Herzig, 2004). Rau et al (2008) observed that communicating with high school vocational students about assignments via mobile Short Message Service (SMS) generally increased student extrinsic motivation without causing higher pressure. Additionally, communication on a digital medium has been shown to enhance a student's likelihood to ask for help from instructors due to a greater

sense of anonymity and decreased intimidation by social cues (Bures et al., 2000). Several studies have indicated that using technology in the classroom adds complexity to learning tasks, which encourages peer coaching and collaboration (Baker et al., 1990; Dwyer, 1994).

The current generation of western K-12 students has been immersed in mobile communication technology since birth. Student familiarity with the use of mobile and touch screen devices is highly prevalent, especially in urban schools. The use of cutting edge consumer technology in the educational setting has great potential for connecting with young students on a more personal level and embracing individualized learning, but is often hard to adopt on an institution-wide basis due to high entry costs and fears of obsolescence. The compact, flexible nature of modern tablet personal computers has the potential to drastically transform both student and instructor immersion. As availability becomes more widespread, educators are beginning to unravel the utility of tablets in the classroom. For example, tablet pcs have been successfully implemented as a mobile presentation platform for instructors in a lecture setting (Anderson et al., 2004). Further integration of tablets into university level courses has allowed students and instructors to share handwritten notes taken on personal tablet devices, fostering increased student participation (Golub, 2004; Anderson et al., 2007). The utility of tablets in engaging young students is beginning to be increasingly recognized (Couse and Chen, 2010).

Although the use of tablets by instructors has become increasingly widespread, there has been little development in the use of class sets of tablets in K-12 Science, Technology, Engineering, and Mathematics (STEM) classrooms. Here we developed a high school Ecology lesson, using the iPad tablet platform in combination with more traditional media, and evaluated changes in student understanding. The primary goal of this study is to detail both the benefits and

limitations of the iPad platform and provide our firsthand account of implementing an iPad-based lesson in a high school Ecology classroom.

VII.2 Study design and lesson plan

VII.2.1 Student demographics

This study was performed at a local Washington state high school (grades 9-12) in the Seattle urban area. Three separate Ecology classes of varying size were given the lesson described below in an effort to examine the effectiveness of tablet-based education in the high school science classroom. Students who are enrolled in this Ecology course are typically in their second year of high school and have taken an introductory Biology course during their first year. Students who are most likely to enroll in Advanced Placement science courses will typically enroll in Chemistry instead of this Ecology course. The majority of students in this study (n=49) were in tenth grade. The gender distribution was nearly equal, with 49% and 51% female and male students, respectively. The high school is made up of a diverse student population, with a school-wide distribution of 37.6% White, 30.2% Black, 22.1% Asian/Pacific Islander, 21.9% Asian, 7.7% Hispanic, and 0.8% American Indian/Alaskan Native students. 41.3% of the student body qualifies for free or reduced-cost meals. 6.2% are enrolled in special education programs and 5.6% of the students are transitional bilingual.

Students involved in this study had taken the Washington State administered High School Proficiency Exam (HSPE) the year prior to being enrolled in Ecology, which is used to gauge grade-level proficiency in reading, writing, math, and science. 67% of the students who participated in this study had below-proficient scores (e.g. 2 or less out of 4) on the science

portion of the HSPE, whereas 33% of the students had satisfactory or above scores (e.g. scored 3-4).

VII.2.2 Learning Goals and Washington State Standards

In Part I, students collected and recorded ecological data in an effort to reconstruct food chain models based on observations and quantitative data. Students explored the concept of energy transfer through trophic levels and the loss of energy via respiration/metabolism.

In Part II, students interacted with a dynamic predator/prey population model in an effort to visualize and identify the linkage between predator and prey populations. Students were able to then identify examples of top-down and bottom up population controls.

Skills:

Data Collection

Models/Data Representation

WA State Standards:

9-11 LS2C Population growth is limited by the availability of matter and energy found in resources, the size of the environment, and the presence of competing and/or predatory organisms.

VII.2.3 Activity Logistics

The lesson plan was conducted over a 2 hour lab period and a debriefing was conducted during the next day's standard 55 minute period (Figure VIII.1).

Part I. Food chain dynamics and trophic energy transfer

The class period began with a warmup exercise to both gauge the initial level of understanding of the students and to promote student engagement early in the class period. Students were asked to explain why a person would or would not gain one pound of body weight if they were to eat one pound of food and to explain some of the ways that their bodies use and store energy. Student responses were later scored on a scale of 0-3 for concept understanding (see Table VIII.1). This score was used as the pre-assessment for the concepts explored in Part I. Students were also asked several similar questions on the lab worksheet, which were scored with the same rubric. The students answered these questions after completing part I of the lesson, and their scores were used as the post-assessment for the Part I concepts.

After the warmup exercise, a brief 5-10 minute lesson on basic food chain construction was given by the instructor. Basic concepts concerning the fixation of energy by autotrophs, the consumption of lower trophic levels by heterotrophs, and the loss of energy between trophic levels via respiration were discussed. Following the short lesson, the students were given the lab worksheet and lab instructions to read over before starting the activity. Students were asked to explain the lab instructions to the class before choosing groups of two to four, depending on the class size. After everyone was clear on the instructions each group was given one iPad tablet (Figure VIII.2).

The iPads were loaded with the *WeallEat* app, a simple game in which the player controls a crab in an effort to avoid predators and consume prey (Figure VIII.3). The crab advances up trophic levels, eventually eating its previous predators. Once eaten by a predator, the app displays how many of each animal type was eaten by the crab and also the final mass of the crab.

Students were asked to use the *WeAllEat* app to collect this data for additional calculations and considerations. Finally, students reconstructed the food chain of the game based on their observations of the different trophic levels.

In its original design this lesson utilized the iPad for every aspect of the lesson (including Part II described below). For example, students would enter their data into pre-formatted Excel spreadsheets and food chain diagrams would be drawn and saved on the iPad using one of many whiteboard apps. However, due to the constraints of the iPad platform and school internet security compliance issues, these parts of the lesson were done on paper, and in the case of part II, the classroom projector (additional discussion of limitations to follow).

Part II. Population Dynamics

After all groups finished the Part I calculations and questions, the iPads were collected and students returned to their desks to complete Part II as a class. The goal of Part II was to explore the influence of predator and prey populations on one another and examine several examples of bottom-up and top-down population controls. The instructor gave a demo of and guided students through the Nutrient-Phytoplankton-Zooplankton (NPZ) model visualization developed at the University of Washington (Banas, 2011). Model results of phytoplankton and zooplankton population sizes, nutrient levels, and detritus sinking and remineralization rates are displayed in a graphic user interface. The user can control numerous parameters and observe how population sizes change. In particular, the instructor guided students through examining the influence of changing phytoplankton and zooplankton maximum growth rates. Top-down and bottom-up population controls were then explained in the context of this model (e.g. bottom-up control occurs when the phytoplankton population size was altered and affected the zooplankton

population and top-down control is when altering the zooplankton population affected the phytoplankton population). Students were also given several other typical examples of predator-prey population dynamics, such as the linkage between kelp, abalone, and sea otter populations.

Students filled out the Part II questions on the lab worksheet while discussing the concepts. Originally, answers to these questions were intended to be used as a pre-assessment for the concepts explored in Part II, and the quiz described below was to be used as a post-assessment. However, the Part II lab questions had minimal utility as a pre-assessment of initial student understanding since they were answered as part of a class discussion guided by the instructor. Alternatively, comparing student performance on the Part II lab questions versus performance on the quiz was a useful measurement of short-term (next day) concept retention.

Part III. Debrief and Final Assessment (55 minute period):

The following day, the instructor gave a formal review of the key concepts discussed during both parts of the lab exercise. Students were then given a quiz, asking them to analyze a scientific plot of predator-prey populations changing over time. This assessment was compared to student scores on the guided Part II questions in the lab worksheet to assess how well students retained information presented to them as a class discussion. Pre and post assessment scores for Part I, on the other hand, were a useful measure of a student's increase or decrease in concept understanding during an interactive, exploratory type lesson. Finally, students were given a questionnaire asking for feedback and to rate certain aspects of the lesson**.**

We present data from these assessments in an effort to gauge the success of this lesson in terms of student learning. However, we do not attempt to quantitatively compare the

effectiveness of our iPad-based lesson to a similar lesson using a more traditional medium. Rather, our primary goal is to outline the benefits and limitations of the iPad for educators who are considering adopting the platform.

VII.3 Results and Discussion

VII.3.1 Assessment of understanding

Initial student understanding of the Part I key concepts was measured at the beginning of the class period with a written warmup exercise (Part I pre-assessment). Similar questions were asked on the lab worksheet during the lesson, and used to gauge changes in student understanding (Part I post-assessment). For both assessments, the student's level of understanding was scored from 0-3 (see I 1). Students generally scored low on the pre-assessment, with an average score of 1.1 (n=49). 22% of the students showed no understanding (e.g. 0 score), 47% showed minimal understanding (e.g. 1 score), 29% showed adequate understanding (e.g. 2 score), and only 2% showed an exceptional level of understanding (n=49).

To assess change in student understanding a student's score on Part I, Question 1 of the lab worksheet was subtracted from their score on the Part I pre-assessment (scores based on the Table VIII.1 rubric). The relationship between a student's initial understanding (e.g. pre-assessment score) was then compared to their relative change in understanding (Figure VIII.1).

Students who showed a strong initial understanding of the concepts (e.g. scored 2-3 on warm up) generally retained the same level of understanding after the lesson. In general, students who had the lowest initial understanding (e.g. scored 0-1 on warm up) showed the most improvement in understanding after the tablet-based lesson (Figure VIII.1). All students who

showed zero initial understanding improved by at least 1 point and generally improved to an adequate understanding (e.g. scored 2 on post-assessment). Students who showed minimal initial understanding (e.g. scored 1 on warm up) varied from showing no improvement to improving by 1-2 points. These results show that the lesson was successful in engaging students at the lower end of the initial understanding spectrum. However, from this alone, it is not possible to determine what role the technological novelty of using iPads played in enhancing student learning.

Responses to question 2 on the lab worksheet were used to assess a student's level of critical thinking. During the data collection and calculation portion of the lab, students were asked to calculate "growth efficiency" as the ratio of their average crab weight to their average weight of food eaten. However, students were never given a formal definition or any instruction on the meaning of growth efficiency previously. Students were then asked to rationalize and explain the meaning of growth efficiency based on their knowledge of how it is calculated and given a score between 0-2 (see Table VIII.2 for rubric).

Critical thinking scores were compared to students' improvement in understanding in order to determine which types of students showed the largest improvements (Figure VIII.2). Those students who received a score of zero on the critical thinking question showed no improvement over their pre-assessment level of understanding. On the other hand, students who performed well on the critical thinking question showed a greater improvement in understanding. These results imply that students who were uncomfortable or unable to answer an unfamiliar question were less likely to change or improve on their previous knowledge of the concepts,

whereas those students who were able to rationalize or at least attempt to solve the unfamiliar question were more likely to change or improve their understanding throughout the lesson.

Part II of the lesson examined predator/prey population dynamics by exploring the NPZ model as a class. Due to constraints of the iPad platform (more discussion below), this part of the lesson was done on the classroom projector with the instructor walking students through the model. Students were closely guided through the questions on the lab worksheet (Part II "pre-assessment"), and generally scored high, with an average score of 5 out of 6 (range=1-6; n=49). Students were given additional lesson debriefing and a quiz (Part II post-assessment) the following day with similar questions. In general, students who scored low on the lab worksheet increased their score on the quiz and those who initially scored well either performed similarly on the quiz or scored one point lower on the quiz (Figure VIII.3). The increase in low performing student scores is likely a result of the additional debriefing given prior to the quiz, however, it would be interesting to see how well students retain information given to them in this type of formal lecture setting alone.

VII.3.2 Student Survey

One to two days after the lab exercise, students were given a questionnaire asking them to rate various aspects of the lesson. The three different class periods ranged in size, with student group sizes ranging from two to four. Students in groups of two to three all responded that they felt like they were able to spend enough time using the iPads in their groups, whereas roughly 10% of the students in groups of four responded that they would have liked more time to use the iPad.

When asked how much fun they had with the activity (from 0-5), student responses were on average 4.5, with a range from 3-5 (n=49). When asked how difficult the lesson content was, student responses were on average 1.6, with a range from 0-3. The above results suggest that student engagement in the activity was very high and, perhaps, this enhanced student engagement resulted in student perception of the content difficulty to be low. In other words, if students have fun they may perceive the lesson content as "easier."

When asked how much they thought they learned, students responded 3.8 on average, with a range from 3-5. Although students perceived the content as "easy," they also acknowledged that they learned a significant amount throughout the lesson. Students rated their preference for a tablet-based lesson compared to a "traditional lesson" as 4.6 on average, ranging from 2-5 and 98% of the students responded that they enjoyed part I of the lab exercise (i.e. tablet portion) more than part II (phytoplankton model demonstration). In general, students responded that their favorite part was playing the game and their least favorite part was performing calculations and answering questions.

Clearly, the students were engaged by the novel experience of using a tablet-pc, and this engagement likely enhanced student involvement in the more rigorous aspects of the lesson. Anecdotally, students who typically showed little to no involvement in regular classroom activities were much more engaged during this activity and showed large improvements in their concept understanding. Contrary to typical lab exercises in this particular classroom, every student entirely completed the lab worksheet and provided meaningful responses to the synthesis questions.

VII.3.3 Logistical Considerations for Instructors

By far the most important consideration when designing a tablet-based lesson is strategizing the desired time-frame for tablet use to maximize student productivity and minimize off-task behavior. In this study, the students in the first class period to participate in the lesson were given more freedom to finish the lab at their own pace. Only a handful of students stayed on task and completed the calculations and questions in a timely manner, whereas many students did not begin any of the calculations until the iPads were taken away from them. Another problem arose when students were asked to calculate averages for their entire group. When performing calculations as a group, typically one student did all of the work and the others engaged in off-task behaviors. The activity was barely finished by the end of the two-hour class period.

The next two class periods to perform the lesson were given more strict instructions on the lesson timeframe. Groups were given only 20 minutes to complete their data collection on the iPad, then given a discreet timeline on when they should finish the calculations and move on to the questions. Student productivity enhanced greatly with these explicit timelines. Interestingly, students in these class periods moved on to the calculations as soon as they were done collecting data (before the 20 minute time limit). Whereas students in the class without discreet time limits were distracted by holding the iPads when they should have been performing calculations, the students in classes with time limits did not need to have their iPads taken away to make progress on the lab worksheet. The class periods with time restrictions were able to finish the lesson with roughly 20-25 minutes of class-time to spare, and were rewarded with free time to explore the other iPad apps. These two class periods were also instructed to calculate averages of their data

only, rather than group averages. Students appeared to work much more diligently and put more thought into answering questions when asked to do so individually.

With the above considerations in mind, an instructor can take additional steps to minimize off-task use of the iPad. There are a handful of apps that allow the instructor to determine which apps or websites students are able to access. For example, *Guided Access* allows an instructor to allow only one designated app to be used. *Padlock* and *KioskPro* are useful apps for limiting users to specific websites. While these and similar apps can enhance student productivity, they may also help the iPad fit within the restraints of school internet security policies discussed below.

VII.3.4 Considerations and Limitations of the iPad platform

The lesson was originally designed to be carried out completely on the iPad, including the calculations (to be performed and plotted in Excel spreadsheets) and part II of the lesson, exploring the NPZ model. However, constraints of the iPad platform and school internet connectivity guidelines made an entirely tablet-based lesson problematic. First, the high school (and most other schools consulted in the area) would not allow the class to connect to wireless internet on the iPads due to internet security issues. Unfortunately, many iPad features and apps require an internet connection, so we were somewhat limited from the start. Ideally, students would have performed all of their work on the iPad and saved it to an online storage space such as Dropbox or Google Drive shared with their instructor.

Second, the iPad platform is somewhat limiting in itself. For example, the NPZ model, developed by UW researchers, is displayed as a Java applet in a web browser. Internet

connectivity issues aside, the iPad restricts viewing both Java and Adobe Flash based web content. There are ways to get around this limitation by using apps such as *Cloud On*, which access remote servers to view the iPad-restricted content. However, testing of many of these apps was unsuccessful, as they are typically designed for viewing videos rather than interactive websites. Many educational tools that are currently being developed or have already been developed by scientists use these older programming languages. Thus, Apple has severely limited the utility of the iPad in an educational setting by restricting the use of Java and Flash-based web content. A tablet-pc with fewer content and application restrictions, such as Android-based, would have fewer potential drawbacks in the educational setting.

It is important that educators are aware of these types of limitations. More importantly, instructors should design lessons and uses for the tablet that amplify the benefits of the platform rather than highlight its limitations. For example, a great tool that could be used every day in the classroom is *Type on PDF*, which allows students to write on pdf copies of lab worksheets, instructions, readings, and more. There are numerous apps with tremendous utility for classroom management such as *Desire 2 Learn* and *Blackboard*. Apps such as *Dropbox* and *Google Drive* are incredibly useful for sharing files such as projects and homework assignments and are typically integrated into other functional apps.

VII.4 Conclusion

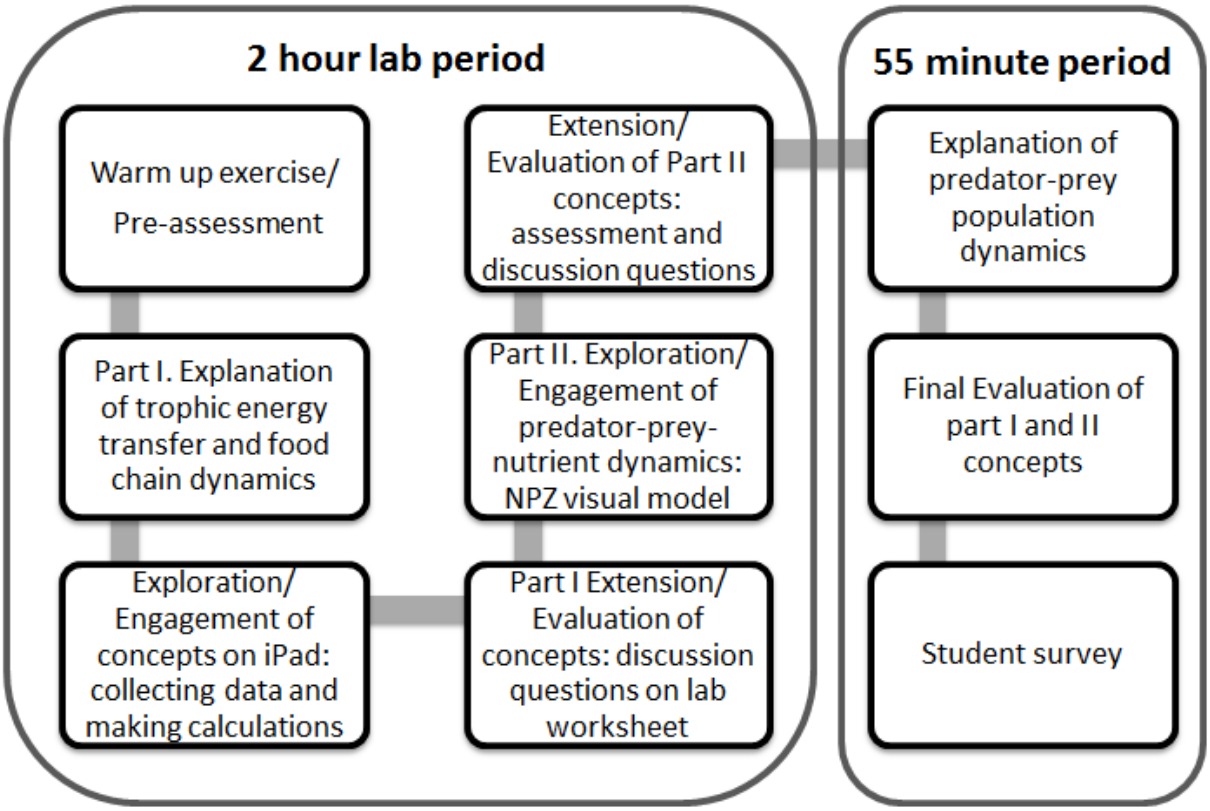
The feedback from students was highly positive with regards to the tablet-based lesson, with most asking when the next iPad lesson would be. Student engagement was anomalously high, especially among those students who typically struggle to participate in regular classroom activities. Here, we took the approach of using a game to collect data and open the floor for

discussion on some basic science concepts. The gaming aspect was highly relatable to most students; however, caution must be taken to ensure that the lesson content is taken seriously using this type of approach. Classroom management is central to a successfully run lesson, regardless of platform. Managing when students have access to the tablets and setting explicit timelines for each part of the lesson proved useful for maximizing student productivity.

Numerous different approaches can be taken to integrate the tablet pc into a K-12 STEM classroom. An institution should assess the different approaches they hope to utilize before purchasing large class sets of tablets. Hardware and software limitations of specific tablet platforms should be considered to determine whether a specific tablet is the right choice for a class set. For example, the camera function can be a nice tool for student projects based on observing visual changes over time or for constructing oral/video presentations. With proper wireless internet connectivity and an appropriate software package, a class set of tablets could also be used to replace desktop computers in the classroom, which are most often used for internet research and word processing. In addition to saving lab counter space, the tablet seems to be more intuitive for the current generation of students. If using tablets as computer replacements, though, it is incredibly important to have an effective theft prevention strategy since they are highly desirable items and much more mobile than a desktop computer.

The utility of a tablet pc in providing novel lessons, such as the one presented here, is mostly limited by the availability of content. There are many free applications such as the game used for this lesson. However, increased development of applications specifically designed for educational purposes would greatly raise the viability of tablet-based education. The lesson presented here used an application built purely for fun as a data generator, but with more

attention to educational applications by software developers, the tablet pc could prove to be a useful source of content in the K-12 STEM classroom.



FigureVII.1 Timeline for a two-part lesson on trophic energy transfer and predator-prey population dynamics. The activity was conducted during a two hour lab period with a debrief and final assessment on the next 55 minute class period.

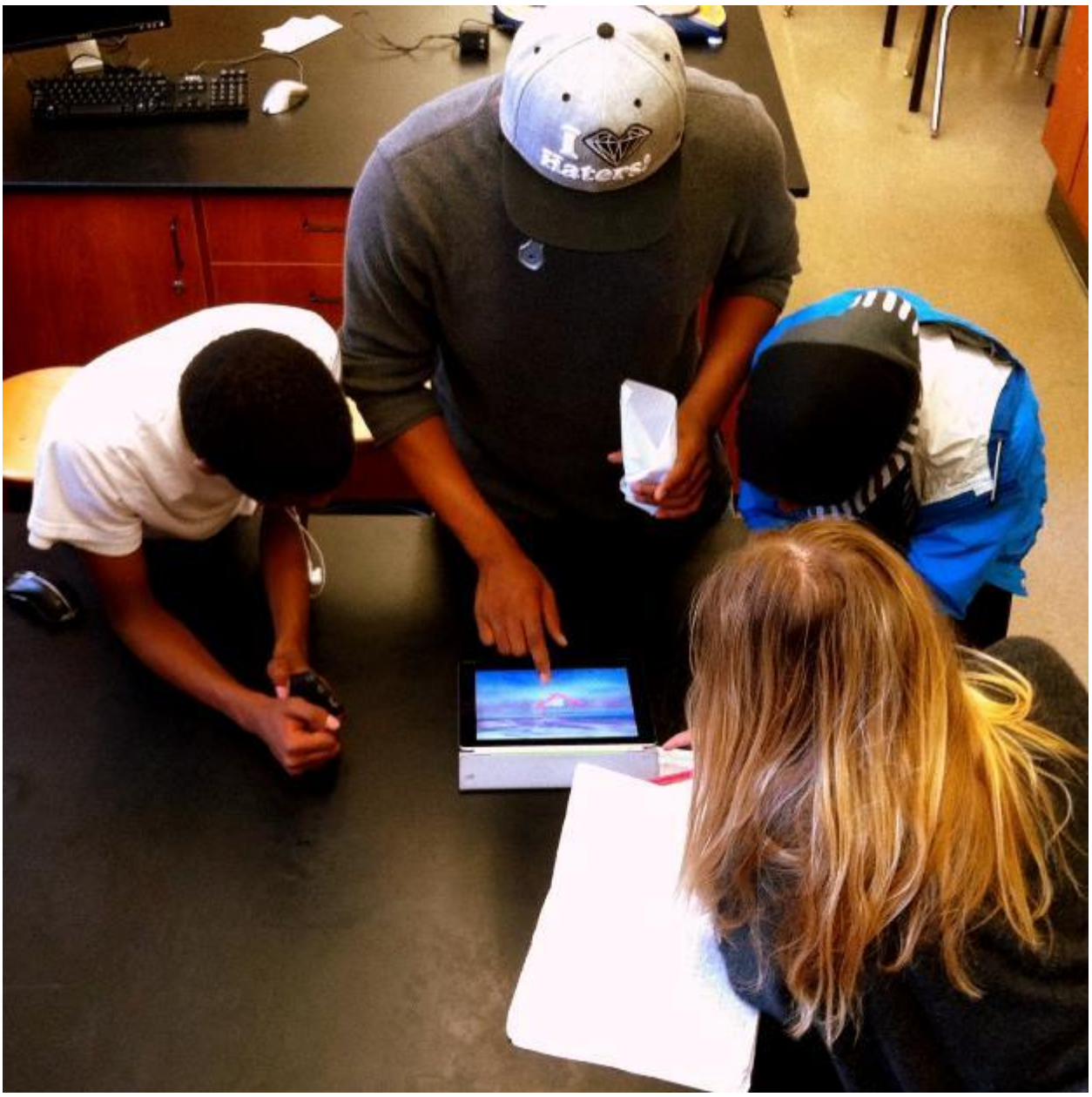


Figure VII.2 Students were given one iPad per group of three to four students.

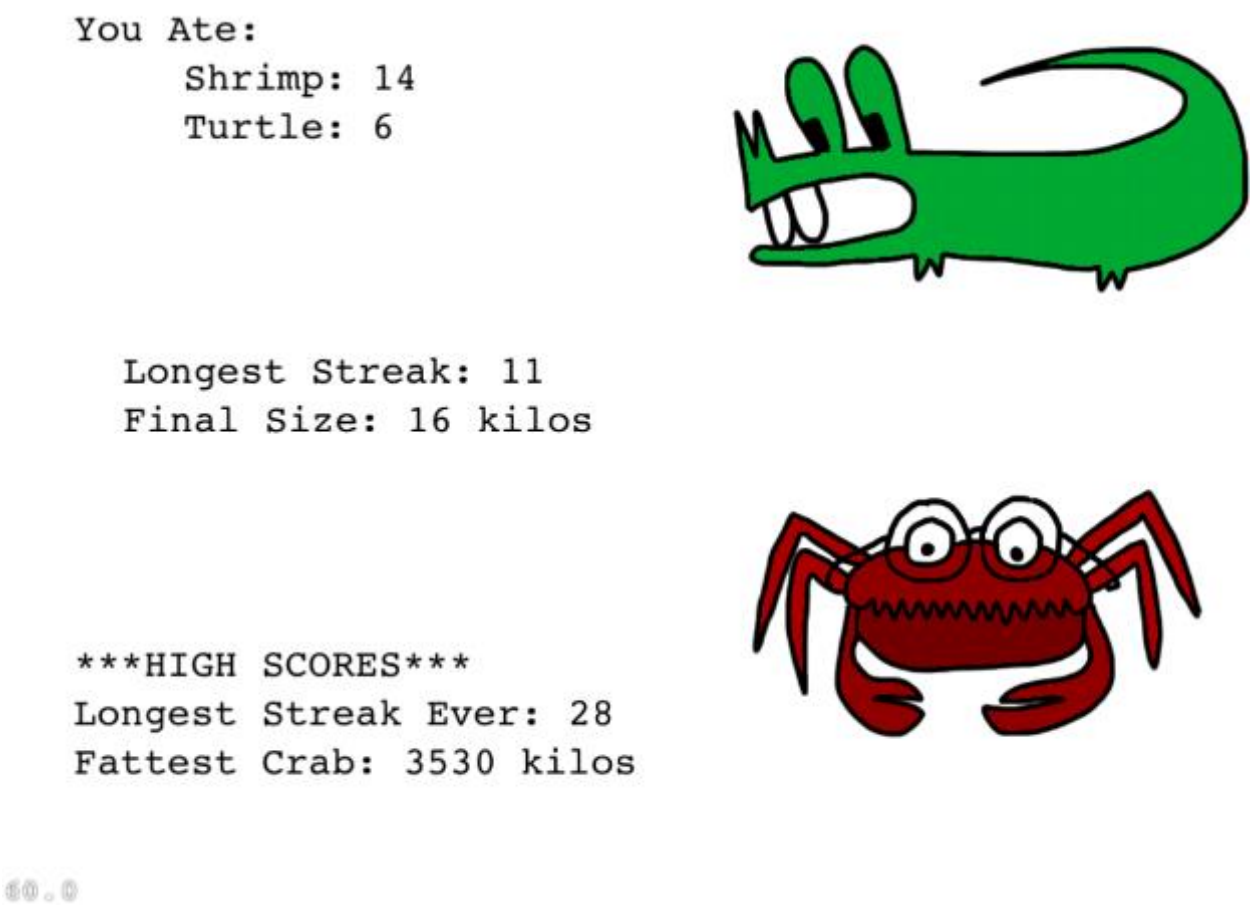


Figure VII.3 During part I of the activity, students collected ecological data while playing the *We All Eat* app.

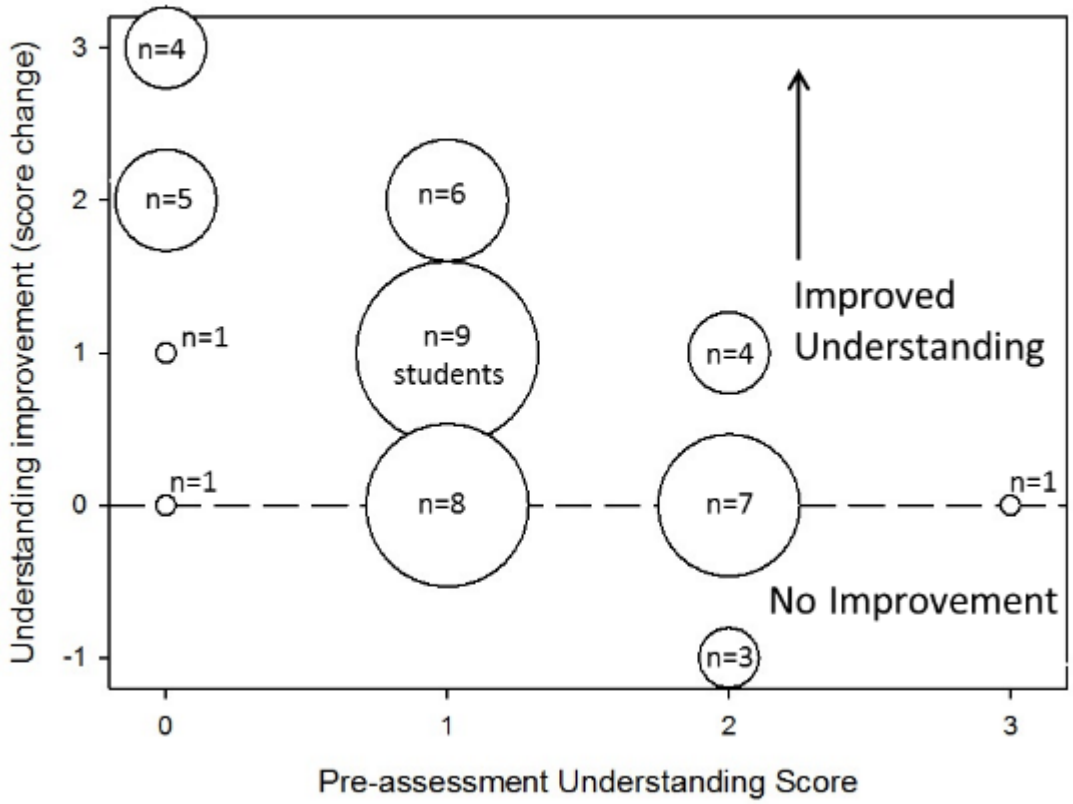


Figure VII.4 The relationship between a student's score on the warm up exercise (pre-assessment) and their improvement in understanding (i.e. difference of post and pre assessment scores). A student's understanding was assessed on a scale from 0-3 (n=49; see TableVIII.1 for rubric scale).

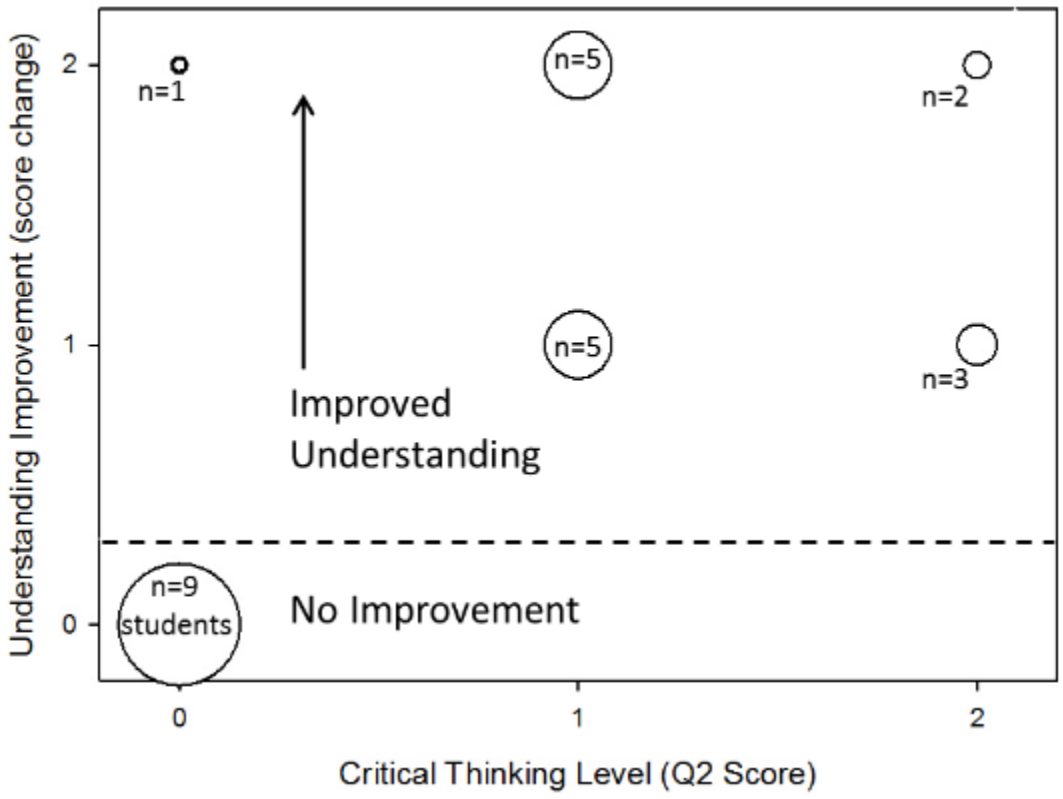


Figure VII.5 The relationship between a student's improvement in understanding (i.e. difference of post and pre assessment scores) and their critical thinking level. Critical thinking levels were determined by a student's ability to rationalize and explain the meaning of "Growth Efficiency," a term that they have never heard before, but were instructed how to calculate during the lesson (n=28; see Table VIII.2 for rubric scale).

Table VII.1 Student score rubric for pre/post assessment

0: Student either made no attempt to answer the question or did not show an understanding that the mass of food consumed does not equal the mass of weight gained by an organism.

1: Student showed a vague understanding that the mass of food consumed does not equal the mass of weight gained by an organism, but is unable to explain why. Student does not mention energy use/storage.

2: Student showed a clear understanding that the mass of food consumed does not equal the mass of weight gained by an organism and that food is utilized by heterotrophs for energy.

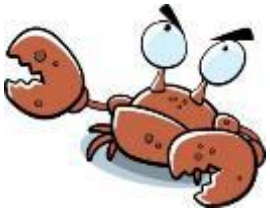
3: Student showed a clear understanding that the mass of food consumed does not equal the mass of weight gained by an organism and that food is utilized by heterotrophs for energy. The student recognized that metabolism/respiration is a sink for energy.

Table VII.2 Student score rubric for critical thinking assessment

0: Student either made no attempt to answer the question or did not display address any of the terms in the Growth Efficiency

1: Student showed a vague understanding that growth efficiency is related to how much biomass an organism fixes relative to the mass of food consumed. Student addressed the terms used to calculate growth efficiency and attempted to rationalize a definition from those terms.

2: Student showed a clear understanding that growth efficiency is related to how much biomass an organism fixes relative to the mass of food consumed and that the difference between biomass consumed and biomass fixed is energy lost to respiration and metabolism. Student was able to rationalize the meaning of growth efficiency by examining the terms used to calculate it.



VII.5 Ipad Food Chain Exploration Lesson Plan

-developed by Nick Ward, UW School of Oceanography

Lesson Length: one 2-hour lab period, and one 55 minute class period for debrief and post assessment.

Learning Goals

Students will collect and record ecological data and reconstruct food chain models based on observations and quantitative data.

Students will understand the link between metabolism and energy loss through trophic cascades and be able to identify top-down and bottom up population controls.

Skills

Data Collection

Models/Data Representation

WA State Standards

9-11 LS2C Population growth is limited by the availability of matter and energy found in resources, the size of the environment, and the presence of competing and/or predatory organisms.

6 Science Strands covered:

Sparking interest and Excitement: Use of the ipad in general and engagement with the We All Eat app

Understanding Scientific Content: Trophic energy transfer and predator/prey population dynamics

Engaging in Scientific Reasoning: Explaining scientific plots

Reflecting on Science: The survey will allow to students to reflect on their own learning process

Using the Tools and Language of Science: Using Scientific models/tools

Identifying with the Scientific Enterprise: Using scientific tools developed locally

Assignments Due

- **Data Collection:** Data collection worksheet

- **Food Chain reconstruction:** InkFlow workbook saved to ipad
- **Synthesis Questions Worksheet:** Completed by the end of the lab period
- **Final Assessment:** Quiz after debrief asking students to analyze a scientific plot

Carry-over from previous lessons

Students will have just completed Owl pellet dissections and an introduction to the unit's central question: *What could have caused the Mouse Plague?*

Technology/Materials

The lesson will be conducted on a class set of (10) ipads in groups of 3-4 students.

Activity Logistics (2 hour Lab period)

Part I. Food Chain and trophic cascade

- Brief introduction to the ipad navigation, the apps that will be used, and the lesson content
- Student Groups start by orienting themselves with the ipad. Then each member of the group will play the *Wealleat app* (1 student plays once, then passes to the next until each student has played 3 times).
- After each turn playing, students record their results on the provided worksheet, performing various calculations.
- Students will reconstruct the food web of the game using the *Ink Flow* app and complete synthesis questions on the worksheet handout.
- Students can freely explore the ipad while waiting for every group to finish part I.

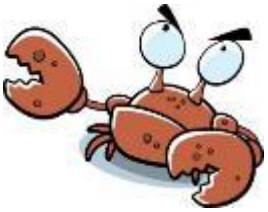
Part II. Population Dynamics

- As a class students navigate to the UW Zooplankton/Phytoplankton Dynamic Model and experiment with adjusting multiple parameters (with guided instructions): http://staff.washington.edu/banasn/
- Students will discuss their observations and answer the part II questions

Further exploration: Students who finish early can answer questions that go into further detail about the model for extra credit.

Debrief/Final Assessment (55 minute period):

Students will review the key concepts in a class discussion, then be given a questionnaire to assess their complete comprehension on the topic in addition to questions such as "on a scale of 0-5 how engaged with this activity were you?" The assessment will ask students to explain a real scientific plot of predator-prey populations.



VII.6 Ipad Food Chain Exploration: Instructions

You will do this activity on the ipad and the handout. Please do not write on this Instruction sheet. You may write your answers on the Questions handout.

Purpose:

Collect, record, and interpret ecological data using the ipad and a predator/prey population model.

Part I. Trophic energy transfer through the food web

Background

As a group, read the following information before starting the lab.

Today you will be using the Ipad to collect and record ecological data. In part I, you will play a game to see how much your crab grows after eating various food sources. You will then draw a diagram of the food chain of the game based on your observations of the **trophic levels**.

Materials and Methods

Each group of 3-4 will share one Ipad. Please answer the questions on the provided handout.

Collecting food chain data

1. Open the "Food Chain Apps" folder on the ipad. Open the "We all eat" app.
2. Each student takes a turn playing the "We all eat" app. After each turn, record your data in the handout (e.g. Weight of crab and # of each animal eaten).

After playing the game ONCE, pass the Ipad to the next student. Each student will play the game a total of 3 times. Be sure to record the data after each run, regardless of your score. No redos ☺

3. After each student has completed the game 3 times, complete the Part I calculations and questions on the handout.
4. After fully completing part I of the handout you are free to explore the different ipad apps. Once all of the groups are done with part I we will work on part II as a class.

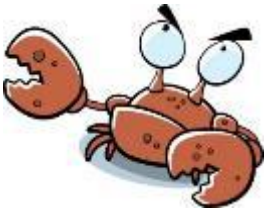
Part II. Predator/Prey Population Dynamics

In Part II, you will use a Zooplankton/Phytoplankton model (developed at UW) to understand the linkage between predator and prey populations. Phytoplankton (P) are marine plants, that use Nutrients (N) and sunlight to grow. Zooplankton (Z) eat phytoplankton for energy. Zooplankton are the predator and

Phytoplankton are the prey. The model also calculates how much Detritus (D) is present. Detritus is made up of dead Phytoplankton and Zooplankton and can be turned back into Nutrients.

1. As a class we will control the Zooplankton (predator) and Phytoplankton (prey) population sizes and observe how the two populations control one another.

2. Answer the Part II questions on the handout



VII.7 Ipad Food Chain Exploration: Questions

Answer these questions after completing the ipad data collection. You may write your answers on this worksheet.

Part I. Trophic energy transfer through the food web

Data Collection. After each run, record how many of each animal you ate and your final crab weight below. After recording your data, pass the ipad to the next student.

	run 1	run 2	run 3
Shrimp eaten			
Turtles eaten			
Gators eaten			
Hippos eaten			
Crab Weight (kg)			

Calculate. Now you will calculate your group's average crab weight and # of animals eaten.

Example calculation: Sally ate 10 shrimp in run 1, 12 shrimp in run 2, and 8 shrimp in run 3.

To calculate the average shrimp eaten by Sally, ***add*** *up* ***all the #s****:* 10+12+8 = 30, *then*

divide *that* ***by the total # of runs***: 30/3 = 10 *shrimp eaten on average*

Group Average Shrimp eaten	

Group Average Turtles eaten	
Group Average Gators eaten	
Group Average Hippos eaten	
Group average Crab Weight (kg)	

Calculate continued…Finally, you will calculate the average weight of food (kg) that your crab ate to determine how efficiently your crab used energy. **Multiply** your group average # of each animal eaten by the weight per animal, then **add** up the **sum** of the total weight eaten.

	Average Weight eaten (kg)
Shrimp eaten_____ x 3 kg/shrimp =	
Turtles eaten_____ x 30 kg/turtle =	
Gators eaten_____x 300 kg/gator =	
Hippos eaten_____ x 3000 kg/hippo =	
Average Total Weight Eaten (kg) =	

To determine how efficiently your crab used the energy stored in its food, calculate your **Growth Efficiency** by **dividing** your group average crab weight by the total weight your crab ate

$$Growth\ Efficiency = \frac{\textbf{Average Crab weight } \_\_\_\_\_(kg)}{\textbf{Average weight eaten } \_\_\_\_\_(\text{kg})} = \_\_\_\_\_$$

Answer Questions 1-6 after completing your We All Eat data collection

1) Why do you think the weight of food eaten is less than the final weight of the crab?

2) In a complete sentence describe what you think "Growth Efficiency" means:

3) If **you** eat 1 pound of food do you gain 1 pound? Why or why not?

4) List 3 ways that your body uses energy:
5) What is one way that your body stores energy?

6) Open the *Ink Flow* app and draw a diagram of the "We all Eat" food chain using the following animals:

Baby Crab (trophic level 1), Medium Crab (trophic Level 2), Giant Crab (trophic Level 3), Godzilla Crab! (trophic Level 4), Gators, Shrimp, Hippos, Squid, Turtles

***Each time the background picture changes, you are on a new "trophic level." If you didn't encounter one of the above animals (e.g. you didn't reach a high enough level) try to guess where it fits in the food chain by process of elimination.

Saving your work

Save your food chain diagram in the Ink Flow app with your period # and at least one group member's name in the filename.

Part II. Predator/Prey Population Dynamics

Answer Questions 7- 11 after observing the Plankton Population Model

7) When the predator population (Z) increases, the prey population (P) _____

Why?

8) When the prey population (P) decreases, the predator population (Z) _____

Why?

9) When the predator population (Z) decreases, the prey population (P) _____

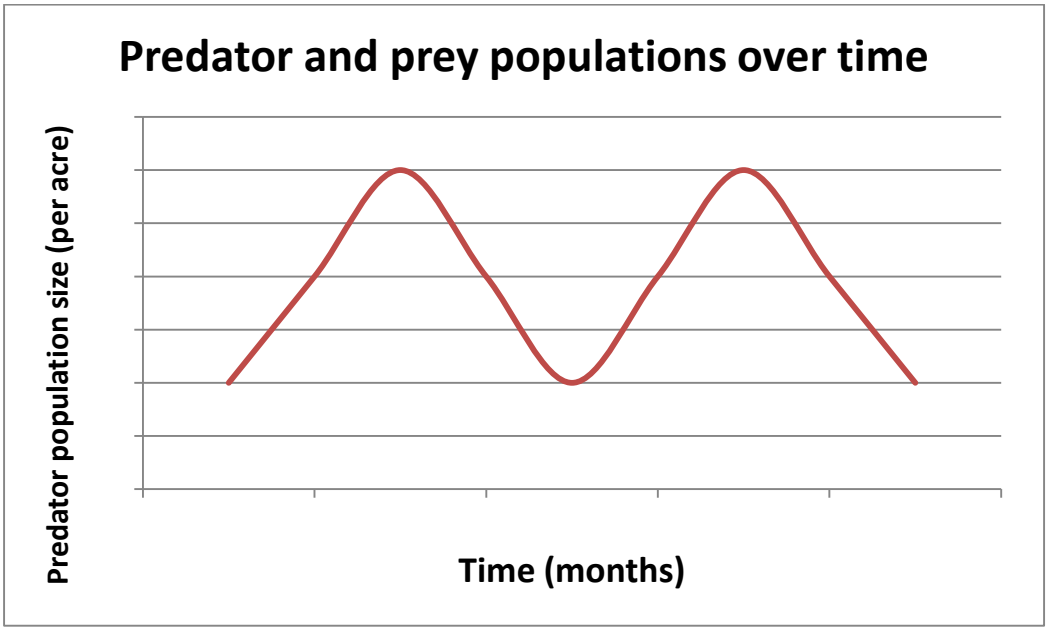
Why?

10) When the population of predators controls the population of prey this is an example of **top-down** or **bottom-up** control (circle one)?

11) When the population of prey controls the population of predators this is an example of **top-down** or **bottom-up** control (circle one)?

12) The graph below shows the population of a hypothetical predator changing over time.

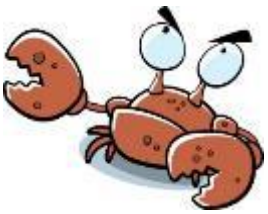
Draw how you think the prey population would change over the same time period on the graph:



Further Exploration . Answering these questions will earn bonus points

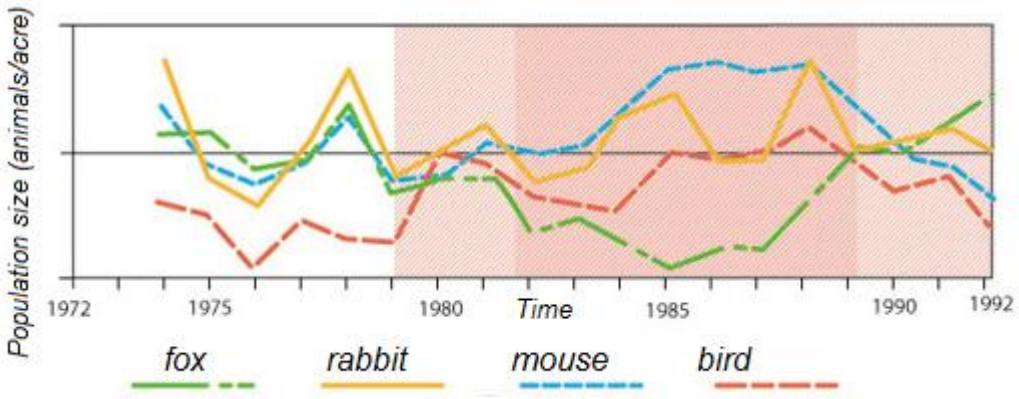
What happens to Zooplankton (Z) and phytoplankton (P) populations when you change the amount of nutrients (N), why?

Detritus (D) can be converted back into nutrients (N). This is called “remineralization.” What happens to the population of phytoplankton (P) when you decrease the sinking rate of detritus, why?



VII.8 Ipad Food Chain Exploration. Synthesis Questions

In complete sentences answer the following questions about the plot below:



1) What do the lines in this plot represent (1 pt)?
2) hat do you think this plot describes (1 pt)?
3) Which animals are predators and which are prey (1 pt)?
4) Why do rabbit, mouse, and bird populations increase from 1983-1987 (2 pts)?
5) Is this an example of **top-down** or **bottom up** population control (circle one) (1pt)?

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