Spatial and temporal variation in stable isotope ratios of primary producers and marine primary consumers in Puget Sound

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Introduction

Organic matter transfer across habitats is a ubiquitous feature of ecosystems (Polis et al. 1997). Because these transfers are critical determinants of the structure and function of recipient ecosystems, research is needed to understand factors that influence the quantity and quality of these resources. For example, stable isotopes (C, N, S, O, H) are often used as tracers to quantify organic matter flows across habitats and to assess ecosystem connectivity. A major assumption with using stable isotopes in this manner is that within an ecosystem or habitat type, the stable isotope ratios of organic matter and the underlying primary producers that contribute to organic matter pools are relatively static in space and time. However, recent studies have shown that primary producer stable isotope ratios within a particular ecosystem are highly variable both spatially and temporally (Finlay 2001, Cloern et al. 2002, Page et al. 2008, Guest et al. 2010, Moore et al. 2011, Dethier et al. 2013). Advances in statistical models to quantify cross-ecosystem linkages allow for this variation in organic matter sources to be incorporated into mixing models (Moore and Semmens 2008, Parnell et al. 2010); yet, the degree of temporal and spatial variability in organic matter (primary producer) sources remains uncharacterized for many systems. Variation in this "isotopic baseline" is not only important to quantify from a food web perspective, but also can be informative with respect to landscape-level differences in biogeochemical and anthropogenic processes (McClelland and Valiela 1998b).

Estuaries are dynamic ecosystems where materials originating in upland portions of watersheds mix with marine waters, and estuarine food webs may be dependent on organic matter from each of these ecosystems (Ruckelshaus et al. 1993, McClelland and Valiela 1998a, Maier and Simenstad 2009, Savage et al. 2012). Stable isotope signatures of filter feeding estuarine consumers will thus reflect both the relative contributions of organic matter sources as well as the underlying spatial and temporal variability within each source. Potential organic matter sources for these estuarine food webs collectively include detritus derived from riverine vegetation (aquatic and terrestrially-derived), salt marsh plants, marine macroalgae and eelgrass, all of which may display variability in isotopic tracers over space and time.

Variation in δ^{13}C ratios of primary producers may be due to photosynthetic processes resulting from fractionation during carbon uptake and assimilation because plants preferentially use the lighter carbon isotope (^{12}C) as well as to variation in δ^{13}C of the pool of available carbon (Farquhar et al. 1989). Terrestrial C_3 plants may contribute significantly to riverine particulate organic matter (POM), particularly in small watersheds (Vannote et al. 1980, Finlay 2001). These plants take up atmospheric carbon through foliar stomata and leaves may vary in δ^{13}C ratios due to a combination of environmental parameters including light, temperature, humidity, soil water, and elevation (Garten and Taylor 1992, Hultine and Marshall 2000). Degree of water loss through transpiration relative to net photosynthesis may also influence δ^{13}C ratios but changes are not consistent across species or systems (Farquhar et al. 1989, Garten and Taylor 1992). For C_3 plants occurring in more saline, tidally influenced environments such as salt marshes, variation in foliar δ^{13}C may be driven by soil salinity and individual plants' response to osmotic stress (Cloern et al. 2002).

Marine macrophyte (seagrasses and macroalage) δ^{13}C ratios are determined by δ^{13}C of dissolved inorganic carbon ($\delta^{13}C_{DIC}$)as well as the relative uptake of dissolved CO_2 and HCO_3, which are influenced by environmental factors such as ambient water temperature, salinity and light intensity as well as by the degree to which carbon concentration mechanisms are used (Simenstad and Wissmar 1985, Grice et al. 1996, Beer et al. 2002, Raven et al. 2002). Other factors such as the degree of ambient respiration and photosynthesis (which affects the quantity of carbon available for uptake by macrophytes) and tidal emersion and subsequent uptake of atmospheric carbon may also be important in driving spatial or temporal variation in macrophyte $\delta^{13}C$ ratios (Clavier et al. 2011).

Variation in $\delta^{15}N$ of primary producers is determined by differences in sources of nitrogen with more enriched values generally indicating more anthropogenically derived nitrogen (Savage 2005, Vander Zanden et al. 2005, Piola et al. 2006). However, the magnitude of the detectable anthropogenic "signal" in primary producers can by highly variable from study to study ranging from up to ~2- 20 ‰ (McClelland and Valiela 1998b, Riera et al. 2000, Savage and Elmgren 2004, Cohen and Fong 2006, Fourqurean et al. 2007). This variation can be complicated by spatial differences in the relative importance of biogeochemical processes such as nitrogen fixation, nitrification and denitrification, all of which can modify the $\delta^{15}N$ of nitrogen available for uptake by primary producers (Mariotti et al. 1984, Kendall et al. 2007). Nevertheless, examining spatial and temporal patterns in $\delta^{15}N$ ratios of primary producers particularly in concert with analysis of freshwater dissolved nitrogen concentrations and $\delta^{15}N$ can provide important insight into the degree to which estuaries are influenced by anthropogenically derived nitrogen (Fry et al. 2003, Cole et al. 2004, Bannon and Roman 2008).

Our objectives were to quantify spatial and temporal variability of $\delta^{13}C$ and $\delta^{15}N$ ratios of primary producers in upland, salt marsh and intertidal environments in Puget Sound, potential freshwater sources of dissolved inorganic nitrogen (DIN) and $\delta^{13}C$ and $\delta^{15}N$ ratios of on estuarine consumer.

Methods

Site descriptions

Our study focused on three catchments (the Samish, Dosewallips and Hamma Hamma rivers) and adjacent shellfish growing areas in Puget Sound (Fig. 1). These watersheds are comparable in size yet have differing land uses, dominant vegetation and geomorphological features. Specifically, the Samish River watershed is more developed, has a lower maximum elevation and a higher proportion of agricultural land use than the two Hood Canal catchments (the Dosewallips and the Hamma Hamma) (Appendix C)(Table 1). The Samish River is also a focus of federal, state and local agencies to reduce fecal coliform pollution (www.skagitcounty.net) for a number of reasons including the economic impact fecal coliform has on oyster production in the Samish River estuary.   Annual nitrogen loadings in each river reflect these land use differences, with much higher loadings in the Samish River than in the Hood Canal rivers (Appendix C) (Mohamedali et al. 2011) (Table 1). Each river forms a delta with the marine waters of Puget Sound, which includes tidally influenced salt marsh as well as a commercially harvested oyster bed (Fig. 1).

Water collection and analysis

Water was collected for POM isotope and dissolved nitrogen analysis in June, August and November from 2-6 locations within each watershed (Fig. 1). Water for POM analysis (8 L) was collected as close to the deepest part of the channel of the stream as possible, transported on ice to the laboratory, and filtered through pre-combusted (450°C for 4 h) Whatman GF/F (0.7µm pore size) glass fiber filters using a low pressure vacuum pump. Up to 3 filters were used from each water grab depending on the degree of accumulation of material on the filter. Filters were placed into acid-washed scintillation vials and frozen at -20°C.   For dissolved inorganic nitrogen (DIN) and nitrate isotopic analysis, water was filtered in the field using hand-held filter syringes with a 0.45µm pore size into 60mL brown plastic HDPE bottles. Water chemistry samples were hand-delivered frozen to the University of Washington Marine Chemistry Lab (MCL). The UW Marine Chemistry Lab uses a modification of the Wood et al. (1967) procedure for the analysis of nitrate and nitrite. Frozen nitrate isotope samples were mailed overnight to the Washington State University Core Isotope Lab, which uses the denitrifier technique (Casciotti et al. 2002, Coplen et al. 2004, Bohlke et al. 2007) to determine the ratios of ^{15}N:^{14}N and ^{18}O:^{17}O using the conversion of NO_3^- to N_2O by the bacteria *Pseudomonas chlororaphis*. Specific standards used were USGS34, USGS35 and IAEANO3. Offshore Puget Sound NO_3 samples were collected opportunistically at depths of 6-20m in June, August, September and December and processed in the same manner as the freshwater samples.

Oyster bed environmental data

Water temperature, salinity and chlorophyll *a* were measured monthly at three locations within each oyster bed at or near high tide using a Seabird CTD cast deployed from a small boat.

Vegetation sampling

To assess the degree to which stable isotope ratios of primary producers varied across our three study sites and across the dry and wet seasons, primary producers that were representative of upland, salt marsh and intertidal ecosystems were collected (Table 2). Because habitat level rather than species level variability was of interest, we grouped primary producer data from each ecosystem (intertidal, salt marsh, upland ecosystems) for all statistical analyses (sensu Cole et al. 2004), data for individual species are reported in Appendix B). Three to five replicates of each species of primary producer were collected in each ecosystem in each watershed in June, late August/early September and November of 2011 (Table 2).

Oyster tissue collection

Six adult oysters (110 mm to 130 mm in length) were haphazardly collected from the primary substratum across three spatially stratified portions (approx. 300 x 300 m) of the shellfish growing area to ensure coverage of the entire bed (18 oysters total per site per sample interval). Collected oysters were placed into clean plastic bags and put on ice immediately until they could be placed in a freezer at -20°C at the end of each sampling day. Oyster mantle tissue, which is more metabolically active than muscle tissue (Fertig et al. 2010), was collected in June, August, November and January (Table 1). Adductor muscle tissue was collected in June, August and January (Table 1).

At Samish Bay, oysters were more patchily distributed and harvest events periodically removed all individuals from a given location. Therefore, to ensure that oysters of the appropriate size could be collected at each sampling location, oysters were relocated into 2m x 2m staked plots in within each sampling strata in August 2011.

Tissue preparation

Adductor muscle and mantle tissue were removed from each oyster, rinsed thoroughly with dilute (10%) hydrochloric acid (HCL) and deionized (DI) water, placed into 50 mL scintillation vials and stored in a minus 20°C freezer. Epiphytes were removed from algal samples by rinsing with DI H_2O and visually inspecting under a dissection microscope to ensure complete removal. Vegetation samples collected from intertidal and salt marsh areas were rinsed with 10% dilute HCl to remove carbonates, followed by a rinse with DI H_2O. All individual samples were lyophilized for 24 h, homogenized to a fine powder with stainless steel scissors and stored in a desiccator. A microbalance was used to weigh 0.5 – 0.8 mg tissue into 5 x 9 mm tin capsules, which were sealed and shipped to Washington State University for analysis. Stable isotopic composition (ratios of ^{13}C: ^{12}C and ^{15}N: ^{14}N) and quantitative elemental composition

(%C, %N) were determined using a Costech ECS 4010 elemental analyzer and a Delta Thermo Finnigan continuous flow mass spectrometer. Delta values are expressed using the standard notation:

$$\text{Heavy Isotope} = \frac{(R_{sample} - R_{standard})}{R_{standard}} \text{ x } 1000$$

where R_{sample} is the ratio of the heavy to light isotope (^{14}C:^{13}C or ^{15}N: ^{14}N) in the sample and $R_{standard}$ is the ratio of ^{14}C:^{13}C or ^{15}N: ^{14}N in Vienna Peedee Belemnite for carbon and atmospheric N for nitrogen. All running standards were calibrated biannually by the Washington State University Isotope Lab to three or more NIST and IAEA certified reference materials

Statistical analyses

To compare primary producer and consumer stable isotope signatures across the three watersheds and across sample intervals, we conducted 2-way ANOVAs using watershed and month as fixed main effects for $\delta^{13}C$ and $\delta^{15}N$ separately (*sensu* Ruesink et al. 2003, Piola et al. 2006, Carlier et al. 2007, Malet et al. 2007, Page et al. 2008). Non-significant interaction terms were removed from the models. Tukey's post-hoc tests were conducted when appropriate. All GLMs were conducted using Systat v. 12. Data were assessed for normality and homogeneity of variances by visually examining probability plots and comparing variances (Quinn and Keough 2002). We also considered both isotopes together, assessing variation in stable isotope signatures across sites and sample intervals using 2 way ANOSIMs and SIMPER analyses (Primer v. 6) to determine the relative contribution of $\delta^{13}C$ and $\delta^{15}N$ in driving differences across watersheds or sampling months.

Results

Freshwater and oyster bed nutrients and POM

Median freshwater dissolved NO_3 concentrations were lower in the Dosewallips and Hamma Hamma rivers than in the Samish River while oyster bed dissolved NO_3 in the Samish River was lower than river NO_3 (Fig. 2). Oyster bed dissolved inorganic nitrogen ($NO_3 + NO_2 + NH_4$= DIN) concentrations were highest in fall and winter, particularly in Samish Bay (Fig. 3d). Mean $\delta^{15}N_{POM}$ in the Samish freshwater (excluding tidally influenced river mouth locations) increased from 1.3‰ in June to 4.0‰ in November, when it was also more variable (Fig. 4a) while mean $\delta^{15}N_{NO3}$ in the Samish was highest in August (5.99‰) and lowest in November (2.9‰) (Fig. 4b). The few (n=5) $\delta^{15}N_{POM}$ samples from the Dosewallips River that were above detection were close to 0‰ in both June and November (Fig. 4b). No $\delta^{15}N_{POM}$ samples from the Hamma Hamma or any $\delta^{15}N_{NO3}$ from Dosewallips or Hamma Hamma were successfully analyzed due to either low accumulation onto filters or low NO_3 concentrations. Dissolved inorganic nitrogen (DIN) concentrations were close to 0 µg/L in both the Hamma Hamma and the Dosewallips rivers while mean concentrations were substantially higher (502 µg/L to 686 µg/L) in the Samish River in all three months (Fig. 4c). The high variability in the Samish River in November was a result of a single sample with a concentration 1,397 µg/L. Mean NO_3 concentrations in the Samish River were very similar to total DIN (485 µg/L to 685µg/L). Offshore Puget Sound NO_3 concentrations ranged from 143 to 330 µg/L and displayed a mean $\delta^{15}N$ of 8.8‰ (Table 3).

$\delta^{15}N$ of upland, salt marsh and intertidal primary producers

Because of our limited success in obtaining freshwater particulate organic matter data in all three watersheds, we limited the extent of our freshwater spatial comparison to upland vegetation. $\delta^{15}N$ of upland vegetation differed across watersheds but not sampling months (Table 4) . Terrestrial plants collected from the Hamma Hamma were less enriched in $\delta^{15}N$ than plants from the Samish (mean difference = 2.18‰) and Dosewallips (mean difference = 1.20‰) (Table 6) (Fig. 5a). δ^{15}N of salt marsh plants changed over time but this change differed by site (significant time × site interaction) (Table 4) such that δ^{15}N of salt marsh plants from the Hamma Hamma and Dosewallips increased between summer and November, whereas Samish Bay decreased slightly from during this same time interval (Fig. 5b). By contrast, δ^{15}N of intertidal macrophytes were similar across sample months but were more enriched in the Samish estuary than the Dosewallips (mean difference = 0.82‰) and Hamma Hamma (mean difference = 1.25‰)(Table 4)(Fig. 5c). Appendix B has data for individual species.

δ^{13}*C of upland, salt marsh and intertidal primary producers*

δ^{13}C of upland vegetation varied across watersheds but not across sample intervals (Table 5)(Fig. 5d). Hamma Hamma and Samish upland vegetation had similar δ^{13}C ratios (mean difference = 0.22 ‰) while Dosewallips upland vegetation δ^{13}C ratios were more deplete than Hamma Hamma (mean difference = 1.43 ‰) and Samish (mean difference = 1.21 ‰), driven in large part by values in August (Table 6) (Fig. 5d). δ^{13}C of salt marsh plants was not significantly different across sites or sample intervals (Fig. 5e) although there was a trend for mean δ^{13}C ratios to be more enriched in Samish Bay than the two Hood Canal sites in June and August (Fig.5e). δ^{13}C of intertidal macrophytes showed a significant effect of both sampling month and watershed (Table 5) (Fig. 5f). Samish Bay was more enriched than both Dosewallips (mean difference = 2.24‰) and Hamma Hamma (mean difference = 3.68‰) (Table 5), particularly in June (Fig.5f). Marine macrophyte δ^{13}C was more also more deplete in June than in August ($p = 0.009$) (Fig. 5f). Appendix B has data for individual species.

δ^{15}*N and* δ^{13}*C of primary consumers*

δ^{15}N of oyster adductor and muscle tissue varied across watershed and sample intervals such that in June and January, Samish Bay oysters displayed more enriched δ^{15}N values (mean differences (Fig.6a,c). While δ^{13}C in oyster tissues also displayed significant watershed × time interactions, there was a clear difference across the sites. Specifically, Samish Bay oysters were Dosewallips and Hamma Hamma for both mantle (Samish vs. Dosewallips mean difference: 2.64‰; Samish vs. Hamma Hamma mean difference 3.14‰) and adductor muscle tissues (Samish vs. Dosewallips mean difference: 2.64‰; Samish vs. Hamma Hamma mean difference: 3.18‰) (Fig. 6b,d). The interaction appeared to be driven by a decrease in δ^{13}C from August to November in oysters from Hood Canal sites that was not observed in the Samish (Fig. 6b,d).

Combined δ^{15}N and δ^{13}C analysis of primary producers and primary consumers

When both isotopes were analyzed together using multidimensional scaling, upland vegetation varied across watersheds but not sample intervals (Table 7; Fig. 7a). Differences in carbon isotopic ratios in Dosewallips upland vegetation were largely responsible for the separation from upland vegetation from Hamma Hamma and Samish rivers, while carbon and nitrogen were similarly responsible for determining isotopic differences between upland plants from the Dosewallips and Samish. By contrast, nitrogen isotope ratios were responsible for separating upland vegetation from the Samish and Hamma Hamma (Table 8). Salt marsh plants stable isotope ratios differed across sample intervals as well as watersheds (Table 7, Fig. 7b) such that salt marsh plants from the Dosewallips exhibited distinct isotopic ratios than the Samish and Hamma Hamma. All site differences in salt marsh plant stable isotopic ratios were mostly a result of nitrogen, particularly for the contrast between Dosewallips and Hamma Hamma (Table 8). For intertidal macrophytes, there was also a significant effect of both watershed and sampling month (Table 7, Fig. 7c). Samish macrophytes were different from both

Dosewallips and Hamma Hamma and SIMPER revealed that these differences were mostly due to carbon (Table 8).

Oyster mantle and adductor tissue were different across sites and sample intervals when both isotopes were considered together (Table 7, Fig. 8). For both tissue types, all three sites were different from one another: differences between Samish Bay and Hood Canal oyster tissue stable isotope ratios were a result of carbon whereas the difference between the two Hood Canal site oysters was due to both carbon and nitrogen (Table 8).

Discussion

We observed spatial and or temporal variation in the nearly all terrestrial and estuarine primary producers collected from three watersheds in Puget Sound with contrasting watershed and land use characteristics. In addition, some of this variation was reflected in stable isotopic ratios of a commercially and economically important consumer, the Pacific oyster. δ^{13}C and δ^{15}N isotope ratios in primary producers may be influenced by abiotic, biotic and anthropogenic factors, creating a heterogeneous patchwork of carbon acquisition mechanisms and nitrogen sources and transformations. Stable isotope ratios of primary consumers partially reflects spatial or temporal variability observed in primary producers, indicating the importance of quantifying the stable isotopic baseline for food web studies. Our results shed some light on these sources of isotopic variation.

Exported riverine particulate organic matter is a combination of terrestrially derived detritus and freshwater algae. The proportion of each of these organic matter (allochthonous and autochthonous) types varies from headwater to estuary such lower portions of a given watershed will receive more direct sunlight and thus be dominated by in-stream or algal production (i.e., autochthonous) (Vannote et al. 1980, Finlay 2001). We also might assume among-watershed variation in the relative importance of organic matter sources due to watershed size, land use, geographic location.. Unfortunately, we were unable to obtain sufficient freshwater POM isotope data to permit comparing across our study watersheds. Instead, we compared the isotopic signatures of the dominant terrestrial vegetation (red alder and willow in the Samish River and red alder and Douglas' fir in the Dosewallips and Hamma Hamma rivers). Despite the elevation and vegetation differences between the Samish River and Hood Canal sites (the Dosewallips and the Hamma Hamma), the main among-site difference in foliar $\delta^{13}C$ ratios was the decline in August in the Dosewallips River that was not observed at the other two sites (Fig. 5). In river systems, network location and river geomorphology (e.g., fast vs. slow water environments) are important factors determining $\delta^{13}C$ of freshwater algae, while in terrestrial habitats factors such as light availability, temperature, humidity, soil water content and water loss through evapotranspiration can affect foliar $\delta^{13}C$ ratios (Garten and Taylor 1992). The same species were sampled at the two Hood Canal watersheds and samples were collected at comparable elevations over time within each watershed, so it is unlikely that watershed-scale differences in environmental conditions (e.g., light, temperature) were responsible for variation in foliar $\delta^{13}C$ ratios we observed (Hultine and Marshall 2000). However, there may have been unmeasured microsite variation in these conditions that contributed to the decline in foliar $\delta^{13}C$ at the Dosewallips.

$\delta^{15}N$ ratios of upland vegetation potentially reflect soil nitrogen cycling, atmospheric deposition, anthropogenic nitrogen in the form of synthetic fertilizers or manure well as the degree to which nitrogen fixation occurs (Helfield and Naiman 2002, Kendall et al. 2007, Burns et al. 2009, Diebel and Vander Zanden 2009). Because both nitrogen fixation and synthetic fertilizers can result in $\delta^{15}N$ ratios of close to 0 ‰, without the use of a second tracer such as oxygen (see discussion below), the specific sources of nitrogen leading to similar upland vegetation foliar δ^{15}N values between the Samish and Dosewallips rivers in August and November cannot be determined from this study. However, given the differences in land use and nitrogen loading between these catchments, it is possible that nitrogen fixation plays more a important role in contributing to soil nitrogen loads in Hood Canal than in the Samish river relative to other sources (Steinberg et al. 2011). The somewhat higher δ^{15}N in June in the Samish River foliar δ^{15}N could be a result of seasonal changes in crop fertilization (either synthetic or organic) or cattle penning.

Salt marshes are thought of as nitrogen sinks due to denitrification and nitrogen recycling through organic matter deposition (Page 1995). Since both of these processes result in nitrogen enrichment, salt marsh plants typically have elevated δ^{15}N ratios relative to other terrestrial plants (Cloern et al. 2002). The highest δ^{15}N ratios were observed in salt marsh plants (9.5‰) from the Samish River estuary and seasonal variation in salt marsh foliar δ^{15}N also differed among the study sites; both of these patterns suggest that processes governing the δ^{15}N ratios of primary producers were variable in both space and time. Because the Samish River foliar δ^{15}N of both upland and salt marsh vegetation was higher in June relative to August and November, it is possible that the same process (e.g., changes in the location of cattle or fertilization practices) affected temporal variation in both types of primary producers. The sharp increase in foliar δ^{15}N of salt marsh plants in the Dosewallips could be due to shifts towards more anoxic conditions that favor denitrification or an increase in anthropogenic nitrogen as a result of rain-driven runoff in the fall months. The increase in oyster bed DIN in the Dosewallips River oyster bed (Fig. 3d) is consistent with the latter hypothesis, however the degree to which individual salt marsh beds receive nitrogen from terrestrial, marine or recycled estuarine sources cannot be determined from this study since the salt marsh ecotone can be regularly inundated by both river and marine water. Inundation of individual salt marshes from fluvial relative to marine sources is likely driven by geomorphology of each river delta.

We observed more enriched δ^{15}N ratios of intertidal macrophytes in Samish Bay, consistent with the observed positive relationship between elevated nitrogen watershed loading and elevated δ^{15}N in estuarine primary producers observed in other systems (Fry et al. 2003, Savage and Elmgren 2004). Like salt marsh plants, intertidal macrophytes in our study have contact with water of both riverine and marine origins, yet as marine primary producers they occur at lower tidal elevations than salt marsh plants and thus are more affected by marine influences. Despite this, variation in δ^{15}N in estuarine macrophytes has been linked to variation in land use and anthropogenic influences in watersheds, particularly in cases when nitrogen loadings are high (McClelland and Valiela 1998b, Riera et al. 2000, Costanzo et al. 2001, Savage and Elmgren 2004, Bannon and Roman 2008). However, the difference in δ^{15}N of intertidal macrophytes between Hood Canal (mean δ^{15}N ~ 7 ‰) and Samish Bay (mean δ^{15}N ~8 ‰) was relatively small, especially in light of the large differences in watershed nitrogen loadings. Previous studies suggest that the δ^{15}N of intertidal macrophytes that are not affected by anthropogenic nitrogen are somewhat lower (4-6 ‰) than what we observed (Riera et al. 2000, Savage and Elmgren 2004), although these estimates are linked to ambient marine δ^{15}N, which may be quite variable. Published values for δ^{15}N of anthropogenically impacted intertidal macrophytes are highly variable, ranging from 8 to 26‰ (McClelland and Valiela 1998b, Riera et al. 2000, Costanzo et al. 2001, Savage and Elmgren 2004, Bannon and Roman 2008). A recent study of subtidal macrophytes in the San Juan Islands reported δ^{15}N ratios of ~4-7‰ (Dethier et al. 2013).

The somewhat elevated $\delta^{15}N$ in Hood Canal intertidal macrophytes may be due the importance of marine derived nitrogen, which can range from 4-15‰ (Casciotti et al. 2002, Kendall et al. 2007, Wankel et al. 2009) or from the transport of enriched nitrogen from other watersheds such as the Skokomish River, which has estimated nitrogen loadings that are intermediate between those of the two Hood Canal rivers we sampled (Dosewallips and Hamma Hamma) and the Samish (Appendix C). Given the high degree of connectivity between the Skokomish and the Dosewallips and Hamma Hamma rivers (Banas et al. *in prep*), hydrologic transport of nutrients from locations with higher watershed nitrogen loadings may partially explain the somewhat higher than expected $\delta^{15}N$ in the Hood Canal intertidal macrophytes. However, it is also well established that marine derived nitrogen comprises a significant portion of the nitrogen in Hood Canal (Mohamedali et al. 2011, Steinberg et al. 2011), thus it is possible that both marine nitrogen and watershed nitrogen contributed to the observed $\delta^{15}N$ values. Furthermore, Banas et al.(*in prep*) found that most of the nearshore water in Hood Canal is of marine origin.   While nitrogen loading in the Samish watershed was high, $\delta^{15}N\text{-}NO_3$ did not approach the very high values typically associated with fecal material (~20-40 ‰)(Riera et al. 2000, Savage and Elmgren 2004, Kendall et al. 2007), which suggests that multiple, differing sources of nitrogen may be important in contributing to the pool of watershed nitrogen (e.g., synthetic fertilizers and manure)(Appendix A1). Our efforts to quantify the marine $\delta^{15}N\text{-}NO_3$ signal in Puget Sound were generally within the accepted range of marine $\delta^{15}N\text{-}NO_3$(Casciotti et al. 2002, Kendall et al. 2007, Wankel et al. 2009). Because we did not travel outside of the Sound to obtain these samples, however, they likely represent water that is more marine influenced than nearshore oyster beds but still a mixture of both terrestrial and marine derived nitrogen. Use of a second tracer (oxygen) did not aid in distinguishing among nitrogen sources (Appendix A1).

Perhaps because of the small among-watershed difference in δ^{15}N of primary producers, δ^{15}N of the benthic primary consumer *C. gigas* tissue did not vary greatly among the study sites with the exception of elevated mantle tissue δ^{15}N ratios in the Samish and Dosewallips which remained high in the Samish but declined over time in the Dosewallips (Fig. 6a,c). This pattern is consistent with the temporal pattern observed in salt marsh plants in the Dosewallips and intertidal macrophytes at both sites (Fig. 5 b,c), suggesting connectivity between the intertidal and salt marsh zones. Adductor tissue, which is less metabolically active and thus likely to have slower turnover, was less variable among the sites although Samish oysters displayed a slight increase in δ^{15}N in adductor tissue in November.

While spatial variation δ^{15}N in intertidal macrophytes was somewhat subtle, spatial variation in δ^{13}C was more pronounced, a finding that was mirrored in the δ^{13}C of *C. gigas* and likely contributed to carbon being the primary driver of dual isotopic differences between the Hood Canal and Samish watersheds. The more depleted δ^{13}C ratios in both intertidal macrophytes and *C. gigas* in Hood Canal could be driven by variation in oyster bed salinity, which was lower in Hood Canal in June, perhaps indicative of summer snowmelt since water salinity can affect $\delta^{13}C_{DIC}$ available for update by primary producers (Simenstad and Wissmar 1985). The pattern of much more enriched δ^{13}C in the Samish Bay was also observed within a single species, *Ulva*, so was likely not due to variation in species composition of macrophytes among the sites (Appendix B). Ruckelshaus et al. (1993) observed macrophytes that ranged from -9 to -14 ‰ in Padilla Bay, WA, which is adjacent to Samish Bay while Dethier et al.(2013) found δ^{13}C that were generally comparable to our observations in Hood Canal (~11 to 18 ‰). While variation in tidal immersion can contribute to variation in $\delta^{13}C$ between subtidal and intertidal macrophytes (Clavier et al. 2011), in our study, macrophytes collected were all intertidally collected so degree of water immersion was likely not the cause of this spatial variation. Very enriched values of $\delta^{13}C$ in macrophytes may suggest a stronger reliance on HCO_3 relative to direct uptake of CO_2 (Raven et al. 2002) in the Samish Bay, which would suggest differences in nearshore carbon chemistry. Local hydrology may also play a role in determining macrophyte $\delta^{13}C$ since water movement affects the boundary layer through which dissolved carbon must diffuse prior to uptake. Faster moving water has a smaller boundary layer and thus potentially permits more macrophyte discrimination between ^{13}C and ^{12}C, resulting in more depleted values where currents are stronger. This may be in part be contributing to the spatial variation we observed since Samish Bay has a higher water retention time than individual river deltas in Hood Canal (Sutherland et al. 2011).

This study underscores the importance of quantifying the isotopic baseline for marine food webs, demonstrating that spatial variation in primary producers is more the rule than the exception, even within are relatively constrained area such as Puget Sound. This variation appears to be driven by a suite of factors that includes watershed land use and land cover, nearshore oceanography and environmental variables such as salinity.

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Table 1. Watershed characteristics

		Samish	Dosewallips	Hamma Hamma
	Area[1] (Km^2)	294	301	218
	Flow[2] (m^3/s)	7	14	14
	Max elevation (m)	1307	2368	2083
Land Use and Land Cover[1]	% Developed	10	1	1
	% Agriculture	15	0	0
	% Natural	75	99	99
	% Deciduous mixed forest	28	2	1
	Simulated nitrogen load (kg/day)[3]	905-955	150-152	113-115

[1] CCAP (Appendix C) [2] USGS [3] Appendix C

Table 2. Species and time intervals of sample collection where S=Samish Bay, D=Dosewallips and H= Hamma Hamma.

	June	**August**	**November**	**January**
Intertidal Macrophytes				
Zostera spp	SDH	SDH	SDH	
Ulva spp	SDH	SDH	SDH	
Gracilaria /Gracilariopsis		DH	H	
Fucus gardneri	DH	D	D	
Laminariales	S	S		
Salt marsh plants				
Salicornia virginica	SDH	SDH	SDH	
Glaux maritima	SD	SD		
Upland Vegetation				
Alnus rubra	SDH	SDH	SDH	
Salix spp	S	S		
Pseudotsuga menziesii	DH	DH	H	
Crassostrea gigas				
Mantle tissue	SDH	SDH	SDH	SDH
Adductor muscle tissue	SDH	SDH		SDH

Table 3. Puget Sound offshore NO_3 concentrations and $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$

	NO_3 (µg/L)	$\delta^{15}N_{NO_3}$ Mean (‰)	$\delta^{15}N_{NO_3}$ SD	$\delta^{18}O_{NO_3}$ Mean (‰)	$\delta^{18}O_{NO_3}$ SD	N
Port Townsend dock Aug	330.16	13.12	----	1.53	----	1
Samish offshore June	220.31	7.91	0.93	5.00	1.00	3
Samish offshore Sep	284.61	14.13	-----	4.71	----	1
Rosario offshore Dec	143.30	6.46	0.60	1.79	0.37	3
Total	213.20	8.80	3.12	3.33	1.81	8

Table 4. Two-way ANOVAs on δ^{15}N of upland vegetation, salt marsh plants, intertidal macrophytes and oyster mantle and adductor tissue across the Samish, Dosewallips and Hamma Hamma watersheds and the months of June, August and November

δ^{15}N	**Source**	**Type III SS**	**df**	**MS**	**F-ratio**	**p-value**
Upland	Watershed	53.81	2	26.91	9.06	**0.00**
	Month	2.81	2	1.41	0.47	0.63
	Watershed * Month	12.84	4	3.21	1.09	0.37
Salt Marsh	Watershed	30.56	2	15.28	11.34	**0.00**
	Month	16.97	2	8.49	6.30	**0.00**
	Watershed * Month	25.43	4	6.36	4.72	**0.00**
Intertidal	Watershed	28.73	2	14.37	19.42	**0.00**
	Month	0.85	2	0.42	0.57	0.57
	Watershed * Month	5.17	4	1.29	1.80	0.13
Oyster adductor	Watershed	5.46	2	2.73	8.75	**0.00**
	Month	1.69	2	0.85	2.71	**0.07**
	Watershed * Month	3.21	4	0.80	2.57	**0.04**
Oyster mantle	Watershed	341.26	2	170.63	387.35	**0.00**
	Month	66.55	3	22.18	50.36	**0.00**
	Watershed * Month	34.93	6	5.82	13.22	**0.00**

Table 5. Two-way ANOVAs on δ^{13}C of upland vegetation, salt marsh plants, intertidal macrophytes and oyster mantle and adductor tissue across the Samish, Dosewallips and Hamma Hamma watersheds and the months of June, August and November

δ^{13}C	Source	Type III SS	df	MS	F-ratio	p-value
Upland	Watershed	28.19	2	14.09	4.53	**0.01**
	Month	14.47	2	7.24	2.32	0.11
	Watershed Month	22.06	4	5.51	1.86	0.13
Salt Marsh	Watershed	8.38	2	4.19	2.14	0.13
	Month	5.42	2	2.71	1.39	0.26
	Watershed Month	3.20	4	0.80	0.39	0.82
Intertidal	Watershed	244.65	2	122.33	13.76	**0.00**
	Month	80.23	2	40.11	4.51	**0.01**
	Watershed Month	39.40	4	9.85	1.11	0.35
Oyster adductor	Watershed	252.64	2	126.32	330.29	**0.00**
	Month	9.52	2	4.76	12.44	**0.00**
	Watershed Month	4.92	4	1.23	3.22	**0.01**
Oyster mantle	Watershed	14.48	2	7.24	27.06	**0.00**
	Month	2.39	3	0.80	2.98	**0.03**
	Watershed * Month	7.36	6	1.23	4.59	**0.00**

Table 6. Tukey's post hoc tests comparing watersheds for models with non-significant interaction terms

		Samish, Dosewallips	Samish, Hamma Hamma	Dosewallips, Hamma Hamma
Upland	$\delta^{15}N$	0.14	**0.00**	**0.05**
	$\delta^{13}C$	0.06	0.91	**0.02**
Marsh	$\delta^{15}N$	na	na	na
	$\delta^{13}C$	na	na	na
Intertidal	$\delta^{15}N$	**0.00**	**0.00**	0.11
	$\delta^{13}C$	**0.00**	**0.00**	0.12

Table 7.Results of two-way ANOSIM tests across upland vegetation, marsh plants, intertidal macrophytes and oyster tissues across the Samish, Dosewallips and Hamma Hamma watersheds and the months of June, August and November.

		R	p
Upland	Watershed	0.14	**0.003**
	Month	0.017	0.306
Marsh	Watershed	0.297	**0.001**
	Month	0.143	**0.031**
Intertidal	Watershed	0.171	**0.001**
	Month	0.108	**0.003**
Oyster mantle	Watershed	0.575	**0.001**
	Month	0.306	**0.001**
Oyster adductor	Watershed	0.616	**0.001**
	Month	0.182	**0.001**

Table 8. Results of the SIMPER test assessing the degree to which carbon and nitrogen drove differences between pairs of sites

	Comparison	R	p	SIMPER %
Upland	Samish, Dosewallips	0.15	**0.01**	51.8 N
	Samish, Hamma Hamma	0.12	**0.05**	73.42 N
	Dosewallips, Hamma Hamma	0.17	**0.01**	70.36 N
Marsh	Samish, Dosewallips	0.49	**0.00**	68.1 N
	Samish, Hamma Hamma	0.09	0.17	56.68 N
	Dosewallips, Hamma Hamma	0.16	**0.08**	76.22 N
Intertidal	Samish, Dosewallips	0.17	**0.00**	89.87 C
	Samish, Hamma Hamma	0.34	**0.00**	90.02 C
	Dosewallips, Hamma Hamma	0.06	**0.11**	93.73 N
Oyster mantle	Samish, Dosewallips	0.728	**0.001**	90.97 C
	Samish, Hamma Hamma	0.924	**0.001**	90.82 C
	Dosewallips, Hamma Hamma	0.119	**0.001**	63.31 C
Oyster adductor	Samish, Dosewallips	0.799	**0.001**	89.6 C
	Samish, Hamma Hamma	0.921	**0.001**	93.36 C
	Dosewallips, Hamma Hamma	0.175	**0.001**	57.73 C

Figure Legends

Figure 1. Map of tissue and water collection locations in Puget Sound (a) with enlargements depicting specific sampling locations in oyster beds (blue), rivers (green) and salt marshes (orange) the Samish River and Bay (b), and the Dosewalllips and Hamma Hamma Rivers (c).

Figure 2. Median nitrate (NO_3) concentrations (ug/L) in the Samish, Dosewallips and Hamma Hamma Rivers and adjacent oyster beds from samples collected in June, August and November 2011.

Figure 3. Monthly mean (+/- SD) Salinity (a), temperature (b), chlorophyll a (c) and dissolved inorganic nitrogen (DIN)(nitrate+nitrite+ammonium) (DIN) in oyster beds in the Samish (red squares), Dosewallips (blue circles) and Hamma Hamma (green triangles)

Figure 4. Mean (±SD) of δ^{15}N-POM (a), δ^{15}N-NO_3 (b) and dissolved inorganic nitrogen (nitrate+nitrite+ammonium) (DIN) in freshwater from the Samish (red), Dosewallips (blue) and Hamma Hamma (green) watersheds (not including river mouths) in June, August and November.

Figure 5. Mean ± SE of δ^{15}N of upland vegetation (a), salt marsh plants (b), intertidal macrophytes (c) and δ^{13}C of upland vegetation (d), salt marsh plants (e), and intertidal macrophytes (f) in the Samish (red solid), Dosewallips (blue dashed), and Hamma Hamma (green dotted) growing areas in June, August and November 2011.

Figure 6. Mean ± SE of δ^{15}N and δ^{13}C of *C. gigas* mantle (a,b) and adductor muscle (c,d) tissues over time in oyster bed adjacent to the Samish (red solid), Dosewallips (blue dashed) and Hamma Hamma (green dotted) watersheds.

Figure 7. δ^{13}C and δ^{15}N ratios of upland vegetation (a), salt marsh plants (b) and intertidal macrophytes (c) in the Samish (red upwards triangles), Dosewallips (blue downwards triangles) and Hamma Hamma (green squares) watersheds and oyster beds in June (1) and August (2), November (3).

Figure 8. δ^{15}N and δ^{13}C ratios of oyster mantle tissue (a), and adductor muscle tissue (b) for the Samish (red), Dosewallips (blue) and Hamma Hamma (green) shellfish growing areas in June (1), August (2), November (3) and January (4).

Figure 1.

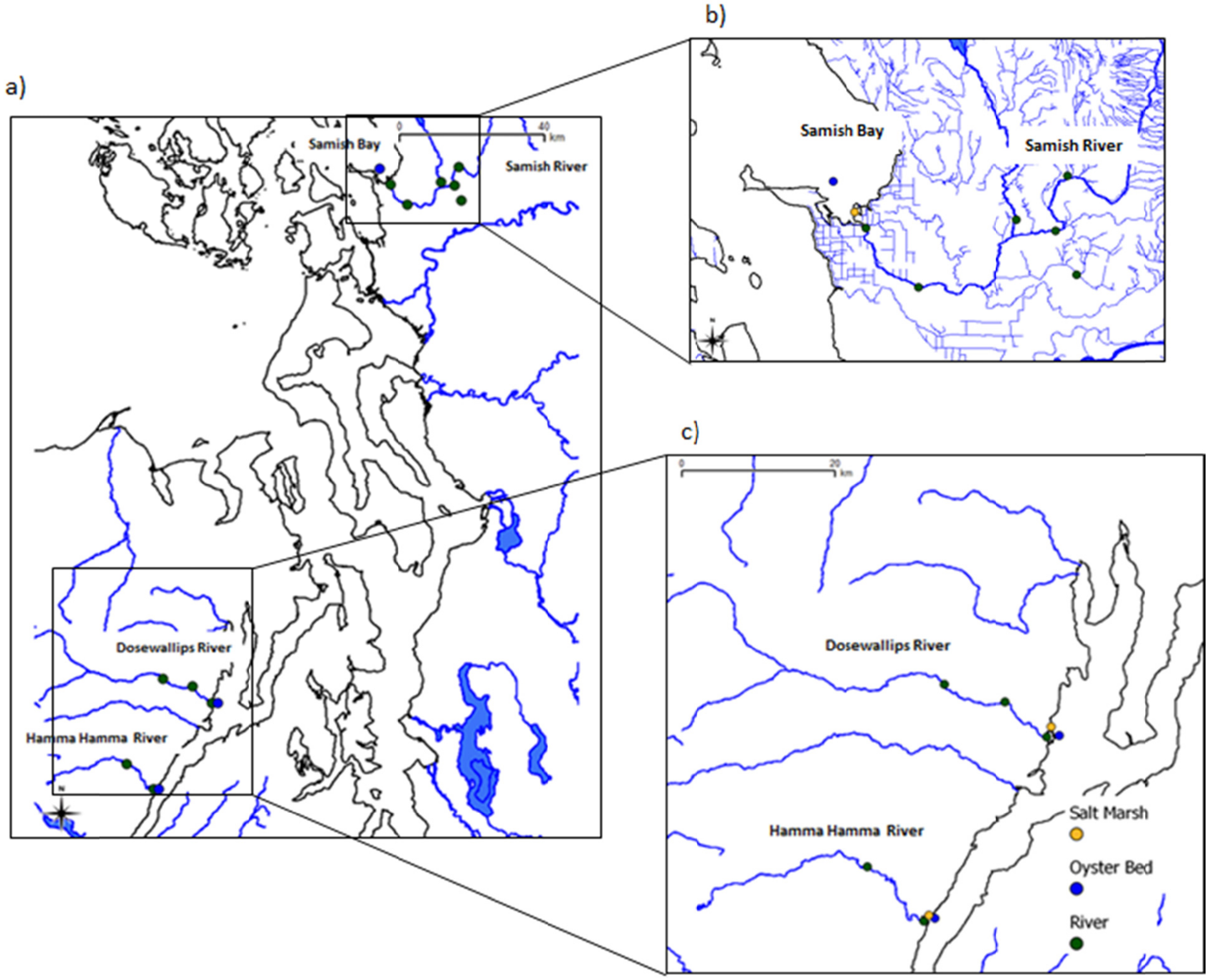
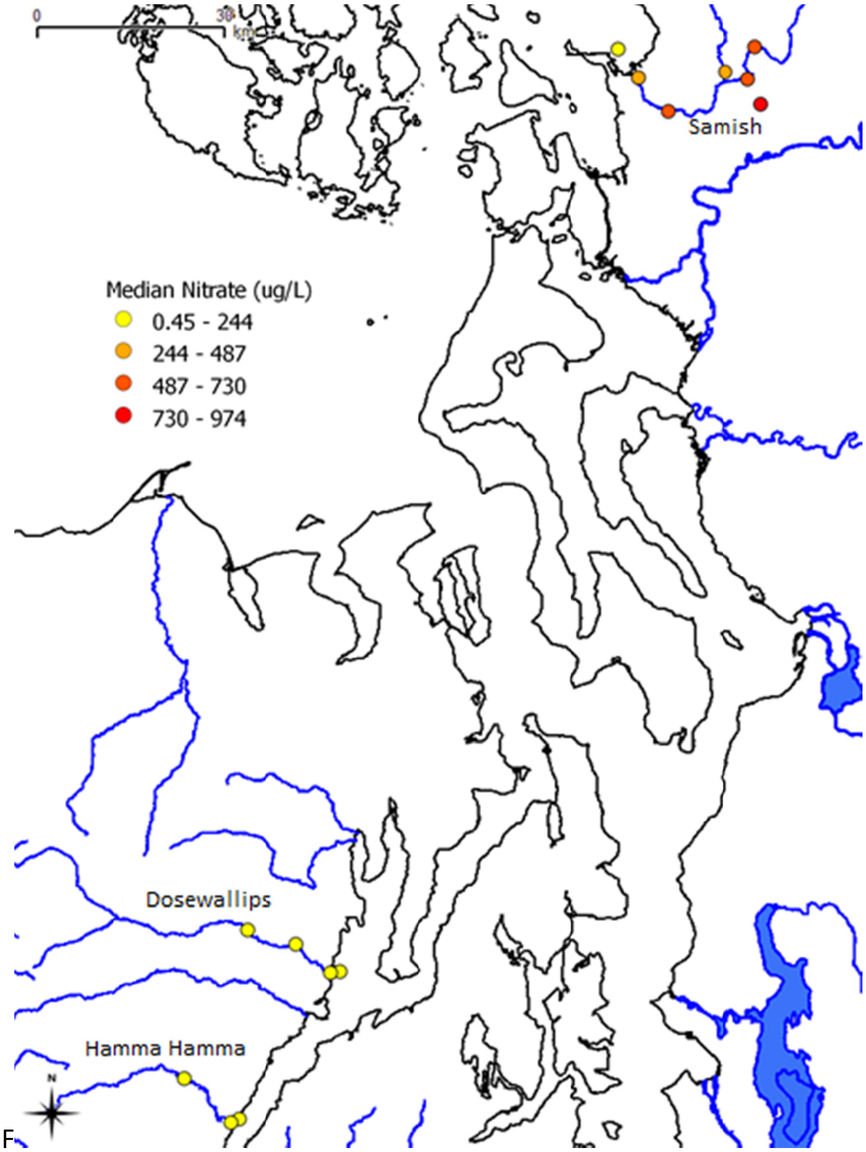

a)
Samish Bay
0
40
km
Samish River
Dosewallips River
Hamma Hamma River
b)
Samish Bay
Samish River
c)
0
20
km
Dosewallips River
Hamma Hamma River
● Salt Marsh
● Oyster Bed
● River

Figure 2.


0
30
km
Samish
Median Nitrate (ug/L)
● 0.45 - 244
● 244 - 487
● 487 - 730
● 730 - 974
Dosewallips
Hamma Hamma
N

F

Figure 3.

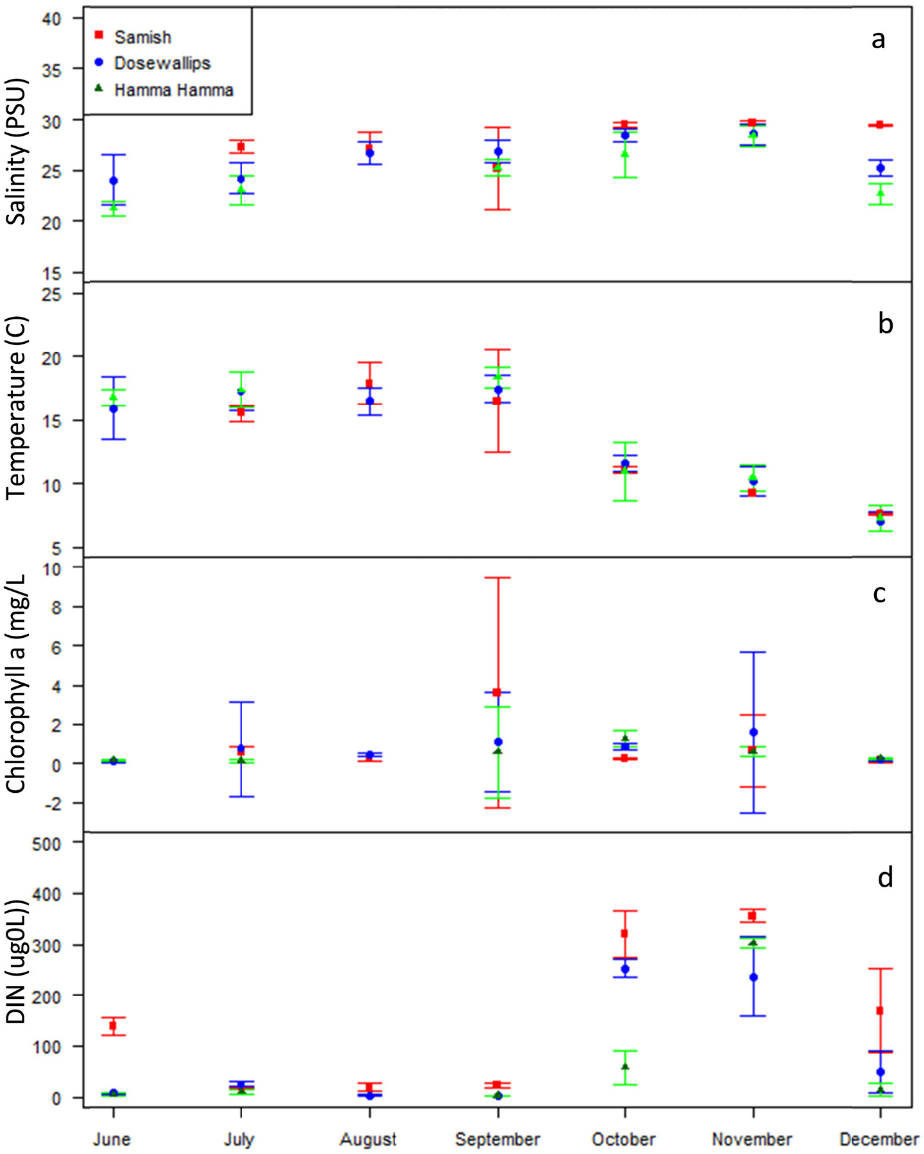
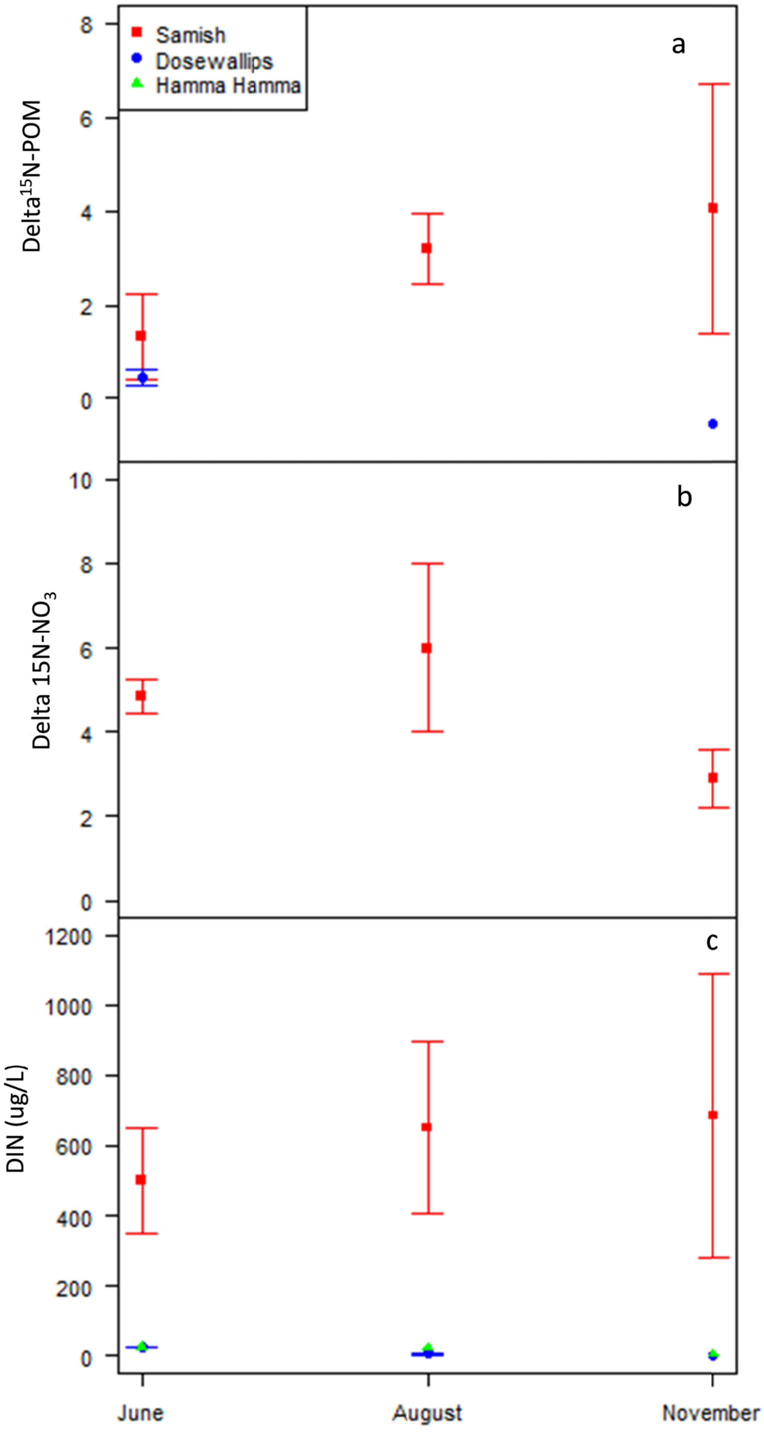

Samish
Dosewallips
Hamma Hamma
a
b
c
d
Salinity (PSU)
Temperature (C)
Chlorophyll a (mg/L
DIN (ug0L))
40
35
30
25
20
15
25
20
15
10
5
10
8
6
4
2
0
-2
500
400
300
200
100
0
June
July
August
September
October
November
December

Figure 4.



■ Samish
● Dosewallips
▲ Hamma Hamma
a
Delta^{15}N-POM
8
6
4
2
0
b
Delta 15N-NO$_3$
10
8
6
4
2
0
c
DIN (ug/L)
1200
1000
800
600
400
200
0
June
August
November

Figure 5.

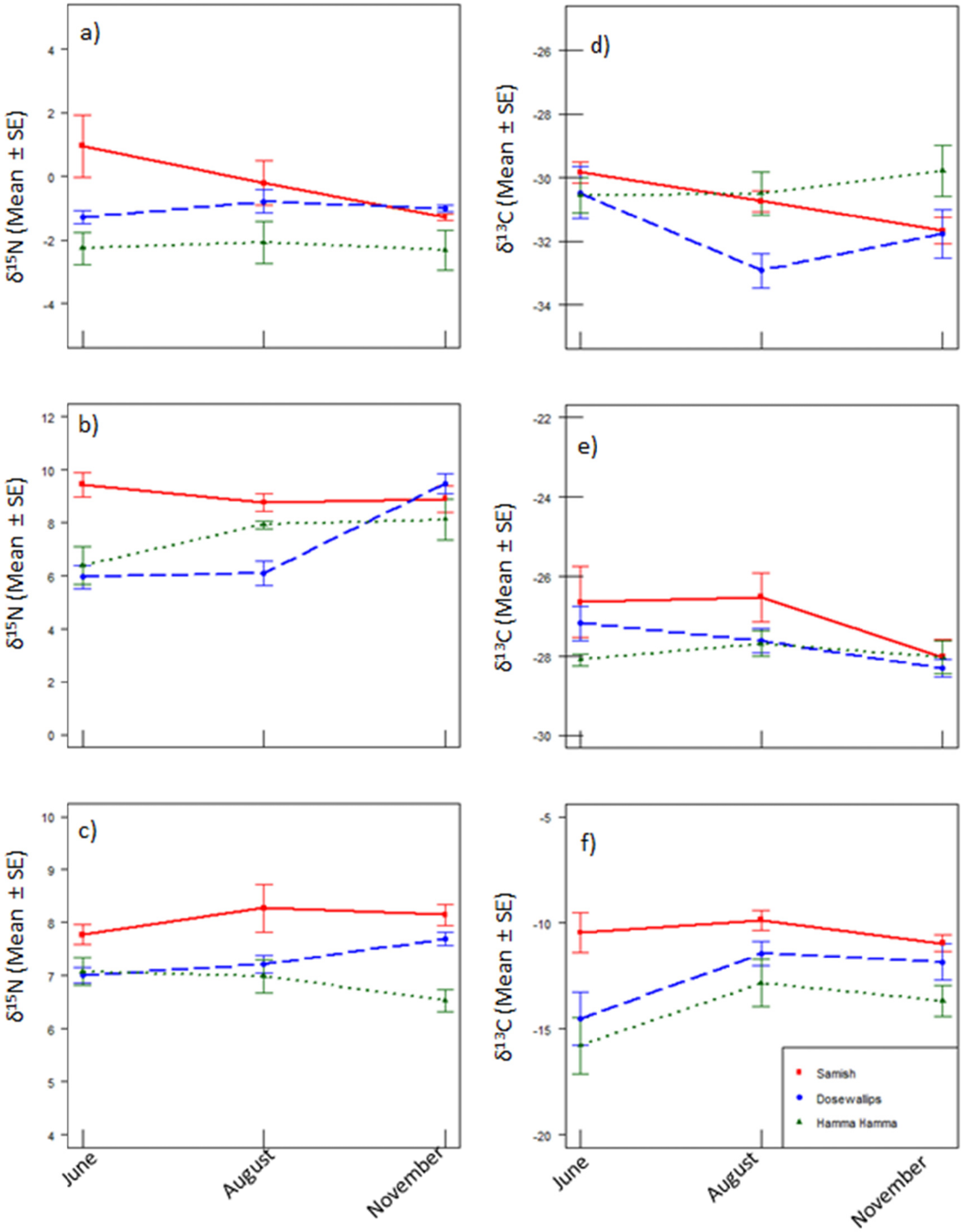
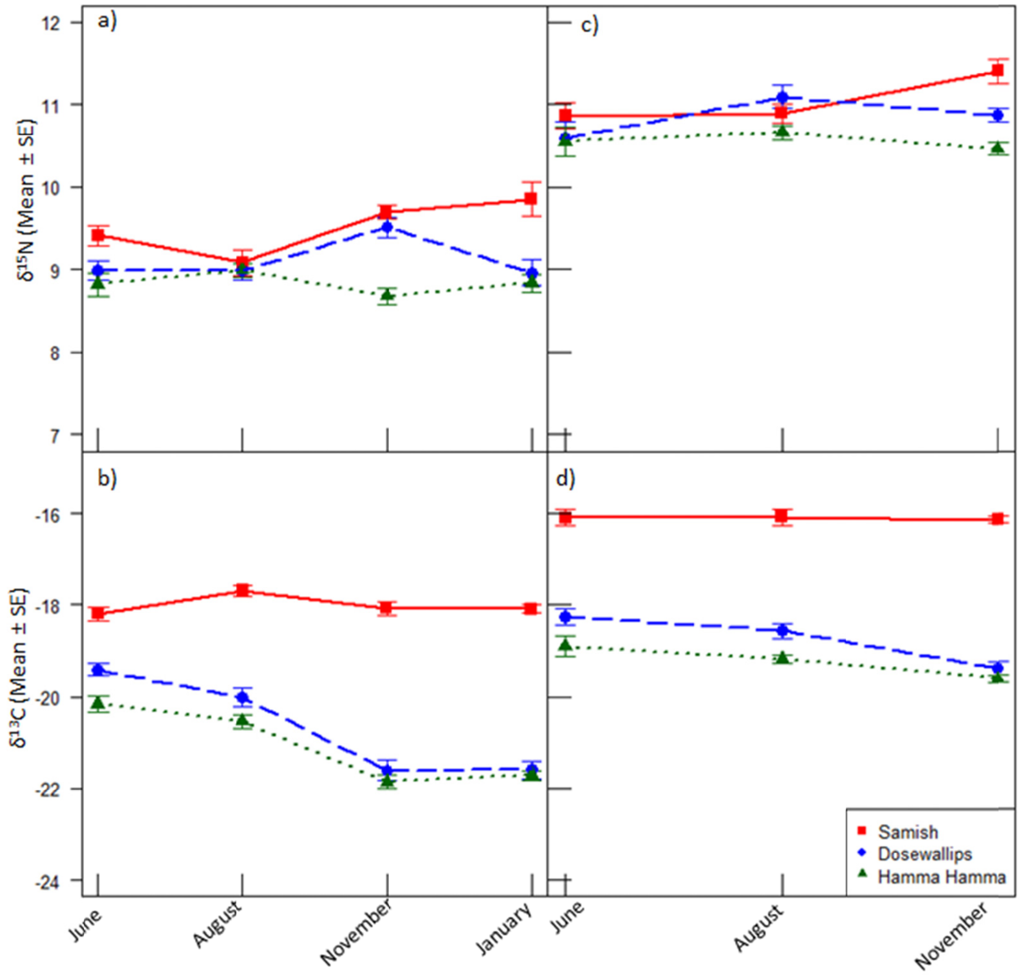

a)
b)
c)
d)
e)
f)
δ15N (Mean ± SE)
δ13C (Mean ± SE)
4
2
0
-2
-4
12
10
8
6
4
2
0
10
9
8
7
6
5
4
-26
-28
-30
-32
-34
-22
-24
-26
-28
-30
-5
-10
-15
-20
June
August
November
Samish
Dosewallips
Hamma Hamma

Figure 6.



a)
b)
c)
d)
δ15N (Mean ± SE)
δ13C (Mean ± SE)
12
11
10
9
8
7
-16
-18
-20
-22
-24
June
August
November
January
June
August
November
■ Samish
◆ Dosewallips
▲ Hamma Hamma

Figure 7.

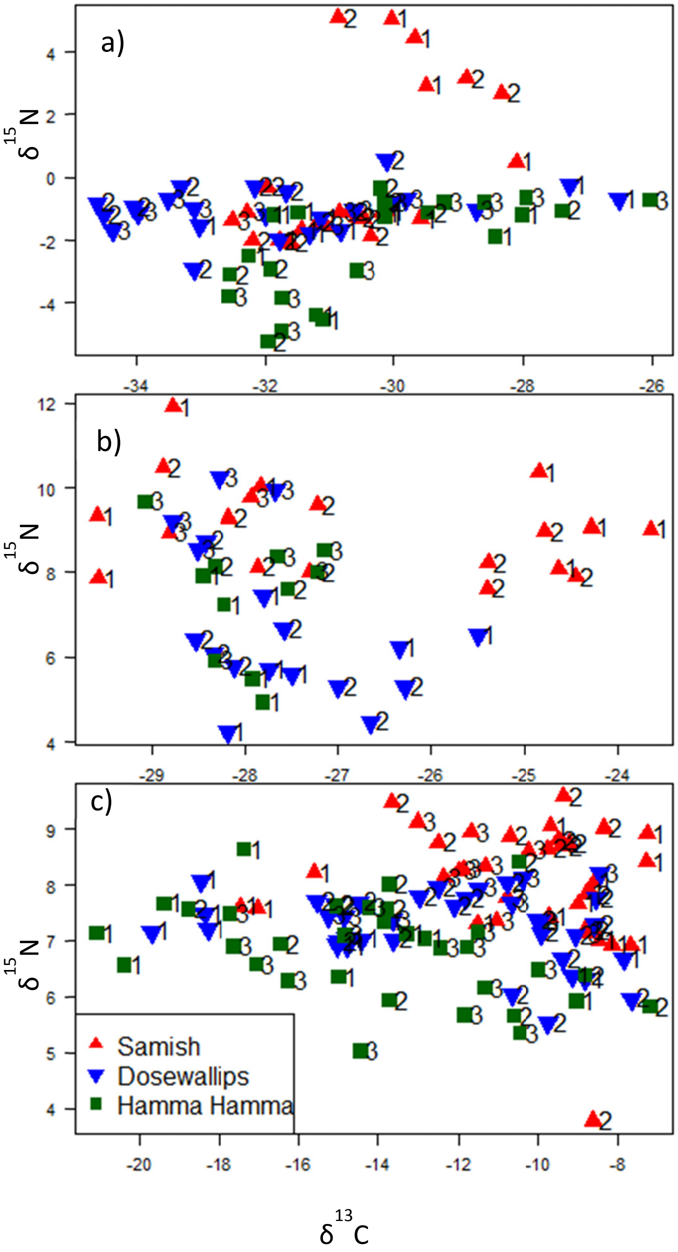
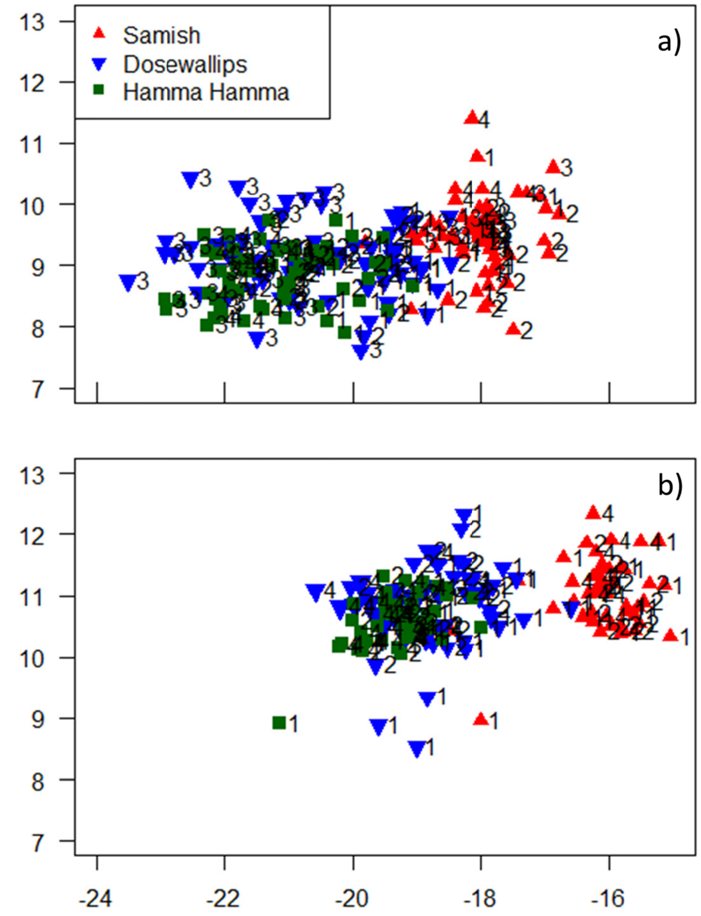
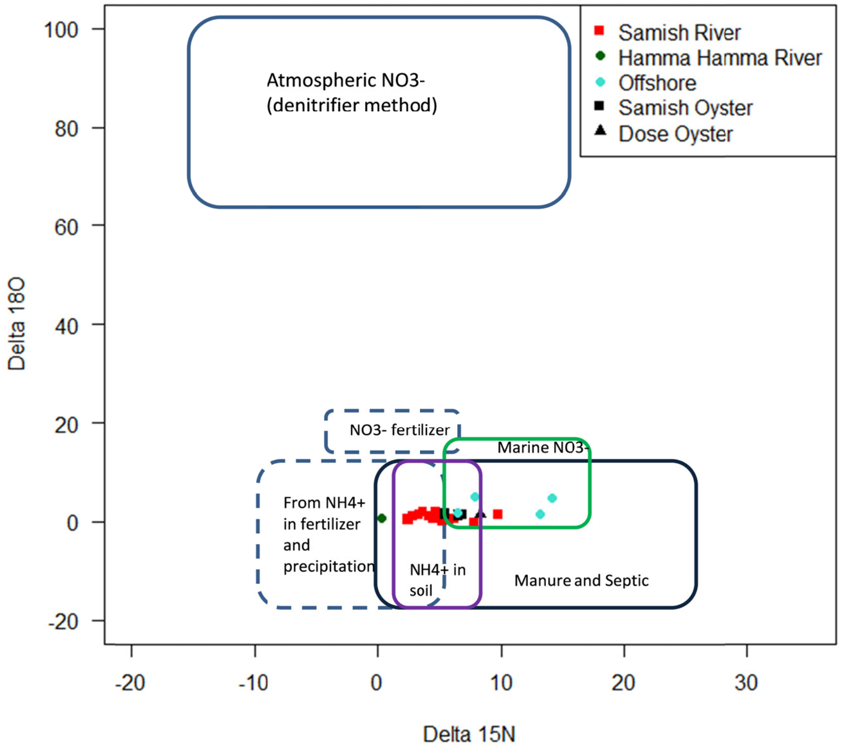

a)
b)
c)
$\delta^{15}N$
$\delta^{13}C$
Samish
Dosewallips
Hamma Hamma
4
2
0
-2
-4
-34
-32
-30
-28
-26
12
10
8
6
4
-29
-28
-27
-26
-25
-24
9
8
7
6
5
4
-20
-18
-16
-14
-12
-10
-8

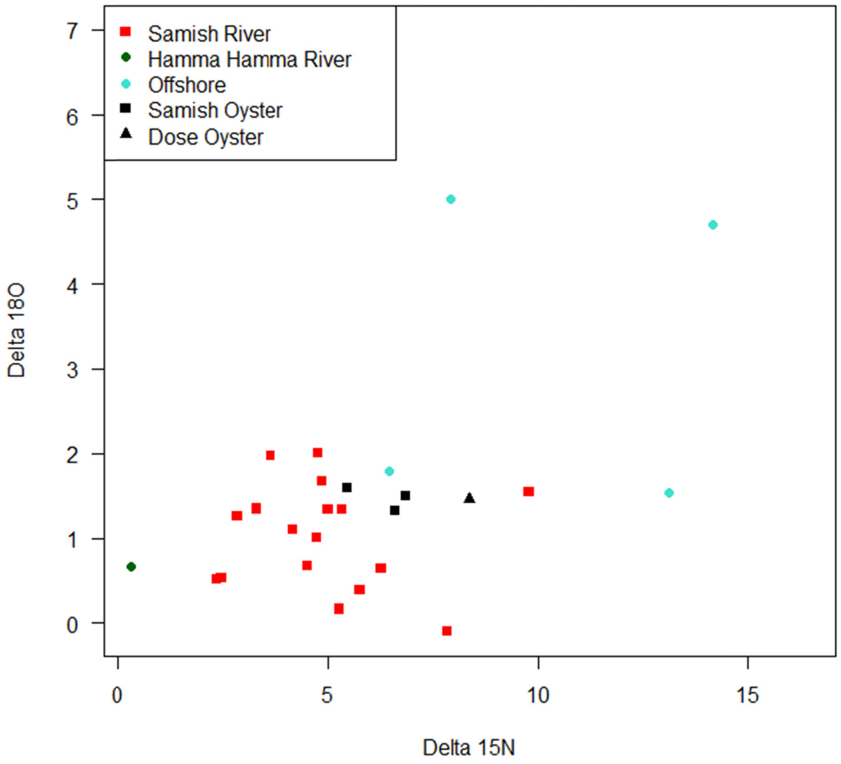
Figure 8.


Samish
Dosewallips
Hamma Hamma
a)
b)
13
12
11
10
9
8
7
-24
-22
-20
-18
-16

Appendix A



A1. Delta 15N and delta 180 values for water nitrates collected in the Samish and Hamma Hamma rivers (including river mouths) and the Samish and Dosewallips oyster beds. Literature values crudely redrawn from Kendall et al. (2007).



A2. Delta 15N and delta 180 values for water nitrates collected in the Samish and Hamma Hamma Rivers and the Samish and Dosewallips oyster beds.

Appendix B. $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios for primary producers and the oyster C. gigas collected from the Samish, Dosewallips and Hamma Hamma watersheds

	June 2011							**August 2011**							**November 2011**						
	Delta C		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**
	Mean	SD	**Mean**	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		Mean	SD	Mean	SD	Mean	SD	
Samish																					
benthic diatoms								-14.45	1.62	7.90	0.77	7.29	1.28	2							
Salix spp	-29.33	0.84	3.21	2.03	22.20	3.63	4	-30.01	1.70	2.64	2.23	23.41	6.00	4							
Alnus rubra	-30.33	0.78	-1.31	0.27	20.91	4.00	4	-31.11	0.69	-1.65	0.39	21.01	2.08	8	-31.67	0.84	-1.29	0.21	23.38	4.32	4
Upland	**-29.83**	0.81	**0.95**	**1.15**	**21.56**	**3.82**	**8**	**-30.56**	**1.19**	**0.50**	**1.31**	**22.21**	**4.04**	**12**	**-31.67**	**0.84**	**-1.29**	**0.21**	**23.38**	**4.32**	**4**
Glaux maritima	-24.35	0.52	9.12	0.93	25.24	10.32	4	-25.01	0.47	8.17	0.58	22.90	2.70	4							
Salicornia virginica	-28.94	0.83	9.77	1.68	9.99	0.63	4	-28.03	0.69	9.36	0.96	14.69	2.93	4	-28.01	0.76	8.90	0.88	21.35	4.07	3
Salt marsh	**-26.64**	0.68	**9.45**	**1.30**	**17.62**	**5.47**	**8**	**-26.52**	**0.58**	**8.76**	**0.77**	**18.80**	**2.82**	**8**	**-28.01**	**0.76**	**8.90**	**0.88**	**21.35**	**4.07**	**3**
Laminariales	-16.70	0.96	7.80	0.36	11.61	0.87	3	-13.66	NA	9.46	NA	17.37	NA	1							
Ulva spp	-9.76	1.25	7.56	0.25	13.03	2.08	4	-10.59	1.40	8.96	0.42	17.01	2.79	4	-10.78	1.58	8.22	0.77	9.71	1.46	4
Zostera japonica	-8.50	0.86	7.47	1.05	13.07	1.20	4	-9.01	0.42	7.12	2.36	14.09	1.80	4	-11.30	1.77	8.21	0.82	16.39	7.04	4
Zostera marina	-7.72	0.76	8.45	0.43	12.62	1.94	3	-8.78	0.51	8.51	0.62	14.40	1.60	3	-10.78	1.02	8.03	0.60	13.80	1.64	4
Intertidal macrophpyte	**-10.67**	0.96	**7.82**	**0.52**	**12.58**	**1.52**	**14**	**-10.51**	**0.78**	**8.51**	**1.13**	**15.72**	**2.06**	**12**	**-10.95**	**1.46**	**8.15**	**0.73**	**13.30**	**3.38**	**12**
Crassostrea gigas **Adductor**	**-16.09**	0.79	**10.87**	**0.64**	**3.49**	**0.51**	**18**	**-16.07**	**0.71**	**10.89**	**0.46**	**3.37**	**0.14**	**16**							
Crassostrea gigas **Mantle**	**-18.19**	0.66	**9.41**	**0.54**	**9.02**	**2.05**	**20**	**-17.68**	**0.45**	**9.08**	**0.61**	**8.34**	**2.69**	**16**	**-18.07**	**0.5748**	**9.6993**	**0.332**	**9.1036**	**2.79935**	**15**

Appendix B. Cont.

	June 2011							**August 2011**							**November 2011**						
	Delta C		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**
	Mean	SD	**Mean**	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		Mean	SD	Mean	SD	Mean	SD	
Dosewallips																					
benthic diatoms	-13.90	1.05	6.50	0.51	12.95	0.88	2	-21.35	NA	4.24	NA	7.09	NA	1	-16.61	NA	6.44	NA	8.79	NA	1
Alnus rubra	-31.98	0.78	-1.50	0.37	17.13	2.32	4	-34.11	0.59	-0.80	0.38	22.72	2.36	4	-29.80	0.80	-0.90	0.17	25.15	3.64	4
Pseudotsuga menziesii	-28.98	2.44	-1.10	0.74	31.95	10.81	4	-31.76	1.25	-0.78	1.50	32.60	7.06	4	-33.74	0.55	-1.12	0.42	37.58	4.65	4
Upland	**-30.48**	1.61	**-1.30**	**0.56**	**24.54**	**6.56**	**8**	**-32.94**	**0.92**	**-0.79**	**0.94**	**27.66**	**4.71**	**8**	**-31.77**	**0.67**	**-1.01**	**0.29**	**31.37**	**4.15**	**8**
Glaux maritima	-25.92	0.59	6.38	0.19	27.41	5.09	2	-27.01	0.79	5.21	0.54	24.98	14.46	4							
Salicornia virginica	-27.81	0.28	5.75	1.31	13.31	4.08	4	-28.22	0.43	6.98	1.20	17.16	2.16	4	-28.31	0.47	9.48	0.76	18.54	4.52	4
Salt marsh	**-26.86**	0.44	**6.06**	**0.75**	**20.36**	**4.58**	**6**	**-27.61**	**0.61**	**6.09**	**0.87**	**21.07**	**8.31**	**4**	**-28.31**	**0.47**	**9.48**	**0.76**	**18.54**	**4.52**	**4**
Fucus gardneri	-14.77	0.26	6.97	0.07	35.95	3.89	4	-14.55	0.68	7.34	0.38	39.38	5.32	4	-13.82	1.69	7.55	0.26	18.25	1.42	4
Gracilaria spp								-12.03	0.95	7.88	0.13	16.69	2.75	4							
Ulva spp	-18.70	0.66	7.48	0.42	19.21	5.51	4	-11.56	3.04	7.62	0.17	29.57	2.18	4	-9.86	0.99	7.83	0.40	8.63	0.83	4
Zostera japonica								-9.37	1.25	6.05	0.48	17.53	0.81	4							
Zostera marina	-8.61	0.67	6.45	0.19	22.93	3.54	3	-9.18	0.67	7.17	0.10	25.66	2.28	3							
Intertidal macrophpyte	**-14.02**	0.53	**6.97**	**0.23**	**26.03**	**4.32**	**11**	**-11.34**	**1.32**	**7.21**	**0.25**	**25.77**	**2.67**	**19**	**-11.84**	**1.34**	**7.69**	**0.33**	**13.44**	**1.12**	**8**
Crassostrea gigas **Adductor**	**-18.25**	0.73	**10.59**	**0.92**	**3.82**	**0.81**	**19**	**-18.57**	**0.62**	**11.09**	**0.57**	**3.32**	**0.08**	**16**							
Crassostrea gigas **Mantle**	**-19.41**	0.61	**8.99**	**0.51**	**8.86**	**2.48**	**21**	**-20.02**	**0.83**	**9.00**	**0.50**	**8.37**	**2.40**	**17**	**-21.61**	**0.9663**	**9.5122**	**0.527**	**7.6648**	**1.37795**	**18**

Appendix B. Cont.

	June 2011							**August 2011**							**November 2011**						
	Delta C		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**	**Delta C**		**Delta N**		**C:N**		**n**
	Mean	SD	**Mean**	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		Mean	SD	Mean	SD	Mean	SD	
Hamma Hamma																					
benthic diatoms								-23.68	NA	5.12	NA	12.37	NA	1							
Alnus rubra	-29.51	1.61	-1.37	0.36	20.73	3.82	4	-29.29	1.32	-0.85	0.35	18.52	2.34	4	-27.94	1.38	-0.76	0.07	24.14	1.68	4
Pseudotsuga menziesii	-31.60	0.55	-3.17	1.61	39.28	3.87	4	-32.13	0.35	-3.77	1.28	42.60	4.88	3	-31.65	0.81	-3.89	0.80	40.81	5.25	4
Upland Veg	**-30.55**	1.08	**-2.27**	**0.98**	**30.01**	**3.84**	**8**	**-30.71**	**0.84**	**-2.31**	**0.82**	**30.56**	**3.61**	**7**	**-29.80**	**1.10**	**-2.33**	**0.43**	**32.48**	**3.47**	**8**
Salicornia virginica	-28.09	0.29	6.39	1.41	13.19	1.42	4	-27.69	0.56	7.91	0.28	17.36	3.02	3	-28.04	0.83	8.11	1.58	29.42	18.70	4
Salt marsh	**-28.09**	0.29	**6.39**	**1.41**	**13.19**	**1.42**	**4**	**-27.69**	**0.56**	**7.91**	**0.28**	**17.36**	**3.02**	**3**	**-28.04**	**0.83**	**8.11**	**1.58**	**29.42**	**18.70**	**4**
Fucus gardneri	-13.74	0.94	6.95	0.43	37.24	6.93	4														
Gracilaria spp								-15.08	1.34	7.36	0.38	15.23	0.36	3	-16.97	0.67	6.58	0.31	10.22	0.87	3
Ulva spp	-19.53	1.61	7.49	0.88	14.05	3.84	4	-15.39	2.91	7.16	1.09	20.61	3.08	3	-15.60	1.85	7.38	0.27	6.78	0.20	3
Zostera	-9.03	NA	5.91	NA	14.62	NA	1														
Zostera japonica								-10.54	0.11	7.03	1.95	17.44	1.07	2	-12.54	1.33	6.47	0.98	8.29	0.87	4
Zostera marina								-8.00	1.14	6.09	0.40	17.08	2.22	2	-10.90	0.84	5.91	0.50	9.76	1.64	4
Intertidal macrophpyte	**-14.10**	1.27	**6.78**	**0.66**	**21.97**	**5.39**	**9**	**-12.25**	**1.37**	**6.91**	**0.95**	**17.59**	**1.68**	**10**	**-14.00**	**1.17**	**6.58**	**0.51**	**8.76**	**0.90**	**14**
C. gigas Adductor	**-18.90**	0.78	**10.55**	**0.60**	**3.73**	**0.97**	**12**	**-19.18**	**0.39**	**10.67**	**0.34**	**3.33**	**0.15**	**17**							
C. gigas Mantle	**-20.16**	0.64	**8.82**	**0.52**	**9.17**	**2.77**	**13**	**-20.53**	**0.62**	**8.99**	**0.37**	**7.37**	**1.75**	**17**	**-21.86**	**0.6622**	**8.68**	**0.431**	**8.6714**	**1.25184**	**18**

Appendix C. (J. Burke, Natural Capital Project)

Water Supply and Total Nitrogen Estimates

We estimated present (YR 2006) and future (YR 2040) water supply and total nitrogen for Hood Canal and North Puget Sound rivers using two tools from the Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST): Reservoir Hydropower Production and Nutrient Retention (Kareiva et al. 2011, Tallis et al. 2011). Both toolsets are ArcGIS models that use spatial and non-spatial parameters (Table 1). Water supply (*cubic meters per year*), in the Reservoir Hydropower Production toolset, was quantified using precipitation, reference evapotranspiration, vegetation type, plant evapotranspiration coefficients, soil depth, plant available water content, land use and land cover, root depth, elevation, and local consumptive water use. Total nitrogen load (*kilograms per year*) was quantified using water yield (water supply + local consumption), land use and land cover, nutrient loading rates, and filtration rates.

We used freely-available GIS data sources for the spatial parameters and conducted a literature review for non-spatial parameters (Table 1). The model results were validated with U.S. Geologic Survey (2011) annual stream discharge data and Washington State Department of Ecology (2011a, 2011b) stream discharge and monthly water quality field data collected for five of the seven watersheds. We calibrated the model using the parameters for nutrient load, root depth, and plant evapotranspiration coefficients based on the range of values obtained from the literature review.

The simulation results for water supply and total nitrogen in Hood Canal and North Puget Sound are presented in Table 2. Model limitations include the lack of groundwater interaction and the need to manually reduce model output results according to values of out-of-basin water use that reduce water supply.

Table 1. Water yield and scarcity and Nutrient Retention input parameters.

Description	Spatial Resolution	Timespan	Purpose	Source
Digital elevation model	30 m	1999	Hydrologic modeling	USGS (2011)
Precipitation	4 km	Annual average; 2005 to 2007	Rainfall amount	PRISM (2011)
Potential Evapotranspiration	4 km	Annual average; 2005 to 2007	Vegetation and developed land potential evapotranspiration	Derived from Annual Precipitation using InVEST tools available at http://www.naturalcapitalproject.org/download.html
Soil Depth	30 m	Varies with soil survey data	Depth to soil restrictive layer	SSURGO (2011) and STATSGO (2011)
Percent Available	30 m	Varies	Percent	Derived from NRCS soil data using USDA Soil

Description	Spatial Resolution	Timespan	Purpose	Source
Water		with soil survey data	available water for vegetation	Viewer, an ArcGIS extension available at http://soils.usda.gov/sdv/
Precipitation	12 km	2035-2045	Predicted rainfall amounts	Maurer et al. (2007); Girvetz et al. (2009)
Potential Evapotranspiration	12 km	2035-2045	Predicted potential evapotranspiration	Maurer et al. (2007); Girvetz et al. (2009)
Land use and land cover	30 m	2006	Vegetation and land use classes	Fry et al. (2011)
Land use and land cover	30 m	2040	Scenarios of future land use and land cover	Bolte and Vache (2010); Version 5
Zhang's Constant = 9	*na*	*na*	Characterize the seasonality of precipitation	Tallis et al. (2011) and best professional judgment
Root Depth	*na*	*na*	maximum root depth for vegetated land use classes	Best professional judgment based on Canadell (1996), Steinberg, P. D. (2001), Smith (1964), Coker (1995), McCain (2004), Antos and Halpern (1997), Topik et al (1986); Rigg and Harrar (1931); Nyvall (2002), James (1993), National Center for Appropriate Technology (2009), James (1993), Kutschera (1960), Ross (1997), and USDA (2012).
Plant evapotransipiration coefficient	*na*	*na*	used to obtain potential evapotranspiration	Best professional judgement based on Allen (2003) and Bandaragoda et al. (2003)
Total Nitrogen Load	*na*	*na*	Nutrient load by land use class	Best professional judgment based on Reckhow (1980), Binkley at al. (1992), Zhu and Mazumber (2007), Compton (2003), Sigleo (2010), Paulson et al. (2004), and Steinberg et al. (2010)
Efficiency, Total Nitrogen	*na*	*na*	Nitrogen filtration by land use class	Nichols (1983), Hunter et al. (2009), Fredriksen (1972), and Correll et al. (1992)
Threshold flow accumulation value	na	*na*	Minimum threshold for stream based on GIS flow accumulation model	Regional; based on perennial hydrology, USEPA and USGS (2005), and best professional judgment
Consumptive Water Use	na	2005-2007	Annual consumptive water use or direct water withdrawal that reduces surface water supply	WRIA 16 Planning Unit (2011) and WRIA 17 Planning Unit (2003)

Table 2. InVEST results for Hood Canal and North Puget rivers. * indicates no validation data available for calibration.

	Discharge (*cubic meters per year*)		**Total Nitrogen (*kilograms per year*)**	
River	**2006**	**2040**	**2006**	**2040**
Dosewallips*	594,364,992	669,198,016	55,638	66,691
Duckabush	413,480,992	469,991,008	37,163	37,968
Hamma Hamma*	485,748,000	557,545,984	42,158	50,855
Skokomish	1,299,154,211	1,523,144,227	133,558	219,152
Nooksack	3,350,899,968	3,521,580,032	2,272,390	2,084,590
Samish	218,916,000	272,867,008	343,116	330,235
Skagit	12,196,899,840	15,460,800,512	1,478,630	1,707,710

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