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

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Summary and Key Findings

Organic matter transfer across habitats is a ubiquitous feature of ecosystems. 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 1998).

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, and potential freshwater sources of dissolved inorganic nitrogen (DIN) and $\delta^{13}C$ and $\delta^{15}N$ ratios of on estuarine consumer.

Our study focused on three catchments (the Samish, Dosewallips and Hamma Hamma rivers) and adjacent shellfish growing areas in Puget Sound (Fig. 1). These catchments 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) (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 (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).

Key findings include the following:

- Median freshwater dissolved NO_3 concentrations were lower in the Dosewallips and Hamma Hamma rivers than in the Samish River while dissolved NO_3 in the oyster beds of the Samish River estuary 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. 3). 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). 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 685 µg/L). Offshore Puget Sound NO_3 concentrations ranged from 143 to 330 µg/L and displayed a mean $\delta^{15}N$- NO_3 of 8.8‰ (Table 3).

- $\delta^{15}N$ of upland vegetation differed across watersheds but not sampling months. 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‰) (Fig. 5a). $\delta^{15}N$ of salt marsh plants changed over time but this change differed by site (significant time × site interaction) such that $\delta^{15}N$ of salt marsh plants from the

Hamma Hamma and Dosewallips increased between summer and November, whereas Samish Bay decreased slightly during this same time interval (Fig. 5b). $\delta^{15}N$ 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‰) (Fig. 5c).

- The highest $\delta^{15}N$ ratios were observed in salt marsh plants (9.5‰) from the Samish estuary and seasonal variation in salt marsh foliar $\delta^{15}N$ also differed among the study sites; both of these patterns suggest that processes governing the $\delta^{15}N$ ratios of primary producers were variable in both space and time. Because the Samish River foliar $\delta^{15}N$ of 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 $\delta^{15}N$ 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 $\delta^{15}N$ ratios of intertidal macrophytes in Samish Bay, consistent with the positive relationship between elevated nitrogen watershed loading and elevated $\delta^{15}N$ 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 $\delta^{15}N$ 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 1998, Riera et al. 2000, Costanzo et al. 2001, Savage and Elmgren 2004, Bannon and Roman 2008). However, the difference in $\delta^{15}N$ of intertidal macrophytes between Hood Canal (mean $\delta^{15}N$ ~ 7 ‰) and Samish Bay (mean $\delta^{15}N$ ~8 ‰) was relatively small, especially in light of the large differences in watershed nitrogen loadings. Previous studies suggest that the $\delta^{15}N$ 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 $\delta^{15}N$, which may be quite variable. 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. 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, $\delta^{15}N$-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).

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

- $\delta^{15}N$ of oyster adductor muscle and mantle tissue varied across watershed and sample intervals such that in June and January, Samish Bay oyster mantle tissue displayed slightly more enriched $\delta^{15}N$ values than the Hood Canal sites while in November, Dosewallips and Samish were both more enriched than the Hamma Hamma (Fig.6a). Muscle tissue showed more similar $\delta^{15}N$ across the sites except for in November, when Samish Bay was somewhat more enriched (Fig. 6c). While $\delta^{13}C$ in oyster tissues also displayed significant watershed × time interactions, there were some consistent difference across the sites. Specifically, Samish Bay oysters were more enriched than 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 site × time interaction appeared to be driven by a decrease in $\delta^{13}C$ from August to November in oysters from Hood Canal sites that was not observed in the Samish (Fig. 6b,d).

- When both isotopes were analyzed together using multidimensional scaling, upland vegetation varied across watersheds but not sample intervals (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. Salt marsh plants stable isotope ratios were different across sample intervals as well as watersheds (Fig. 7b) such that Dosewallips was different from both Samish and Hamma Hamma while Samish and Hamma Hamma were similar. All site differences in salt marsh plant stable isotopic ratios were driven primarily by nitrogen. For intertidal macrophytes, there was also a significant effect of both watershed and sampling month. Samish macrophytes were different from both Dosewallips and Hamma Hamma and these differences were mostly due to carbon (Fig. 7c).

- Oyster mantle and adductor tissue were different across sites and sample intervals when both isotopes were considered together (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.

- Spatial variation in $\delta^{13}C$ in *C.gigas* was quite pronounced, a finding that mirrored variation observed in intertidal macrophyte $\delta^{13}C$ and likely contributed to carbon being the primary driver of dual isotopic differences between the Hood Canal and Samish watersheds. The more depleted $\delta^{13}C$ 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 uptake by primary producers due to carbon biogeochemical cycling (Simenstad and Wissmar 1985, Fry 2002).

- When dual isotopic (N and O) methods were used to assess dissolved nitrogen sources across the study sites, our data fell within the published ranges of isotopic overlap among the sources (soil nitrogen, fertilizer, and septic) (Kendall et al. 2007) so it was not possible to distinguish among them (Fig. 9). Sampling more watersheds that encompass a more diverse range of nitrogen loads and land uses throughout an entire annual cycle combined with more precise estimates of site-specific nitrate end member values may aid in future endeavors of this nature. We were unable to compare nitrate sources across the watersheds since there was insufficient dissolved nitrate in Hood Canal rivers for isotopic analysis. However, given the differences in land use and nitrogen loading between catchments we studied, it is likely that nitrogen fixation plays a proportionately bigger role in contributing to nitrogen loads in Hood Canal than in the Samish River relative to other sources (e.g. manure, fertilizer) despite the higher cover of deciduous forest in the Samish River (Table 1).

- We observed spatial and or temporal variation in most 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. $\delta^{13}C$ and $\delta^{15}N$ 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.

- 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 a 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	

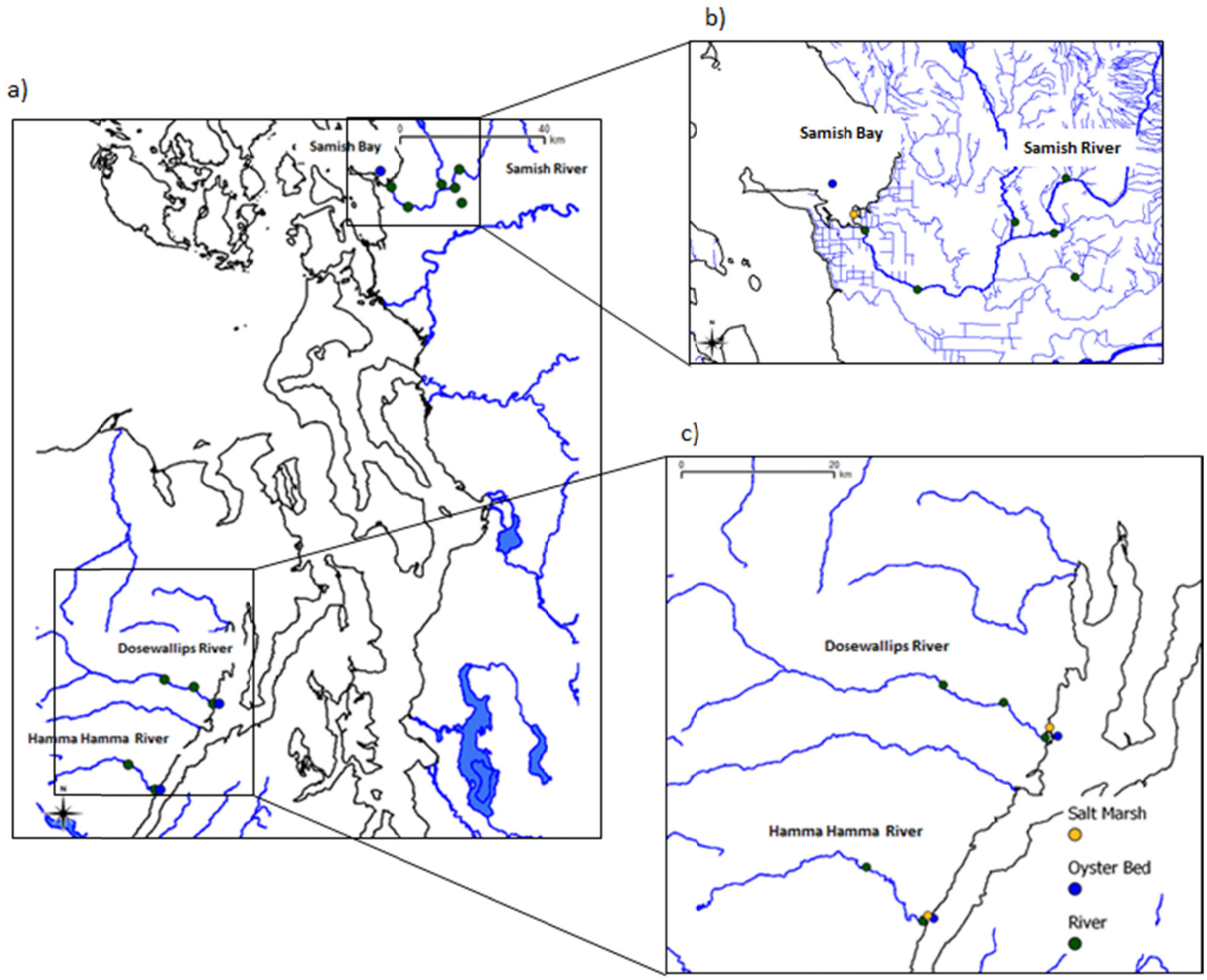

Fig. 1 Map of tissue and water collection locations in Puget Sound (a) with enlargements depicting specific sampling locations in oyster beds (blue), rivers (green) and salt marshes (orange) the Samish River and Bay (b), and the Dosewalllips and Hamma Hamma Rivers (c).

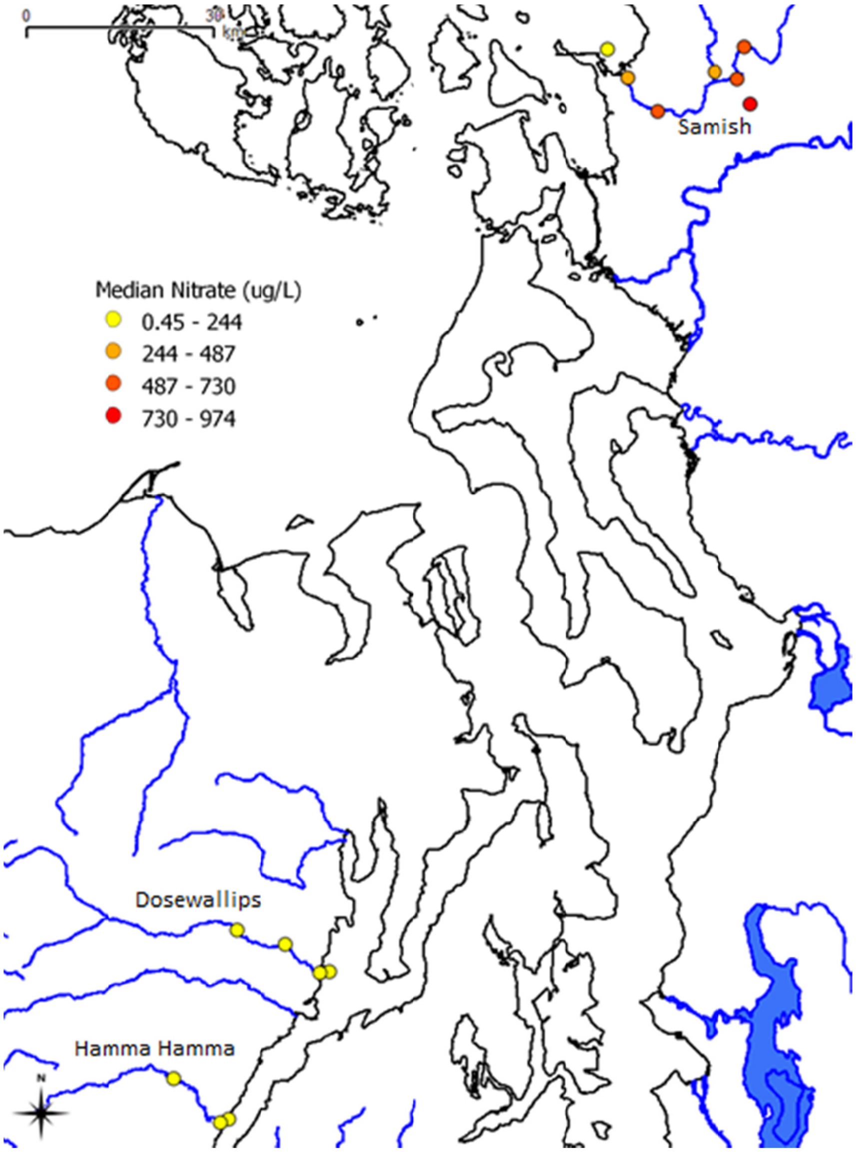

Fig.2. Median nitrate (NO_3) concentrations (ug/L) in the Samish, Dosewallips and Hamma Hamma Rivers and adjacent oyster beds from samples collected in June, August and November 2011.

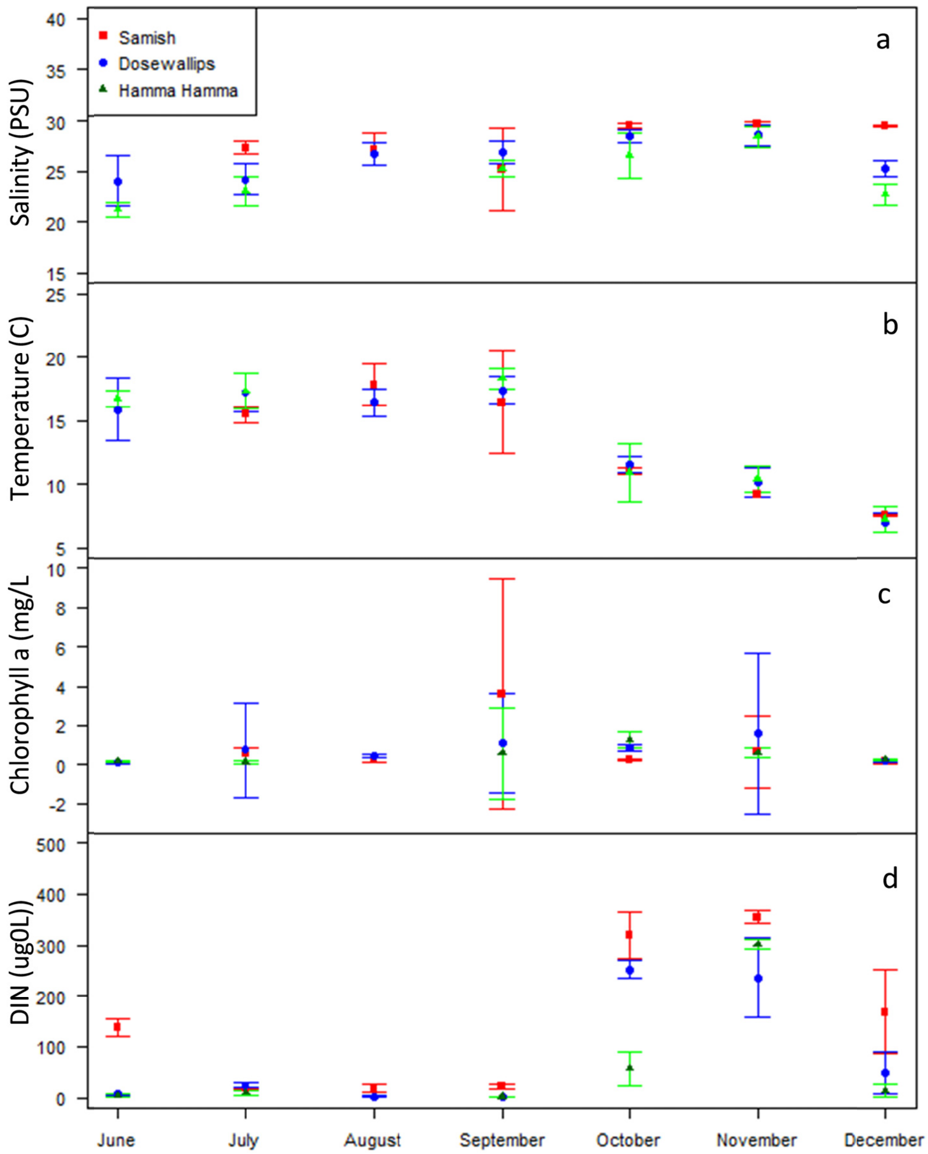

Fig. 3. Monthly mean (+/- SD) Salinity (a), temperature (b), chlorophyll a (c) and dissolved inorganic nitrogen (DIN)(nitrate+nitrite+ammonium) (DIN) in oyster beds in the Samish (red squares), Dosewallips (blue circles) and Hamma Hamma (green triangles)

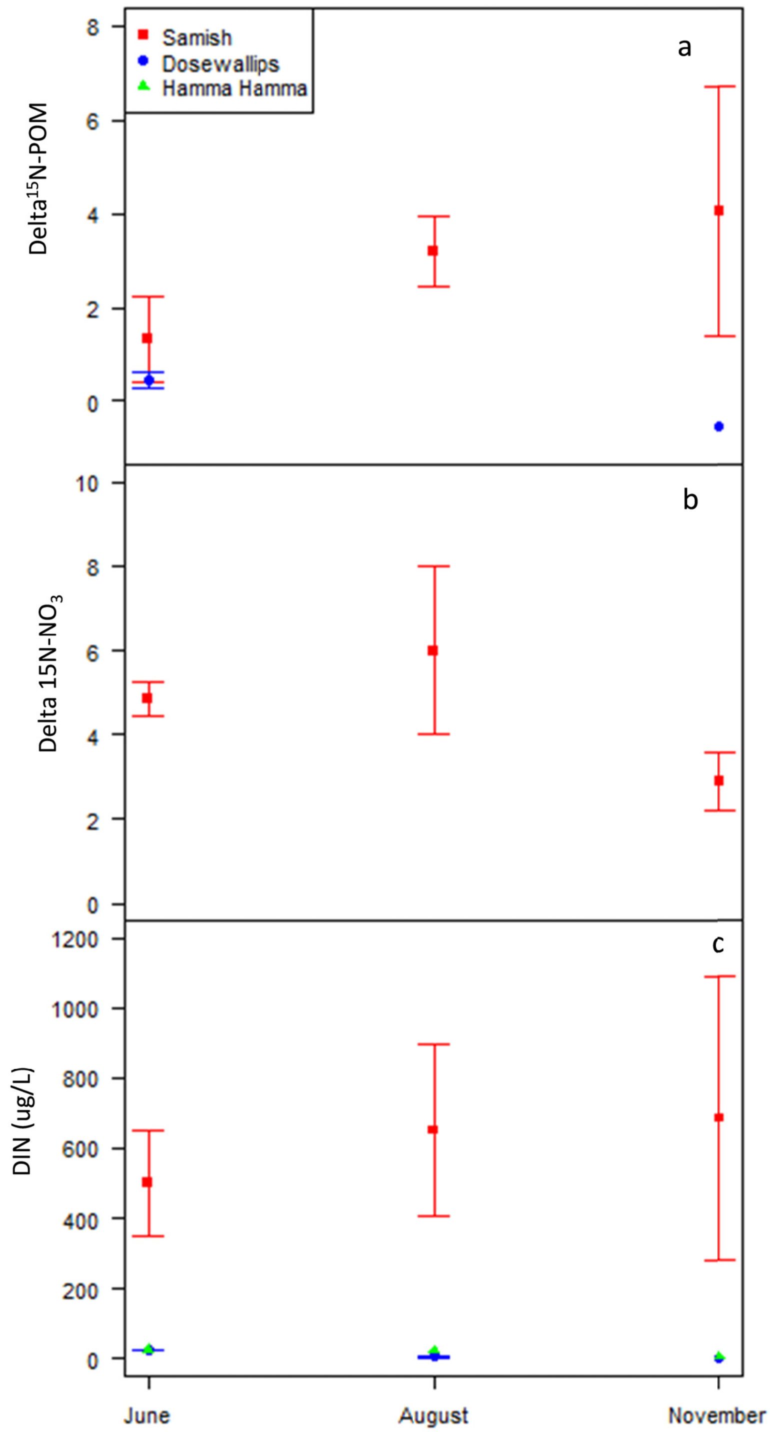

Fig. 4. Mean (±SD) of δ^{15}N-POM (a), δ^{15}N-NO_3 (b) and dissolved inorganic nitrogen (nitrate+nitrite+ammonium) (DIN) in freshwater from the Samish (red), Dosewallips (blue) and Hamma Hamma (green) watersheds (not including river mouths) in June, August and November.

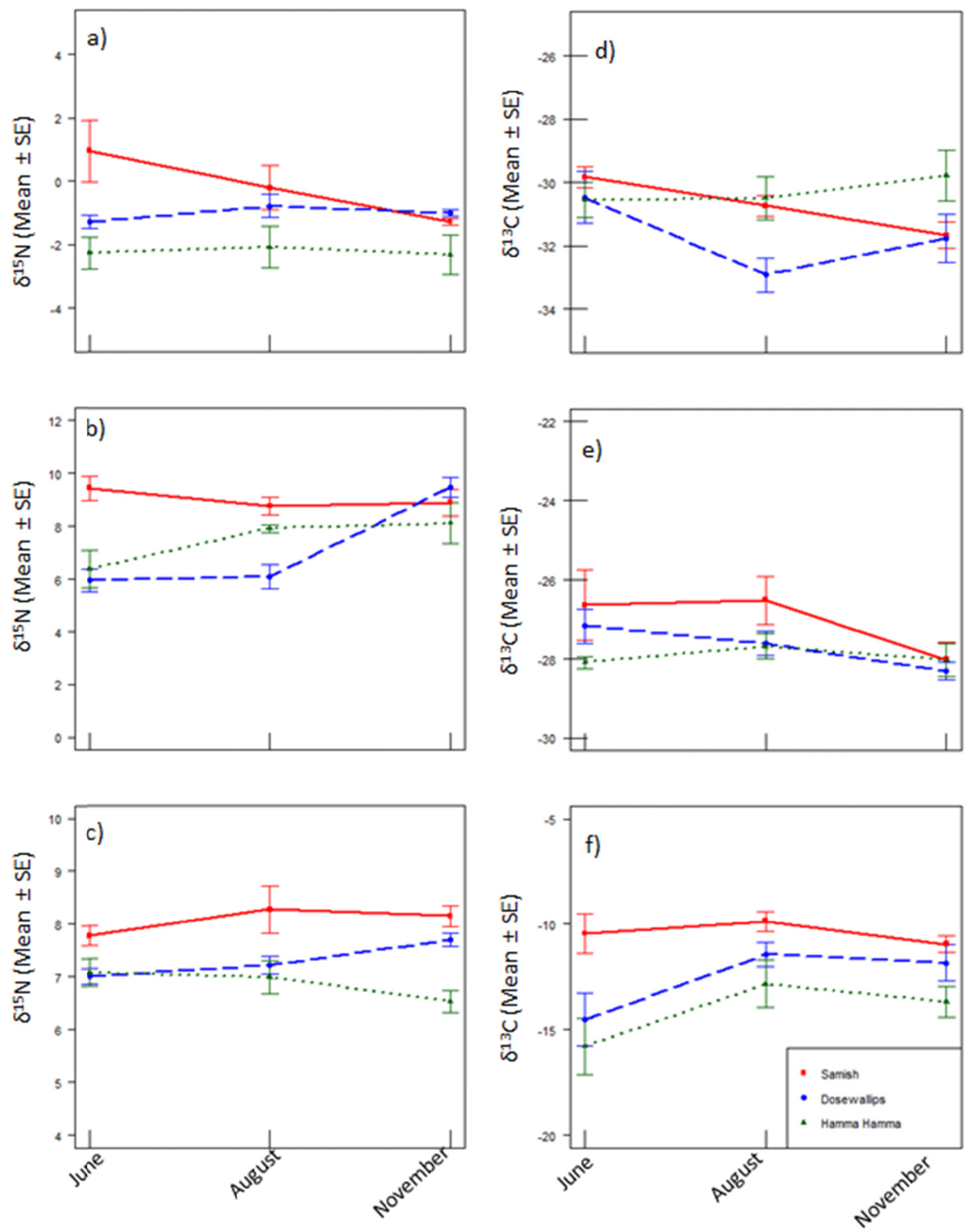

Fig. 5. Mean ± SE of $\delta^{15}N$ of upland vegetation (a), salt marsh plants (b), intertidal macrophytes (c) and $\delta^{13}C$ of upland vegetation (d), salt marsh plants (e), and intertidal macrophytes (f) in the Samish (red), Dosewallips (blue), and Hamma Hamma (green) growing areas in June, August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

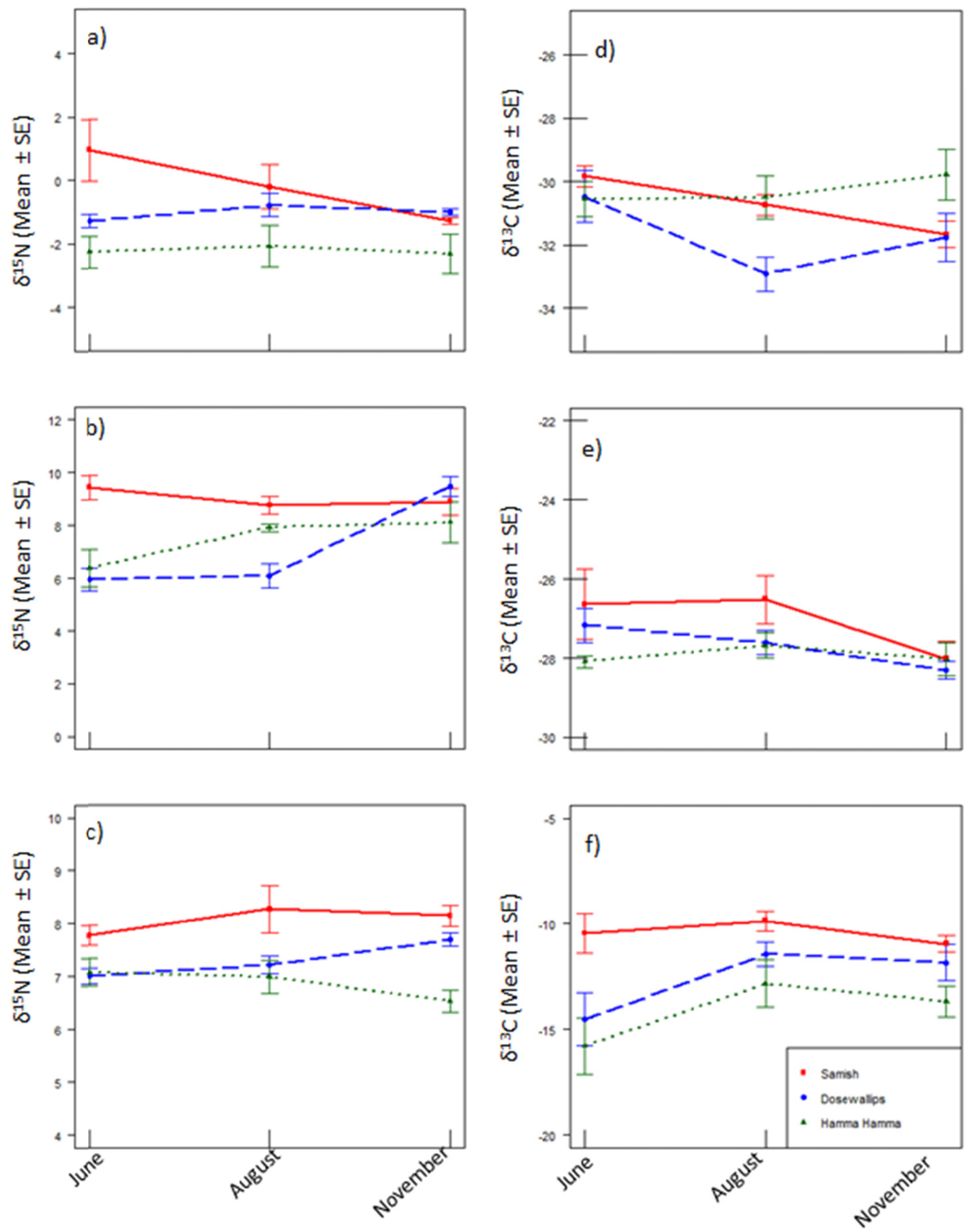

Fig. 6. Mean ± SE of $\delta^{15}N$ and $\delta^{13}C$ of *C. gigas* mantle (a,b) and adductor muscle (c,d) tissues over time in oyster bed adjacent to the Samish (red solid), Dosewallips (blue dashed) and Hamma Hamma (green dotted) watersheds.

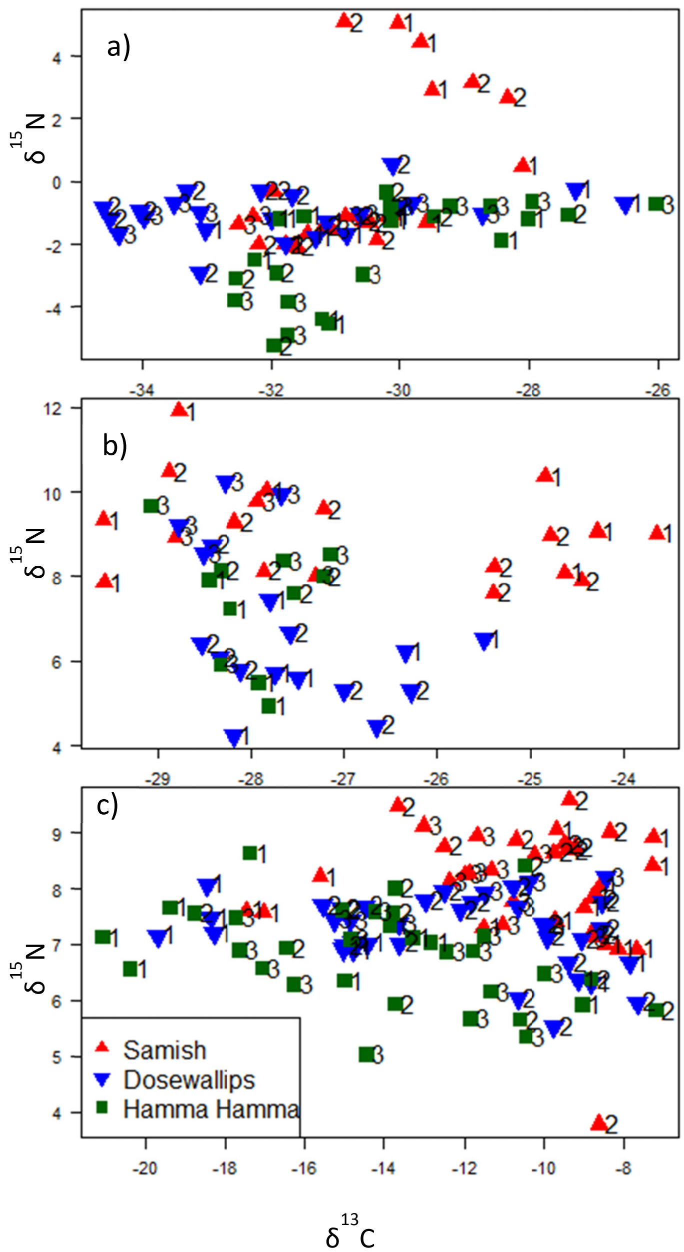

Fig. 7. $\delta^{13}C$ and $\delta^{15}N$ ratios of upland vegetation (a), salt marsh plants (b) and intertidal macrophytes (c) in the Samish (red upwards triangles), Dosewallips (blue downwards triangles) and Hamma Hamma (green squares) watersheds and oyster beds in June (1) and August (2), November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

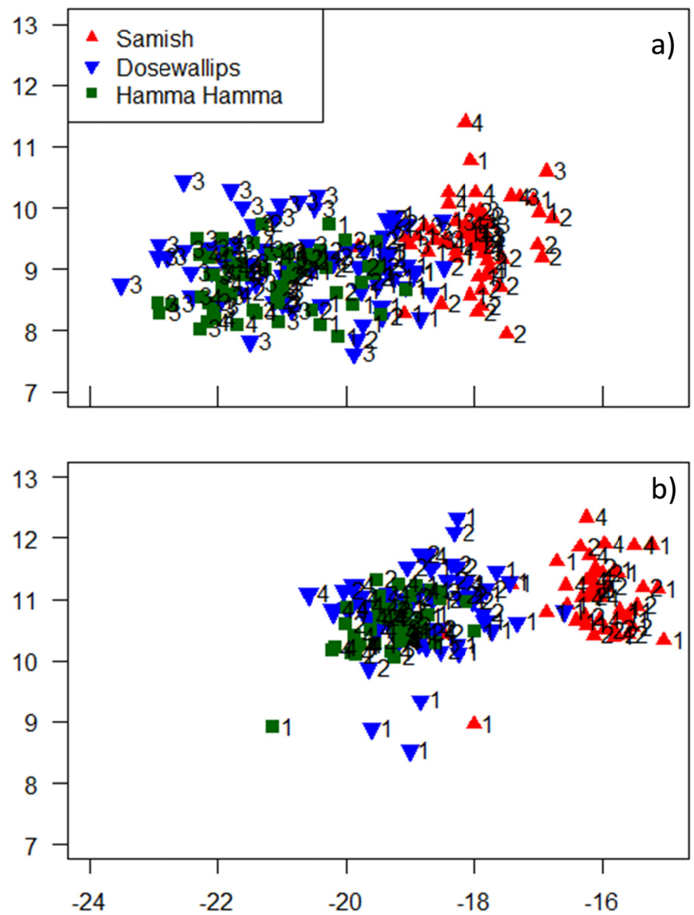

Fig. 8. $\delta^{15}N$ and $\delta^{13}C$ ratios of oyster mantle tissue (a), and adductor muscle tissue (b) for the Samish (red), Dosewallips (blue) and Hamma Hamma (green) shellfish growing areas in June (1), August (2), November (3) and January (4).

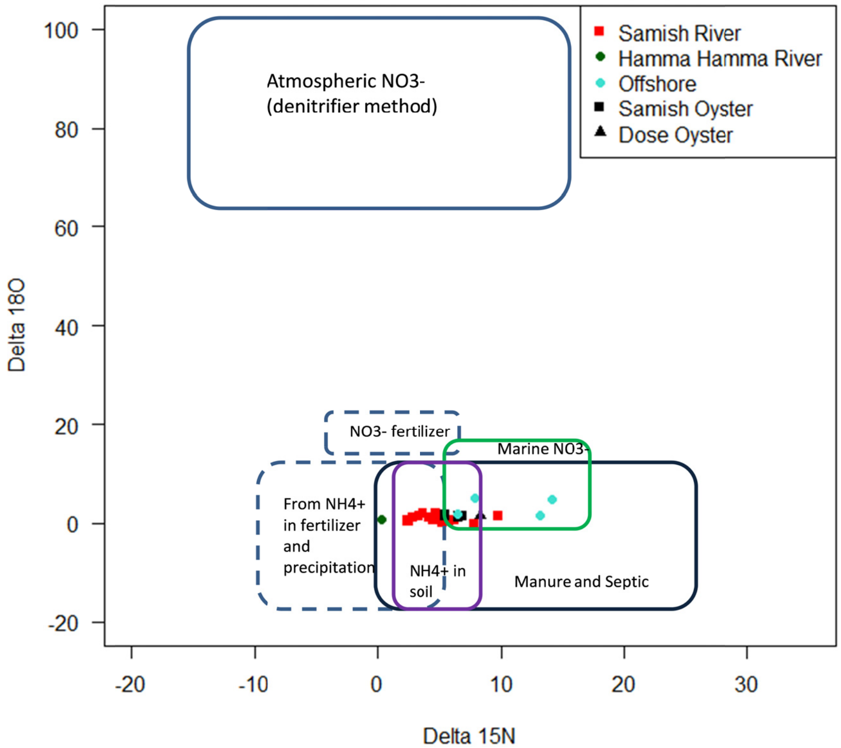

Figure 9. Delta 15N and delta 18O values for water nitrates collected in the Samish and Hamma Hamma rivers (including river mouths) and the Samish and Dosewallips oyster beds. Literature values crudely redrawn from Kendall et al. (2007).