Estuarine organic matter subsidizes shellfish beds in Puget Sound, WA

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Abstract

Organic matter from both autochthonous and allochthonous sources may provide energy and nutrients to nearshore food webs through consumption by filter feeding bivalves. In Puget Sound, where shellfish provide a variety of ecological, economic and cultural services, the degree to which shellfish rely on these subsidies may be important for their management and the management of near-shore food webs in general. We quantified the relative importance of terrestrial, near-shore and marine organic matter sources to both the diets of oysters and to the composition of nearshore particulate organic matter (POM) across three shellfish growing areas and adjacent river basins by analyzing the $\delta^{13}C$ and ^{15}N ratios of oyster tissue and primary producers from intertidal, offshore, salt marsh and upland habitats across the dry and wet seasons. Using the Bayesian mixing model, MixSIR, our results indicate that much of the organic matter consumed by shellfish originates from intertidal macrophytes (seaweeds and eelgrass) and salt marsh plants. We tested the robustness of this assertion to two different trophic fractionation estimates, finding comparable proportionate contributions to shellfish diets of the organic matter sources we included in the model (upland vegetation, salt marsh plants, intertidal macrophytes, phytoplankton and benthic diatoms). Oyster bed POM was also composed largely of salt marsh plants and intertidal macrophytes. Importantly, spatial variation in stable isotope signatures of consumers was not driven substantially be differing contributions of diet sources but rather variation in isotopic signatures of the sources themselves, underscoring the importance of including such variability in mixing models.

Introduction

The importance of cross-ecosystems subsidies is well accepted, effectively establishing a landscape perspective on food webs as mosaics connected through the spatial transfer of nutrients, organic matter, or organisms (Polis et al. 1997). Despite this recognition, it is also becoming increasingly apparent that the manner in which ecosystems are connected is not always intuitive and subject to vary over space and time (Francis et al. 2011, Holtgrieve and Schindler 2011). Nearshore ecosystems in particular are complex and dynamic habitats that are subject to trophic subsidies and physical forcing from terrestrial and marine environments (Dame and Allen 1996) and understanding the nature of this connection has proven to be context-dependent, driven by factors such as species examined, season, availability of different carbon sources and degree of freshwater influence (Page and Lastra 2003, Connolly et al. 2005). Freshwater flow and proximity to different organic matter sources can influence the growth and biomass of nearshore consumers (Ruckelshaus et al. 1993, Riera and Richard 1996, Ruesink et al. 2003). For example, in a location with little or no freshwater influence, wild bivalves were estimated to consume primarily marine phytoplankton, with lower but significant contributions of salt marsh vegetation and benthic diatoms (Riera 2007). By contrast, bivalves in estuaries receiving some direct riverine input displayed more depleted (more terrestrial) $\delta^{13}C$ signatures, particularly in time periods of high freshwater flow (Riera and Richard 1997, Marin Leal et al. 2008) and along a salinity gradient (Bucci et al. 2007). Ruckelshaus et al. (1993) found that the diet of bivalves reflected their distribution with respect to the dominant primary producers within an estuary. As such, the relative importance of allochthonous and autochthonous organic matter to nearshore bivalves may depend on the quantity and quality of freshwater and marine fluxes.

Organic matter available to estuarine food webs is comprised of phytoplankton and benthic microalgae as well as detritus that may originate from terrestrial, estuarine or marine sources. The relative abundance of these components will likely shift in space and time based on phytoplankton productivity, macrophyte standing crops and input of organic matter from terrestrial ecosystems mediated by freshwater flow. Suspension-feeding bivalves filter ambient water by ingesting a combination of phytoplankton, resuspended benthic microalgae and detritus originating from riparian vegetation, salt marsh vegetation or marine macrophytes (Dubois et al. 2007, Marin Leal et al. 2008). Many filter feeders are capable of selectively ingesting their food based on both particle size and food quality (Baldwin and Newell 1995, Ward and Shumway 2004, Beninger et al. 2007) such that the diet of these organisms reflects the interaction between food availability and preferences of individual shellfish. As sedentary filter feeders, nearshore bivalves consume particulate organic matter and thus are potential indicators of the strength of terrestrial-marine coupling at a given location.

Despite the inherent challenges in determining the relative importance of marine and terrestrial organic matter subsides to estuarine food webs, estuarine habitats must be considered through the lens of their connectivity with upland and offshore ecosystems since estuaries are subject to natural and anthropogenic influences originating in these adjacent ecosystems. Our objective was to use stable isotopes to quantify the relative importance of these connections in three watersheds that drain into Puget Sound, WA, USA (Fig. 1).

Puget Sound is a dynamic, marine-influenced estuary (Moore et al. 2010, Sutherland et al. 2011) receiving freshwater from 15 major rivers as well as many smaller drainages, all of which serve as conduits linking land and sea. In a companion paper Banas et al. (*in prep*) showed a high degree of connectivity within Puget Sound via freshwater from major rivers

within the basin as well as with the Fraser River in Canada, particularly in summer and fall. Thus, although Puget Sound is strongly influenced by marine hydrology, the influence of rivers on nearshore ecosystems is potentially important and non-local, driven in part by the size of the river basin, and the entrainment capacity or lifespan of the transported particles (Banas et al. *in prep*). While the importance of marine derived nutrients to freshwater habitats is well established (Naiman et al. 2002) the importance of terrestrially derived food sources to nearshore habitats remains largely unexplored in this region (but see Simenstad and Wissmar 1985).

In Puget Sound, shellfish are a widely harvested component of the nearshore food web, and as ecosystem engineers and filter feeders provide ecosystem services that include substrate formation, nitrogen reduction, erosion protection and increased water clarity (Grabowski et al. 2012). Despite the economic and ecological value of shellfish, little is known about the sources of organic matter that sustain oysters and other filter feeders in Puget Sound. Furthermore, recent efforts to implement ecosystem-based management in Puget Sound have emphasized the importance in understanding links between land and sea (Ruckelshaus et al. 2009).

Stable isotope ratios are a tool for distinguishing among organic matter sources in bivalve diets (Simenstad and Wissmar 1985, Ruckelshaus et al. 1993, Page and Lastra 2003, Riera 2007, Marin Leal et al. 2008, Lefebvre et al. 2009b) because of the distinct isotopic ratios different diet items can display, in this case, primary producers (Fry and Sherr 1984, Cloern et al. 2002). We sought to explore the relative importance of terrestrial, marine and estuarine primary producers as food for oysters in three target shellfish growing areas in Puget Sound. We used a Bayesian mixing model (MixSIR) (Moore and Semmens 2008) to measured stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of primary producers, particulate organic matter (POM) and Pacific Oyster (*Crassostrea gigas* Thunberg) tissue at the mouth of three river basins.

Methods

We assessed the isotopic content of *C. gigas* tissue and that of potential diet items both at their likely origins and as particulate organic matter (POM) (sensu Kwak and Zedler 1997, Riera 2007, Malet et al. 2008) at oyster beds beds at the mouth of three river basins (Samish, Hamma Hamma and Dosewallips) in Puget Sound (Fig. 1a) . We collected oyster tissue in June 2011, August 2011 and January 2012 and potential diet items in June 2011, August 2011 and November 2011. Oyster bed POM was collected monthly (Table 1).

Site descriptions

The Samish Bay , which is adjacent to Samish River watershed is located in the northern basin of Puget Sound (Fig. 1b). Average annual discharge is approximately 7 m^3/s (USGS). Oysters at this site were outplanted as juveniles by local oyster growers since natural recruitment of *C. gigas* does not typically occur in this portion of Puget Sound. Oysters included in this study were distributed patchily across an area of 1 km^2, which was further divided into three approximately 300 x 300 m subareas.. The Dosewallips and Hamma Hamma river deltas drain the Olympic Mountain range on the west side of Hood Canal (Fig. 1c). The Dosewallips oyster bed extends approximately 2 km north of the river outlet and 0.5 km offshore, resulting in an available sampling area of approximately 1 km^2. In the Hamma Hamma river, access restricted 0.07 km2 of the available ~0.5km^2 growing area. The rivers are comparable are in flow, with average discharges of ~ 14 m^3/s (USGS).

Oyster tissue collection

Six adult oysters of standardized size (110 mm to 130 mm in length) were haphazardly collected from three stratified portions of the shellfish growing area to ensure coverage of the entire bed (12-18 oysters total per site at each sample interval). For consistency across sites,

only oysters growing on the primary substratum were collected. Oysters from each portion of the bed were placed into clean plastic bags and put ice immediately following collection and frozen at -20°C at the end of each sampling day.

In the Samish Bay growing area, harvest periodically removes all individuals from a given location; therefore, in early August 2011, 30 oysters were relocated within each subarea and placed in 2 m x 2 m staked plots. Subsequent collections were made from these staked plots. This ensured that oysters of the appropriate size could be located during subsequent sampling events.

Oyster tissue preparation

Prior to dissection, oyster shell length and width (mm) and wet weight (g) were recorded and all individual oysters were rinsed thoroughly with dilute (10%) hydrochloric acid (HCL) and deionized (DI) water. The adductor muscle tissue was removed and placed into 50 mL glass scintillation vials and then stored in a minus 20°C freezer. 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 precise amounts (0.5 – 0.8 mg) of tissue which was then placed into 5 x 9 mm tin capsules and placed into a sealed 96 well tray to be shipped to the Washington State University Core Laboratory 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.

Vegetation sampling

To determine the relative contribution of terrestrial and marine sources to oyster diets, a suite of potential food sources were collected. These included marine sources including phytoplankton (marine POM), macroalgae, benthic diatoms and *Zostera* spp and terrestrial sources including leaf material from riparian vegetation and salt marsh plants (Table 2). Collections for all tissue-based sources (e.g., leaves, macroalgae) followed the methodology described above for oyster tissue. Epiphytes were removed from algal samples by rinsing with DI H_2O and visually inspecting under a dissection microscope to ensure complete removal. 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. Benthic diatoms were collected using 15 cm x 15 cm nitex (20 µm mesh size) squares placed onto the substrate during day time low tides and allowed to accumulate diatoms for 1-2 hours (*sensu* Cloern et al. 2002, Howe and Simenstad 2011). Diatoms were rinsed from nitex with DI H_2O and allowed to settle in sterile 250 ml glass beakers (1-3 h) to separate diatoms from inorganic particulates, which may have been present. Diatoms were carefully removed using sterile 20 ml disposable pipettes, re-suspended in DI H_2O, then filtered onto Whatman 47mm GF/F filters. Samples were then oven dried (60° C for 12 hours), exposed to 12N HCl vapor in a glass desiccator for 4 h at room temperature to remove carbonates (Lorrain et al. 2003), placed in a fume hood (3 h) and then a drying oven (12 h 60º C) to remove excess HCl and water. Particulate matter was scraped from filters and processed in the

same manner as oyster tissue. To ensure the samples met the minimum detection limits for C and N of the mass spectrometer, larger amounts of material (10-12 mg) were submitted for analysis.

Particulate Organic Matter sampling

Water was collected from oyster beds using small boats at or near high tide from 1 m below the water surface from each portion of the oyster bed at each study location using a 5 L Niskin grab sampler. Samples were then immediately filtered onto precombusted (450°C for 4 h) Whatman 47 mm GF/F filters using a low pressure (<15 psi) vacuum pump until visible particulate accumulation occurred. Filters were folded using sterile forceps, placed into pre-combusted glass scintillation vials and frozen at -20°C. When shipboard vacuum pumping was not feasible, water samples were transported on ice and filtered no more than 8 h after collection. Once filtered, POM samples were processed in the same manner as the benthic diatom samples (above).

Environmental data

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.

Site comparisons

We used one-way ANOVA to evaluate among-basin differences in isotope values for intertidal macrophytes, terrestrial vegetation, salt marsh plants and oysters. Tukey's post-hoc test was used to make pairwise comparisons among sites when appropriate. All GLM analyses were done using Systat v. 10.

Mixing model - Oysters

We used the Bayesian mixing model MixSIR (Moore and Semmens 2008) to estimate the likely proportionate contributions of difference food sources to oyster diets at each of the sites

and at each time period. This analysis permitted us to both incorporate source variability as well as to generate probability distributions of likely diet contributions. Potential diet items were: phytoplankton (POM collected from offshore locations in Puget Sound), benthic microalgae, terrestrial vegetation which consisted of leaves of red alder (*Alnus rubra)*, Douglas fir (*Pseudotsuga menziesii*) and willow tree (*Salix* spp), salt marsh vegetation (*Salicornia virginica* and *Glaux maritima*) and marine macrophytes (intertidal *Zostera spp.* and macroalgae *Ulva spp*, *Fucus gardneri, Gracilaria* spp and *Laminaria* spp*)*.

The degree to which consumers become enriched relative to their food sources (fractionation) is perhaps the biggest source of uncertainty associated with using stable isotope ratios to infer consumer diets since fractionation *in situ* is likely to vary as a function of physiological condition of the consumer, food availability, growth rates and ambient temperature (Fry 2006). To partly address the potential variability and uncertainty in fractionation, we used two fractionation scenarios. We took the approach of Lefebvre et al. (2009a) by using both the estimate for *C. gigas* whole body derived in Dubois et al. (2007) of Δ 1.85 for $\delta^{13}C$ and Δ 3.79 for $\delta^{15}N$ as well as a corrected estimate for muscle tissue by adding the Dubois et al. (2007) fractionation to the difference between whole body and muscle tissue derived from McCutchan et al. (2003). This resulted using in a second trophic enrichment factor of Δ 2.9 for $\delta^{13}C$ and Δ 4.7 for $\delta^{15}N$(Lefebvre et al. 2009a). We used 1.1 as the standard deviation for both estimates (Dubois et al. 2007). We used temporally pooled location-specific values for terrestrial vegetation, intertidal macrophytes and salt marsh plants. This permitted us to incorporate temporal variability in end members into the mixing model (Dethier et al. 2013). For phytoplankton and benthic diatoms, we pooled data over both space and time since individual samples sizes were low.

Mixing model – POM

To estimate the contribution of the different primary producers to POM collected in oyster beds, we used the same set of sources described above for oysters. We assumed no fractionation between the sources (primary producers) and POM. Low quantities of organic material accumulated onto filters combined with analytical difficulties resulted in generally low sample sizes, particularly in Hood Canal. Because of this, we pooled data from all intervals. Dosewallips and Hamma Hamma data were pooled for the mixing model analysis. No POM data were obtained from the months of October and November at any of the three sites.

Results

Environmental data

Water temperature in the Samish Bay ranged from CTD profile averages of 14.3°C in August, to 7.6 °C, and 17.5 °C and 17.8 °C in August and 7.0 °C and 7.3 °C in December for Dosewallips and Hamma Hamma, respectively (Fig. 2a). With exception of September, when weather conditions forced sampling in the Samish Bay closer to the river, water over oyster beds was ~4 to 50 % more saline in Samish Bay than Hood Canal sites in all months (Fig. 2b). Lower salinity in Hood Canal in June and December potentially reflect increases in river flow from snowmelt and rainfall, respectively. Mean chlorophyll *a* was generally low at all three sites, with the highest values observed in July, September and November (Fig. 5c).

Stable isotope values of consumers and primary producers

In general, primary producers isotope ratios were more enriched at the Samish river compared to the Hood Canal sites. For example, Samish Bay intertidal macrophytes were more enriched than Hood Canal sites in $\delta^{13}C$ (Dosewallips vs. Samish, $p= 0.015$; Hamma Hamma vs. Samish, $p <0.001$) and $\delta^{15}N$ (Dosewallips, $p <0.001$; Hamma Hamma, $p <0.001$) while isotope

levels of macrophytes showed a trend towards being more enriched in the Dosewallips than in the Hamma Hamma ($\delta^{13}C$, $p = 0.08$; $\delta^{15}N$, $p = 0.08$) (Fig. 3) (Appendix A). There was weak evidence that marsh plants from the Samish were slightly more enriched in $\delta^{13}C$, and plants from Hamma Hamma the most deplete ($F_{2,45} = 2.77$, $p=0.07$) (Fig. 3)(Appendix A). Salt marsh plant $\delta^{15}N$ was also different across sites ($F_{2,45} = 11.44$, $p <0.001$) such that the two Hood Canal sites were similar ($p = 0.51$) while Samish salt marsh plants were more enriched in $\delta^{15}N$ than Dosewallips ($p <0.001$) and Hamma Hamma ($p = 0.014$) Fig. 3) (Appendix A). Upland vegetation $\delta^{13}C$ differed across sites ($F_{2,68} = 4.28$, $p =0.018$) such that Dosewallips vegetation was less enriched than both Hamma Hamma ($p = 0.019$) and showed a non-significant trend towards being less enriched than Samish vegetation ($p = 0.08$)(Fig.3) (Appendix A). $\delta^{15}N$ of upland vegetation was also different across sites ($F_{2, 68} = 9.98$, $p <0.001$) such that Hamma Hamma was less enriched than both Samish ($p < 0.001$) and Dosewallips ($p = 0.048$) (Fig. 3)(Appendix A).

Oyster adductor tissue $\delta^{13}C$ ratios were different across sites ($F_{2,142} = 277.19$, $p <0.001$) with Samish being more enriched than both Dosewallips ($p< 0.001$) and Hamma Hamma ($p < 0.001$) while Dosewallips was more enriched in $\delta^{13}C$ than Hamma ($p <0.001$)(Fig. 3) (Appendix A). Oyster adductor muscle $\delta^{15}N$ ratios were also different across sites ($F_{2,142} = 7.04$, $p =0.001$) such that Hamma Hamma oysters were less enriched than both Samish ($p < 0.001$) and Dosewallips oysters ($p = 0.04$) (Fig. 3) (Appendix A).

Mixing model-Oysters

Using both trophic enrichment factors, $\delta^{13}C$ and $\delta^{15}N$ of oyster adductor muscle tissue fell within the ranges of their potential diet sources at all three sites (Fig. 4 a-f), indicating that we likely that we sampled all major groups of diet sources. Because of the overlap of isotopic

values of *C. gigas* across sample intervals (Fig. 4), we pooled values across time periods. Distributions for source contributions were unimodal and with generally informative central tendencies with the possible exception of diatoms and phytoplankton, both of which had wide distributions (Fig. 5a-f). Results from trophic enrichment factors reported using Dubois et al. (2007) (Fig. 5 a-c) and Lefebvre et al. (2009a) (Fig. 5 d-f) were comparable, suggesting that the relative contributions of organic matter sources were fairly robust to uncertainty in trophic fractionation, with the exception of an increase in the estimated contribution of upland vegetation at all three site, in particular Samish Bay (Fig. 5a, 5d). While all three sites showed median values of approximately 30% for combined contribution of benthic diatoms and phytoplankton, they showed somewhat different relative contributions of the other primary producers (Fig. 6). Both Hood Canal sites had high combined contributions of terrestrial vegetation and salt marsh plants, driven largely by salt marsh plants (~40%), suggesting particularly strong coupling between salt marsh and lower intertidal habitats in the Dosewallips and Hamma Hamma river estuaries (Fig. 9). Samish Bay more reliance on autochthonous intertidal primary producers(38-40%) and somewhat lower contribution from (18-25%) with approximately 7% of oyster diets originating from terrestrially-derived detritus using fractionation factors from Dubois et al. (2007) and 17% using Lefebvre et al. (2009a) (Fig. 6a,b). Median contributions of organic matter sources were similar for oysters collected in June, September and January 2011 for both sets of trophic enrichment factors (Appendix B).

Mixing model - POM

Oyster bed POM $\delta^{13}C$ varied markedly from June to December in the Samish Bay oyster bed, with the most enriched (marine-influenced) ratios occurring in July and August (-17 to -14‰) and the most depleted occurring in June and December (-20‰) , potentially indicating

freshwater influence from both snowmelt (June) and rain (December) (Fig. 2e). The $\delta^{13}C_{POM}$ values for Hood Canal sites were generally more depleted (terrestrially-influenced)(-22 to -24 ‰) than the Samish Bay (-18 to -19 ‰) in both June and December (Fig 2e). The Hamma Hamma oyster bed POM was somewhat less enriched in $\delta^{15}N$ (5‰) relative to the Samish Bay (7 ‰) and Dosewallips (6‰) shellfish growing areas in June, the only month with sufficient material for analysis from all three sites (Fig. 2d).

Contributions of different primary producers to nearshore (oyster bed) POM were relatively similar to the contributions to oyster diets, despite different timeframes inherent to the sampling of both oyster tissue, which integrates over several months, and POM, which is a punctuated sampling of organic matter available to filter feeders at a given place and time. Most notably, both salt marsh plants and intertidal macrophytes were estimated to contribute to the nearshore POM although macrophytes appeared to be less important component of the POM in Hood Canal (5%) than in Samish Bay (30%) (Figs. 6, 7). This may in part be driven by lack of POM data in Hood Canal in July, September and October, resulting in an under-representation of POM sampling in the summer months in Hood Canal relative to Samish Bay.

Discussion

The degree to which nearshore food webs are linked to upland, offshore and estuarine ecosystems is key to both understanding and managing these food webs. We found that for three sites in Puget Sound, WA, benthic consumers relied heavily on autochthonously derived carbon from intertidal macrophytes (eelgrass and macroalgae) and on exported salt marsh plant organic matter, with lower contributions of phytoplankton, benthic diatoms and upland vegetation. This reliance on organic matter source other than phytoplankton seems reasonable given both the low chlorophyll *a* concentrations observed in the oyster beds at all three sites (Fig. 2c) and the

observation that salt marsh vegetation, eelgrass beds and macroalgae are highly productive and can serve as important sources of organic matter for nearshore benthic food webs (Simenstad and Wissmar 1985, Ruckelshaus et al. 1993, Tallis 2009, Howe and Simenstad 2011). While both eelgrass and macroalgae are relatively abundant in Samish Bay proper (E. Howe unpublished data), it is also adjacent to one of the largest continuous beds of eelgrass in Puget Sound, Padilla Bay (WDNR report), which may help explain the high proportion of intertidal macrophytes contributing to oyster diets (Fig. 6) and POM (Fig. 7) in Samish Bay. Furthermore, the oceanographic transport of particles predicted by (Banas et al. *in prep*) suggests strong connectivity between Samish and Padilla Bays, providing a mechanism for transport of organic matter between these two habitats. Hood Canal is more dominated by salt marshes than eelgrass beds, driven largely by the expansive Skokomish River delta (Simenstad and Wissmar 1985), which is also predicted to influence the Hamma Hamma and Dosewallips regions of Hood Canal via freshwater transport (Banas et al. *in prep*). This linkage is borne out by the high contribution of salt marsh plants in oyster diets (Fig. 6) and nearshore POM (Fig. 7) for the Hood Canal sites.

Because we found a relatively high (~25%) contribution of intertidal macrophytes to Hood Canal oyster diets and only 5 % to the nearshore POM, it is possible that either the Hood Canal oysters are consuming resources in a manner disproportionate to its availability (displaying food preferences)(Beninger et al. 2007) or that the low sample sizes of nearshore POM in Hood Canal led to an underrepresentation of macrophytes. Sampling the nearshore POM at a higher frequency and for at least one full annual cycle would help distinguish among these two scenarios.

Despite the differences in contributions of salt marsh plants and intertidal macrophytes and spatial differences in stable isotope ratios in the consumers themselves, the relative

295 contribution of organic matter sources to oyster diets were more similar across sites than we
296 expected given the spatially distinct consumer isotopic values... This result suggests that the
297 spatial pattern in consumer stable isotope ratios were partly due to differences in the stable
298 isotope values of diet items, rather than differences in the proportion consumed of a spatially
299 homogenous pool of primary producers. This finding underscores the importance of
300 understanding variation in the underlying isotope baseline when making inferences about
301 consumer diets (Vander Zanden and Rasmussen 2001, Post 2002, Solomon et al. 2011, Dethier et
302 al. 2013).

303 *Conclusions*

304 Nearshore oyster beds in Puget Sound appear to rely on organic matter originating in estuarine
305 habitats as well as salt marshes, with lower contributions of organic matter from phytoplankton,
306 benthic diatoms and upland vegetation. There was some evidence that the relative importance of
307 these sources may vary among river deltas within Puget Sound. These findings provide further
308 evidence for strong physical, biological and chemical linkages between adjacent ecosystems.
309 That oysters were highly dependent on energy from adjacent salt marsh habitats as well as
310 autochthonous production suggests that changes (e.g., land conversion, shoreline modifications)
311 in these source habitats may affect the secondary productivity of the estuarine ecosystem. This is
312 supported by several lines of evidence, including commensurate spatial variation in $\delta^{13}C$ and
313 $\delta^{15}N$ ratios of benthic consumers and primary producers, hydrologic connectivity of major
314 organic matter sources to the shellfish beds we studied and the strong contribution of these
315 sources to independently sampled particulate organic matter at high tide above oyster beds. This
316 evidence for local-scale connectivity between estuarine habitats (intertidal shellfish beds and salt
317 marshes) adds to the emerging picture of the Puget Sound ecosystem as a heterogeneous mosaic

318 of interconnected habitats, emphasizing the need for management that transcends ecosystem
319 boundaries and recognizes such linkages.

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Table 1. Timeframe for field sample collections (2011/2012)

Collection Type	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan
Oyster tissue (adductor muscle)	X			X				X
Oyster bed POM	X	X	X	X	X	X	X	X
Offshore POM	X			X			X	
Intertidal Macrophytes	X		X			X		
Salt marsh plants	X		X			X		
Upland vegetation	X		X			X		

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. Mean (±SD) temperature (a), salinity (b), chlorophyll *a* (c), Delta ^{15}N-POM (d), and Delta ^{13}C-POM (e), in submerged oysters beds in the Samish (red squares), Dosewallips (blue circles) and Hamma Hamma (green triangles) shellfish growing areas.

Figure 3. Mean (+/-) SD carbon and nitrogen stable isotope ratios of oysters and primary producers collected at the Samish (red), Dosewallips (blue) and Hamma Hamma (green) shellfish growing areas and adjacent salt marsh and upland habitats. Oyster stable isotope ratios are corrected for trophic enrichment using the muscle-specific fractionation values from Lefebvre et al. 2009a (Δ2.9 for carbon and Δ4.7 for nitrogen).

Figure 4. Carbon and nitrogen stable isotope ratios of individual oysters, oyster bed POM and mean (+/-SD) potential diet items. Oyster values are adjusted using trophic enrichment factors from Dubois et al 2009: (Δ1.85 for carbon and Δ3.79 for nitrogen) in the a)Samish, b) Dosewallips and c) Hamma Hamma growing areas and Lefebvre et al. 2009: (using Δ2.9 for carbon and Δ4.7 for nitrogen) in the d)Samish, e) Dosewallips and f) Hamma Hamma growing areas.

Figure 5. Estimated proportion contribution of diet items using the trophic enrichment factor from Dubois et al. 2009: (Δ1.85 for carbon and Δ3.79 for nitrogen) in the a)Samish, b) Dosewallips and c) Hamma Hamma growing areas and Lefebvre et al. 2009: (using Δ2.9 for carbon and Δ4.7 for nitrogen) in the d)Samish, e) Dosewallips and f) Hamma Hamma growing areas .

Figure 6. MixSIR results showing contributions to oyster diets pooling across all sample intervals using trophic enrichment factors from a) Dubois et al. 2007 and b)Lefebvre et al. 2009 for the Samish, Dosewallips and Hamma Hamma growing areas.

Figure 7. MixSIR results showing median contributions to oyster bed POM

Figures

Figure 1.

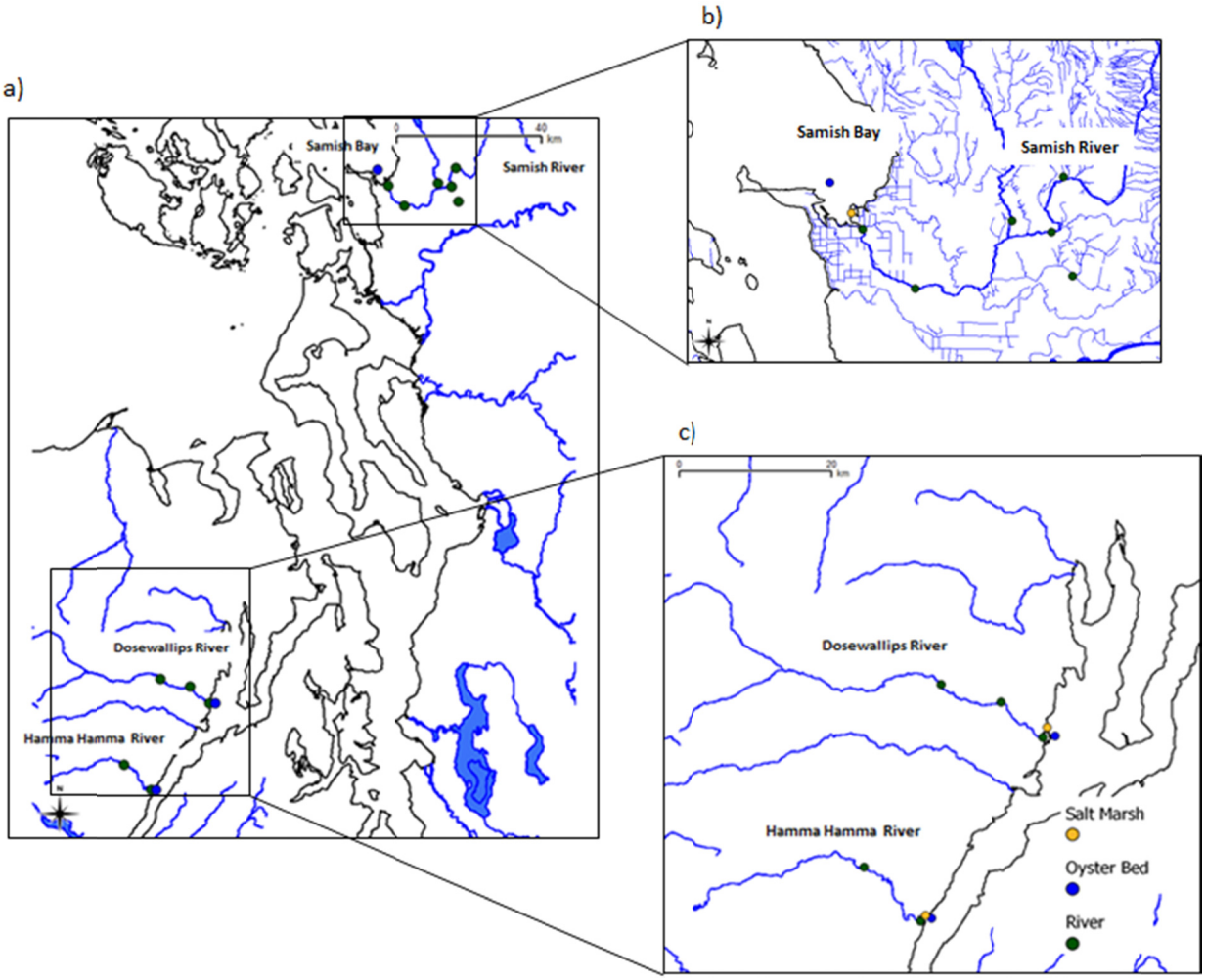


Figure 2.

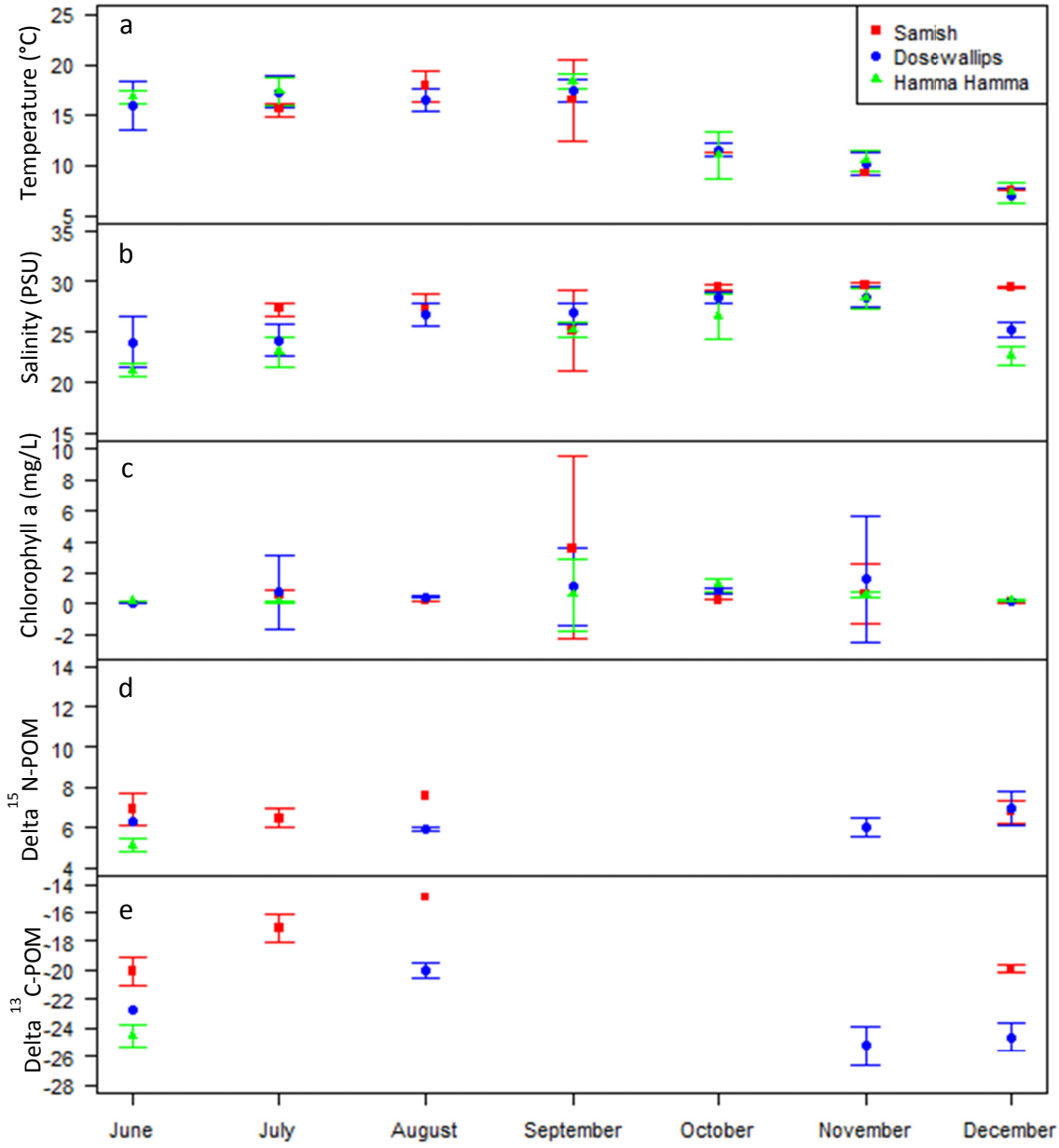

a
b
c
d
e
Samish
Dosewallips
Hamma Hamma
Temperature (°C)
Salinity (PSU)
Chlorophyll a (mg/L)
Delta 15 N-POM
Delta 13 C-POM
June
July
August
September
October
November
December

Figure 3.

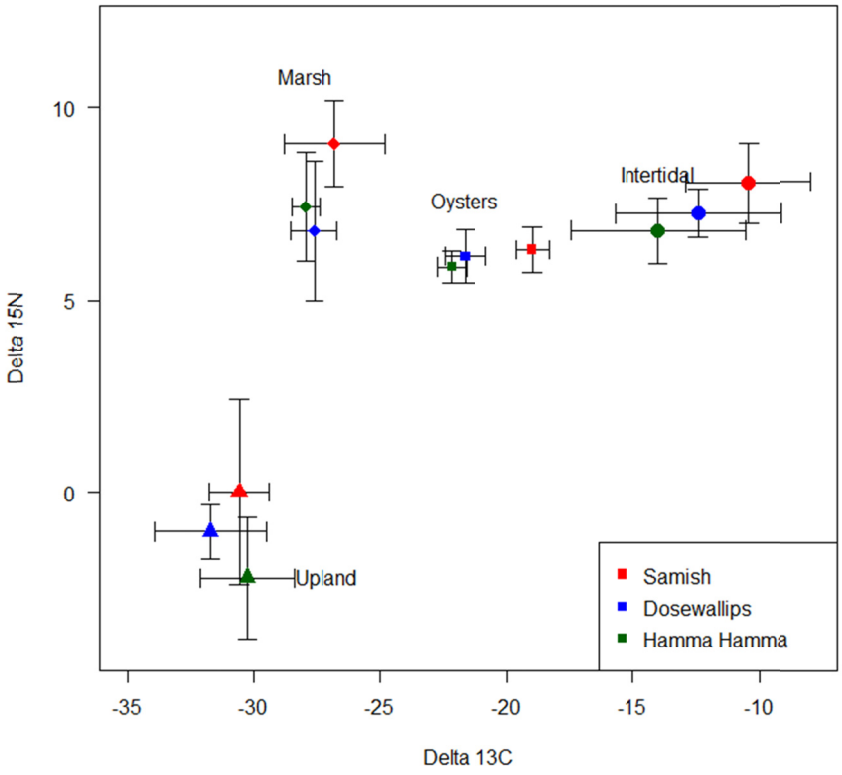

Marsh
Oysters
Intertidal
Upland
Samish
Dosewallips
Hamma Hamma
Delta 15N
Delta 13C
10
5
0
-35
-30
-25
-20
-15
-10

Figure 4.

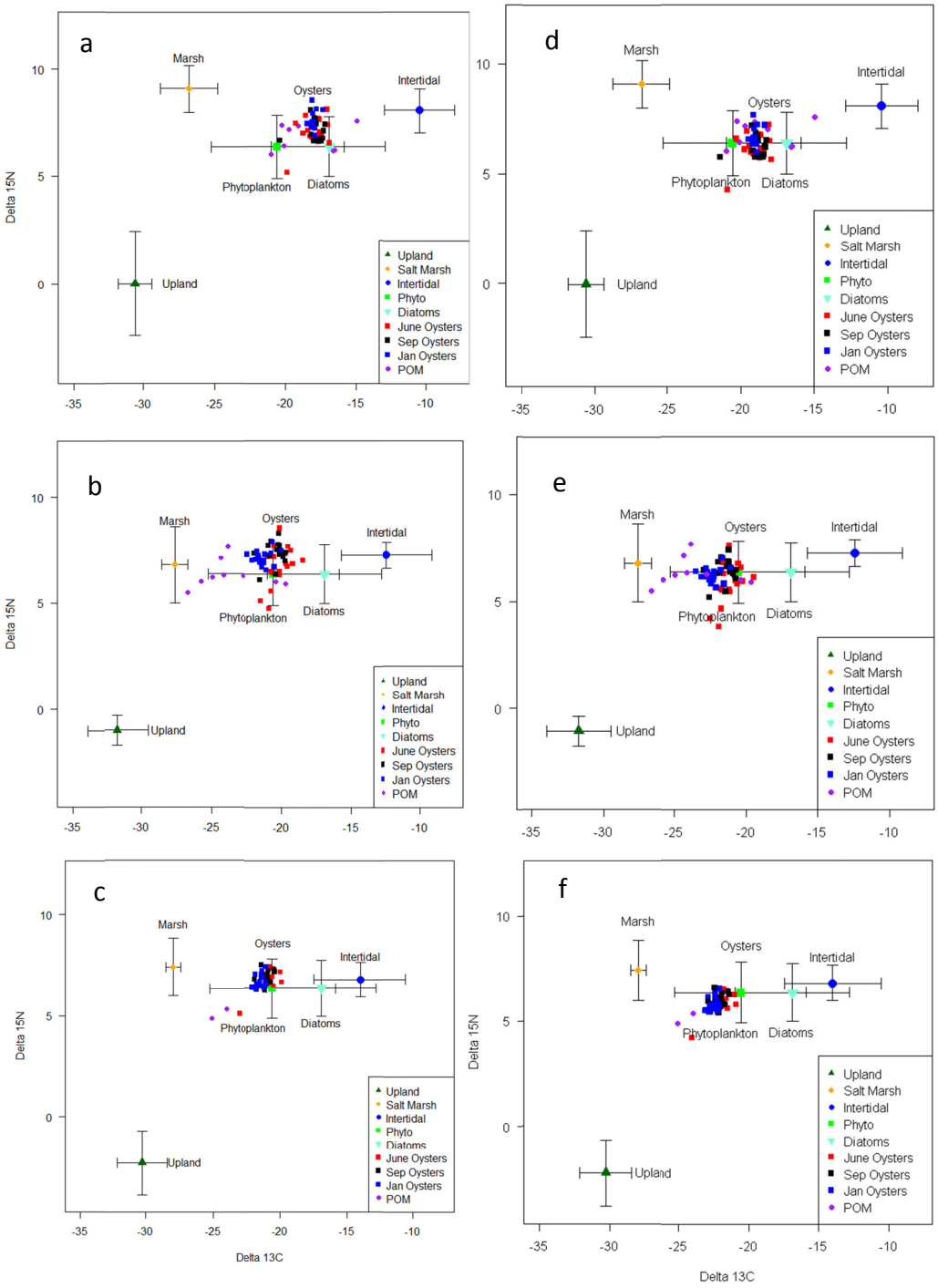

a
b
c
d
e
f
Marsh
Oysters
Intertidal
Phytoplankton
Diatoms
Upland
Delta 15N
Delta 13C
Upland
Salt Marsh
Intertidal
Phyto
Diatoms
June Oysters
Sep Oysters
Jan Oysters
POM

Figure 5.

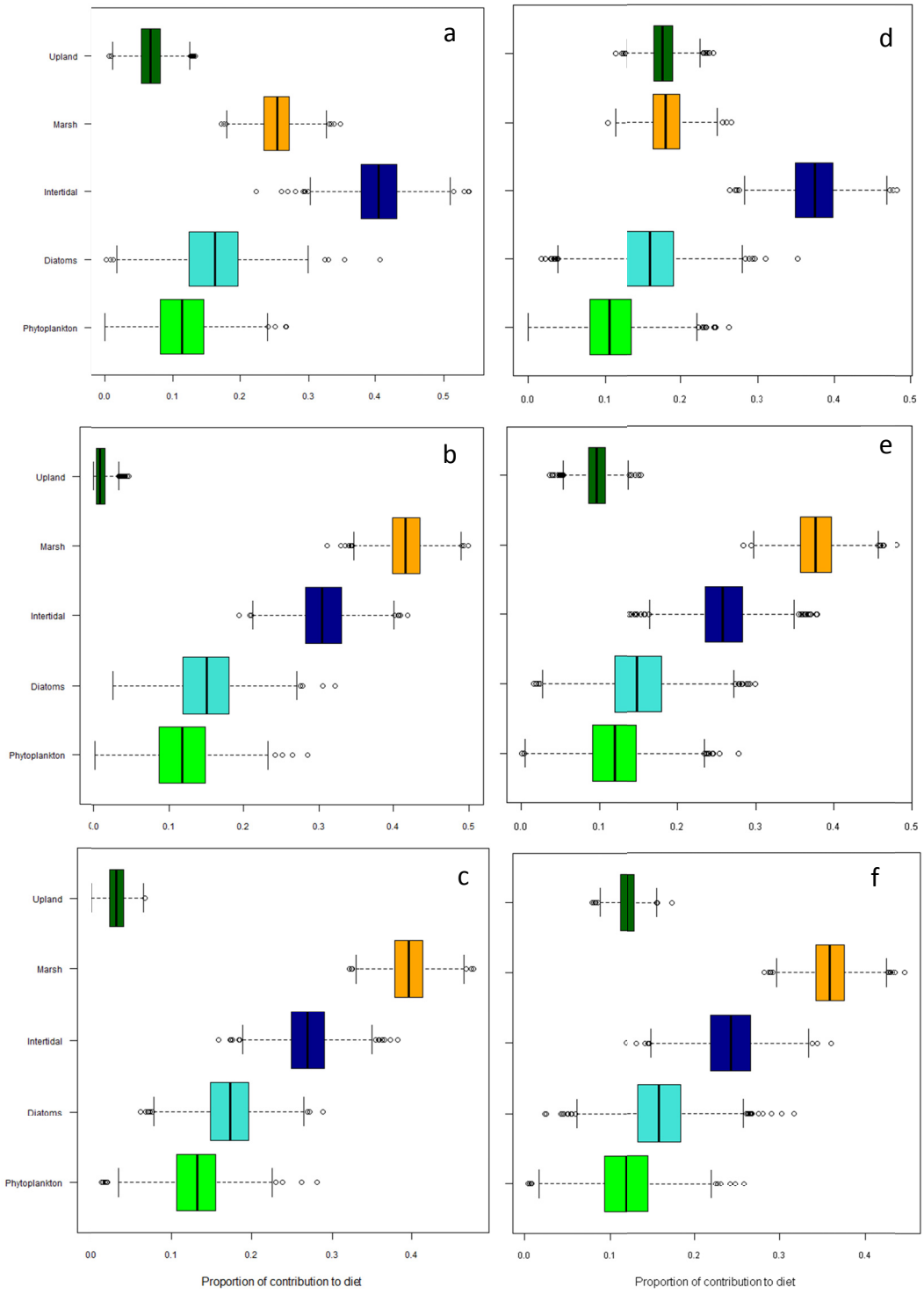

a
b
c
d
e
f
Upland
Marsh
Intertidal
Diatoms
Phytoplankton
0.0
0.1
0.2
0.3
0.4
0.5
Proportion of contribution to diet

Figure 6.

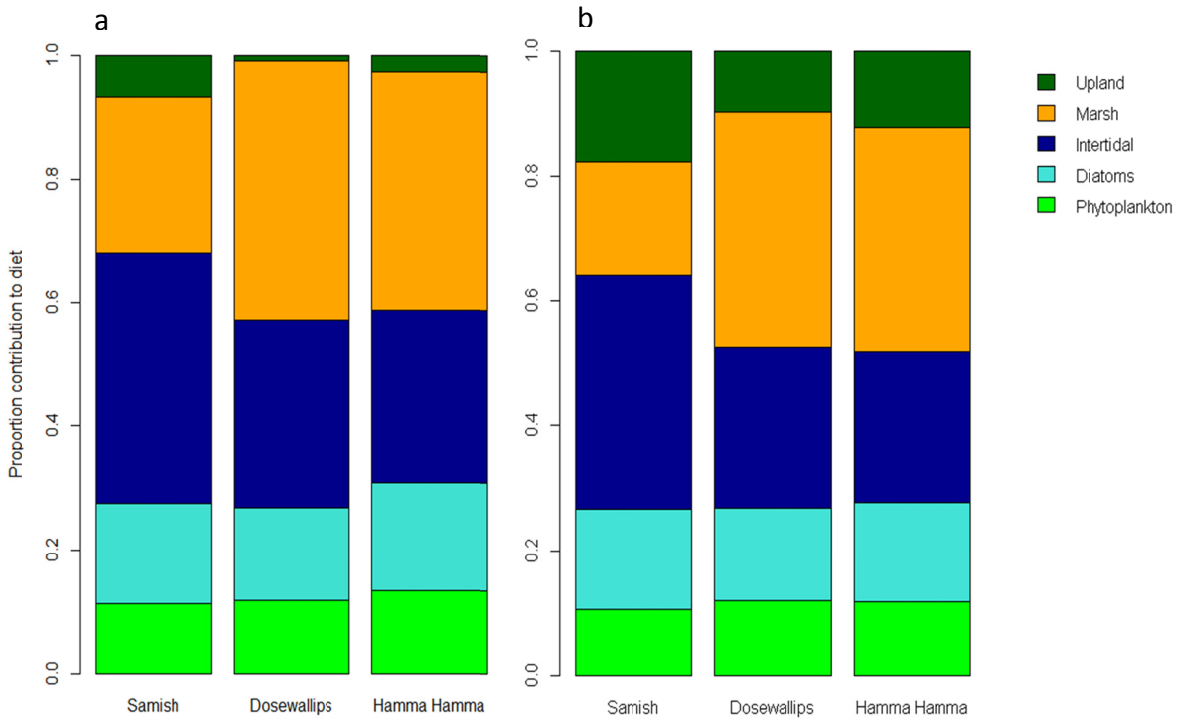
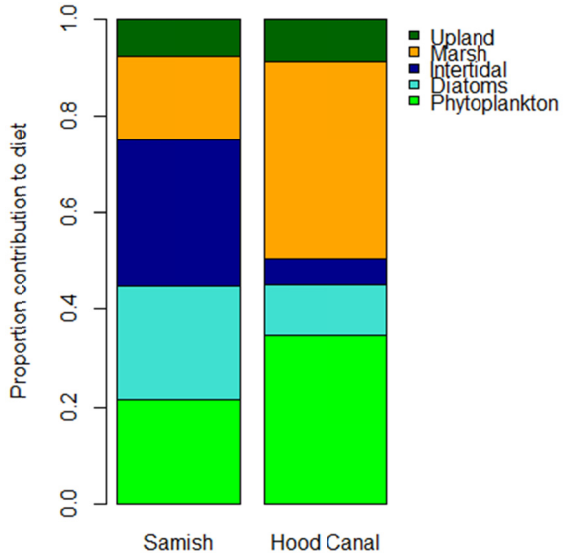

a
b
Proportion contribution to diet
0.0
0.2
0.4
0.6
0.8
1.0
Samish
Dosewallips
Hamma Hamma
Upland
Marsh
Intertidal
Diatoms
Phytoplankton

Figure 7.


Proportion contribution to diet
0.0
0.2
0.4
0.6
0.8
1.0
Samish
Hood Canal
Upland
Marsh
Intertidal
Diatoms
Phytoplankton

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Appendix A. $\delta^{13}C$ and $\delta^{15}N$ ratios for primary producers and *C. gigas* collected from three shellfish growing areas and adjacent upland habitats in Puget Sound (Samish, Dosewallips and Hamma Hamma).

	Delta 13C						Delta 15N						C:N						Sample Size		
	Samish		Dosewallips		Hamma Hamma		Samish		Dosewallips		Hamma Hamma		Samish		Dosewallips		Hamma Hamma		Samish	Dosewallips	Hamma Hamma
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Primary Producers																					
Upland	**-30.59**	**1.19**	**-31.73**	**2.21**	**-30.27**	**1.84**	**-0.01**	**2.40**	**-1.03**	**0.70**	**-2.24**	**1.58**	**21.99**	**3.67**	**27.85**	**8.77**	**30.51**	**10.73**	**24**	**24**	**23**
Pseudotsuga menziesii			-31.49	2.51	-31.76	0.61			-1.00	0.92	-3.60	1.19			34.04	7.63	40.74	4.41		12	11
Alnus rubra	-31.05	0.85	-31.97	1.95	-28.91	1.49	-1.47	0.36	-1.07	0.43	-0.99	0.39	21.57	3.18	21.67	4.35	21.13	3.48	16	12	12
Salix spp	-29.67	1.29					2.92	2.00					22.81	4.64					8		
Salt marsh	**-26.81**	**2.00**	**-27.62**	**0.92**	**-27.96**	**0.57**	**9.07**	**1.11**	**6.80**	**1.81**	**7.43**	**1.43**	**18.70**	**7.57**	**19.49**	**8.41**	**20.23**	**12.78**	**19**	**18**	**11**
Glaux maritima	-24.68	0.58	-26.65	0.87			8.64	0.88	5.60	0.74			24.07	7.09	25.79	11.50			8	6	
Salicornia virginica	-28.36	0.82	-28.11	0.43	-27.96	0.57	9.38	1.19	7.40	1.91	7.43	1.43	14.80	5.30	16.34	4.09	20.23	12.78	11	12	11
Oyster bed	**-10.42**	**2.46**	**-12.42**	**3.28**	**-14.00**	**3.45**	**8.05**	**1.03**	**7.26**	**0.63**	**6.81**	**0.86**	**13.73**	**3.29**	**23.33**	**9.71**	**15.74**	**9.46**	**38**	**38**	**33**
Fucus gardneri			-14.38	1.05	-13.74	0.94			7.28	0.35	6.95	0.43			31.19	10.29	37.24	6.93		12	4
Gracilaria spp			-12.03	0.95	-16.03	1.40			7.88	0.13	6.97	0.53			16.69	2.75	12.72	2.81		4	6
Laminariales	-15.94	1.71					8.21	0.88					13.05	2.97					4		
Ulva spp	-10.37	1.37	-13.37	4.35	-17.11	2.80	8.24	0.76	7.64	0.35	7.36	0.75	13.25	3.69	19.14	9.46	13.84	6.24	12	12	10
Zostera spp					-9.03						5.91						14.62				1
Zostera japonica	-9.60	1.65	-9.37	1.25	-11.87	1.46	7.60	1.49	6.05	0.48	6.66	1.19	14.52	4.11	17.53	0.81	11.34	4.80	12	4	6
Zostera marina	-9.26	1.56	-8.90	0.68	-9.93	1.71	8.30	0.55	6.81	0.41	5.97	0.44	13.63	1.69	24.30	3.06	12.20	4.11	10	6	6
Consumer																					
Crassostrea gigas	-16.09	0.66	-18.74	0.82	-19.27	0.57	11.01	0.58	10.84	0.69	10.56	0.42	3.40	0.34	3.51	0.54	3.44	0.52	45	53	47

Appendix B. Results from MixSIR showing median contributions of organic matter sources to oyster diets using trophic enrichment factors from a) Dubois et al. (2007) and b) Lefebvre et al. (2009a) in shellfish beds adjacent to the Samish, Dosewallips and Hamma Hamma rivers for oysters collected in June, September and January of 2011.

a)

Site	Source	June	September	January
Samish	Upland	8.90	8.24	4.35
	Marsh	21.67	21.89	28.38
	Intertidal	41.64	40.61	41.24
	Diatoms	16.03	16.82	14.53
	Phytoplankton	11.77	12.44	11.50
Dosewallips	Upland	2.15	1.16	1.86
	Marsh	37.62	41.46	44.27
	Intertidal	33.05	33.30	27.29
	Diatoms	16.13	13.35	14.34
	Phytoplankton	11.05	10.74	12.24
Hamma Hamma	Upland	2.74	2.16	3.93
	Marsh	35.41	38.34	39.73
	Intertidal	29.83	28.72	26.63
	Diatoms	18.34	17.64	17.16
	Phytoplankton	13.68	13.14	12.55

b)

Site	Source	June	September	January
Samish	Upland	19.02	18.09	13.34
	Marsh	15.62	16.01	22.33
	Intertidal	37.5	36.46	35.66
	Diatoms	15.78	16.63	15.74
	Phytoplankton	12.08	12.21	12.01
Dosewallips	Upland	11.41	6.79	10.07
	Marsh	33.57	38.88	40.47
	Intertidal	28.73	25.48	22.17
	Diatoms	16.04	15.43	14.27
	Phytoplankton	10.24	12.95	12.65
Hamma Hamma	Upland	11.58	10.97	13.21
	Marsh	33.05	36.18	36.96
	Intertidal	25.15	24.25	22.80
	Diatoms	16.88	15.98	15.01
	Phytoplankton	13.34	12.33	11.88