

Estuarine organic matter subsidizes shellfish beds in Puget Sound, WA

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Summary and Key Findings

Nearshore ecosystems are complex and dynamic habitats that are subject to trophic subsidies and physical forcing from both terrestrial and marine environments (Dame and Allen 1996). While it is accepted that estuaries couple terrestrial and marine environments, understanding the nature of this connection has proven context-dependent, driven by factors such as availability of different carbon sources and degree of freshwater influence (Ruckelshaus et al. 1993, Riera and Richard 1996, Ruesink et al. 2003).

Freshwater flow and proximity to different organic matter sources can influence the growth and biomass of nearshore filter feeders (Ruckelshaus et al. 1993, Riera and Richard 1996, Ruesink et al. 2003). Specifically, the relative importance of allochthonous and autochthonous organic matter to nearshore bivalves may depend on the quantity and quality of freshwater and marine fluxes.

Despite the inherent challenges in determining the relative importance of marine and terrestrial organic matter subsidies to estuarine food webs, estuarine habitats must be considered through the lens of their connectivity with both upland and offshore ecosystems since they are subject to natural and anthropogenic influences originating from 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 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*).

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 primary producers can display (Fry and Sherr 1984, Cloern et al. 2002). We explored 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of primary producers, particulate organic matter (POM) and Pacific Oyster (*Crassostrea gigas* Thunberg) tissue at the mouth of three river basins.

Key findings include the following:

- Stable isotope ratios of intertidal macrophytes differed across the three sites in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ such that Samish Bay intertidal macrophytes were more enriched than both Hood Canal sites in both in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 2). There was weak evidence that marsh plants from the Samish were slightly more enriched in $\delta^{13}\text{C}$ than Hood Canal sites, and plants from Hamma Hamma the most deplete (Fig. 2). Samish salt marsh plants were more enriched in $\delta^{15}\text{N}$ than Dosewallips and Hamma Hamma (Fig. 2). Upland vegetation $\delta^{13}\text{C}$ differed across sites such that Dosewallips vegetation was less enriched than plants from the Hamma Hamma, and showed a non-significant trend towards being less

enriched than Samish vegetation (Fig.2). $\delta^{15}\text{N}$ of upland vegetation from the Hamma Hamma was less enriched than both Samish and Dosewallips (Fig. 2).

- Oyster adductor tissue $\delta^{13}\text{C}$ ratios were different across sites with Samish being more enriched than both Dosewallips and Hamma Hamma while Dosewallips was more enriched in $\delta^{13}\text{C}$ than Hamma Hamma (Fig. 6). Oyster adductor muscle $\delta^{15}\text{N}$ ratios from Hamma Hamma oysters were less enriched than oysters from the Samish and Dosewallips (Fig. 2).
- Using two different trophic enrichment factors, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of oyster adductor muscle tissue fell within the ranges of their potential diet sources at all three sites (Fig. 3 a-f), indicating that we likely sampled all major diet sources. Because of the overlap of isotopic values of *C. gigas* across sample intervals (Fig. 3), 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. 4a-f).
- Results from trophic enrichment factors reported using Dubois et al. (2007) (Fig. 4 a-c) and Lefebvre et al. (2009a) (Fig. 4 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 sites, in particular Samish Bay (Fig. 5a,b).
- 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. 5). Both Hood Canal sites had high combined contributions of terrestrial vegetation largely from salt marsh plants (~40%), suggesting particularly strong coupling between salt marsh and lower intertidal habitats (Fig. 5). Samish Bay showed more reliance on autochthonous intertidal primary producers (38-40%), and somewhat lower contribution from salt marshes (18-25%), with approximately 7-17% of oyster diets originating from terrestrially-derived detritus (using fractionation factors from Dubois et al. (2007), Lefebvre et al. (2009a) (Fig. 5a,b).
- Nearshore oyster beds in Puget Sound appear to rely on organic matter originating from a variety of habitats including primarily intertidal and salt marsh, but also pelagic, benthic and upland vegetation. There was some evidence that the relative importance of these

sources may vary among river deltas within Puget Sound. This is supported by several lines of evidence, including commensurate spatial variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of benthic consumers and primary producers, hydrologic connectivity of major organic matter sources to the shellfish beds we studied and the strong contribution of these sources to independently sampled particulate organic matter at high tide above oyster beds.

- 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 contributions of the marine and freshwater organic matter sources were more similar across sites than we expected given the spatially distinct consumer isotopic values. This result suggests that the spatial pattern in consumer stable isotope ratios was partly due to differences in the stable isotope values of diet items, rather than differences in the proportion consumed of a homogenous pool of primary producers. This finding underscores the importance of understanding variation in the underlying isotope baseline when making inferences about consumer diets (Vander Zanden and Rasmussen 2001, Post 2002, Solomon et al. 2011, Dethier et al. 2013).
- Our findings provide further evidence for strong physical, biological and chemical linkages between adjacent ecosystems. Specifically, oysters were highly dependent on energy from adjacent salt marsh habitats as well as autochthonous production; thus a change (e.g., land conversion, shoreline modifications) in these source habitats may affect the secondary productivity of the estuarine ecosystem.
- This evidence for local-scale connectivity between estuarine habitats (intertidal shellfish beds and salt marshes) adds to the emerging picture of the Puget Sound ecosystem as a heterogeneous mosaic of interconnected habitats, emphasizing the need for management that transcends ecosystem boundaries and recognizes such linkages.

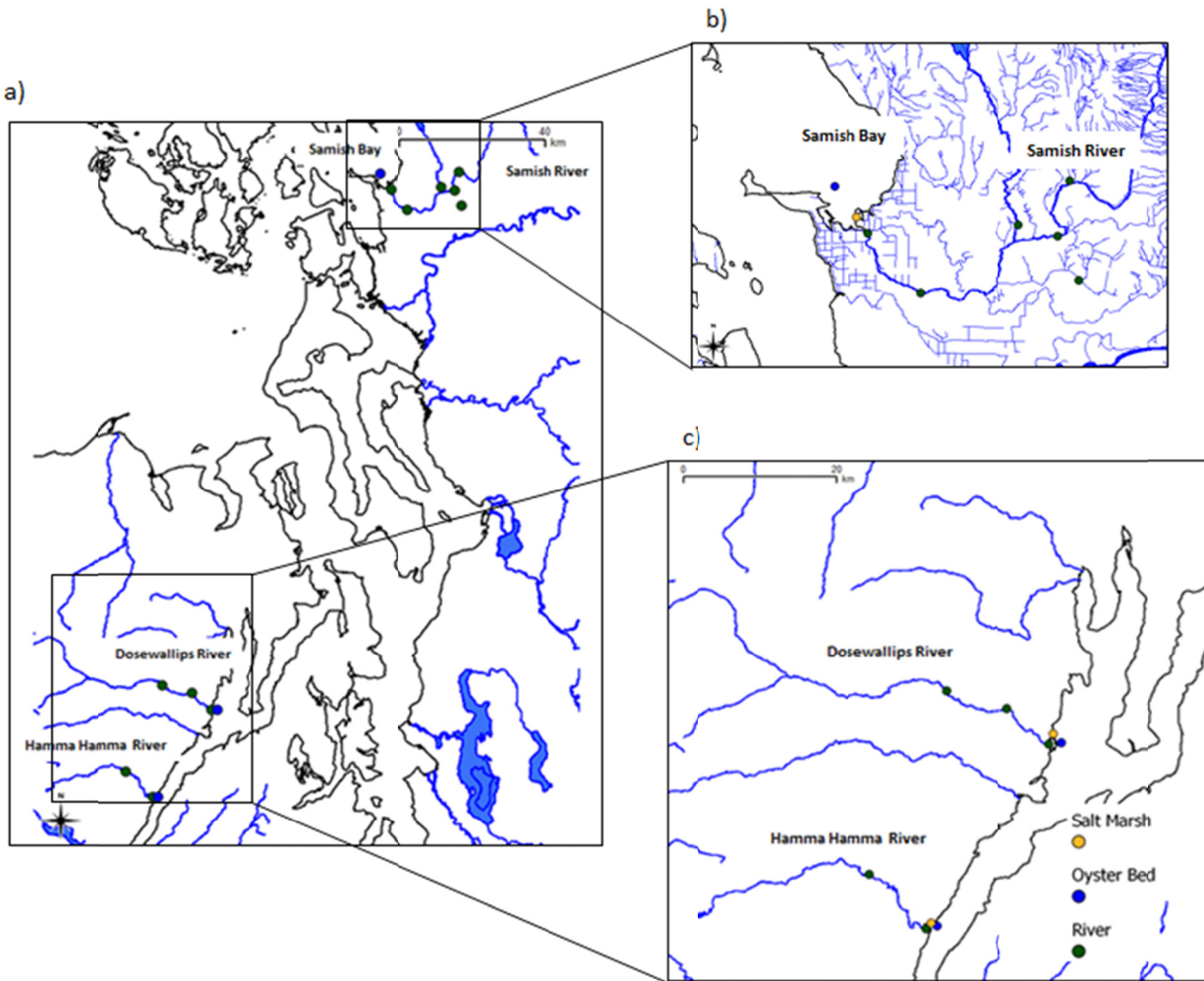


Fig. 1 Map of tissue and water collection locations in Puget Sound (a) with enlargements depicting specific sampling locations in oyster beds (blue), rivers (green) and salt marshes (orange) the Samish River and Bay (b), and the Dosewallips and Hamma Hamma Rivers (c).

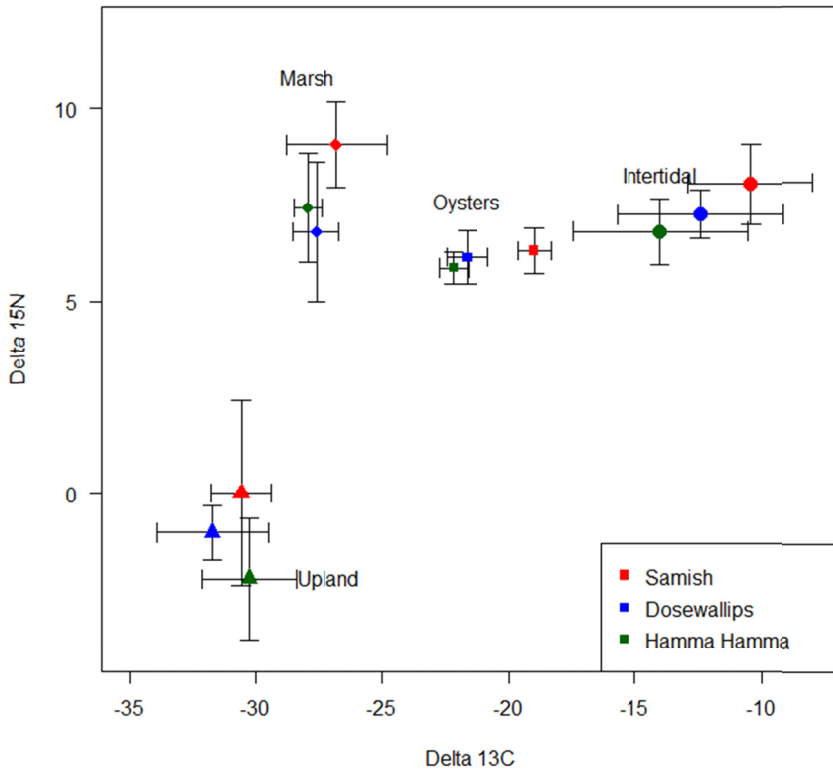


Figure 2. Mean (+/-) SD carbon and nitrogen stable isotope ratios of oysters and primary producers collected at the Samish (red), Dosewallips (blue) and Hamma Hamma (green) shellfish growing areas and adjacent salt marsh and upland habitats. Oyster stable isotope ratios are corrected for trophic enrichment using the muscle-specific fractionation values from Lefebvre et al. 2009a ($\Delta 2.9$ for carbon and $\Delta 4.7$ for nitrogen).

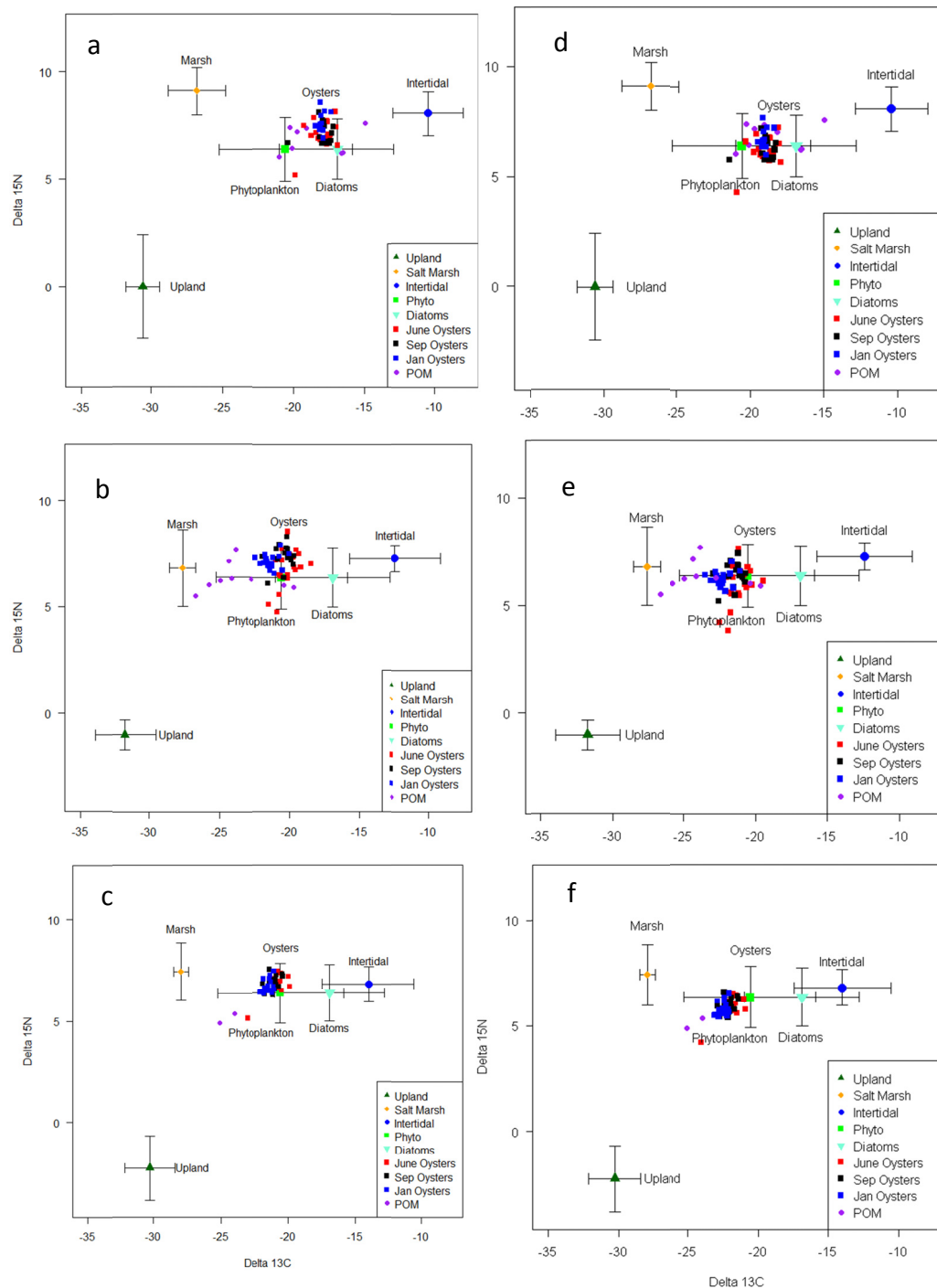


Figure 3. Carbon and nitrogen stable isotope ratios of individual oysters, oyster bed POM and mean (\pm SD) potential diet items. Oyster values are adjusted using trophic enrichment factors from Dubois et al 2009: ($\Delta 1.85$ for carbon and $\Delta 3.79$ for nitrogen) in the a) Samish, b) Dosewallips and c) Hamma Hamma growing areas and Lefebvre et al. 2009: (using $\Delta 2.9$ for carbon and $\Delta 4.7$ for nitrogen) in the d) Samish, e) Dosewallips and f) Hamma Hamma growing areas .

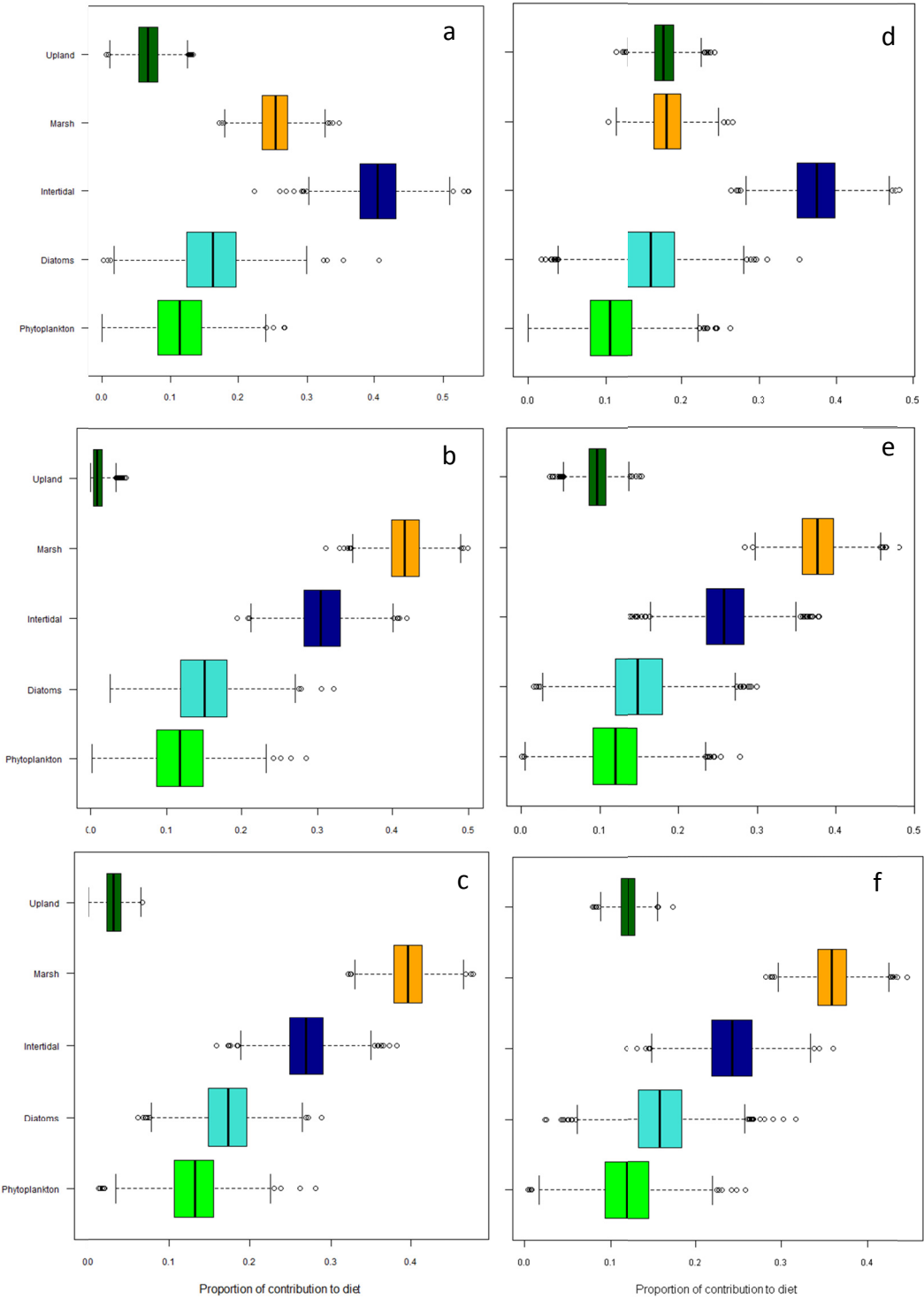


Figure 4. Estimated proportion contribution of diet items using the trophic enrichment factor from Dubois et al. 2009: ($\Delta 1.85$ for carbon and $\Delta 3.79$ for nitrogen) in the a) Samish, b) Dosewallips and c) Hamma Hamma growing areas and Lefebvre et al. 2009: (using $\Delta 2.9$ for carbon and $\Delta 4.7$ for nitrogen) in the d) Samish, e) Dosewallips and f) Hamma Hamma growing areas .

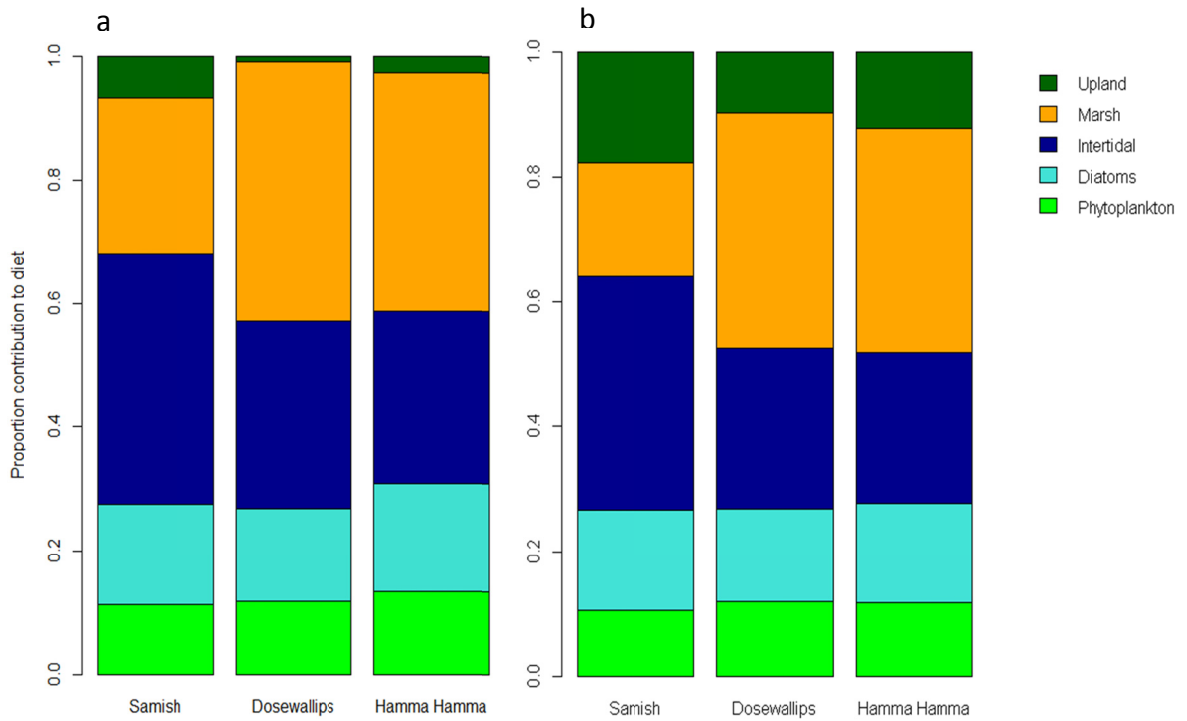


Figure 5. MixSIR results showing contributions to oyster diets pooling across all sample intervals using trophic enrichment factors from a) Dubois et al. 2007 and b) Lefebvre et al. 2009 for the Samish, Dosewallips and Hamma Hamma growing areas.

Literature Cited

- Banas, N. S., L. L. Conway-Cranos, D. A. Sutherland, P. MacCready, P. Kiffney, and M. Plummer. *in prep.* Patterns of river influence and connectivity among subbasins of puget sound, with application to bacterial and nutrient loading. in preparation.
- Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the san francisco bay estuarine system. *Limnology and Oceanography* **47**:713-729.
- Dame, R. F. and D. M. Allen. 1996. Between estuaries and the sea. *Journal of Experimental Marine Biology and Ecology* **200**:169-185.
- Dethier, M., E. Sosik, A. Galloway, D. Duggins, and C. Simenstad. 2013. Addressing assumptions: Variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. *Marine Ecology Progress Series* **478**:1-14.
- Dubois, S., B. Jean-Louis, B. Bertrand, and S. Lefebvre. 2007. Isotope trophic-step fractionation of suspension-feeding species: Implications for food partitioning in coastal ecosystems. *Journal of Experimental Marine Biology and Ecology* **351**:121-128.
- Fry, B. and E. Sherr. 1984. Delta 13c measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**:13-47.
- Lefebvre, S., C. Harma, and J. L. Blin. 2009a. Trophic typology of coastal ecosystems based on delta c-13 and delta n-15 ratios in an opportunistic suspension feeder. *Marine Ecology-Progress Series* **390**:27-37.
- Lefebvre, S., J. C. Marín Leal, S. Dubois, F. Orvain, J.-L. Blin, M.-P. Bataillé, A. Ourry, and R. Galois. 2009b. Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in two shellfish culture dominated ecosystems. *Estuarine, Coastal and Shelf Science* **82**:415-425.
- Marin Leal, J. C., S. Dubois, F. Orvain, R. Galois, J. L. Blin, M. Ropert, M. P. Bataille, A. Ourry, and S. Lefebvre. 2008. Stable isotopes (delta c-13, delta n-15) and modelling as tools to estimate the trophic ecology of cultivated oysters in two contrasting environments. *Marine Biology* **153**:673-688.
- Moore, J. W. and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* **11**:470-480.
- Moore, S. K., N. J. Mantua, J. A. Newton, M. Kawase, M. J. Warner, and J. P. Kellogg. 2010. A descriptive analysis of temporal and spatial patterns of variability in puget sound

- oceanographic properties (vol 80, pg 545, 2008). *Estuarine Coastal and Shelf Science* **87**:174-174.
- Page, H. M. and M. Lastra. 2003. Diet of intertidal bivalves in the ria de arosa (nw spain): Evidence from stable c and n isotope analysis. *Marine Biology* **143**:519-532.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**:703-718.
- Riera, P. 2007. Trophic subsidies of *crassostrea gigas*, *mytilus edulis* and *crepidula fornicata* in the bay of mont saint michel (france): A delta c-13 and delta n-15 investigation. *Estuarine Coastal and Shelf Science* **72**:33-41.
- Riera, P. and P. Richard. 1996. Isotopic determination of food sources of *crassostrea gigas* along a trophic gradient in the estuarine bay of marennes-oleron. *Estuarine Coastal and Shelf Science* **42**:347-360.
- Ruckelshaus, M., T. Essington, and P. Levin. 2009. Puget sound, washington, USA. Pages 201-226 *in* K. McLeod and H. Leslie, editors. *Ecosystem-based management for the oceans*. Island Press, Washington, DC.
- Ruckelshaus, M. H., R. C. Wissmar, and C. A. Simenstad. 1993. The importance of autotroph distribution to mussel growth in a well-mixed, temperate estuary. *Estuaries* **16**:898-912.
- Ruesink, J. L., G. C. Roegner, B. R. Dumbauld, J. A. Newton, and D. A. Armstrong. 2003. Contributions of coastal and watershed energy sources to secondary production in a northeastern pacific estuary. *Estuaries* **26**:1079-1093.
- Simenstad, C. A. and R. C. Wissmar. 1985. Delta c13 evidence of the origins and fates of organic carbon in estuarine and nearshore food webs *Marine Ecology-Progress Series* **22**:141-152.
- Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. Vander Zanden, and B. C. Weidel. 2011. Terrestrial, benthic, and pelagic resource use in lakes: Results from a three-isotope bayesian mixing model. *Ecology* **92**:1115-1125.
- Sutherland, D. A., P. MacCready, N. S. Banas, and L. F. Smedstad. 2011. A model study of the salish sea estuarine circulation. *Journal of Physical Oceanography* **41**:1125-1143.
- Vander Zanden, M. J. and J. B. Rasmussen. 2001. Variation in delta n-15 and delta c-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* **46**:2061-2066.