Detrital shadows:
Evaluating landscape and species effects on detritus-based food web connectivity in Pacific Northwest estuaries

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Abstract

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Estuaries are inherently open systems, linking together terrestrial, aquatic, and marine ecosystems. With fluid, permeable transitions (ecotones) marking the boundaries between these ecosystems, estuaries subsidize coastal food web productivity through the mediation of nutrient, material, and energy flux across ecosystem boundaries. Mechanisms governing the strength and scale of estuarine detritus-based food web connectivity, however, are poorly understood. For example, early estuarine descriptions suggest that extensive mixing and large-scale transport of organic matter occurs within estuarine systems, while recent evidence in estuarine detritus-based food webs has shown strong spatial gradients in the sources of organic matter assimilated by consumers across a diversity of scales. This suggests food webs are spatially compartmentalized in some estuaries, but strongly connected in others. Given that estuaries have experienced extensive structural and hydrological alterations over the past century, research describing the mechanisms of estuarine-supported subsidies is necessary if we are to provide informed guidelines for the conservation and restoration of estuaries and estuarine functions. In this dissertation, I describe the role of landscape context, consumer feeding mode, and consumer life histories in shaping the scale of food web connectivity in Pacific Northwest estuaries with implications for conservation and restoration strategies. Specifically, I examine detritus-based food webs, using multiple stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) in combination with a Bayesian stable isotope mixing model to trace food web connections between organic matter (OM) sources and estuarine consumers.

Chapter 1 identifies several factors influencing the degree of food web connectivity in Pacific Northwest estuaries. Both fluvial discharge and consumer feeding mode strongly influenced the strength and spatial scale of food web linkages observed in the estuarine systems we examined. To a lesser degree, seasonal shifts, and other estuary-specific landscape characteristics, such as marsh area or particle transport speed, can also influence the degree of food web linkages across space and time, often accounting for unexpected patterns in food web connectivity. Chapter 2 assesses whether passive (hydrologic) and active (behavioral) processes are the primary mechanism by which trophic energy flows across estuarine ecotones. We specifically compare passive OM transfer by estuarine circulation to the active trophic relay of OM via nekton movement by comparing isotopic and diet compositions of resident (bay pipefish, *Syngnathus leptorhynchus*) and transient (English sole, *Parophrys vetulus*) fishes in two estuaries with contrasting freshwater inflows. Our results indicate that both OM movement and organism movement enhance connectivity in Pacific Northwest estuaries. In the estuary exhibiting high fluvial discharge, water-advection plays a critical role in large-scale OM transport and delivery to adjoining ecosystems, while trophic relay by organisms may provide the more important vector of food web connectivity in the estuary exhibiting little to no fluvial discharge. The two mechanisms, however, certainly work in tandem to enhance food web connectivity across estuarine ecotones. In Chapter 3, I examine patterns in food web connectivity associated with restoration efforts within the Skokomish River estuary. We conclude that increasing ecosystem capacity for detritus production by restoring emergent marsh ecosystems can bolster support for detritus-based food webs, and that restoration actions that enhance connectivity across estuarine ecotones may achieve functional equivalency more rapidly than restoration projects exhibiting limited connectivity to the surrounding landscape.

Together, the results presented in this dissertation demonstrate how physical and biological factors interact to affect food web connectivity in estuarine ecosystems. In light of the current, altered state of the world’s estuaries, restoration and conservation actions addressing ecosystem capacity and connectivity may effectively promote ecosystem function.

Contents

Table of Figures

Table of Tables

Preface

"The message is clear: ecosystems are closely bound to one another, be they pelagic and intertidal zones, farms and the sea, forest and river, or ocean and desert" (Polis et al. 1997)

Spatial food web subsidies

Ecologists have long recognized that there is considerable flux of materials across the boundaries of all ecosystems such that no ecosystem exists in complete isolation of the surrounding landscape (Vanni et al. 2004). However, this hasn't always been the case in terms of the flow of trophic energy. Early food web theorists tended to consider food webs in isolation, not only arbitrarily delimiting boundaries on the organisms considered, but examining food webs in the absence of landscape or spatial context (Polis et al. 2004a). As a result, food web theorists often conflicted with the convictions of field ecologists who, more often than not, regularly observed patterns and processes predicted to be extremely rare. Rooted in a strong, field-based understanding of the ecosystems in which they worked, Gary Polis, Wendy Anderson, and Robert Holt pushed for the marriage of landscape ecology and food web ecology in their seminal paper, *Towards an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs* (Polis et al. 1997)*,* noting that flows of energy, materials, or organisms from one habitat to another could strongly influence the structure and dynamics of food webs.

Highlighting the ubiquitous nature of the concept, the phenomenon of spatially subsidized food webs has been described across a wide variety of ecosystems at various spatial scales, including aquatic ecosystems (Duggins et al. 1989, Bustamante and Branch 1996, Menge 2004, Mumby et al. 2004), across the land-water interface (Polis and Hurd 1995, 1996b, a, Sanchez-Pinero and Polis 2000, Nakano and Masashi 2001, Wilkinson et al. 2005, Winder et al. 2005), and between terrestrial ecotones (Edwards and Sugg 1993, Candesso et al. 2004). Polis et al. (1997) define ecosystem subsidies as those systems wherein the resource (i.e. prey, detritus, nutrients, etc.) is donor-controlled and is transferred from one habitat to a recipient inhabiting another habitat. Cross-boundary resource subsidies are further defined to increase productivity of the recipient, but may alter other consumer-resource dynamics in the recipient ecosystem. In general, resources flow from areas of high productivity to areas of low productivity, with allochthonous materials subsidizing consumers in adjacent, less productive ecosystems (Polis and Hurd 1995, 1996b, a). However, the direction in which a subsidy flows between ecosystems is not always unidirectional nor fixed. For example, reciprocal food web subsidies have been observed in forested stream ecosystems as a result of temporal complementarity in resource productivity between stream and forest ecosystems; the stream ecosystem supplements forest bird, bat and spider populations during the autumn and spring when terrestrial productivity is low, and the forest ecosystem supplements stream organisms with terrestrial insects and leaf detritus when the summer forest canopy reduces stream productivity (Nakano and Masashi 2001). In these and other studies, spatial subsidies of resources are not necessarily continuous through time, but rather track temporal patterns associated with biological (e.g., primary productivity) or physical processes (e.g., fluvial discharge, ocean upwelling) that comprise the subsidy (Nakano and Masashi 2001, Menge 2004, Mortillaro et al. 2011).

A variety of mechanisms mediate the transfer of energy-laden resources across ecotones and ecosystems. Physical forces, such as wind, river currents, and tidal flushing, are important vectors for the flow of nutrients and detritus (Menge 2004, Witman et al. 2004). Biological forces can be equally as important, including but not limited to direct trophic transfers galvanized through organism mobility associated with ontogenetic and feeding migrations, or through the deposition of guano or carrion (Sanchez-Pinero and Polis 2000, Mumby et al. 2004, Vanni and Headworth 2004, Willson et al. 2004, Winder et al. 2005).

The effect of food web subsides on community dynamics is widespread and variable, stretching across multiple spatial and temporal scales, as well as ecosystems. The degree and importance of spatial food web subsidization depends upon multiple intersecting factors that include landscape patterns, physical processes, and biological interactions, all of which are subject to specific spatial and temporal scales. Local food web structure or the trophic role of a particular recipient consumer, for example, may exert just as strong of an influence on community dynamics as the permeability of habitat boundaries or the relative productivity of trophically connected habitats (Polis et al. 1997). The central theme, however, is that food web subsidies across organism habitats or ecosystems are widespread and important to the continuing function of populations and ecological relationships. In many cases, allochthonous subsidies contribute substantially to ecosystem stability (Huxel and McCann 1998, Huxel et al. 2002, Takimoto et al. 2002, Anderson and Polis 2004, Caraco and Cole 2004, Lobry et al. 2008).

Food web connectivity versus compartmentalization

Traditionally, the term *food web connectivity* has been used to describe the number of connecting nodes within a trophic web. Highly connected food webs, termed 'reticulate' food webs, are characterized by multiple organisms within a trophic web, each displaying multiple trophic connections to other organisms (Pimm and Lawton 1980). In contrast, the term *food web compartmentalization* has referred to subgroups (modules) of taxa in which many strong interactions occur within subgroups, but few, weak interactions occur between subgroups (Krause et al. 2003).

The effects of food web connectivity, complexity, and compartmentalization on ecosystem stability have stimulated an intense, evolving discussion since the 1970's (May 1973, Paine 1980, Pimm and Lawton 1980, Pimm 1984, Huxel and McCann 1998, Huxel et al. 2002). Early on, spatial considerations associated with food web compartments were ignored, perhaps leading to the general conclusion within the theoretical modeling literature that compartments beget ecosystem stability, while highly connected or complex food webs lead to destabilization (May 1973, Pimm and Lawton 1980, Pimm 1984). Field ecologists, however, had difficulty identifying compartments in true food webs. They also had difficulty relating food web structure to ecological stability, leading Paine to proclaim that "ecological stability remains a frustrating issue, and to a field ecologist, ties between model and reality at times appear remote" (Paine 1980). Later on, field ecologists noted that empirical observations of ecosystem instability were more prevalent in less-connected systems (Huxel et al. 2004), further arguing that increasing connectivity enhances ecosystem stability via the portfolio effect (Huxel et al. 2002, Hooper et al. 2005), perhaps because the myriad of links in a highly connected system provide alternative pathways of food web support that absorb disturbance and variability in resource availability..

The incorporation of spatial landscape context into food web theory produced two fundamental shifts in the ongoing discussion. Firstly, food web modules or compartments were no longer an artifact of modeling exercises. Empirical evidence of food web compartments was repeatedly observed in the field once researchers broadened the spatial scope of their work to include food web relationships occurring across ecosystem boundaries (Raffaelli and Hall 1992, Krause et al. 2003, Guimera et al. 2010). The phenomenon is exemplified by Grenier's (2004)study of the salt marsh song sparrow, *Melospiza melodia* *samuelis*, habitat use in California salt-marshes, wherein food webs were found to be divided horizontally and vertically into compartments by habitat, with the marsh plain being trophically separate from the lower marsh bench and tidal channels. This study aligned well with early food web models, which suggested that food web compartments, if they existed, could only be detected across major habitat divisions, such as the aquatic-terrestrial transition (Pimm and Lawton 1980). While now well established that trophic connections occur across multiple landscape scales and ecosystem transitions, previous food web theory had been built using a closed-system approach, such that aquatic food webs were considered in isolation from terrestrial food webs. The incorporation of spatial context allowed field experiments to dovetail with food web theory by promoting an open-system approach, folding seemingly disparate “parts” into a broader ecosystem “whole”.

Secondly, the incorporation of spatial landscape context into food web field experiments documented that food web relationships among compartments could exert profound influence on the dynamical stability of those compartments (Polis et al. 1997, Polis et al. 2004b). For example, the community dynamics of desert island communities in the Sea of Cortez are unequivocally linked to marine ecosystems through only two trophic conduits—seabird guano and drift of marine algae (Polis and Hurd 1995, 1996b, a, Polis et al. 2004b). This contrasts strongly with earlier theory derived from models which defined compartments as subsystems with few or weak linkages among them. Echoing the sentiments of Paine (1980), more recent evidence in the field of food web ecology in the landscape context shows that regardless of whether food webs are strongly connected in a reticulated manner, or loosely connected in a more compartmentalized systems, the strength and timing of those linkages are what matters most (Polis et al. 1997, Nakano and Masashi 2001).

With the push to examine food web structure within the landscape context, the term *food web connectivity* began to be used to describe food web linkages, or interactions, that connect organisms across space. In this way, *food web connectivity* describes spatial trophic connections among *food web compartments*, thereby eliminating the oppositional nature traditionally embedded between the two terms, and instead considering the larger food web system at hand. Because the influence of a spatial resource subsidy on community dynamics can occur across multiple spatial and temporal scales, and depends upon the strength, timing, and complexity of interactions among food web components (Polis et al. 1997), viewing food webs within their spatial context allows for the consideration of gradients of food web connectivity between compartments.

Gradients in food web connectivity can be measured by the *length* or *strength* of the trophic linkage. The transport and assimilation distance, or length, of the food web connection across an ecotone reflects the spatial extent of connectivity between adjacent ecosystems, and has only been explicitly examined between seagrass and mangrove ecosystems in Australia (Guest and Connolly 2004, Guest *et al.* 2004, Guest and Connolly 2006). Long transport and assimilation distances, however, do not necessarily indicate strong connections between adjacent ecosystems, as the strength of ecosystem connections refers to the proportional contribution of allochthonous primary producers to consumer diets. Differential strengths of food web connectivity may occur as a result of resource availability (i.e., detrital transport distances), food quality, or organism feeding selectivity. It is this latter context— the consideration of the length and strength of food web connections among spatially distinct compartments within the estuarine landscape— in which I use the terms *connectivity* and *compartmentalization* in this dissertation.

Subsidies and connectivity in the estuarine ecosystem

The concept of spatial food web subsidies is especially pertinent to estuaries because they are inherently open, energetic systems, connected to terrestrial, aquatic, and marine ecosystems. The boundaries between land and water are exceptionally fluid, permeable, and transient. Consequently, materials and organisms flow easily between estuarine and adjacent ecosystems, with water as the primary vector of transport (Polis et al. 1997).

The extraordinary productivity of estuarine marsh ecosystems and their potential contribution to the productivity of neighboring ecosystems has long been recognized in the field of ecology (Teal 1962). In fact, the concept of spatial subsidies underlines Odum's 1968 "estuarine outwelling hypothesis", in which he suggested that excess production in estuaries is transported downstream where it promotes coastal productivity (Odum 1980). The direction of transboundary energy flows, however, is highly variable among estuaries. Some estuaries import, while other export (Odum 1980). Regardless of directionality, ecosystem connectivity is critical for estuaries. This concept is captured in the newly emerging paradigm in estuarine ecology: "an estuary is an ecosystem in its own right, but cannot function indefinitely on its own in isolation. [It] depends largely on other ecosystems, possibly more so than . . . other ecosystems" (Elliott and Whitfield 2011). The statement emphasizes the importance of spatial subsidies in estuarine ecosystems, for without support from adjacent ecosystems, an estuary ceases to function.

While estuaries are characterized by fluid, permeable "boundaries", termed ecotones (Holland 1990), strong physical gradients in salinity and inundation regimes result in discrete spatial assemblages of estuarine primary producers (Bertness and Pennings 2000). In some cases, the discrete spatial ranges of vegetation assemblages have been observed to propagate up the food web (Deegan and Garritt 1997, Hsieh et al. 2002, Guest and Connolly 2004, Guest et al. 2004, Connolly et al. 2005, McMahon et al. 2005, Wozniak et al. 2006), indicating that spatial food web compartmentalization of organic matter sources can translate to spatial compartmentalization in higher trophic levels. However, because organic matter is readily transported by water currents, the spatial range (termed "detrital shadow" in this dissertation) of available plant-specific detritus generally exceeds the spatial boundaries of any particular primary producer assemblage (Gordon et al. 1985). The range of the detrital shadow, however, appears to vary considerably among estuaries. Some estuaries exhibit broad-scale linkages on the order of tens to hundreds of kilometers (Gordon et al. 1985, Gordon and Goni 2003), while others are characterized by extremely confined (meter scale) spatial scales of organic matter movement and assimilation (Guest and Connolly 2004, Guest et al. 2004). We suggest that variability in the spatial scale of food web connectivity across estuaries stems from the interaction of physical and biological forces.

Physically, estuaries receive daily energy pulses with the ebb and flow of tides, making them tidally subsidized, fluctuating water level ecosystems (Odum et al. 1995). Tidal amplitude and pulsing frequency, however, vary significantly by location and over time. Some estuaries are subject to microtidal conditions, with less than 1 m difference between high and low tide, while others are subject to macrotidal conditions, with a tidal range greater than 14 meters. Additionally, tidal inundation time varies depending on whether diurnal, semi-diurnal, or mixed semi-diurnal tides prevail. Both tidal range and inundation regime potentially affect the transport of organic matter moving across the land-water interface.

Tidal pulsing, however, is not the only physical forcing mechanism in estuaries. In contrast with fringing, pocket, or embayment type marshes, estuarine marshes associated with estuarine deltas may also experience high amplitude energy pulses during periods of high freshwater discharge (Day *et al.* 2000). As water is the principal vector for the transport of organic matter across estuarine ecotones (Polis *et al.*

1997), differences in pulsing frequencies and magnitudes of fluvial forcing may lead to a difference in the timing and extent of organic matter transport and assimilation between river-influenced estuarine marshes and non-fluvial marsh types (Eldgridge *et al.* 2005). Variability in physical forcing mechanisms may thus partially explain why recent evidence in estuarine detritus-based food webs has shown strong gradients in the sources of organic matter assimilated by consumers across diverse spatial scales (Deegan and Garritt 1997, Guest et al. 2004, McMahon et al. 2005, Howe and Simenstad 2007, Richoux and Froneman 2007, Grimaldo et al. 2009, Howe and Simenstad 2011).

In addition to the influence of physical mechanisms on food web connectivity, spatial food web connectivity also depends on biological processes. Organisms exhibiting small feeding ranges, feeding specialization, and strong site fidelity tend to exhibit spatial compartmentalization (McMahon et al. 2005), while those with large feeding ranges, generalist feeding, migratory behavior, or weak site fidelity display less trophic compartmentalization (Melville and Connolly 2003). Furthermore, an organism's degree of spatial compartmentalization can change with seasonal or ontogenetic shifts in feeding behavior and movement, as organisms expand or contract feeding and movement ranges in response to juvenile outmigration, annual spawning migrations, or food availability (Hansson et al. 1997). Food web connectivity can also be enhanced by a series of predator-prey interactions that trophically relay organic matter source contributions through space (Kneib 2000). While food web compartmentalization can and does propagate from organic matter sources to upper level consumers, spatial and temporal scales in the feeding behavior, availability, and movement of organisms must coincide. If those scales do not match, compartmentalization of food webs increasingly dissipates with increasing trophic status (Vizzini and Mazzola 2006). As a result, "a system may be compartmented at one trophic level, but reticulate at the next" (Pimm and Lawton 1980).

How strongly, and by what mechanisms, spatial food web compartmentalization is expressed across the estuarine landscape is the central question of this dissertation. Why do some estuaries exhibit broad scale mixing with relatively homogenous sources of organic matter supporting the food web throughout, while others exhibit extremely confined scales of organic matter transport, giving rise to sharp contrasts in food web support from one location to the next?

Estuaries in the landscape context: anthropogenic changes

Estuaries are some of the more degraded ecosystems on earth, having been subjected to a litany of anthropogenic alterations. Although comparatively recent on the scene of estuarine occupation, since Euro-American settlement in the mid 19th century, Pacific Northwest estuaries have experienced rapid and extreme structural and hydrological modifications. Most strikingly, tidal wetland area, which includes estuarine and tidal freshwater environments, has been reduced to 17-19% of its historical extent (Collins and Sheikh 2005, Simenstad et al. 2011). Like elsewhere, the tidal wetlands of Puget Sound have largely been diked and reclaimed for agriculture, industrial, and urban development, unquestionably representing a major reduction in the types and abundance of organic matter sources once available to nearshore Puget Sound's detritus based food webs.

In addition to tidal marsh loss, upstream alterations have also occurred. Dams, levees, and landuse change have fragmented the landscape in ways that are detrimental to estuarine organisms and processes dependent on spatial subsidies. Hydroelectric dams, for example, not only interrupt and modify the downstream flux of sediments, organic matter, and organisms, but they also affect the flow regime of the river, muting seasonal flood pulses that historically blurred the boundary between fluvial and terrestrial ecosystems (Poff et al. 1997). Further hardening of the boundary between aquatic and terrestrial ecosystems, kilometers of levees now line lowland river channels and estuarine delta fronts (Hood 2004, Collins and Sheikh 2005). Levees prevent the river from connecting with historical floodplain wetlands and off-channel aquatic habitats, thus cutting off important transboundary connections known to enhance the condition of secondary consumers, such as Chinook salmon (Limm and Marchetti 2009, Mortillaro et al. 2011). Levees also simplify channel edges by reducing the fractal irregularity or folding of the edge (Polis et al. 1997). In some cases, entire distributary channels are filled or blocked by levees, further reducing the amount of edge available for transboundary energy transfers. The loss of 'interaction edges' represents yet another avenue by which anthropogenic alterations have reduced the potential for spatial food web subsidies in estuarine ecosystems, as the ratio of "edge" to "interior" has been documented to be a powerful determinant of both ecosystem's and organism's ability to access and respond to allochthonous resources (Polis and Hurd 1996b, Polis et al. 1997).

As detailed in the chapters following, freshwater inflow, tidal dynamics, residence time, transboundary interaction potential, and proximity and flux of different organic matter sources can all influence food web connections. These factors affect how and where organic matter is transported and deposited, and whether or how organisms can access it. Historic alterations to natural estuarine ecosystems have disrupted the mechanisms and corridors that once maintained spatial resource subsidies in Pacific Northwest estuaries. As a result, estuarine productivity is more isolated from key consumer resources. Without an understanding of the mechanisms by which food web connections are made and sustained in estuaries, restoration actions addressing this key issue are difficult to employ.

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Chapter 1. Detrital shadows: stable isotopes reveal estuarine food web connectivity depends on fluvial influence and consumer feeding mode

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Abstract

To examine whether the highly compartmentalized food webs documented in microtidal estuaries with little freshwater discharge apply under different landscape settings, this paper quantifies the strength and spatial scales of detritus-based food web (source) connectivity among adjacent ecosystems and across different degrees of fluvial forcing under macrotidal conditions. We used stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) in combination with a Bayesian multiple source mixing model to trace primary producer contributions to suspension- and deposit-feeding bivalve consumers (*Mytilus trossulus, Macoma nasuta* or *balthica*) transplanted to specific locations within and across a gradient of up to three estuarine ecotones: emergent marsh:mudflat, mudflat:Japanese eelgrass (*Z. japonica*), Japanese:native eelgrass (*Z. marina*). The study was conducted in five Pacific Northwest estuaries, ranging from small embayments with little freshwater influence to large river deltas. Compared with the results from earlier studies, we found that both fluvial discharge and consumer feeding mode strongly influenced the strength and spatial scale of food web linkages observed in the estuarine systems we examined. To a lesser degree, season affected the degree to which the estuarine consumers depended on organic matter (OM) sources from certain ecosystems. The diets of filter-feeding *Mytilus* mussels displayed strong cross-ecosystem connectivity in all estuaries, indicating homogenization of detrital sources within the water column of each estuary. Comparisons among estuaries, however, showed decreasing marine influence on mussel diets in the more fluvial estuaries. In contrast, the diets of benthic deposit-feeding *Macoma* clams indicated stronger compartmentalization in food web connectivity as compared to mussels, especially in the largest river delta where clam diets indicated complete trophic disconnection from marsh OM sources. Benthic deposit-feeders thus suggest that the deposition of OM is patchy across space, and less homogenous than the suspended detritus pool. Our results indicate that estuary-specific environmental drivers other than fluvial discharge, such as marsh area or particle transport rate, also influence the degree of food web linkages across space and time, often accounting for unexpected, variable patterns in food web connectivity. As such, anthropogenic transformations of the estuarine landscape that alter river hydrology or availability of OM sources can potentially disrupt natural food web relationships at the landscape scale, especially for sedentary organisms which cannot track their food sources.

Key words: stable isotopes, food web, landscape ecology, connectivity, estuary, detritus, scale

Introduction

Despite suggestions that extensive mixing and large-scale transport of organic matter (OM) occurs within estuarine systems and across fluvial-estuarine-coastal ecotones (Teal 1962, Odum 1980), more recent evidence from estuarine detritus-based food webs has shown strong gradients in the sources of OM assimilated by consumers across diverse landscape scales (Gordon et al. 1985, Deegan et al. 1990, Deegan and Garritt 1997). These emerging results challenge prior concepts of the scale of food web connections across estuarine ecotones, as they infer greater compartmentalization of food webs in relation to landscape setting than previously considered. It is now becoming evident that in some landscape settings, the spatial scale of OM movement and assimilation can be extremely confined. Several recent studies have documented that estuarine wetland consumers inhabiting fringing and non-fluvial embayment marshes feed exclusively on locally produced OM, indicating that the transport and assimilation of OM can be confined to a few tens of meters or less (Hsieh et al. 2002, Grenier 2004, Guest et al. 2004a, Guest et al. 2004b, Guest and Connolly 2006). However, the aforementioned studies have all occurred in microtidal environments or in areas receiving little to no riverine inputs, both landscape setting factors which may minimize detrital transport distances. Thus, to address differences in the scale of food web connectivity previously documented in estuaries, and given that little work has been devoted towards distinguishing the scale of food web compartmentalization in relation to landscape setting, this paper quantifies the strength and spatial scales of connectivity among different ecosystems and across different gradients of fluvial forcing.

Receiving daily energy pulses with the ebb and flow of tides, estuarine marshes and intertidal flats can be described as tidally subsidized, fluctuating water level ecosystems (Odum 1980, Odum et al. 1995). However, tidal pulsing is not the only physical forcing mechanism in estuarine ecosystems. In contrast with fringing, pocket, or embayment estuaries, estuarine deltas may also experience high amplitude energy pulses during periods of high freshwater discharge (Day et al. 2000). As water is the principal vector for the transport of OM across ecosystem boundaries in estuarine systems (Polis et al. 1997), the difference in pulsing frequencies and magnitudes may lead to a difference in the timing and extent of OM transport and assimilation between river-influenced and non-fluvial estuaries (Eldridge et al. 2005).

In this paper, we evaluate food web connectivity in five Pacific Northwest estuaries representing a gradient of physically contrasting dynamics by describing the extent to which ecosystem-specific OM supports estuarine consumers across ecotones. Our use of *ecosystem* here is analogous to that of a *patch*, and refers to the distinct vegetative zones wherein specific organic matter sources are found within an estuarine mosaic. Additionally, the use of *ecotone* is analogous to *landscape boundary* or *transition zone,* in line with the definitions currently used in aquatic-terrestrial ecotone ecology (Holland 1990). Given that ecotones are distinguished by the strength of the interactions between adjacent ecological systems, and therefore can occur at broad or fine spatial scales (Risser 1990), we evaluate the strengths[1] and lengths[2] of food web connections across estuarine ecotones through a manipulative translocation experiment in which we use multiple stable isotopes to trace connections between consumer organisms and primary producers. The spatial and temporal context within which we quantify food web source connections strengthens the currently weak understanding of variation in food web connectivity across diverse landscape scales and features, and across a gradient of contrasting estuarine fluvial forcing.

It is important to recognize the difference between food web connectivity length and strength. The transport and assimilation distance, or length, of the food web connection across one or more ecotones reflects the spatial extent of connectivity across adjacent ecosystems, and has only been explicitly examined between seagrass and mangrove ecosystems in Australia (Guest and Connolly 2004, Guest et al. 2004a, Guest et al. 2004b, Guest and Connolly 2006). Long distances of transport and assimilation, however, do not necessarily indicate strong connections among ecosystems, as the strength of ecosystem connections refers to the proportional contribution of allochthonous primary producers to consumers across ecotones. Differential strengths of food web connectivity may occur as a result of both resource availability (i.e., detrital transport distances or available biomass), and organism feeding selectivity (van Oevelen et al. 2006). Selectivity of certain consumers may indicate reduced connectivity strengths across ecotones, while opportunistic, generalist feeders may indicate stronger food web connections (van Oevelen et al. 2006, Marczak et al. 2007). It is therefore critical to recognize that diverse and autonomous food web pathways can occur within the same system.

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[1] The strength of food web connections refers to the proportional contribution of a particular OM source to a consumer organism's diet or ultimate diet.

[2] The length of food web connections refers to the distance between where an OM source is produced on the landscape and where it can be detected in food web consumer organisms.

The ecosystems of focus in this paper are characterized by a dominant vegetation type, and include intertidal emergent marshes, mudflats, and seagrass beds. Because estuarine primary producers often tend to occur in discrete assemblages (Bertness and Pennings 2000), food web availability of specific primary producer OM coincides with the discrete spatial ranges of the vegetation assemblages (Deegan and Garritt 1997, Guest et al. 2004a, Guest et al. 2004b, McMahon et al. 2005, Guest and Connolly 2006, Wozniak et al. 2006). However, given that detritus is readily transported by water currents, the spatial range ("detrital shadow") of available producer-specific detritus generally exceeds the spatial boundaries of any particular primary producer assemblage (Gordon et al. 1985). Assimilation of OM by consumers can therefore occur across gradients of food web ecotones, where the diets of consumers inhabiting the ecotone may reflect contributions from adjacent ecological systems, but the diets of consumers inhabiting the deep interior of a specific ecosystem may primarily reflect contributions from immediately adjacent primary producers (Guest et al. 2004a, Guest et al. 2004b).

The degree of food web connectivity, or compartmentalization, has broad implications for food web stability, as the flux of energy and nutrient resources across ecosystem boundaries can exert major impacts on adjacent food webs (Huxel and McCann 1998, Huxel et al. 2002, Guimera et al. 2010). Thus, assessing the role of fluvial discharge in enhancing or diminishing food web connectivity, both spatially and temporally, carries broad implications to basic and theoretical science, as well as applied sciences involved with coastal resource protection, restoration, and management. Preserving ecosystem connectivity in order to maintain ecosystem functions and support migratory populations has recently been adopted by food web theorists, who suggest that ecosystem connectivity is critical for maintaining trophic support of consumer organisms. This assertion is evidenced by the recent characterization of spatially-subsidized food webs across aquatic, marine, and terrestrial ecotones (Polis et al. 1997, Marczak et al. 2007, Uesugi and Murakami 2007, Valentine et al. 2007). More specifically, the importance of river-estuary connectivity has been demonstrated for maintaining a variety of estuarine ecosystem functions, including the deposition of both fine and coarse sediments which prevents deltaic marsh subsidence (Day et al. 2000), as well as the increase of estuarine primary productivity due to nutrient influx (Boyton et al. 1982). However, not all estuarine marshes adjoin with major river systems. The lack of a riverine connection often results from anthropogenic causes, such as the construction of levees or the rerouting of river channels, but fringing, pocket, and embayment marshes also have little natural connection with river systems. Nevertheless, ecosystem connectivity among adjacent upland and intertidal ecosystems likely plays a significant role in maintaining ecosystem functions within non-fluvial estuaries (Roman et al. 2000). Here, we describe the first quantitative effort to compare the length and strength of ecosystem connectivity across a gradient of system types.

In this study, we extend the scope of the Australian mangrove-saltmarsh studies in three ways. Firstly, we not only assess the spatial extent of connectivity, but we also quantify the strength of cross-ecosystem food web connections to terrestrial, pelagic, and riverine sources of OM. Secondly, we examine food web connectivity using consumer indicators representative of two different detritus-feeding pathways, a benthic deposit-feeding clam and a suspension-feeding mussel. Thirdly, we test for differences in strengths and lengths of food web connections among estuaries representing a gradient of fluvial discharge in order to investigate landscape setting influences on food web connectivity. We quantify the strengths and lengths of food web connections among consumers and ecosystems by differentiating stable isotope signatures of dominant food web components at a number of spatial and temporal scales through an experimental manipulation study focusing on OM transport and assimilation across ecotones.

Conceptual Model and Objectives

Because water is the principle vector of organism and particle transport in intertidal estuarine ecosystems (Holland et al. 1990, Polis et al. 1997), we hypothesized that ecosystem connectivity, as reflected in relative food web source contributions to consumer diets—the detrital shadow— will lengthen and strengthen in more fluvially-dominated sites as a result of strong pulses in freshwater discharge which physically enhance the transport and mixing of OM and organisms across adjoining ecosystems. In contrast, those estuaries dominated more by tidal pulsing, such as embayment and fringing wetlands, should exhibit more compartmentalized food webs with shortened distances of OM transport and assimilation across ecosystem boundaries. To further verify the importance of fluvial forcing, we further hypothesized that connectivity (increased compartmentalization) would be reduced within fluvial estuarine systems during periods of low freshwater discharge.

While even generalist consumer organisms exhibit some measure of feeding selectivity (Charles and Newell 1997, Kreeger and Newell 2001, Huang *et al.* 2003, van Oevelen *et al.* 2006), we also designed the study to address examine whether a higher degree of compartmentalization occurs in benthic deposit- feeders as compared to filter feeders because they require the settling and retention of OM onto the benthos.

Approach

We examined differences in food web connectivity in relation to fluvial forcing by comparing five Pacific Northwest estuaries representing a gradient in the magnitude of fluvial discharge (Figure 1; Table 1). We conducted an experimental translocation study in which the filter-feeding bay mussel, *Mytilus trossulus*, and the benthic-deposit feeding clams, *Macoma nasuta* and *Macoma balthica,* were used as bioindicators to determine food web connections across a gradient of up to three estuarine ecotones—(1) emergent marsh: mudflat, (2) mudflat:Japanese eelgrass (*Z. japonica*), and (3) Japanese:native eelgrass (*Z. marina*). The two bivalve species were selected for study because they are widely present throughout Puget Sound and are tolerant of a wide range of environmental conditions. In the few locations where *M. nasuta* could not survive, *Macoma balthica,* also a benthic deposit-feeding bivalve, was used as a surrogate indicator (hereafter referred to both species as *Macoma* spp.). In order to capture seasonal differences associated with fluvial discharge, and peak primary production and senescence, we replicated field collections every six months. Recent evidence suggests that seasonal variability in food source availability, as reflected in consumer diets, plays a large role in regulating ecosystem subsidy direction and the importance of cross-ecosystem connectivity (Nakano and Masashi 2001, Sabo and Power 2002, Marczak et al. 2007, Uesugi and Murakami 2007).

We employed stable isotope geochemistry (δ^{13}C, δ^{15}N, and δ^{34}S) in combination with Bayesian multiple source mixing models to assess the spatial extent and strength of dominant primary producer contributions to the estuarine food web. Stable isotope analysis has emerged as the leading technique in determining food web pathways, as it is a relatively direct method of tracking energy across trophic levels (Peterson et al. 1986, Peterson and Howarth 1987, Hobson and Wassenaar 1999). Early studies used the technique to describe large-scale differences in food web support (Peterson 1999), but more recent work has been able to characterize more discretely defined ecosystems on the scale of several meters to a few kilometers (Deegan and Garritt 1997, Connolly et al. 2004, Connolly et al. 2005a, Connolly et al. 2005b, Wozniak et al. 2006, Howe and Simenstad 2007). We thus used stable isotopes to establish the relationship between the OM assimilated by consumer organisms and the location of translocated consumers across the estuaries. We particularly focused on differences between the original location wherein estuarine and marine OM was produced, and the ultimate location to where it was predominantly transported and subsequently consumed. Hence, for each major ecosystem providing an isotopically distinct OM source, we examine the spatial extent and connective strength of the detrital shadow as it moves into the food web.

The main questions we address are:

(1) How does the length and strength of the detrital shadow, as reflected in consumer organism assimilation of OM sources, vary across the gradient of estuaries between fluvial and embayment marsh types?

(2) Over what spatial and temporal scales are estuarine consumers trophically connected to primary producers originating from different ecosystems within a single estuary?

(3) How do patterns of connectivity differ between indicator organisms representative of different consumer feeding modes (benthic-deposit vs. filter feeder)?

Materials and Methods

Study sites and design

The study was conducted from March 2007 through April 2010 in five northern Puget Sound estuaries (Figure 1). The five estuaries experience dramatically different freshwater flow regimes from one another (Table 1), with Skagit Bay receiving the most fluvial inputs, and Mud Bay in Lopez Sound receiving the least. The majority of freshwater discharge occurs during winter rains, although the Skagit and Stillaguamish rivers also drain snowmelt from the Cascade Mountains in early spring. All five estuaries are shallow (<3 m depth), macrotidal (>3 m tide range), and exhibit a seaward continuum of emergent marsh, mudflats (with occasional occurrence of Japanese eelgrass), and native *Z. marina* eelgrass beds, commensurate with a typically well-mixed salinity gradient except during peak freshwater outflow of the larger rivers.

Primary producer collection

Dominant sources of carbon were collected for an isotopic baseline at each of the five estuaries, including riverine particulate OM, marine phytoplankton, benthic microalgae, macroalgae, and vascular plants. River-borne particulate OM (rPOM) was collected using a 0.5 m, 30-µm mesh plankton net. These samples reflect an integrated signature of allochthonous terrestrial and river-produced OM, thereby enabling the incorporation of upstream vegetative sources in the estuarine food web. Marine phytoplankton was collected with a 0.5-m, 30-µm plankton net towed behind a small boat. Samples of benthic microalgae (diatoms) inhabiting the intertidal and shallow subtidal sediments were collected using a method adapted from Cloern *et al.*(2002). Triangular 0.25-m^2, 20-µm mesh Nitex screens were distributed haphazardly on exposed mudflats. Screens were pulled and rinsed clean after 2-4 h exposure, depending on ambient light levels and visual assessments of diatom migration into the screens. Samples of benthic microalgae and phytoplankton were filtered through 100-µm sieves in order to remove larger detritus fragments and organisms, and examined under a dissecting scope to ensure that the majority of the sample was composed of live algal cells, before vacuum filtering onto pre-combusted (500°C, 4 h) 0.2-µm Whatman GF/F glass fiber filters and freeze-dried for 24 h.

During the growing season, four replicates of vascular plants and macroalgae were obtained from the apical foliage of each dominant species in each estuary (Table 2). Vegetation and algae samples were bagged, kept cool on ice in the field, and frozen until processing in the lab. Samples were washed thoroughly in deionized water, rinsed with 5% HCl to remove soil carbonates, and rinsed again with deionized water to neutral pH (Cloern et al. 2002). Samples were freeze-dried for 24 h, then ground to a fine powder using a mechanical mill.

Bioindicator organism translocation

To ensure that baseline isotope signatures were similar across estuaries, *Mytilus* and *Macoma* were collected from single source populations in Cultus Bay, WA on the southern end of Whidbey Island. To assess growth, clams were individually labeled with waterproof ink and measured for length and weight before and after incubation in the field. Clams and mussels were then translocated along 100-m cross-ecotone transects at each of the five estuaries to assimilate available OM sources during seasonal high and low freshwater discharge. Bivalves used to characterize the low discharge period were outplanted in early spring (March and April), and allowed to feed *in situ* for five months before collection in early fall (August and September). Bivalves used to examine the high discharge season were outplanted in early fall, allowed to feed for seven months before collection the following spring. In Skagit and Padilla bays, bivalves were deployed for three years (2007-2010); bivalves were deployed were collected for two years in the remaining estuaries (2008-2010).

The 100-m cross-ecotone transects were located at each vegetative transition, extending 50 m on either side of the transition point, generally perpendicular to shore (Figure 2). Three ecotone transition points occur in Padilla and Port Susan bays—marsh:mudflat, mudflat:Japanese eelgrass, and Japanese eelgrass:native eelgrass. Japanese eelgrass does not exist in Skagit Bay or Lopez Sound, nor is it distributed in sufficient quantity in Samish Bay to fit the sampling design. Thus, only two ecotones were included in these estuaries. Within each estuary, transects were nested by region and stratified along the shoreline to capture important landscape features at the estuary scale (e.g., north and. south distributary channels of the Skagit River delta). Three regions were examined in Padilla and Skagit Bays, while two regions were sampled in the remaining estuaries, for a total of 29 cross-ecotone transects in 12 regions.

Mussels and clams were translocated to positions at increasing distances of 2 m, 10 m, and 50 m from the vegetative transition point (both sides; six positions total) on each cross-ecotone transect. Five mussels were placed in each cage (1-cm plastic mesh), and the cage was secured 10 cm above the substrate on a combination of rebar, PVC pipe, or cinder blocks. Five clams were placed in each of two plastic kitchen colanders (Inomata® 23-cm diameter, ~ 2-mm mesh) at each of the six aforementioned positions along each transect (12 total colanders). A 0.5-cm mesh netting zip-tied to the colander was used to ensure that clams did not exit the colander due to disturbance by burrowing crabs or by scouring. Colanders were located 0.5 m apart from one another, and 0.5 m away from each mussel station. Colanders were anchored in place by U-shaped iron rebar stakes and buried in the sediment such that the colander rim was even with the original sediment surface.

Tissue preparation and isotope analysis

Recovered mussels and clams were frozen until processing in the lab. Specimens were rinsed thoroughly in deionized water to remove sediments, then adductor and mantle tissue was removed for isotope analysis. The tissue of all organisms collected from each transplant container (mussel cages and clam colanders) was combined in order to have enough material for isotope analysis. Thus, at each transect position there is one mussel signature and two clam signatures. According to the methods of Arrington and Winemiller (2002), samples were freeze-dried for 48 h and ground to a fine powder using a Wig-L-Bug® dental mill and a stainless steel vial and ball pestle. Samples were weighed into tin capsules for isotope processing of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Isotope analyses were performed by Washington State University's Stable Isotope Core lab using a Costech Analytical ECS 4010 elemental analyzer connected via a gas dilution to a Thermofinnigan Delta PlusXP mass spectrometer.

Isotopic ratios are expressed in δ notation, which indicates the enrichment (+) or depletion (-) of the heavy isotope relative to the light isotope compared with the standard:

$\delta X(‰) = [R_{sample}/R_{standard})-1] \times 10^3$

where X = ^{13}C, ^{15}N, or ^{34}S, and R = ^{13}C/^{12}C, ^{15}N/^{14}N, or ^{34}S/^{32}S. Sulfur isotopic ratios are relative to the VCDT (Vienna Canon Diablo Troilite) standard. The standard used for carbon was VDDB (Vienna Peedee belemnite), and atmospheric nitrogen was used as the standard for δ^{15}N.

Mixing Model Diet Estimations

We used Semmens et al. (2009) hierarchical Bayesian mixing model to estimate the proportional contribution of OM sources to bivalve diets. The model is an extension to the stable isotope mixing model, MixSIR, discussed by Moore and Semmens (2008), and allows for the estimation of individual diet heterogeneity rather than assuming that all consumers within the sampled population assimilate food resources in the same relative proportions. The model requires the following types of information: 1) the mean and variance for each OM source isotope signature, 2) the mean and variance of fractionation for each isotope, 3) the isotope signature for each consumer, and 4) the hierarchical assignments of each consumer. In this study, samples were analyzed on both the individual and group level. Individuals were grouped by estuary, flow period, species, and transect location (i.e., cross-ecotone type). While the model can theoretically incorporate a wide array of food types, we found it difficult to achieve model convergence using the full suite of OM sources available in each estuary, likely because sources incorporated in the model must display different enough isotopic signatures from one another to be distinguishable as a separate source. Acceptable separation of producers was thus tested using a nearest neighbor distance test (NND^2) in the SOURCE model (Lubetkin and Simenstad 2004), wherein producers exhibiting a NND^2 value less than 0.1 were pooled together in a single category. Categories of combined primary producers varied by estuary, but NND^2 tests did not require the pooling of OM sources originating from different ecosystems.

Fractionation rates used in the model differed for the two bivalve species. The clam-specific mean and variance of fractionation values utilized in *Macoma* spp. mixing models was 0.8 0.09 ‰ for δ^{13}C, and 3.4 0.1024‰ for δ^{15}N (Yokoyama et al. 2005). *Mytilus* sp. fractionation values of 2.17 0.09‰ for δ^{13}C and 3.78 0.10 for δ^{15}N were obtained from Dubois et al. (2007). Currently, no bivalve-specific sulfur fractionation rates exist in the published literature. Thus, the more generic fractionation value of 0.5 0.31‰ was used for δ^{34}S in both the *Macoma* and *Mytilus* mixing model runs (McCutchan et al. 2003). Non-informative priors were used for each OM source. Gibbs sampling was performed for each model using two parallel chains in JAGS (Plummer 2003). Following a burn-in phase of 19000 vectors, 20000 remaining vectors were sampled (retaining every other sample). Convergence and diagnostic statistics were performed using the CODA package (Best et al. 1995).

Hydrodynamic characterization

Near-bed hydrodynamic flow was characterized for each of the five estuaries by deploying a small (1.5 m high), instrumented ReefProbe tripod at each location for 48 h of stable conditions during the low flow period. In Skagit Bay, tripods were deployed at the mudflat:native eelgrass ecotone off the mouths of both the north and south distributary channels. Skagit Bay deployments captured both spring and neap tidal cycles. Due to time constraints associated with required equipment, tripods deployed in the remaining estuaries were only located at the mudflat:native eelgrass ecotone during a spring tidal cycle during the low flow period.

The ReefProbe (Ogston et al. 2004) includes Sontek Acoustic Doppler Velocimeter (ADV) Ocean Hydra instrumentation to measure 3-D flow in a small sampling volume at a fixed distance from the probe in conjunction with a pressure sensor, two D&A Instruments optical backscatter sensors (OBS), and a SeaBird conductivity/temperature sensor (CT). All instruments log data in a burst mode to collect both wave and current information. The ADV and OBS were placed near (~20 cm above) the seabed to sample boundary layer flows, sediment resuspension, and transport. A Fiber Optic Backscatter System (FOBS) and an Acoustic Backscatter System (ABS) were deployed with the ADV equipment in order to collect detailed suspended-sediment concentration data. This information, combined with the wave and current data, enable examination of sediment flux throughout the system.

Statistical analyses

Data were analyzed using Microsoft Excel® (univariate statistics), R (mixing model), and Primer 6® and PERMANOVA+ (multivariate statistics) software. We performed F-tests to test for normality and variance of isotope signatures by site and species, and then used *t*-tests ($\alpha=0.05$) to separately test for differences in $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ between species within estuaries.

Multivariate analyses were used to compare overall consumer organism isotope signatures and estimated OM source assimilation patterns derived from the Bayesian mixing models. The mixing model estimates posterior probability distributions describing the proportional contribution of each OM source to a given population of consumers. After conferring with the model authors regarding appropriate summary metrics, the median value was extracted from these distributions for use in statistical analyses (Eric Ward; pers. comm.). Because these data are proportional, all mixing model output was square-root transformed prior to further analyses (Schafer et al. 2002). Similarity matrices for mixing model output were constructed using the Bray-Curtis similarity coefficient, while those created for isotope data used Euclidean distance (Clarke and Warwick 2001). We used multidimensional scaling (MDS) in combination with analysis of similarity (ANOSIM), and permutational multivariate analysis of variance (PERMANOVA) to visualize and test for differences among factors. PERMANOVA was used to examine the effect for each estuary of flow period, transect, ecotone, and location along each transect on OM support for *Macoma* spp. and *Mytilus* sp. separately, using Type III partial sums of squares and 999 permutations. Each factor was nested in the previous factor, such that no interaction effects were examined. ANOSIM was used to examine differences between species within each estuary. Each ANOSIM analysis calculates a p-value similar to that of an ANOVA, with values of $p < 0.05$ indicating significant differences, and an R statistic scaled between -1 and 1, with the biological importance of the difference becoming greater as R approaches unity. Values greater than 0.4 are considered biologically important (Clarke and Warwick 2001). PERMANOVA provides an F-statistic and p-value to test for significance. Similarity percentages (SIMPER), analogous to parametric post-hoc tests, were then used to determine which primary producer groups were responsible for diet differences. For all cross-estuary comparisons, OM sources were categorized into five groups: marsh, benthic diatoms, macroalgae, eelgrass, and phytoplankton. For within-estuary comparisons, OM sources were kept the same as those input into the mixing model, and thus vary among estuaries.

In order to identify the key environmental variables that underlie diet variation within and across the five study estuaries, we used Primer's DISTLM and dbRDA to analyze the relative importance of mean monthly discharge, the areal coverage of *Zostera* spp. in each system, the areal coverage of emergent marsh in each system, the seasonal discharge period (winter high discharge period versus summer low discharge period), the distance between the marsh fringe and *Zostera marina* landward ecotone (proxy for delta size), transect location, mean flow velocity, acoustic backscatter, maximum salinity, net range of transport (NRT), and the scale ratio (ratio of net range of transport to distance) as predictors of *Macoma* spp. and *Mytlius* sp. assimilation (Table 3). With the exception of transect location, explanatory variables were obtained at the estuary level, such that response variables (clam and mussel diets) had a many to one relationship with predictive variables. In order to account for and evaluate the effect of the dramatic difference in delta size among study estuaries, the distance between the marsh:mudflat (transect 1) and mudflat:*Z. marina* (transect 2 or 3, depending on the estuary) ecotones was used as a proxy for delta size and included in the redundancy analysis. Given the increased probability of greater ranges of particle transport in the more fluvial estuaries, the ratio of the NRT to distance between ecotones was used to scale the estuaries to one another in terms of size and particle transport ability.

DISTLM implements a distance-based-redundancy-analysis routine, described as a multivariate multiple regression that is constrained to find linear combinations of the predictor variables which explain the greatest amount of variation in the data (Anderson et al. 2008). As with multiple regression, variance explained by a given model can be determined, and the significance of the explanatory variables tested. The DISTLM routine was used to partition variance and build models, while the dbRDA routine was used to view the fitted model using an eigen-analysis of the fitted data (Anderson et al. 2008). DISTLM modeling was conducted using the step-wise selection procedure which begins with a null model, then adds a variable that will increase the selection criterion. The most parsimonious model was chosen using a distance-based multivariate analogue to the univariate AIC selection criterion.

To compare the degree of food web compartmentalization among estuaries, we quantified spatial changes in the strength of marsh and *Z. marina* detritus assimilation at the cross-ecosystem scale in each estuary. The seaward transport distance and assimilation strength of marsh detritus was assessed by examining the change in proportional contribution of this OM source to consumers transplanted to the most seaward (mudflat- *Z. marina*) ecotone, and by measuring the distance from the marsh:mudflat ecotone to the mudflat:*Z.marina* ecotone. The landward transport distance and assimilation strength of *Zostera* detritus was assessed by examining the change in proportional contribution of this OM source to bivalves transplanted to the most landward (marsh-mudflat) ecotone. In a cross-estuary comparison, we used univariate linear regression models (stepwise, bidirectional selection) to separately identify environmental variables associated with the seaward transport and assimilation of marsh detritus, and the landward transport and assimilation of *Z. marina* detritus.

Results

Hydrodynamic characterization

Particle transport speeds were highest in Skagit Bay, but also reached high velocities in Samish Bay (Table 4). The net and maximum range of particle transport was greatest in Skagit Bay, followed by Samish Bay, and least in Lopez Sound. Similarly, salinites ranged most widely in Skagit Bay, and least in Lopez Sound. In contrast, suspended particle concentration, as measured by acoustic backscatter, was highest in Skagit Bay, followed by Lopez Sound. Decreasing particle concentrations otherwise followed decreasing influence of fluvial discharge.

Isotope Signatures of OM Sources and Consumers

Isotopic signatures of OM sources— Over the five separate estuarine OM baselines, excluding *Distichlis spicata,* a C_4 plant, marsh-associated primary producers were more depleted in δ^{13}C (range of mean δ^{13}C across estuaries: -26.33 to -28.73‰), more variable in δ^{34}S (range: -3.03 to 19.91‰), and similar in δ^{15}N (range: 1.64 to 10.41‰) as compared to the marine OM components, including benthic diatoms, *Zostera* spp., macroalgae, and phytoplankton (Table 2). *Zostera marina* was the most enriched δ^{13}C source in all estuaries. Benthic diatoms exhibited the most depleted δ^{34}S values (-5.10 to -17.17‰), and phytoplankton displayed the most enriched (δ^{34}S = 21.73 0.77‰) when compared with other sources. The most enriched δ^{15}N occurred in Samish Bay *Salicornia virginica* (10.41 ± 1.32‰) and *Distichlis spicata* (10.04 ± 0.81‰), both marsh-associated primary producers.

Macoma species comparisons— *M. balthica* were substituted for *M. nasuta* where salinities were extremely low. Despite a significant, but small difference in isotope signatures (ANOSIM, R = 0.808, p = 0.001, absolute difference: δ^{13}C = 0.28‰, δ^{15}N = 2.98‰, δ^{34}S = 1.74‰), there was no significant difference in mixing model diet estimations between these two bivalves when they were allowed to feed under identical conditions (ANOSIM control experiment conducted on 103 individuals in Skagit Bay, R = 0.025, p = 0.33). Thus, we assume observed spatial and temporal differences in mixing model estimates between these two species are attributable to changes in OM entering the diet, and not due to interspecific metabolic differences or diet preferences.

Isotopic signatures of consumer indicators— Isotopic signatures of *Macoma* spp. were significantly different from *Mytilus* sp. among all estuaries, flow periods, and transects (ANOSIM, R = 0.776, p = 0.001), as were the estimated diets (ANOSIM, R = 1, p = 0.001). *Macoma* spp. were significantly more enriched in δ^{13}C and δ^{15}N than *Mytilus* sp., but depleted in δ^{34}S (Figure 3, Appendix I). The range of *Macoma* spp. mean δ^{13}C was most depleted in Skagit Bay, the most fluvial estuary, and most enriched in Lopez Sound (Appendix I). Mean δ^{15}N values were most depleted in Port Susan Bay, and most enriched in Samish Bay (Appendix I). Mean δ^{34}S values ranged between were most depleted in Lopez Sound and most enriched in Port Susan Bay (Appendix I). Differences in isotope signatures were greater between consumer indicators in Lopez Sound and Padilla Bay (SIMPER, mean distance^2 = 97.57 and 90.43, respectively), but less in Port Susan and Samish Bays (SIMPER, mean distance^2 = 38.44 and 68.32, respectively). In Skagit Bay, the consumer indicator isotope signatures were moderately distinct in comparison to the other estuaries (mean distance^2 = 72.16).

Estimated diets of consumer indicators

Parallel with source isotope results, estimated compositions of source OM to *Macoma* spp. and *Mytilus* sp. diets differed (ANOSIM, R = 1, p= 0.001) within each estuary. When data were combined across all spatial and temporal sampling stratifications, mean diet similarity was significantly higher among *Mytilus* sp. (90.17 ± 4.68) as compared to *Macoma* spp. (82.60 ± 5.61, *t-test* on SIMPER % similarity values: t = 2.3, $p < 0.05$), indicating that *Mytilus* sp. diets were more consistent than *Macoma* spp. within each estuary.

Overall Macoma spp. *diets— Macoma* spp. generally consumed a combination of OM derived from benthic diatoms, macroalgae, and eelgrass in each of the five estuaries, with the exception of Skagit Bay, where clams assimilated very little eelgrass detritus OM (Table 5, Figure 4a). Marsh detritus contributed most in the intermediately fluvial sites, Samish Bay (high flow (HF): 25 ± 6%, low flow (LF): 48 ± 15%) and Port Susan Bay (HF: 25 ± 15.6%, LF: 14 ± 7.1%), but in diminished proportions in the most fluvial site, Skagit Bay (HF: 10.3 ± 16.5%, LF: 8.1 ± 15.3%), and Padilla Bay (HF: 4.1 ± 0.1%, LF: 5.5 ± 0.01%, Table 5). No marsh contribution was estimated for clams in Lopez Sound. Phytoplankton only contributed measurably to clam diets in Port Susan Bay (HF: 27.6 ± 10.6%, LF: 11.5 ± 18.6%) and Samish Bay (HF: 2.2 ± 0%). However, river POM did not contribute measurably at any site, even Skagit Bay (0.0000006%). The contribution of OM from macroalgae to clam diets was most prominent in the least (Lopez Sound, HF: 43.2 ± 17.1%, LF: 63.2 ± 7.6%) and most (Skagit Bay, HF: 54.9 ± 26.4%, LF: 64.8 ± 12.7%) fluvially-influenced sites.

Overall Mytilus sp. diets—Compared to *Macoma* spp., *Mytilus* sp. usually consumed an equal or greater variety of OM sources within a given estuary and seasonal flow period spp. (Table 5; Figure 4b). In the less fluvial estuaries, *Mytilus* sp. assimilated primarily phytoplankton during both the high and low flow periods (Table 5, Figure 4b, Lopez Sound, HF: 83.1 ± 1.4%, LF: 80.7 ± 4.2%, Padilla Bay, HF: 48.6 ± 2.8%, LF: 39.2 ± 1.7%, Samish Bay HF: 55.6 ± 5.5%, LF: 43.0 ± 4.7%). Average phytoplankton contributions to mussel diets decreased in the more fluvial estuaries (Port Susan Bay, HF: 27.6 ± 7.0%, LF: 21.4 ± 11.2%, Skagit Bay, HF: 15.6 ± 6.4%, LF: 17.1 ± 9.9%), where mussels assimilated greater amounts of marsh detritus (Port Susan Bay, HF: 45.2 ± 6.1%, LF: 43.8 ± 2.9%, Skagit Bay, HF: 45.9 ± 6.4%, LF: 42.2 ± 5.2%). Generally, *Mytilus* sp. assimilated more macroalgae during the low flow period and in the more fluvial sites. Benthic diatoms (<10%) and eelgrass (<10%) contributed minimally (<10%) to mussel diets, with the exception of Lopez Sound, where benthic diatoms comprised 13- 20% of mussel diets.

Skagit Bay— Macoma spp. diet varied more extensively across space than time. Clams in the marsh:mudflat ecotone had primarily assimilated marsh detritus (34-36%) and benthic diatoms (19-21%, Table 5). At the mudflat:eelgrass ecotone further out the intertidal gradient, clams assimilated more macroalgal OM (70-71%) and benthic diatoms (28-29%). Marsh detritus did not contribute to *Macoma* sp. diets in this ecotone. Estimates of *Mytilus* sp. diets did not show a strong spatial or seasonal shift. During both seasons and across both ecotones, marsh detritus consistently comprised 39-50% of mussel diets and macroalgae comprised 34-48%. In the lower elevation ecotone phytoplankton comprised 18-22% of mussel diets. Phytoplankton contributed < 1% to mussels transplanted to the marsh:mudflat ecotone.

Port Susan Bay—Marsh detritus in *Macoma* spp. diets during the high flow period diminished from the marsh:mudflat ecotone (39 %), to the mudflat:*Z. japonica* (28 %) and mudflat:*Z. marina* (3%) ecotones (Table 5). During the low flow period, marsh detritus contributions increased at the outer two ecotones (18%) compared to the marsh:mudflat interface (6%). Clams transplanted into the mudflat:*Z. marina* ecotone consumed higher proportions (78-81%) of *Zostera marina* OM than those at the marsh:mudflat ecotone (26-40%) during the low flow period. Compared to the clams, *Mytilus* sp. did not show dramatic shifts in diet over space or time, although phytoplankton consumption was estimated to be higher in the mudflat:*Z. marina* ecotone (33-38% vs. 6-23%). Marsh detritus consistently contributed 40-55% to mussel diets across space and time, whereas *Z. marina* contributed < 5% to mussel diets with the exception of the outermost ecotone during the low flow period (24%).

Samish Bay—Macoma sp. diets were dominated by marsh detritus during the low flow period (48-49%), and eelgrass during the high flow period (43-50%), but showed no differences across the different ecotones (Table 5). *Mytilus* sp. consumed a more variable diet as compared to *Macoma* spp., but also showed no major diet shifts across space. *Mytilus* sp. diets were dominated by phytoplankton (42-58%), with secondary contributions from eelgrass (5-10%), macroalgae (3-25%), and marsh (6-10%). The contribution of macroalgae OM to *Mytilus* sp. diets increased during the low flow period, while contributions of eelgrass OM and phytoplankton decreased.

Padilla Bay— The estimated diet compositions of *Macoma* spp. were more consistent across space than time (Table 5). Their diets during high flow were dominated by macroalgae OM (34-35%) and *Zostera* spp. (29-38%), followed by benthic diatoms (20-31%) and marsh detritus (4-5%). The largest observed spatial shift occurred between the marsh:mudflat ecotone and the mudflat:*Z. japonica* ecotone during the high flow, where there was a 12% decrease in benthic diatom support. During the low flow period, *Macoma* spp. derived 52-57% of their diet from *Zostera* spp. OM, followed by benthic diatoms (19-23%), and macroalgae (9-24%). Marsh detritus decreased from 7.87 ± 1.1% to 0.11 ± 0.0% from the marsh:mudflat ecotone to the mudflat:*Z. marina* ecotone, and was accompanied by an increase of macroalgae support from 9.48 ± 1.5% to 24.27 ± 2.8%. *Mytilus* sp. diets varied little across space and time, and were dominated by phytoplankton (39-50%), followed by macroalgae (15-30%), marsh detritus (17-25%), *Zostera* spp. (5-12%), and benthic diatoms (0-2%).

Lopez Sound—Macoma spp. derived their diet from a mixture of macroalgae (34-56%), *Zostera marina* (23-52%) and benthic diatoms (15-20%) during high flow. Macroalgae was more prevalent in clam diets at the marsh:mudflat ecotone, while *Z. marina* contributions were stronger at the mudflat:*Z. marina* ecotone (Table 5). Macroalgal contributions to clam diets increased during low flow (57-72%), while *Z. marina* contributions diminished. *Mytilus* sp. largely depended on phytoplankton (80%) and benthic diatoms (20%), with no major spatial or seasonal shifts.

Effects of landscape setting on food web connectivity

Diet comparisons among estuaries —Differences in the type and strength of OM assimilated by both *Macoma* spp. and *Mytilus sp.* were significantly different among the five estuaries (Macoma: ANOSIM R = 0.832, p < 0.001; Mytilus: ANOSIM R = 0.822, p < 0.001) (Figure 5a). With the exception of Lopez x Skagit Bay (R = 0.243, p < 0.001), all pairwise cross-estuary tests for differences in *Macoma* spp. diets were significant and biologically important (0.508 < R < 0.969). SIMPER results indicate that estuaries experiencing similar fluvial discharge conditions generally exhibit lower percent dissimilarity in diet composition as compared to estuaries with strong physical differences in freshwater flow (Table 6). For example, clams in the two least fluvial estuaries—Padilla Bay and Lopez Sound—had the most similar diets (% dissimilarity = 26.9%) to one another, whereas clams feeding in Lopez Sound and Port Susan Bay, a strong fluvial system, show distinct differences in diet (% dissimilarity = 67.9%) from one another. Clam diets in the most fluvial system—Skagit Bay—however, do not follow this pattern. Diets were much more similar to the estuary with no or little freshwater discharge (% dissimilarity = 26.9%) than to Port Susan Bay, the next largest river system in this study (% dissimilarity = 79.6%). While similar proportional assimilation of OM sources by *Macoma spp.* occurred between Lopez Sound and Skagit

Bay, and between Lopez Sound and Padilla Bay, as evidenced by overlap in MDS space (Figure 5a), significant overlap was not observed for any other estuary combinations.

There was no biologically important difference in *Mytilus sp.* diet composition between the two most fluvial sites, Port Susan and Skagit Bays (R: 0.338, $p < 0.001$) but all other pairwise comparisons were significant and important ($0.678 < R < 1$, $p < 0.001$). Unlike *Macoma spp.* diets, the two most similar estuaries with respect to mussel diets were the two most fluvially-influenced sites—Skagit and Port Susan Bay (% dissimilarity = 17.9%)—and the two least similar sites were those sites with the greatest contrast in fluvial discharge, Skagit Bay and Lopez Sound (% dissimilarity = 72.1%). While overlap in the estimated diets of *Mytilus sp.* across the estuaries is greater than with *Macoma spp.* they generally fall in order from least to most fluvially-influenced in multidimensional space, with the exception of Padilla and Samish Bays (Figure 5b).

Temporal differences in OM support for both *Macoma sp.* and *Mytilus sp.* were significant in all five estuaries (Table 7), as evidenced by the seasonally-associated shift observed in MDS space (Figure 6). Overall, *Macoma spp.* diets were significantly more dissimilar between flow periods than *Mytilus sp.* diets (*Macoma spp.*: 22.62 ± 7.19%, *Mytilus sp.*: 12.52 ± 4.97%, $t = 2.58$, $p = 0.03$). Diets shifted most in Port Susan Bay and least in Padilla Bay.

Environmental drivers of cross-estuary diet variability — When examined individually for *Macoma* spp. across the five estuaries, marginal tests of explanatory variables revealed that the net range of transport explained the most variation (49%) associated with diet variability, followed by mean monthly discharge (35%), acoustic backscatter (28%), maximum salinity (23%), marsh area (12%), and mean flow velocity (11%); *Zostera* area, scale ratio, seasonal flow period, and transect each explained less than 5% of total variation. The only variable without a significant relationship with the data was *Zostera* area; all other variables were significant at the $p < 0.001$ level. Sequential tests of explanatory variables indicate six drivers of diet variability, with the main sources of variation explained by the net range of transport, mean flow velocity, and *Zostera* area (Table 8). Together, the predictor variables included in the best model explain 78.4% of the total variation present in the *Macoma* spp. diet data. Axis 1 of the dbRDA analysis explains 74.7% of total diet variation (95.2% of fitted variation) and was most associated with acoustic backscatter and/or mean monthly discharge (variable correlation = 0.93, multiple partial correlation with axis = -0.904), while axis 2 explains 6.9% of total variation (8.8% of fitted) and was most associated with the ecotone where a given consumer was translocated within the estuary (multiple partial correlation = -0.66). In contrast to our hypothesis regarding fluvial discharge effects on diet, the diets of clams from Lopez Sound and Skagit map closely to one another in dbRDA ordination space (Figure 7a).

In contrast to our results for *Macoma* spp., *Mytilus sp.* diets from Lopez Sound and Skagit Bay did not map closely to one another. Rather, the estuaries map across axis 1 in order of most to least fluvially-influenced (Figure 7b). Marginal tests for *Mytilus* sp. show that, when considered alone, maximum salinity explained the most variation (61%) associated with diet variability, followed by estuary size (57%), minimum salinity (52%), marsh area (50%), mean flow velocity (46%), mean monthly flow (39%), scale ratio (37%), and net range of particle movement (30%). The only variable without a significant relationship with the data was seasonal flow period; all other variables were significant at the $p < 0.001$ level. Sequential tests of explanatory variables indicated six significant drivers of diet variability, with the main sources of variation explained by maximum salinity, mean monthly flow, and marsh area (Table 8). Together, these predictor variables explain 87.5% of the variation present in *Mytilus* sp. diet data. Axis 1 of the dbRDA analysis explains 78.7% of total diet variation (90% of fitted variation), and was most associated with marsh area (multiple partial correlation = -0.77) and maximum salinity (multiple partial correlation = 0.47). Axis 2 explains 9 % of total variation (10.7% of fitted), and was most associated with the net range of particle transport (multiple partial correlation = 0.58) and marsh area (multiple partial correlation = 0.59).

Diet component drivers of cross-estuary diet variability — Both indicator species illustrated distinct patterns in the proportional contributions of individual OM sources. When bubble plots were overlaid atop dbRDA ordinations (Figure 8), the strength of dietary dependence on particular OM sources assimilated by *Macoma* spp., exhibits two discernible patterns aligned with the gradient of axis 1. Firstly, when bubble size indicates marsh detritus, data points for consumers translocated to eelgrass beds in Skagit Bay, and all locations in Lopez Sound are absent, indicating a lack of marsh detritus in these locations. The remaining bubble points indicate increasing marsh detritus in *Macoma* spp. diets with increasing fluvial discharge and acoustic backscatter (Figure 8a). Secondly, with the exception of Lopez Sound, macroalgae shows the opposite pattern in *Macoma* spp. diets, indicating decreasing contributions as fluvial discharge and acoustic backscatter increases, (Figure 8c). Less evident patterns were observed for benthic diatoms, phytoplankton, and *Z. marina*. Marsh detritus and macroalgae contributions to *Mytilus* sp. increase with increasing freshwater influence (as measured by fluvial discharge and maximum salinity) and acoustic backscatter (Figure 8f, 8h). The proportions of benthic diatoms and phytoplankton contributing to *Mytilus* sp. diets increased as fluvial influence and backscatter decrease (Figure 8g, 8j). The relationship between fluvial discharge, acoustic backscatter and *Zostera* spp. indicates increased assimilation on this food source under intermediate flow conditions, but diminished assimilation in estuaries with either very high (Skagit Bay) or absent (Lopez sound) freshwater discharge (Figure 8i).

Spatial scales of food web connectivity: cross-ecosystem, cross- ecotone, within-ecotone

Within estuary comparisons of food web connectivity — Within each estuary, food web connectivity was examined at three spatial scales; within-ecotone, cross-ecotone and cross-ecosystem. We hypothesized that within one half of a cross-ecotone transect, OM diet support might shift such that organisms transplanted to a position 2 m from the vegetative transition point might reflect a diet based on more allochthonous sources as compared to those organisms transplanted to a more interior ecosystem position, 50 m from the ecotone transition. However, PERMANOVA results for both *Macoma* spp. and *Mytilus* sp. indicate little food web compartmentalization at fine spatial scales. We found no change in OM diet support at the within-ecotone scale in any of the study locations (Table 7). Additionally, even in the embayment type estuaries with no fluvial influence, we found little evidence of food web compartmentalization at the cross-ecotone scale, where organisms inhabiting either side of the cross-ecotone transect were compared to one another. Except for one significant pair-wise *post-hoc* test among mussels feeding across the marsh:mudflat interface during the high flow period in Port Susan Bay, no significant differences in the type and strength of OM supporting consumers was observed across ecotones, regardless of the vegetative ecosystems at the transition point (Table 7).

Patterns of OM diet contributions indicate that food web connectivity is compartmentalized for both *Macoma* spp. and *Mytilus* sp. at the cross-ecosystem spatial scale (Figure 6). Significant differences in OM diet contributions to *Macoma* spp.*,* were observed in clams inhabiting the marsh:mudflat ecotone and those translocated to the mudflat:*Z. marina* ecotone in all estuaries but Samish Bay (Table 7). Similarly, OM diet support was significantly different among *Mytilus* sp. at the cross-ecosystem scale in all estuaries but Lopez Sound (Table 7). In Padilla and Port Susan Bay, a third ecotone transition occurs due to the presence of *Z. japonica* in the system. In Padilla Bay, *Macoma* spp. diets differed between the mudflat:*Z. japonica* and *Z. japonica:Z. marina* transects during high flow conditions, and between the marsh:mudflat and *Z. japonica:Z. marina* transects and mudflat:*Z. japonica* and *Z. japonica:Z. marina* during low flow conditions (H: t=11.11, p= 0.001, L:1&3: t=16.44, p=0.013, 2&3: t=49.15, p=0.001).

Food web compartmentalization among estuaries

To compare the degree of food web compartmentalization among estuaries, we quantified spatial changes in the seaward transport distance and assimilation strength of marsh detritus to the most seaward (mudflat:*Z. marina*) ecotone, and the landward transport distance and assimilation strength of *Zostera* detritus to bivalves transplanted to the most landward (marsh-mudflat) ecotone.

Seaward ecotone connectivity — Based on *Macoma* spp. as an indicator, cross- ecosystem food web connections to the marsh ecosystem were most evident in Samish Bay (1.39 km, marsh contribution to diet = 44.4 ± 12.8%), followed by Port Susan Bay (2.4 km, 12.8 ± 7.7%) and Padilla Bay (1.32 km, 1.9 ± 1.8 %). During the high flow period, marsh detritus was equally assimilated at the cross-ecosystem scale by *Macoma* spp. in Padilla and Samish Bays (Table 5). Assimilation strengths diminished between the marsh:mudflat ecotone and the mudflat:*Z. marina* ecotone in Port Susan (Δ= -36%) and Skagit Bays (Δ= -57%). During the low flow period, marsh detritus assimilation strength diminished with increasing distance from shore in Padilla (Δ= -8%) and Skagit bays (Δ= -34%). In Port Susan Bay, marsh detritus contributions to *Macoma* spp. diets increased between the marsh:mudflat and mudflat:*Z. japonica* ecotones (Δ= +12%), but remained the same at the mudflat:*Z. marina* ecotone. In Samish Bay, marsh detritus OM was similar at the cross-ecosystem scale (Δ = -2 %). At the mudflat:*Z. marina* ecotone, no marsh detritus was assimilated by *Macoma* spp. in Skagit Bay (4.04 km) or Lopez Sound (0.351 km).

In contrast, *Mytilus* sp. show strong cross-ecosystem food web connectivity to marsh ecosystems in Skagit (42.2 ± 4.4%) and Port Susan bays (40.1 ± 1.2%), followed by Padilla (18.4 ± 1.0%), and Samish bays (9.7 ± 2.0%). Mussels in Lopez Sound did not appear to consume any marsh detritus. Diminished assimilation strengths of marsh detritus across space were observed during both high and low flow periods in Skagit Bay (Δ= -10%, -12 % respectively) and the low flow period in Padilla Bay (Δ= -14%). Little shift in marsh detritus contributions to the mussels' diet were observed between the marsh:mudflat and mudflat:*Z. marina* ecotones during the high flow period in Padilla Bay (Δ = 1 %). In Port Susan Bay, no major shifts in marsh detritus support were observed between marsh:mudflat and mudflat:*Z. marina* ecotones (HF: Δ= -3%, LF: Δ= 0%), but marsh detritus contributed more to *Mytilus* sp. diets in the middle ecotone during the high flow period (HF: Δ = +12-15%). No spatial shift in marsh support was observed during the either flow period in Samish Bay and Lopez Sound.

Landward ecotone connectivity — Strong assimilation of *Z. marina* detritus by *Macoma* spp. was observed in Samish Bay (47.65 ± 5.1 %), Padilla Bay (47.4 ± 15.2 %) and Port Susan Bay (31.6 ± 9.8 %), followed by significantly less in Lopez Sound (10.0 ± 11.9 %), and Skagit Bay (7.2 ± 6.5 %). Diminished assimilation of *Z. marina* detritus between the mudflat:*Z. marina* ecotone and the marsh:mudflat ecotone by *Macoma* spp. was observed in Lopez Sound (HF: Δ= -29%, LF: Δ= -10%), Padilla Bay (HF: Δ= -9%), Samish Bay (HF: Δ= -4%) and Port Susan Bay (HF: Δ= -14%, LF: Δ= -45%). *Z. marina* contributions to clam diets increased at the marsh ecotone in Skagit Bay during the high flow period (Table 5).

In comparison to *Macoma* spp., *Mytilus* sp. indicated weaker connectivity to *Z. marina* at the marsh:mudflat ecotone. The strongest assimilation strengths were observed in Padilla Bay (11.7 ± 1.0%). *Mytilus* sp. transplanted to Skagit Bay, Port Susan Bay, Samish Bay and Lopez Sound assimilated *Zostera* spp. detritus for less than 2% of their diets at the marsh:mudflat ecotone. The only evidence of decreasing landward assimilation strength of *Z. marina* by *Mytilus* sp. occurred in Port Susan Bay during the low flow period (LF: Δ= -24%). *Z. marina* OM was assimilated relatively evenly into diets across space in the other estuaries and flow periods, with the exception of Padilla Bay, where *Z. marina* diet contributions increased 6%.

Environmental drivers of cross-estuary diet variability— The proportional contribution of seaward flowing marsh detritus to *Macoma* sp. diets at the mudflat:*Z. marina* ecotone was negatively associated with net range of transport (-0.559), and positively associated with maximum transport particle speed (0.811), high flow period (0.098), and scale ratio (0.062)(F = 451.65, $p < 0.001$, $R^2 = 0.791$). At the same location, assimilation of marsh detritus by *Mytilus* sp. was positively associated with maximum range of transport (0.023), high flow period (0.168), and mean monthly discharge (0.002) (F = 102.56, $p < 0.001$, $R^2 = 0.758$). The landward transport and assimilation of *Zostera* detritus assimilated by *Macoma* spp. at the marsh:mudflat ecotone was negatively associated with maximum salinity (-0.047) and particle backscatter (-0.015) (F= 251.07, $p < 0.001$, $R^2 = 0.694$), while *Mytilus* sp. assimilation of *Zostera* detritus was positively associated with the area covered by *Z. marina* in the estuary (0.894), and negatively associated with mean monthly discharge (-0.293), maximum particle transport speed (-0.580), and the seasonal high flow period (-0.553) (F = 1049.60, $p < 0.001$, $R^2 = 0.973$).

Effect of season on cross-ecosystem food web connectivity

In order to determine how seasonal shifts in freshwater discharge influence food web connectivity, we compared the percent dissimilarity in consumer diets between the marsh:mudflat and *Z. marina:*mudflat ecotones within each estuary during the low and high discharge periods. We found that spatial dissimilarity in *Macoma* spp. and *Mytilus* sp. diets was greater in the more fluvial estuaries. *Macoma* spp. diets in the marsh:mudflat and mudflat:*Z. marina* ecotones differed by more than 25 % in Port Susan and Skagit Bays, but by less than 17 % in the remaining estuaries. Overall, spatial dissimilarity in diets did not significantly increase during the low flow period for either *Macoma* spp. (HF: 17.85 ± 13.72%, LF: 23.54 ± 22.02%, t = 2.12, p = 0.52) or *Mytilus sp.* (HF: 10.50 ± 8.04%, LF: 18.03 ± 12.83%*, t* = -2.12, p = 0.15). However, when examined by individual estuary, spatial differences in diet were greater during the low flow period for five of nine cross-ecotone comparisons of *Macoma* spp. diets, and six of nine comparisons of *Mytilus* sp. diets (Figure 9). Seasonal shifts in spatial diet similarities were not restricted to those estuaries subject to changes in fluvial discharge, but the magnitude of the spatial diet shifts was generally greater in more fluvial systems (Figure 9). For both *Macoma* spp. and *Mytilus* sp.*,* the greatest spatial dissimilarity in diets occurred during the low flow period in Port Susan Bay (Figure 9). The diets of clams feeding along the marsh:mudflat ecotone were 69% and 50% different from the diets of those feeding at the mudflat:*Z. japonica* and mudflat:*Z. marina* ecotones, respectively. The diets of mussels feeding at the marsh:mudflat ecotone were 20% and 45% different from those feeding at the mudflat:*Z. japonica* and mudflat:*Z. marina* ecotones, respectively. Finally, *Macoma* spp. diets did not display significantly greater dissimilarity across space as compared to *Mytilus* sp. when all estuaries and flow periods were combined (*Macoma* spp.: 20.69 ± 18.04%, *Mytilus* sp.: 18.03 ± 11.09%, *t*= 1.69, p = 0.21), but specific cross-ecotone transect comparisons in specific estuaries revealed that *Macoma* spp. were more dissimilar across space than *Mytilus* sp. in 14 of 18 comparisons.

Discussion

In this study, we identify several factors affecting the strength and length of food web connectivity in five Pacific Northwest estuaries with contrasting fluvial discharge and landscape settings. Both fluvial discharge and consumer feeding mode strongly influenced the strength and spatial scale of food web connectivity across intertidal ecotones. To a lesser degree, seasonal shifts affected the degree to which indicator consumers depended on OM sources from certain ecosystems. Finally, estuary-specific landscape characteristics other than fluvial discharge, such as marsh area or particle transport speed, also influenced the degree of food web connectivity across space and time, often accounting for patterns contrary to our hypotheses and expectations. Our findings suggest that the strength and length of food web connectivity among different OM sources across estuarine ecotones not only depends on the availability of OM sources in each estuary, but also on the consumer indicator examined, and the system's OM transport and retention potential.

Cross-estuary differences in food web connectivity

At the coarsest scale of comparison, in which we compared estuaries to one another, our results clearly indicate differences along the gradient of embayment to delta estuaries. We originally hypothesized that OM support would be most similar among estuaries exhibiting similar river discharge regimes because physical forces governing the biomass, transport, and retention of OM sources would be more likely to align as a function of fluvial forcing. SIMPER results support this hypothesis when *Mytilus* sp. were used as indicators, as the most fluvial system (Skagit) and the least fluvial system (Lopez) had the least similarities in terms of diet support, while pairs of estuaries within each end of the fluvial spectrum exhibited the greatest similarities in diet support.

The most notable pattern in *Mytilus* sp. diet support among estuaries was the steady decrease in phytoplankton contributions with increasing fluvial discharge, and the concurrent increase in assimilation of marsh detritus. This general pattern follows numerous other diet studies in which decreased phytoplankton contributions in the upper regions of estuaries or during years of high river discharge are complemented by an increase in marsh or terrestrial OM contributions (Kasai and Nakata 2005, Vinagre et al. 2011). The positive correlation between marsh detritus contributions to estuarine consumer diets and fluvial influence also coincides with Sakamaki et al.'s (2010) interpretation of $\delta^{13}C$ results from 20 PNW estuaries representing differing watershed sizes. They document increasing evidence of enriched $\delta^{13}C$ in surface sediments and benthic macrofauna in the larger watersheds, implying that watershed area, which generally scales with larger river discharge (Galster 2007), is positively linked to the relative abundance of terrestrial POM available in the estuary and, consequently, in consumer diets.

Observed *Mytilus* sp. diet patterns across the five estuaries also corroborate the emerging estuarine paradigm that diets of estuarine organisms are derived from localized (kilometer scale) primary productivity, which influences the relative abundance of OM sources available to consumers along the estuarine gradient (Deegan and Garritt 1997, Kasai and Nakata 2005, Weinstein et al. 2005, Howe and Simenstad 2011, Vinagre et al. 2011). In our study estuaries, however, changes and combinations in primary producers occur at much finer spatial scales than described in the studies above, likely because the five PNW estuaries we examined are short in comparison to many estuaries of the world. For example, the Chesapeake extends a distance of 300 km from the Atlantic Ocean to the Susquehanna River, whereas salinity intrusion only reaches an upstream distance of 3.2 km in the Skagit River (Yang and Khangaonkar 2009), and the upper limit of tidal influence is at river kilometer 13 (Hood 2004). We thus compared consumer diets across a gradient of estuary types, rather than along the salinity gradient within one estuary in order to examine how river discharge affects the distribution of different OM sources available to estuarine consumers at the estuary scale. The results of our distance-based linear modeling suggest that *Mytilus* sp. diets among the five estuaries are primarily influenced by factors affecting the transport (maximum salinity, mean monthly discharge, net range of transport, and seasonal flow period) and magnitude (marsh area) of OM source inputs in the estuary. These results for *Mytilus* sp. suggest that fluvial discharge among estuaries, much like position along the estuarine gradient, can be used to predict the assimilation strengths of different detritus sources by benthic filter feeders at the estuary scale. This predictive ability is largely made possible by the documented relationship between salinity and estuarine primary producer communities (Crain et al. 2004, Engels and Jensen 2009, Tuxen et al. 2011).

However, predicting OM diet sources based on fluvial discharge is not without uncertainty. Distance-based redundancy analysis revealed that the relationship between diet and environmental drivers switches the expected order of the Padilla and Samish estuaries along axis 1, which was most strongly related to maximum salinity and marsh area. This suggests actions that modify the area of marsh ecosystems in an estuary could alter the strength of diet linkages between the marsh ecosystem and estuarine consumers. In this case, however, both estuaries have similarly small remnants of tidal marsh ecosystems, but direct freshwater inputs are greater in Samish Bay. Yet, analysis indicates that *Mytilus* sp. diets in Padilla Bay are more similar to the more fluvial systems than are the diets from Samish Bay. Specifically, marsh detritus is a larger contributor to Padilla Bay *Mytilus* sp. diets. One explanation for this pattern is the potential for the Skagit River plume to extend northward through the navigation channel that connects to Padilla Bay. Observed salinities suggest greater freshwater influence in Padilla Bay than expected. Furthermore, sedimentation studies indicate that due to high current speeds (1.4 m s-1), sediment is readily transported up the navigation channel into southern Padilla Bay (Johannessen 2010). Given these lines of evidence, it is likely that the Skagit sediment plume augments the availability of marsh detritus available to consumers in Padilla Bay, causing the switch observed in the redundancy analysis.

In contrast to *Mytilus* sp. results, patterns of OM assimilation by *Macoma* sp. suggest more complex relationships among estuaries with different fluvial influences. Whereas increasing fluvial discharge coincided with an increase in marsh detritus in *Mytilus* sp. diets, *Macoma* sp. display a hump-shaped (unimodal) relationship, in which clams feeding in estuaries with intermediate discharge consume the most amount of marsh detritus, but those in estuaries with the least and most discharge assimilate this marsh detritus to a lesser extent. This pattern mirrors Connell's (1979) intermediate disturbance hypothesis, in which too little physical disturbance allows biological competition to drive down diversity, but too much disturbance decreases diversity because few species can tolerate the stressful physical conditions. In the case of benthic deposit- feeding *Macoma* sp. assimilation of marsh detritus, it appears that too little fluvial influence in a system prevents the transport of marsh detritus across the estuarine landscape, thereby cleaving this potential food web linkage. For example, although marsh ecosystems exist at the non-fluvial Lopez study site, neither *Mytilus* sp. or *Macoma* sp. diets assimilate marsh detritus in more than trace amounts, suggesting that this food resource is unavailable to detritivores even under a macrotidal regime. On the other hand, strong fluvial hydrodynamics may export marsh detritus completely beyond the estuarine delta, as high velocity currents prevent settlement and retention of fine particulate OM (Zhu et al. 2011). As a result, suspended marsh detritus may effectively bypass benthic-deposit feeders inhabiting the deltas of strongly fluvial systems. We suggest this phenomenon occurs in Skagit Bay. Individuals inhabiting the marsh fringe, where current velocities are slow (Yang and Khangaonkar 2009), acquired 36% of their diets from marsh detritus. In contrast, individuals feeding on the outer edge of the delta, where velocities are much higher, consumed no marsh detritus despite the fact that it is available in the system (as evidenced by Skagit *Mytilus* sp. diet results). This assertion is further supported by DistLm results indicating that across the five estuaries, *Macoma* sp. diets are sensitive to factors potentially affecting the distribution and retention of OM, including mean monthly discharge, net range of transport, acoustic backscatter, maximum salinity, and mean flow velocity, as well as the organism's location within any one estuary. As a result, graphical depiction of dbRDA results indicates *Macoma* sp. diets in the Skagit estuary are more similar to *Macoma* sp. diets in the non-fluvial site, Lopez Bay, than any other estuary.

Cross-ecosystem connectivity

In addition to diet differences among estuaries, our study design also enabled us to examine spatial gradients in OM transport and consumer assimilation within each estuarine delta. By tracking the percent dissimilarity in consumer diets among transects in each estuary, we found that food web compartmentalization occurred at the cross-ecosystem scale, with the exception of *Mytilus* sp. in Lopez Sound and *Macoma* sp. in Samish Bay. Given that distances between study transects range between 350 m and 4 km, this represents a meso-scale spatial shift in diet contributions as compared to other studies documenting micro-scale shifts at the meter scale (Guest and Connolly 2004, Guest et al. 2004b, Guest et al. 2006), and macro-scale shifts over tens to hundreds of kilometers along the estuarine salinity gradient or between estuarine deltas and adjacent coastal basins (Gordon et al. 1985, Deegan and Garritt 1997, Machas et al. 2003, Vinagre et al. 2008, Howe and Simenstad 2011, Vinagre et al. 2011).

Unlike the complete compartmentalization observed by Guest et al. (2004) across the mangrove:saltmarsh ecotone, we found compartmentalization at the cross-ecosystem scale to be only partial, and usually did not fully eclipse connectivity in OM sources across the estuarine landscape. We used assimilation of marsh and *Z. marina* detritus to examine differences in seaward and landward OM transport lengths and assimilation strengths across the five estuaries. With the exception of only the least fluvially-influenced estuary (Lopez Sound), we observed assimilation of marsh detritus at the mudflat:eelgrass ecotone, and *Z. marina* detritus at the marsh:mudflat ecotone in each estuary by at least one consumer indicator species. This suggests the spatial extent of each OM source's detrital shadow fully encompasses the area of each estuary's delta. Thus, in order to examine how fluvial forcing and landscape structure influences food web linkages within estuaries, we focused on the strength of food web linkages rather than the lengths. Specifically, we examined the extent to which compartmentalization varied among estuaries as evidenced by spatial shifts in the percent dissimilarity in source contributions to consumer diets across ecotones. We hypothesized that: 1) less spatial dissimilarity in diet support would occur in estuaries with greater fluvial discharge (indicating greater connectivity); 2) greater spatial dissimilarity in diet support would occur during the low flow period when the food web is likely to be more spatially compartmentalized due to lesser physical forcing; 3) greater seasonal shifts in spatial dissimilarities in diet support would occur in the more fluvial estuaries; and, 4) *Macoma* sp. diets would exhibit greater spatial dissimilarity than *Mytilus sp.* diets.

In direct conflict with our first hypothesis, we observed greater spatial diet dissimilarity in the more fluvial estuaries as compared to the embayment estuaries. With *Macoma* sp., the greatest extent of cross-ecosystem connectivity occurred in the intermediately fluvial estuaries, including Padilla and Samish Bays, while the strongest compartmentalization was observed in Port Susan and Skagit Bays, the two most fluvial systems. For *Mytilus* sp., the pattern was less pronounced, but spatial diet differences were greater in the more fluvial systems. One explanation for the occurrence of larger spatial diet differences in the more fluvial estuaries may simply relate to estuary size. Longer transport distances are required to distribute OM across the full suite of ecotones in the more fluvial estuaries. Given the documented localized nature of estuarine food web support (Deegan and Garritt 1997, Connolly et al. 2005a, Howe and Simenstad 2011), it follows that consumers located 4 km from one another would exhibit greater diet differences than consumers located 350 m from one another. However, we observed a nearly 1:1 relationship between the distance from the marsh to eelgrass ecotones and the maximum spatial range of particle transport in each estuary during a spring tidal series ($y = 0.96x + 652.7$, $R^2 = 0.75$). Thus, while larger river systems are associated with larger deltas across which OM must be transported, the ability of the system to transport OM across greater intertidal gradients and OM source ecotones increases as well. Therefore, spatial shifts in the composition of the detrital pool should scale in relation to system size, meaning that the magnitude of spatial diet shifts should not change across estuaries of various sizes. In fact, when spatial diet shifts in marsh support are normalized by distance between ecotones, *Mytilus* sp. exhibit extremely similar levels of change among the four fluvial estuaries. When *Macoma* sp. diets are normalized by distance, spatial diet shifts in marsh support are similar in the three intermediately fluvial estuaries, but greater change in diets are still reflected in Skagit Bay. Because consumers did not assimilate marsh detritus in Mud Bay, no change was observed in this location. This suggests that fluvial forcing is an important mechanism for spatially integrating the detrital pool within an estuary, but identifies Skagit Bay as an anomaly wherein something interrupts the scale relationships among estuary size, OM transport distance, and consumer assimilation.

As mentioned earlier, marsh detritus did not support consumers in any location in Mud Bay on Lopez Island. This suggests that macrotidal exchanges are insufficient to transport marsh detritus into the intertidal in the absence of fluvial discharge, although it is easily capable of integrating *Z. marina* detritus across a system, perhaps because wave action augments tidal forcing. The complete lack of connectivity to the marsh was unexpected in this estuary, given that inundation of the marsh plain occurs during high spring tides.

Our results support our second hypothesis that lower freshwater flows during the summer decrease food web connectivity at the cross-ecosystem scale. With the exception of Skagit Bay, we observed greater food web compartmentalization during the low flow period, indicating that less fluvial forcing may decrease the spatial extent of detrital transport and consumer assimilation within an estuary. This phenomenon has also been observed in Portugal's Tagus River estuary, where freshwater flow regulates the degree of food web connectivity between nursery grounds for juvenile sole (Vinagre et al. 2011). During average flow conditions, two geographically distinct nursery areas exhibit low connectivity, as evidenced by their isotopically distinct C and N signatures. The separation of isotope signatures, however, begins to diminish and overlap during periods of increased flow associated with above normal rainfall, indicating a higher degree of food web connectivity across the estuarine landscape. The degree of food web connectivity for estuarine organisms can thus be regulated by changes in fluvial discharge on both seasonal and inter-annual time scales.

Following our expectation to see greater seasonal shifts in the degree of cross-ecosystem connectivity in the more fluvial systems, our data suggest the flow gradient across estuaries is the dominant scale at which fluvial forcing determines spatial change in diets. Within an estuary, season plays a minor role in regulating the degree of food web connectivity at the cross-ecosystem scale. Although we expected to see greater seasonal shifts in food web compartmentalization of estuaries with the greatest fluvial discharge, the largest seasonal diet shift did not occur in the Skagit estuary. Rather, the largest seasonal diet shifts were observed at the marsh:mudflat interface in the Port Susan Bay estuary. However, this observation does align with patterns in seasonal flow variation; the largest seasonal shift in fluvial discharge in any of the estuaries evaluated occurs in the Stillaguamish River ($\Delta = 32\ m^{3s-1}$). Unlike the Skagit River, the Stillaguamish River flowing into Port Susan Bay does not receive a spring freshet in the early summer. Freshwater discharge emanating from the Stilliguamish therefore remains low once the winter rains cease. In contrast, the spring freshet characterizing the Skagit River in early June resembles winter high flow conditions. The low flow conditions in Skagit Bay, under which food web compartmentalization emerges, only persist between late July and September.

Despite strong seasonal shifts in fluvial discharge, seasonal shifts in the degree of compartmentalization may not become apparent for two reasons. Firstly, tissue turnover rates in clams and mussels may operate on timescales longer than shifts in fluvial discharge (Tieszen et al. 1983, Hesslein et al. 1993, MacAvoy et al. 2001, Bearhop et al. 2002, Barnes and Jennings 2007, Sweeting et al. 2007, Suring and Wing 2009, Buchheister and Latour 2010). If so, by the time clams and mussels begin to reflect isotopic distinction across space, high flows return with the fall rains, once again integrating the detrital pool across the estuary. Secondly, the rate of detrital consumption may be slow enough that detritus-feeders fail to deplete the pool of detritus distributed across the estuary during high flow conditions. In this case, the shortened period of decreased detrital transport in the Skagit estuary may not affect the types of detritus available to consumers as compared to the less fluvial systems, wherein long periods of low freshwater discharge could eventually result in the depletion of marsh detritus in the outer reaches of the estuary.

Finally, our data support our fourth cross-ecosystem hypothesis; *Macoma* sp. exhibited greater spatial diet dissimilarity between the marsh:mudflat and mudflat:*Z. marina* ecotones as compared to *Mytilus* sp. in all estuaries. This suggests that interpretation of food web connectivity depends on consumer feeding mode, and is confirmed by dbRDA model results which suggest the diets of the two indicator consumers respond to different environmental drivers. Specifically, the assimilation of outflowing marsh detritus by *Macoma* spp. was regulated by maximum flow speed, net range of transport, mean monthly flow, and location within the estuary. Thus, the ability of benthic-deposit feeding clams to assimilate marsh OM depends of a combination of factors affecting the transport and assimilation of fine detrital particles from the marsh. As a result, the diets of benthic deposit feeders are more likely to reflect patchiness of OM transport, deposition, or retention across space. The assimilation of landward flowing *Z. marina* OM by *Macoma* spp. responded negatively to salinity and particle backscatter. Given that increased freshwater flow often coincides with increased turbidity (Nichols 1977), these two variables suggest that fluvial discharge mediates tidal actions, preventing the transport and deposition of eelgrass OM to the marsh fringe. It thus appears that food web connectivity behaves similar to, if not commensurate with, estuarine sedimentary dynamics, which operate according to fluvial processes under high river discharge, but become complicated by a tidal component under low discharge (van den Berg et al. 2007). Suspension-feeding *Mytilus* sp.*,* on the other hand, reflected positive relationships between outflowing marsh detritus and particle transport range, particle transport speed, mean monthly flow, and marsh area. *Mytilus* sp. thus respond to the availability of a source in the system, as well as the efficiency with which that source is transported across space. Because *Mytilus* sp. do not require the deposition of OM to the benthic boundary layer, they reflect a more spatially homogenous consumption pattern. Like *Macoma* spp., the assimilation of marine OM sources by *Mytilus* sp. are also negatively associated with increased fluvial discharge. Additionally, as *Z. marina* availability in the system increases (area), *Mytilus* sp. assimilate more of this OM source regardless of freshwater discharge.

At the cross-ecosystem scale, *Macoma* spp. displayed an unexpected positive relationship with maximum particle transport speed, indicating that as flow velocities increase in an estuary, *Macoma* spp. living on the outer delta consume more marsh detritus. At the same time, as net range of transport increases, *Macoma* spp. assimilation of marsh detritus decreases. These relationships appear to be at odds with one another, and may be driven by a series of anomalous circumstances in Samish Bay. Firstly, particle transport speeds in Samish may be artificially higher than the other systems because the equipment tripod was placed close to the mainstem channel of the Samish River. The tripods in the other estuaries were placed in areas subject to sheet flow or near one of hundreds of shallow distributaries on the outer edge of the deltaic fan, as in the Skagit. Thus, our documented particle transport speeds are likely more strongly influenced by river velocity in Samish Bay as compared to the other estuaries. Secondly, during the low flow period, the Bayesian mixing model estimates of marsh detritus assimilation by *Macoma* spp. in Samish Bay are extremely high. The combination of high marsh assimilation and high velocity in Samish likely drives the redundancy model relationships between marsh support and environmental drivers. This relationship may be further compromised by a possible OM source omission. *Macoma* sp. in Samish Bay exhibit extremely high δ^{15}N signatures during the low flow period. While *Salicornia virginica* also exhibit high δ^{15}N signatures in Samish Bay, it is possible that another source of N^{15} enriched OM is available. The Samish River has one of the highest nitrogen concentrations in the area, largely because the watershed basin is dominated by agricultural land (Inkpen and Embry 1998). While artificial fertilizers tend to exhibit low δ^{15}N signatures, bovine manure effluent ranges between +2‰ and +30‰ (Lefebvre et al. 2007). Effluent from several dairies upstream in the Samish frequently shut down shellfish operations in the bay due to high fecal coliform levels, and likely serves as the enriched nitrogen source of OM consumed by *Macoma* spp. downstream. However, because effluent was not included as a source in the mixing model, it is possible that the mixing model estimatesof *Macoma* spp. OM sources in Samish Bay erroneously suggest extremely high dependence on marsh detritus. The combination of a missed OM source and placement of the tripod likely drive the unexpected relationship between maximum particle speeds, net range of particle transport, and marsh detritus assimilation by *Macoma* spp..

Cross-ecotone connectivity

Our results indicate that recent models of finitely compartmentalized food webs (Guest and Connolly 2004, Guest et al. 2004b) does not apply to Pacific Northwest estuaries, regardless of freshwater influence. In all cases, no significant shifts in OM support were observed at the cross-ecotone level, implying that the PNW macrotidal forcing is strong enough to integrate OM at the 100 m scale. This supports a major paradigm in estuarine ecology, which observes that northern hemisphere estuaries generally display structural and functional components on a larger scale than southern hemisphere estuaries (Elliott and Whitfield 2011). The difference in scales has been largely attributed to differences in fluvial discharge between the two hemispheres. However, our results indicate that even in those PNW estuaries receiving little to no freshwater discharge, the scales of food web connectivity still exceed those reported in Moreton Bay, Australia. This suggests that macrotidal conditions may accentuate the spatial scale of detrital food web connectivity, especially given that PNW estuaries experience a 4 m tidal range, while those in the Australian study experience a 1- 2 m range. We previously described the 1:1 relationship between the distance from the marsh to eelgrass ecotones and the maximum spatial range of particle transport in each estuary ($y = 0.96x + 652.7$, $R^2 = 0.75$). The intercept (652.7 m) may indicate the scale of food web integration caused by tidal forcing in PNW estuaries, although we did not test this specifically.

Cross-ecosystem trophic and other energy exchanges also depend on the permeability of ecosystem boundaries, the structural complexity of the landscape, and the productivity disparity between the two ecosystems (Polis and Hurd 1995, 1996b, a, Holt 2002). The original Guest et al. (2004) study documenting fine-scale carbon movement (< 2 m) took place across two highly structured ecosystems; a densely vegetated saltmarsh and a mangrove forest. Increased structure, such as stem density, can inhibit cross-ecosystem exchange by decreasing water velocities, which in turn facilitates sedimentation and retention of organic particles (Chen et al. 2007). In less structured ecotones, such as the seagrass:mudflat interface we investigated, boundaries are more easily transgressed and the degree of food web compartmentalization tends to diminish. Indeed, Connolly et al. (2005a) observed an expansion of cross-ecosystem carbon movement (> 100 m) indicating that mudflat animals located hundreds of meters away from seagrass beds relied heavily on seagrass detritus OM. This scale of carbon movement more closely reflects our observations in PNW estuaries, perhaps because we quantified food web connectivity at vegetated:mudflat ecotones as well. In addition to boundary permeability, another explanation for the reliance on allochthonous carbon in adjacent mudflats further coincides with food web subsidy theory which suggests that strong food web subsidies are more likely to occur between areas exhibiting large differences in productivity (Polis and Hurd 1996b, a, Polis et al. 1997). Thus, the fine-scale compartmentalization of saltmarsh:mangrove food webs may not only reflect less permeable boundary conditions than mudflat:seagrass ecotones, but a more equal level of productivity. Because each of our study ecotones was positioned adjacent to a mudflat, the productivity disparity may have increased the spatial scale at which we observed food web connections.

Finally, the spatial scale of food web connectivity may also be a result of consumer indicator choice rather than estuarine conditions and landscape structure. The crab species used by Guest et al. (2004) consumed a mixture of sediment detritus, benthic microalgae, and large (> 125 µm) detrital fragments. Food consumption by *M. nasuta* and *M. trossulus* is restricted to much smaller particles (20 -30 µm) (Beecham 2008), including phytoplankton, benthic microalgae, and highly conditioned organic particles. These particles are not only more easily transported due to their small size, but also have been in the detrital pool for a longer period of time, thereby increasing their probability of having been transported across greater distances. Thus, even though the crabs are more mobile (although restrict their movement to < 2 m) than our mussels and clams, their feeding behavior may functionally restrict their spatial connectivity to production in adjacent ecosystems (Guest et al. 2006). However, detritus-feeding gastropods collected in the same locations as the saltmarsh crabs only exhibited slightly greater connectivity, assimilating carbon sources 2-15 m away (Guest et al. 2004b). It is therefore unlikely that consumer indicator choice alone explains the larger scale of OM transport and assimilation observed in this study.

Consumer feeding mode

In addition to fluvial discharge and estuarine settings, observed differences between mussel and clam OM support suggests food web connectivity also depends on consumer organism feeding mode. The trophic relay concept (Kneib 2000) suggests the physical movement of organisms can facilitate connectivity across ecosystem boundaries by transferring energy obtained in one ecosystem and depositing it in another via metabolic waste deposition, death and decay. For sedentary organisms, however, connectivity with spatially distant ecosystems relies on the transport and distribution of detritus. Thus, generalist sedentary species feeding in the same location should reflect similar food source dependencies. However, differences in feeding mode and position can result in differential access to the detrital pool (Grall et al. 2006, Le Loc'h et al. 2008). Thus, because our indicator species represent different feeding modes, one deposit feeding on the sediment surface and the other suspension feeding from the water column, some amount of food web segregation was built into our study. The considerable strength of segregation between the two species, however, was unexpected, and provides further insights on the influence of landscape setting on trophic connectivity.

Spatial feeding separation achieved through vertical positioning among benthic suspension feeders has been shown to segregate food sources in other systems (Le Loc'h and Hily 2005, Le Loc'h et al. 2008). Le Loc’h et al. (2008) described strong differences in the trophic functioning between benthic communities in the Bay of Biscay, wherein suprabenthos organisms were found to rely on recent sedimenting of POM, while the benthic deposit feeders and partly-benthic suspension feeders relied on a conditioned benthic detrital food source. While mussels were not included in the suprabenthos described by Le Loc’h, the cages in which transplanted mussels were placed in this study were attached considerably higher above the sediment surface than the clams. Specifically, differences in vertical positioning may explain why benthic deposit-feeding clams consumed considerably more benthic diatoms compared to mussels in every estuary, season, and location. Because benthic diatoms occupy surficial sediments, their presence in mussel diets requires resuspension through turbulent flow into the water column. The minimal presence of this high quality food source (Miller et al. 1996) in mussel diets suggests either little resuspension of OM from the sediment surface occurs in these estuaries, or the available biomass of benthic diatoms that could be resuspended is small compared to other OM sources.

Within a specific estuary, the diets of suspension-feeding mussels exhibited less variability across space as compared to the diets of benthic-deposit feeding clams. This pattern was especially pronounced in Skagit and Port Susan bays, where clam diets differed by ~50 % at the cross ecosystem scale, but mussel diets only differed by 30- 40 %, respectively. When specifically considering the contribution of marsh detritus to Skagit and Port Susan bays’ consumers, the pattern becomes clearer. Clams inhabiting the marsh fringe assimilate this food source for ~30-40 % of their diet, while those inhabiting the outer edge of the delta are trophically disconnected from the marsh ecosystem (this pattern is true only during the high flow period in PSB). Mussels, on the other hand, consistently assimilated on marsh detritus for 30-50 % of their diets. This observation suggests a more integrated and spatially homogenized detrital pool in the water column as compared to that entrained in surface sediments. The disparity in marsh OM contributions between clams and mussels becomes less distinct in estuaries with less fluvial discharge, suggesting increasing similarity between benthic and water column detritus pools as physical forcing diminishes. Strong hydrological forces, such as wind, wave and tidal currents, can resuspend and winnow away fine-grained sediments associated with OM (Zhu et al. 2011). This phenomenon may explain the lack of assimilated marsh detritus by benthic- deposit feeders in the outer Skagit and PSB deltas, as these zones are subjected to substantial fetch from prevailing winter southerlies and by strong tidal currents (Finlayson 2006, Yang and Khangaonkar 2009). In the other study estuaries, the lack of strong tidal currents and exposure to southerly winds may result in less resuspension and consequent export of OM, a situation that would increase OM similarity between the water column and sediments.

In addition to affecting the availability of OM sources, strong hydrological forces also affect bivalve feeding efficiencies and behaviors. Clearance rate studies of suspension feeders extracting OM from the water column have shown that feeding efficiency can decrease under high flow conditions (>15-17 cm s^{-1} (Wildish and Miyares 1990, Sobral and Widdows 2000). Benthic deposit- feeders are further affected by flow velocities because, in order to access detrital material, they require water velocities to be low enough that OM settles out and becomes entrained in the sediments (<5-12 cm s^{-1})(Sobral and Widdows 2000, Flindt et al. 2007). Additionally, benthic deposit feeders such as *Macoma nasuta* feed by extending their siphons across the sediment surface. As flow velocities increase, *M. nasuta* decrease their feeding radius in order to reduce drag on their siphons; under high enough velocities, some individuals have been observed to stop feeding entirely (Levinton 1991). Maximum particle speeds recorded under low flow, spring tidal conditions in Samish, Port Susan, and Skagit Bays all exceed the 15 cm s^{-1}high flow delineation (44. cm s^{-1}, 19.4 cm s^{-1}, and 23.7 cm s^{-1}, respectively). It is thus likely that clams feeding at the outer edges of the deltas cease feeding, or severely reduce their feeding during the maximum ebb tide, precisely the period of time when marsh detritus is being transported across the tidal flats. If *Macoma nasuta* transplanted to the outer delta edges feed most effectively during high tide, it follows that their diets reflect marine sources of OM support.

In comparison, mussels not only maintain feeding efficiency at higher flow velocities (Sobral and Widdows 2000), but turbulence and eddies created by the mussel cages may have reduced flow velocities and retained OM particles immediately surrounding the enclosures, a phenomenon documented in naturally occurring mussel and oyster beds (Nelson et al. 2004, Jones et al. 2011) and in experimental filtration rate studies (Riisgard 2001). As a result, mussels may show less spatial contrast in OM assimilation because they are able to take advantage of detritus exported from the marsh ecosystem during ebbing tides. In environments such as Skagit and Port Susan Bay, where sandy sediments are constantly reworked and OM is poorly retained, high OM transport speeds may render potential marsh-derived OM unavailable to benthic-deposit feeding consumers. Thus, the tenet that water movement acts as the principle vector of OM transport and spatial integration in estuarine environments may well be true, but the functional organism response to high energy environments may create spatially disjunct food webs in an otherwise integrated ecosystem. Thus, when considering an organism's role in ecosystem functioning, behavioral and anatomical organ features must be considered in concert with an organisms' position, both vertical and geographical, in the environment.

Conclusion: Implications for delta restoration and management

Aquatic ecosystems are subject to a litany of anthropogenic alterations, many of which have implications for food web connectivity. Our data suggest that alterations in estuarine and coastal hydrodynamic regimes may play a particularly important role in regulating food web connectivity across estuarine landscapes. Dams, levees, and water diversions are well known to dramatically alter a wide array of ecosystem functions, often disrupting ecosystem connectivity by decreasing freshwater flow (Polis et al. 1997, Naiman and Bilby 1998, Amoros and Bornette 2002). However, our results from the Skagit River estuary imply that increased flow velocity can be just as disruptive to system connectivity as alterations that mute the historical hydrograph. Just as decreased freshwater flow reduces a river's ability to transport terrestrial and marsh detritus across space, high flow velocity can produce disjointed connectivity by exporting OM beyond the estuarine delta, depositing fine particulate detritus in deeper subtidal troughs, coastal basins, or nearby pocket bays.

Increased flow velocities can result from river channelization, in which levees prevent surges in freshwater discharge from dissipating across the floodplain, as well as by disconnecting distributary channels in river deltas, forcing the full discharge of a river to flow through fewer outlets (Syvitski et al. 2005, Pinter et al. 2010). Distributary channels usually divide up the discharge from the mainstem, thereby reducing the velocity of the effluent along with its ability to transport sediment away from the coast (Syvitski et al. 2005). With reduced momentum of seaward flowing plumes, more suspended sediment and associated OM is trapped near the river mouth. As a result, rivers with intact distributary networks display muddy deltas and diffusive plumes, while single channel rivers display jet-like plumes and deltas composed of coarser sediments (Syvitski et al. 2005). As such, disconnecting distributaries from the mainstem of a river can decrease consumers' access to detritus in estuarine deltas by enhancing export of fine materials through high velocity seaward plumes.

In the case of the Skagit River delta, levee construction, marsh conversion, and distributary channel obstruction have reduced the area of estuarine wetlands by ~89% (Hood 2004). The north channel of the Skagit River is highly channelized with no distributary channels, and displays a jet-like plume, while the south channel splits into five main distributary sloughs, although many of these are constrained by levees as well. The Skagit, unlike the other estuaries in Puget Sound, contains very little muddy sediments associated with the delta, suggesting unnatural conditions upstream. Usually, large rivers produce muddy deltas and prodeltas, with sand content less than 20% (Syvitski et al. 2005). This is largely the case in Samish, Padilla, and Port Susan Bays (Bulthuis 1996, Heatwole 2006, Liebman 2008), but is clearly not the case in the Skagit, where the delta is dominated by coarse, sandy deposits (Webster et al. in press). Our data indicate that the lack of fine sediments and OM deposition on the delta is not due to a lack of these materials in the system, as evidenced by *Mytilus* sp. diets, which establish the availability of marsh detritus in the water column of Skagit Bay. Rather, *Macoma* sp. diets suggest high river flow velocity exports fine particulate OM beyond the immediate estuarine system, functionally separating detrital marsh resources from benthic-deposit feeders inhabiting the estuarine delta. Studies examining sediment dynamics in Skagit Bay support this contention, as they describe very little OM accumulation on the delta's surface (Webster et al. in press), instead finding fine sediment deposits in the subtidal trough adjacent to the Skagit River delta, as well as in the deep channels to the north and south of Skagit Bay (A. Ogston, UW Oceanography, E. Grossman, USGS, pers. comm.).

Marsh detritus and fine sediments also accumulate in pocket estuaries and muddy embayments along the eastern side of Whidbey Island at the distal margin of the Skagit Riever outflow (personal observation). Preliminary stable isotope analysis of *Macoma balthica* inhabiting Dugualla Bay, located directly across the Whidbey Channel trough from the north fork of the Skagit River, indicates that clams in this muddy location obtain 60.6% of their diet from marsh detritus (Howe, unpublished data). These clams consume nearly twice as much marsh detritus as those inhabiting the marsh ecosystems themselves, providing evidence that marsh detritus constitutes a prominent trophic subsidy to benthic deposit- feeders in Skagit Bay, just not those inhabiting the delta itself. While OM transported away from the Skagit delta certainly has the potential to fuel detritus-based food webs in surrounding areas, organisms inhabiting the delta experience decreased trophic connectivity to marsh and terrestrial food sources. If decreased connectivity results in decreased productivity, then estuarine-dependent organisms may be adversely affected. In the PNW, estuarine productivity is critical to a number of threatened and commercially important species, such as anadromous salmonids (*Oncorhynchus* spp.) and Dungeness crabs (*Metacarcinus magister*), which extensively forage in shallow estuarine deltas during their juvenile life stage (Healy 1982, Simenstad et al. 1982). Given that many such estuarine predators rely on a suite of benthic-deposit feeders (Holsman et al. 2003, Hurst et al. 2007), which in turn rely on the deposition of OM, changes in flow velocity within an estuarine delta can theoretically result in cascading food web effects.

In addition to the effects of flow velocity on trophic connectivity, landscape alterations that decrease interaction across the fluvial-terrestrial interface may also act to decrease food web connectivity. In the case of estuaries, reducing the number of distributary channels not only eliminates the complexity of natural dendritic distributary networks, but also reduces channel sinuosity and the ratio of "edge" to "interior" by restricting the linear distance of the fluvial:terrestrial ecotone. Because water is the principle vector of OM transport in estuarine ecosystems (Polis et al. 1997), maintaining a low interior to edge ratio (interior/edge) increases the opportunity for OM exchange across ecosystem boundaries (Forman and Godron 1986, Ingegnoli 2002). The lower the interior/edge ratio, the more edge is exposed to water movement, thereby potentially enhancing food web connectivity across the landscape through OM transport (Witman et al. 2004). Cross-boundary exchange, however, is not only affected by the amount of interface along the ecotone. The type of ecotone boundary, manifested in the permeability of the boundary, can also have profound effects on food web connectivity (Witman et al. 2004). Leveed river systems, as seen in the Skagit, represent low permeability boundaries because water and organism movement is restricted between adjacent ecosystems by the levee structure. When coupled with decreased interface area, in the case of decreased distributary channel networks, leveed river systems become vulnerable to disruptions in food web connectivity at the landscape scale. Although more work is needed to test this hypothesis, we suggest that the historical reduction of marsh area, distributary channel number, and ecotone permeability via levee construction cumulatively disrupt food web connectivity across the Skagit River delta.

Finally, our data show that indicator estuarine consumers assimilated a combination of OM sources year round, including seagrass, marsh macrophytes, macroalgae, benthic diatoms, and phytoplankton. This indicates that estuarine consumers can draw on the full mosaic of ecosystems within the estuarine landscape with respect to food. However, temporal and spatial variability in the assimilation of these sources infers variability in source availability. As a result, we observed temporal variability in the degree of food web connectivity across the estuarine landscape. As with temporal subsidies in stream ecosystems (Nakano and Masashi 2001, Takimoto et al. 2002), the timing of spatial food web convergence or divergence may play an under-appreciated role in regulating secondary productivity in estuarine systems. With the exception of Skagit Bay, where extremely high flow velocities act to disconnect food web connectivity, our data suggest spatial food web convergence occurs during the winter under high flow conditions. Increased trophic connectivity from marsh to other estuarine ecosystems during the winter and early spring may provide essential OM resources to detritivores inhabiting estuarine deltas and shorelines during a time when the productivity of marine macroalgae, phytoplankton, and diatoms is low (Admiraal and Peletier 1980, Newton and Van Voorhis 2002, Nelson et al. 2003). Because detritus is less bioavailable compared to marine algae (Sobczak et al. 2002), and is thus a less desirable food source, it has been argued that efforts to restore estuarine food webs should focus on phytoplankton production as opposed to detrital inputs (Jassby et al. 2003). Our data, however, suggest that pelagic sources of OM become less available to consumers under high flow conditions, creating opportunity for detritus emanating from estuarine marsh and terrestrial ecosystems during certain periods of the year to subsidize detritus-based consumers. For example, *Mytilus* sp. diets showed decreasing dependence on phytoplankton with increasing river discharge across estuaries. Thus, although detritus may not provide as energetic a resource as marine phytoplankton or microphytobenthos, it may provide a critical energy subsidy during periods of high flow and turbidity in estuarine systems when suspended particulates fluxing from rivers limit light availability for algal growth.

While it is true that many organisms do not invest in growth during the winter period of high fluvial discharge (Henry and Cerrato 2007), others, such as juvenile salmon, rely in the early spring on estuarine food resources for rapid growth prior to ocean migration (Beamer et al. 2005). Many of the important prey organisms to juvenile salmon are benthic detritivores (Cordell et al. 1999). These detritivores benefit from increased availability of high quality (i.e., fresh) detritus within estuarine deltas, especially during the winter when marine primary productivity is low (Simenstad and Wissmar 1985). Sustaining natural seasonal periods of high fluvial discharge may therefore represent a key component for restoration planners to consider, as these conditions act to increase food web connectivity to marsh ecosystems at critical periods of the year. However, if flow velocities increase too much as a result of estuarine delta alterations, as they appear to in the Skagit, detritus can be exported beyond the delta before deposition. This decreases connectivity to marsh and terrestrial detrital sources for benthic-deposit feeding consumers in deltaic habitats, potentially limiting tertiary productivity in the estuary. Restoration efforts seeking to strengthen estuarine detrital food webs should thus include mitigation of upstream alterations to natural river flow in order to ensure detrital deposition across the estuarine delta.

In conclusion, by coupling spatial patterns internal to each study estuary with the dichotomy of dietary response reflected in *Macoma* spp. and *Mytlius* sp. diets across the fluvial gradient among five different estuaries, we were able to identify landscape-scale patterns of food web connectivity. Our results suggest that food web connectivity is related to environmental drivers governing the availability and transport of OM sources. Specifically, the diets of benthic deposit- feeding organisms correspond most directly to those factors affecting OM source, transport and deposition regimes, while the diets of filter-feeding organisms respond more strongly to the extent of OM transport, the availability of various sources within the systems, and the fluvial discharge through the estuary. As such, alterations to flow due to climate variability, allocation of water resources, or landscape restructuring can thereby shift natural patterns in food web convergence or divergence across the estuarine landscape, especially for sedentary organisms which cannot track their food sources. More positively, our results suggest restoration efforts that increase the availability of detritus sources and restore natural hydrological regimes may have significant impacts on restoring the historic balance of OM types and connectivity to detritus-feeding organisms in estuarine ecosystems.

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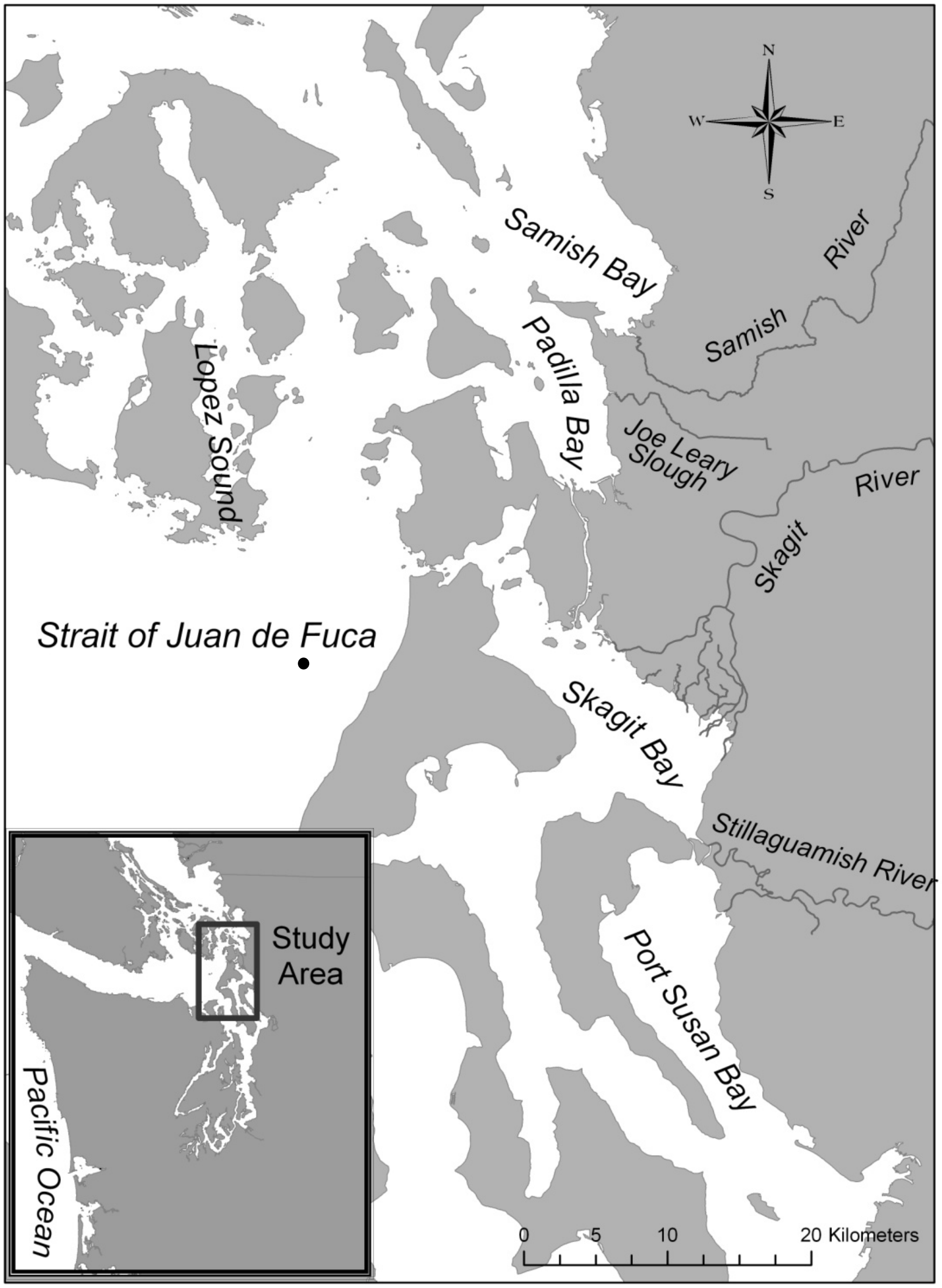


Figure 1.1. Site map of study estuaries and associated freshwater inputs in Puget Sound, Washington, USA.

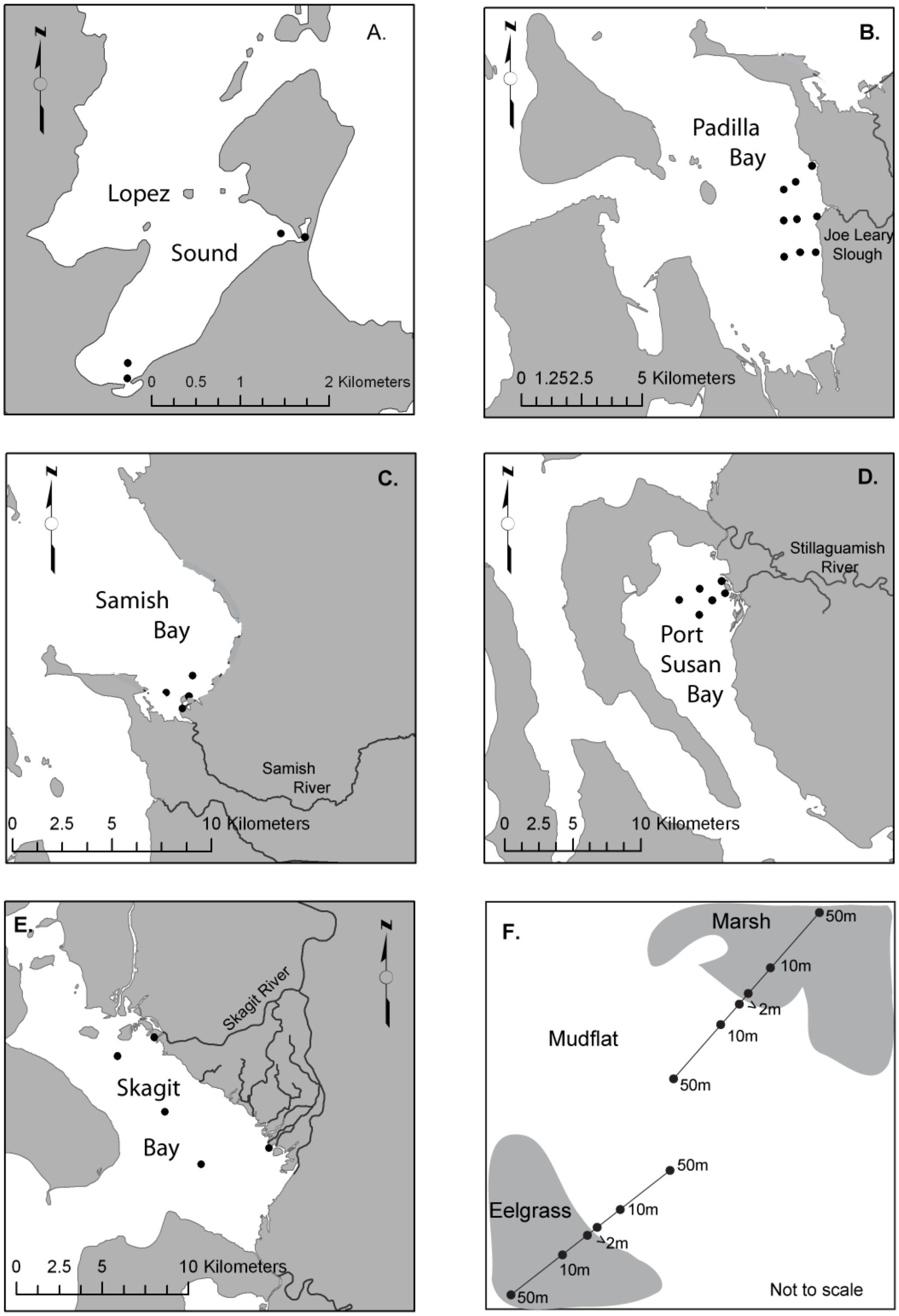
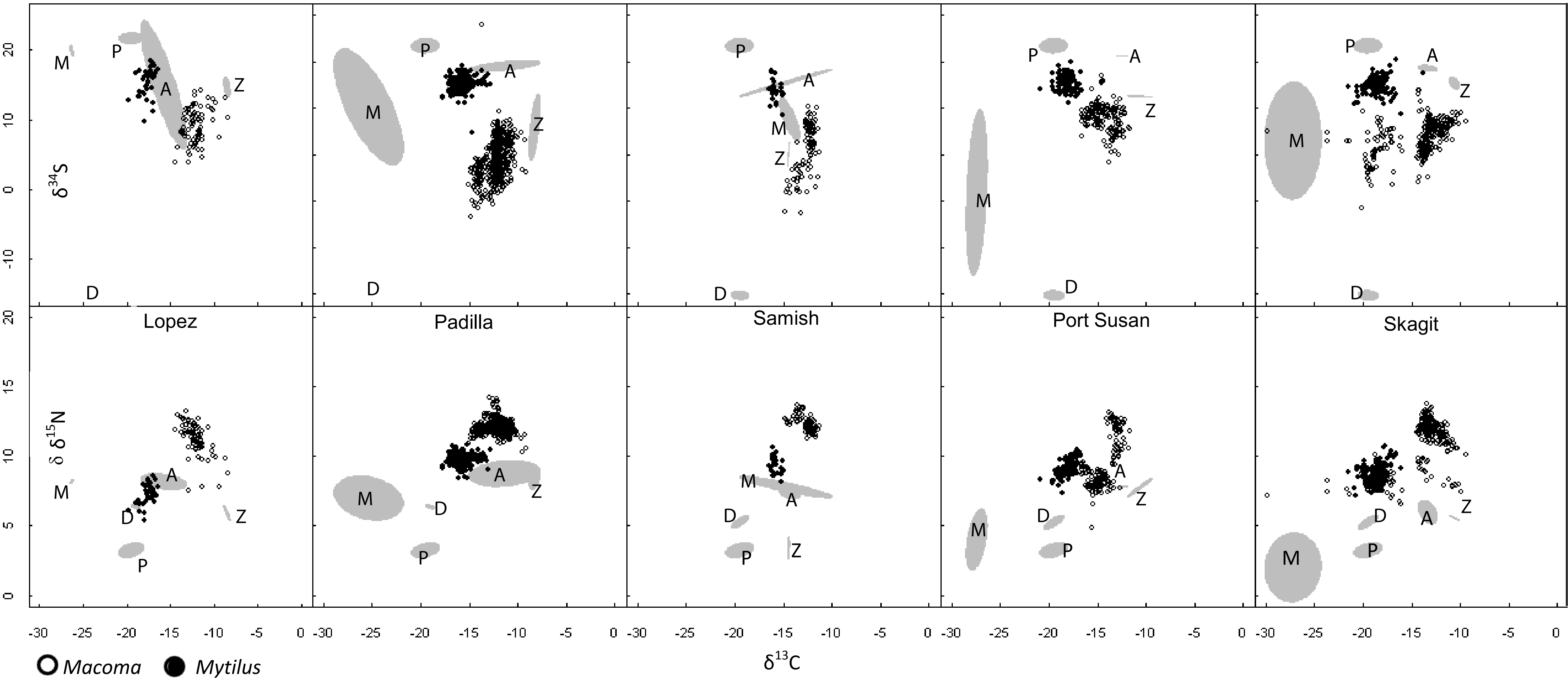


Figure 1.2. These maps show the positions of transect locations in each of the study estuaries, as well as a schematic of the sampling design for transplanted organisms at the marsh:mudflat and mudflat:eelgrass ecotones (F).

Figure 1.3. Dual isotope plots of δ13C and δ15N (below) and δ13C and δ34S (below) of consumer bioindicators, Mytilus sp. and Macoma spp., and the five major OM source categories (Grey ellipses indicate the 95% confidence intervals around the mean isotope value). M: Marsh detritus, P: phytoplankton, Z: Zostera spp., D: benthic diatoms, A: marine macroalgae.



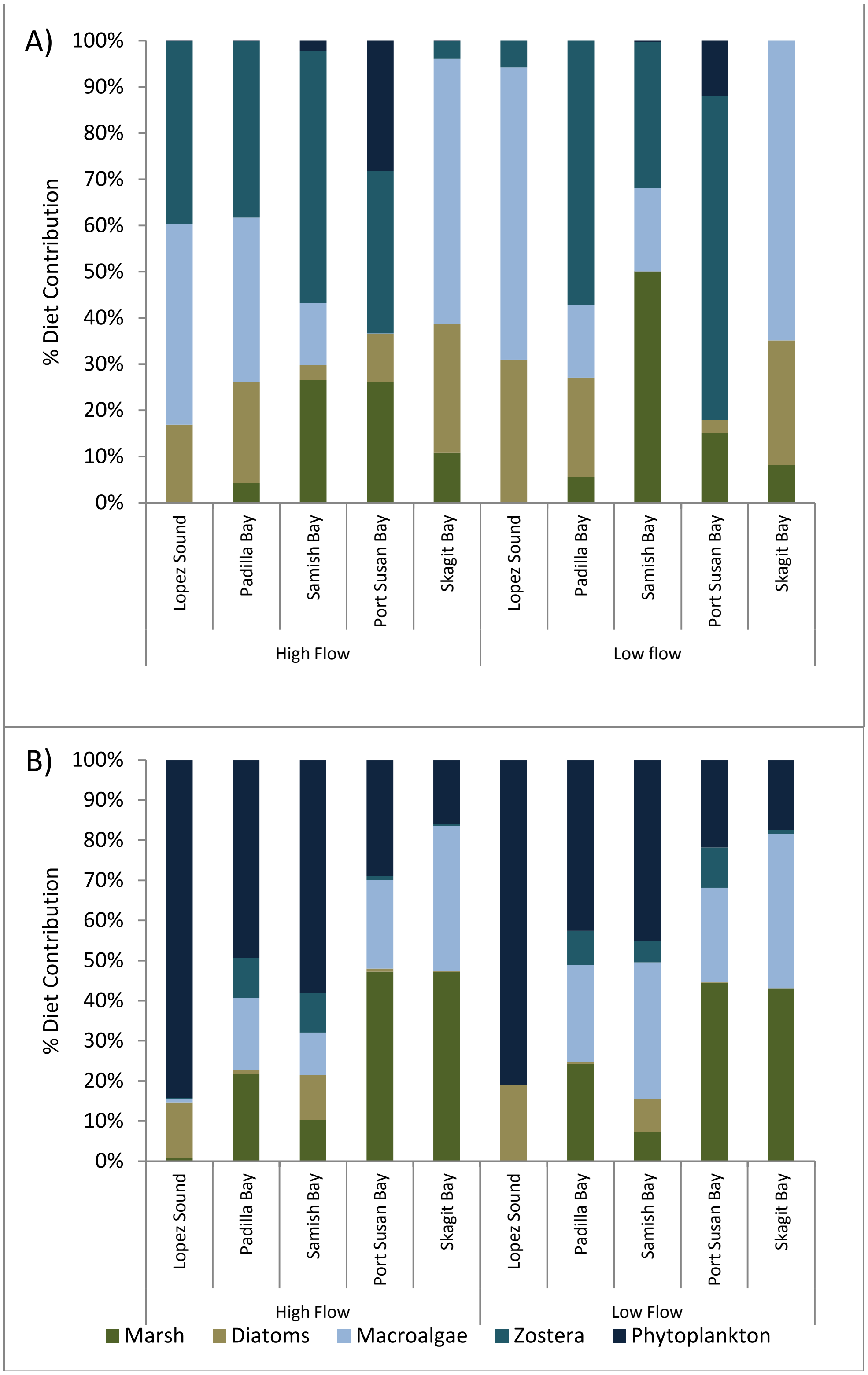


Figure 1.4. Estimated proportions of OM sources to A) *Macoma* spp. and B) *Mytilus* sp., averaged across transects within each estuary.

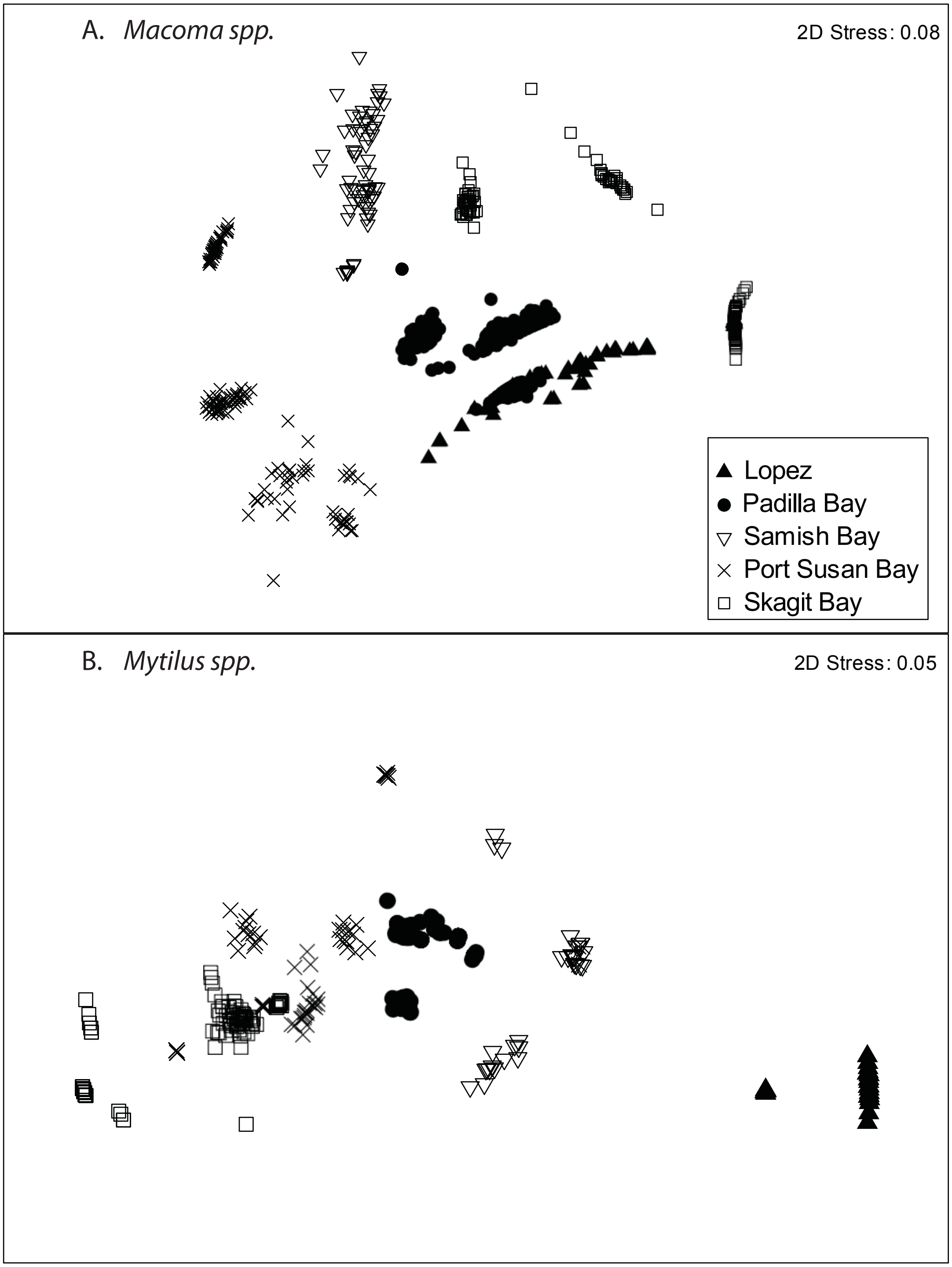


Figure 1.5. MDS ordination of Bayesian mixing model estimates of A) *Macoma* spp. and B) *Mytilus* sp. diet compositions by estuary, based on Bray-Curtis similarities from square-root transformed data.

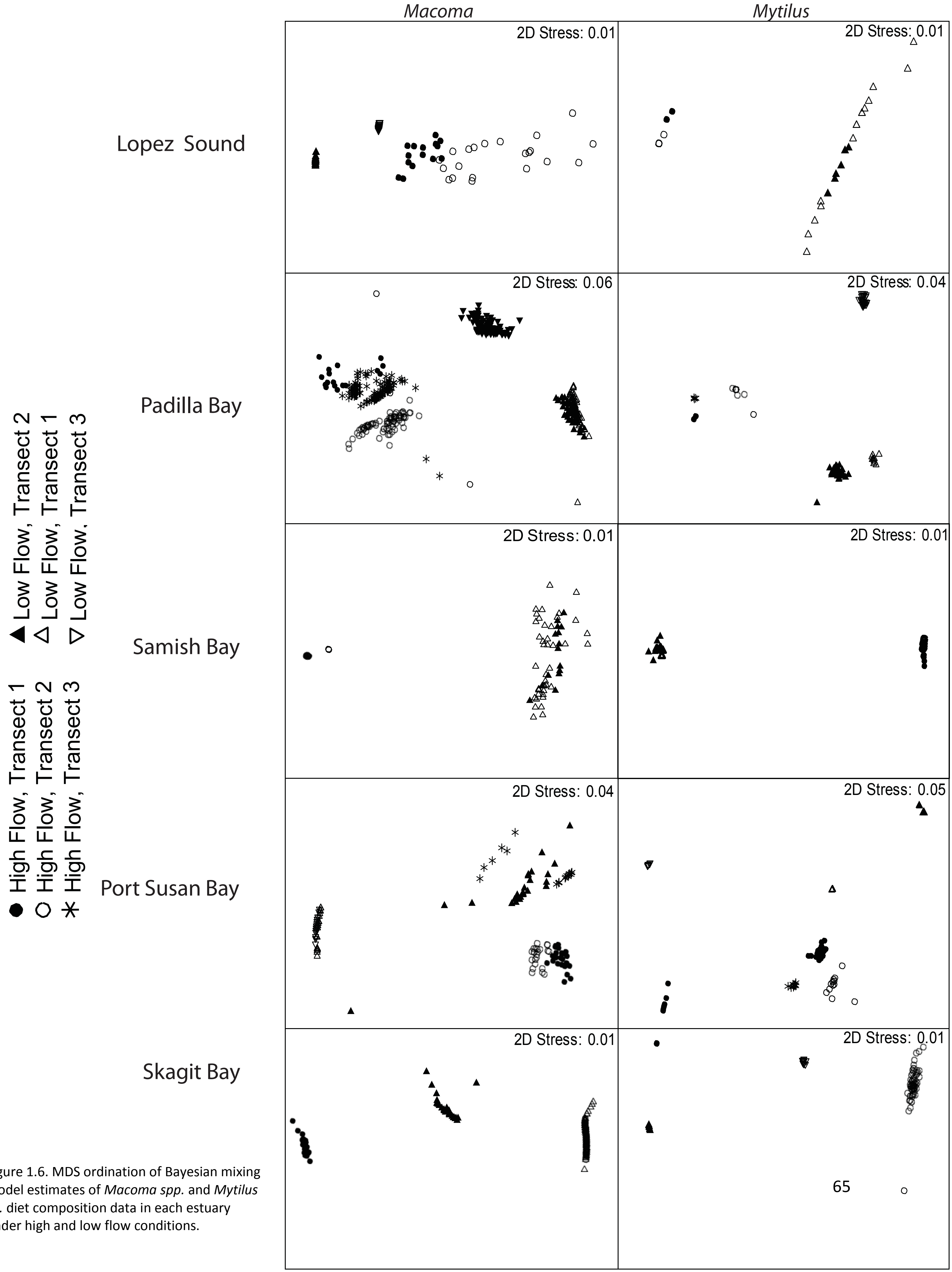


Figure 1.6. MDS ordination of Bayesian mixing model estimates of *Macoma spp.* and *Mytilus sp.* diet composition data in each estuary under high and low flow conditions.

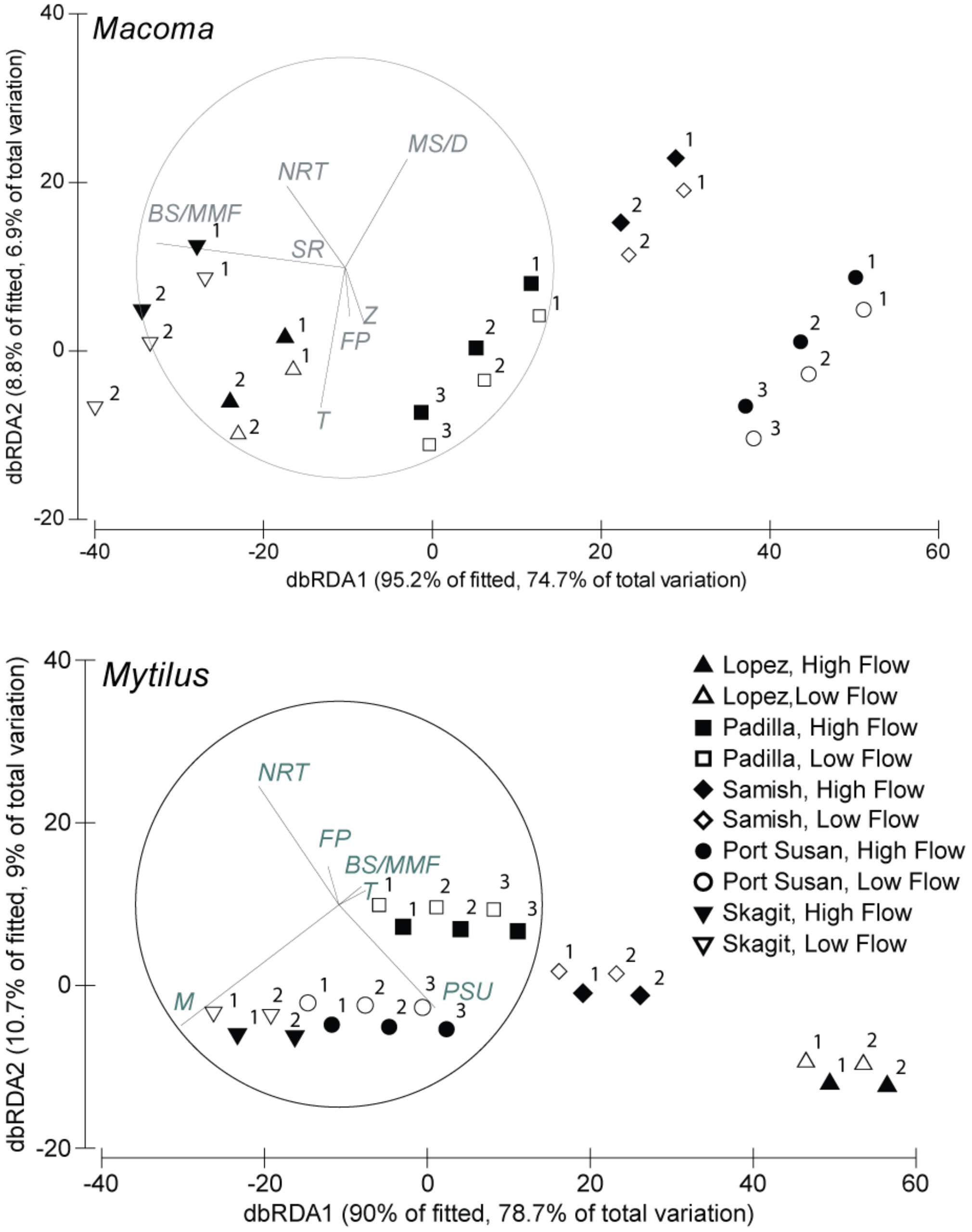


Figure 1.7. Distance-based RDA (dbRDA) ordination for the fitted models of Bayesian mixing model estimates of *Macoma* spp. and *Mytilus* sp. diet composition in five estuaries during low and high flow (based on Bray-Curtis distance measure after square-root transformation). Numbers refer to transect number. NRT = net range of particle transport, FP = Seasonal flow period, BS = backscatter (proxy for particle concentration), MMF = mean monthly river discharge (flow), PSU = practical salinity units, M = marsh area, T = transect, SR = scale ratio, Z = *Zostera marina* area, MS = mean particle transport speed, D= distance between the marsh:mudflat ecotone and the mudflat:*Z. marina* ecotone.

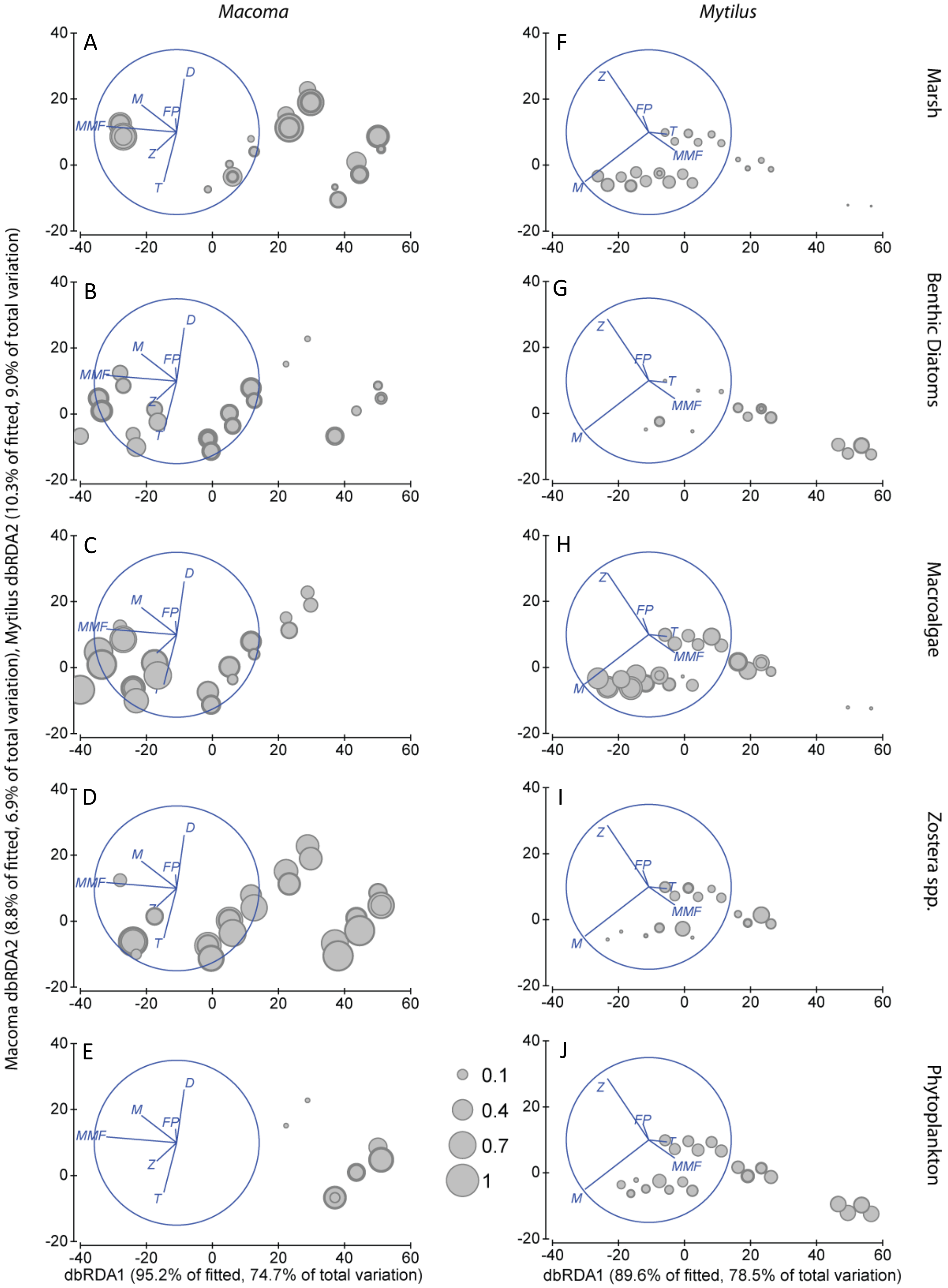


Figure 1.8. Distance-based redundancy analysis (dbRDA) ordination for the fitted model of *Macoma* spp. and *Mytilus* sp. diet composition (based on Bray-Curtis similarity after square-root transformation). The vector labeled “MMF” also reflects backscatter, as the two were highly correlated.

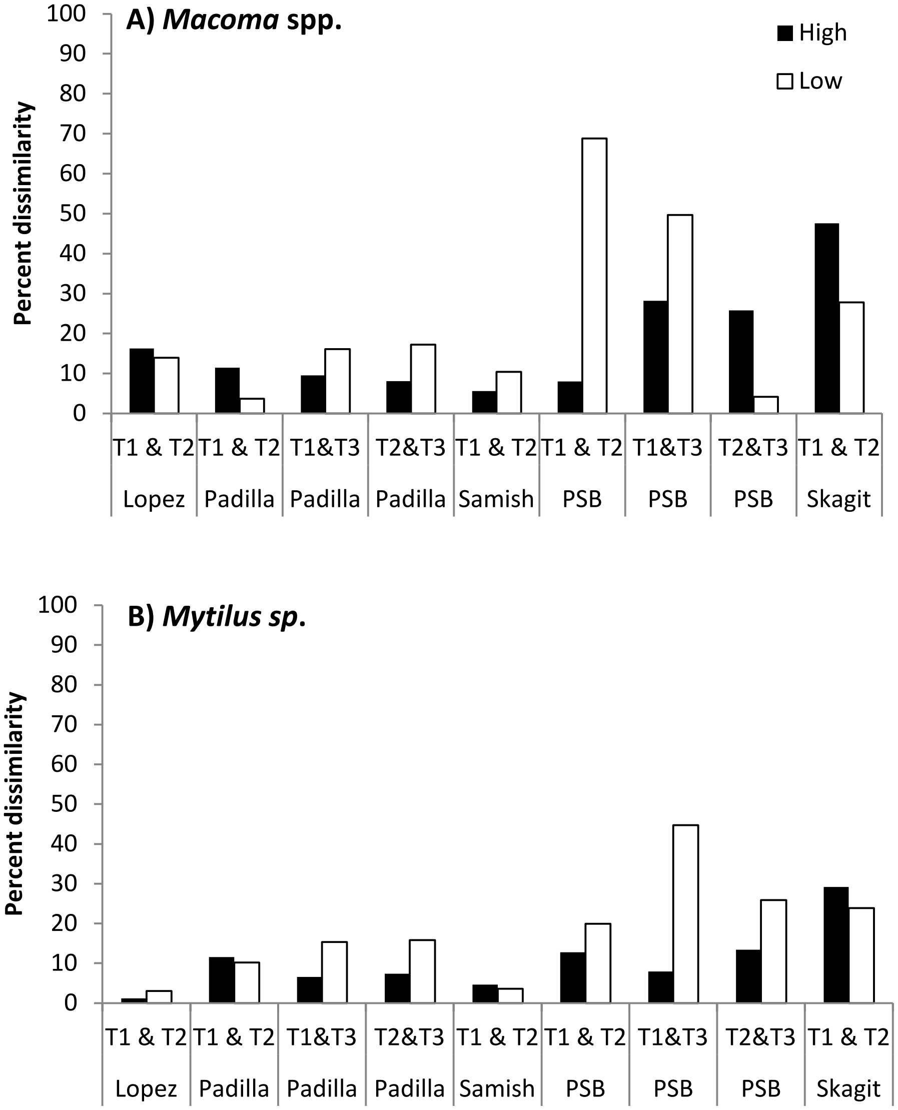


Figure 1.9. SIMPER results of the percent dissimilarity in A) *Macoma* spp. and B) *Mytilus* sp. Bayesian mixing model diet estimations between indicator species transplanted to different transects across three estuarine ecotones during high and low seasonal freshwater discharge: T1 = marsh:mudflat; T2 = mudflat:*Z. japonica*; and T3= *Z. japonica:Z. marina*. Dissimilarity in diet represents change in OM assimilation across space, either due to the type or amount of OM sources entering consumer diets.

Table 1.1. Physical characteristics for the five study estuaries.

Estuary	Freshwater Source	Mean annual discharge (m^3s^{-1})*	Salinity (psu)	Tidal Range (m)	Bay Area (km^2)	Watershed Area (km^2)	Marsh Area (km^2)	Eelgrass Area (m^2)	Max. distance across ecosystem gradient (m)
Lopez Sound	(insignificant)	-	29.9-30	2.6	2	19	0.002	0.21	352
Padilla Bay	Joe Leary, No Name, Big Indian sloughs	0.2	15-30	3.66	45	93	0.58	31.71	1320
Samish Bay	Samish River, Edison Slough	7	10-29.5	3.66	40	228	0.37	18.58	1394
Port Susan Bay	Stillaguamish River	105	10-29.5	2.2-3.5	120	1800	4.62	3.79	2400
Skagit Bay	Skagit River	468	18-28	3.17	308	8,544	78.20	10.96	4041

* USGS 2011, waterdata.usgs.gov

Table 1.2. Mean and standard deviation of organic matter source categories used in mixing model analyses, expressed in standard δ notation in pars per thousand (‰). Many plant species were combined into a single category because the similarity of their isotope signatures violated SOURCE's NND^2 minimum value of 0.1. "Scrub Shrub" includes *Salix spp., Lonicera involucrata, Myrica gale, Deschampsia caespitosa*, and *Potentilla anserina*. "Marsh complex" includes *Carex lyngbyei, Juncus balticus,* and *Schoenoplectus americanus*, *Schoenoplectus acutus, Schoenoplectus maritimus, Cotula coronopifolia,* and *Glaux maritima. Ulva spp.* includes *Ulva intestinalis* and *Ulva fenestrata.* ASG= *Atriplex patula, Salicornia virginica,* and *Glaux maritma.*

Estuary	**OM Source**	**δ^{13}C**	**(SD)**	**δ^{15}N**	**(SD)**	**δ^{34}S**	**(SD)**
Lopez Sound	*Salicornia virginica*	-26.33	0.18	8.20	0.14	19.91	0.79
	benthic diatoms	-19.04	0.46	6.38	0.15	-17.16	0.50
	Macroalgae	-16.44	2.74	8.12	0.90	19.39	1.83
	Zostera marina	-8.57	0.37	5.96	0.53	14.71	1.48
	phytoplankton	-19.52	1.41	3.37	0.63	21.73	0.77
Padilla Bay	*Atriplex, Salicornia, Glaux*	-26.57	1.2	7.16	1.73	18.17	1.87
	Ceramium sp.	-14.70	1.05	9.82	0.32	19.63	0.25
	benthic diatoms	-19.04	0.46	6.38	0.15	-17.16	0.50
	Distichlis spicata	-14.87	0.27	5.84	0.48	2.82	4.52
	Triglochin maritima	-25.67	0.75	6.92	0.37	4.63	1.27
	Ulva spp. & epiphytes	-10.35	3.62	8.44	0.81	19.53	0.68
	Zostera japonica	-8.57	0.52	7.74	0.48	9.78	2.58
	Zostera marina	-8.08	0.54	8.92	0.65	15.01	2.45
	phytoplankton	-19.52	1.41	3.37	0.63	21.73	0.77
Samish Bay	*Salicornia virginica*	-28.73	0.88	10.41	1.32	18.69	1.40
	Distichlis spicata	-14.49	0.13	3.21	0.81	10.10	1.28
	benthic diatoms	-19.52	1.03	5.28	0.51	-5.10	0.53
	Zostera japonica	-8.51	1.04	7.62	1.23	15.72	0.23
	Zostera marina	-7.72	0.76	8.45	0.47	11.95	1.64
	Ulva spp.	-10.41	1.54	7.11	0.24	19.08	0.37
	Ceramium spp.	-19.07	1.08	8.50	0.24	16.39	0.44
	phytoplankton	-19.52	1.41	3.37	0.63	21.73	0.77
Port Susan Bay	*Potentilla-Typha*	-28.35	0.70	1.64	1.94	5.92	7.10
	Schoenoplectus spp.	-26.55	0.51	5.33	0.89	14.97	2.14
	Triglochin-Carex	-27.51	1.11	5.16	1.20	-3.03	4.75
	Distichlis spicata	-16.62	4.27	3.21	0.80	9.62	1.42
	benthic diatoms	-19.52	1.03	5.28	0.51	-5.10	0.53
	Zostera marina	-10.61	1.26	7.77	0.67	16.28	0.06
	Ulva spp.	-12.45	0.53	7.82	0.08	20.67	0.02
	Phytoplankton	-19.52	1.41	3.37	0.63	21.73	0.77
Skagit Bay	river POM	-25.83	0.51	1.95	0.96	-2.58	1.74
	scrub-shrub	-27.75	2.16	2.96	1.19	13.40	1.32
	Typha sp.	-27.24	0.63	4.78	0.82	13.86	1.99
	Distichlis spicata	-14.49	0.13	3.21	0.81	9.62	1.28

	marsh complex	-27.70	2.03	3.05	1.78	9.18	6.25
	benthic diatoms	-19.52	1.03	5.28	0.51	-5.10	0.53
	Ulva spp.	-13.32	1.05	6.17	0.80	19.27	0.38
	Zostera marina	-10.74	0.50	5.62	0.16	17.81	0.68
	phytoplankton	-19.52	1.41	3.37	0.63	21.73	0.77

Table 1.3. Data and sources included in DistLM and dbRDA analysis.

Data Type	Data Source	Site
Mean annual discharge (1929-2010)	USGS waterdata	Skagit, Stillaguamish, Samish Rivers
Mean annual discharge (2003)	Washington Dept. of Ecology, District 2005	No Name Slough, Padilla Bay
Salinity	ReefProbe tripod sampling	All sites
Suspended particle concentration (acoustic backscatter)	ReefProbe tripod sampling	All sites
Flow velocity (particle speed)	ReefProbe tripod sampling	All sites
Current direction	ReefProbe tripod sampling	All sites
Particle transport range	ReefProbe tripod sampling	All sites
Areal *Z. marina* coverage	Washington Dept. of Natural Resources Jeff Gaeckle, unpublished data	All sites
Areal marsh coverage	The Nature Conservancy D. Heatwole, unpublished data	Port Susan Bay
	Padilla Bay National Estuarine Research Reserve, Bulthuis and Shull 2006	Padilla Bay
	People for Puget Sound, Dean et al. 2000, and Skagit River System Cooperative, McBride et al. 2006	Skagit Bay
	Digitization from aerial photographs	Samish Bay, Lopez Sound

Table 1.4. Current, suspended particle concentration, transport, and salinity measurements obtained from hydrodynamic tripod sampling for each of the five estuaries during the low flow period 2009 (June through August).

Estuary	Mean particle transport speed (cm/s)	Max. particle transport speed (cm/s)	Back-scatter	Min. salinity (psu)	Max. salinity (psu)	Net range of transport (km)	Max. range of transport (km)
Lopez Sound	1.5	4.8	133	30.4	30.7	0.64	0.64
Padilla Bay	3.7	14.6	113	27	28.9	1.84	1.84
Samish Bay	8.1	44.4	120	24.2	29.8	1.85	3.14
Port Susan Bay	6.3	19.4	125	24.1	28.8	0.36	2.02
Skagit Bay, North Fork	11.10	23.7	148	17.3	26.7	4.75	4.75
Skagit Bay, South Fork	12.3	51.2	146	18.6	27.4	2.49	7.03

Table 1.5. Bayesian mixing model median estimates and interquartile ranges (IQR's) of proportional OM source contributions to bivalve diets. Transect 1 = marsh: mudflat ecotone, Transect 2 = mudflat: *Z. marina* ecotone.

	Macoma								*Mytilus*							
	High Flow				Low Flow				High Flow				Low Flow			
	Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2	
	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR
LOPEZ SOUND																
Marsh	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.00	0.00	0.00	0.00
Benthic Diatoms	**0.21**	0.04	0.14	0.03	**0.28**	0.01	**0.33**	0.01	0.14	0.01	0.13	0.01	0.17	0.02	0.20	0.02
Macroalgae	**0.56**	0.09	**0.34**	0.06	**0.72**	0.01	**0.57**	0.03	0.01	0.02	0.01	0.03	0.00	0.00	0.00	0.00
Zostera	**0.23**	0.08	**0.52**	0.07	0.00	0.00	0.10	0.03	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Phytoplankton	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.81**	0.02	**0.84**	0.04	**0.82**	0.02	**0.80**	0.02
SAMISH BAY																
S. virginica	0.05	0.06	0.10	0.01	0.05	0.02	0.06	0.02	0.05	0.02	0.05	0.02	0.05	0.03	0.04	0.03
D. spicata	**0.21**	0.04	0.16	0.08	**0.45**	0.05	**0.42**	0.04	0.05	0.10	0.05	0.07	0.02	0.07	0.02	0.08
Benthic Diatoms	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.13	0.04	0.10	0.02	0.07	0.04	0.09	0.04
Z. japonica	0.02	0.01	0.01	0.00	**0.30**	0.06	**0.28**	0.07	0.01	0.10	0.01	0.10	0.03	0.03	0.03	0.03
Z. marina	**0.48**	0.05	**0.52**	0.08	0.02	0.03	0.02	0.03	0.09	0.10	0.07	0.09	0.02	0.03	0.02	0.04
Ulva spp.	0.08	0.04	0.05	0.07	0.08	0.07	0.10	0.08	0.06	0.03	0.07	0.04	0.09	0.04	0.09	0.04
Ceramium sp.	0.08	0.11	0.07	0.08	0.06	0.03	0.08	0.04	0.04	0.02	0.03	0.02	**0.25**	0.08	**0.20**	0.09
Phytoplankton	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.01	**0.52**	0.08	**0.58**	0.06	**0.42**	0.06	**0.45**	0.08
SKAGIT BAY																
River POM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scrub shrub	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	**0.20**	0.10	0.00	0.01	0.06	0.12
Typha sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.54**	0.06	**0.24**	0.05	**0.48**	0.03	**0.31**	0.06
D. spicata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Marsh	**0.36**	0.11	0.00	0.00	**0.34**	0.03	**0.00**	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.02	0.04
Benthic Diatoms	**0.21**	0.08	**0.29**	0.01	**0.19**	0.03	**0.30**	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Ulva spp.	0.14	0.43	**0.71**	0.01	**0.46**	0.03	**0.70**	0.01	**0.43**	0.04	**0.34**	0.05	**0.48**	0.02	**0.34**	0.07
Z. marina	0.13	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.02
Phytoplankton	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.06	0.00	0.00	0.23	0.06

Table 1.5. cont. Note the change of format to accommodate 3 transects in these estuaries. Transect 1 = marsh: mudflat ecotone, Transect 2 = mudflat: *Z. japonica* ecotone. Transect 3 = *Z. japonica:Z. marina* ecotone.

	Macoma											
	High Flow						Low Flow					
	Transect 1		Transect 2		Transect 3		Transect 1		Transect 2		Transect 3	
	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR
PADILLA BAY												
Atriplex-Salicornia	0.00	0.02	0.01	0.03	0.00	0.01	0.08	0.15	0.10	0.05	0.00	0.00
T. maritima	0.03	0.04	0.03	0.04	0.03	0.02	0.00	0.34	0.00	0.11	0.00	0.01
D. spicata	0.01	0.03	0.01	0.01	0.01	0.01	0.00	0.05	0.00	0.01	0.00	0.01
Benthic Diatoms	**0.31**	0.01	**0.20**	0.00	**0.21**	0.00	0.19	0.00	**0.20**	0.00	**0.23**	0.00
Ulva spp.	0.03	0.02	0.03	0.03	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Ceramium sp.	**0.31**	0.02	**0.32**	0.01	**0.31**	0.03	0.09	0.00	0.09	0.00	**0.24**	0.00
Z. japonica	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Z. marina	**0.28**	0.04	**0.38**	0.04	**0.37**	0.05	**0.57**	0.23	**0.60**	0.08	**0.52**	0.01
Phytoplankton	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PORT SUSAN BAY												
D.spicata	**0.39**	0.06	**0.28**	0.07	0.03	0.05	0.02	0.19	0.00	0.00	0.00	0.00
Potentilla-Typha	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.05	0.00	0.00
Schoenoplectus	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Triglochin-Carex	0.00	0.01	0.00	0.01	0.00	0.00	0.04	0.09	0.18	0.06	0.18	0.02
Benthic Diatoms	0.06	0.03	0.06	0.03	**0.21**	0.04	0.09	0.18	0.00	0.00	0.00	0.00
Ulva spp.	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Z.marina	**0.26**	0.04	**0.38**	0.04	**0.40**	0.05	**0.37**	0.04	**0.78**	0.03	**0.82**	0.02
Phytoplankton	**0.25**	0.05	**0.24**	0.05	**0.35**	0.05	**0.38**	0.15	0.00	0.01	0.00	0.00

	Mytilus											
	High Flow						Low Flow					
	Transect 1		Transect 2		Transect 3		Transect 1		Transect 2		Transect 3	
	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR
PADILLA BAY												
Atriplex-Salicornia	0.04	0.06	0.07	0.02	0.05	0.01	0.16	0.05	0.18	0.02	0.07	0.04
T. maritima	0.19	0.05	0.12	0.02	0.17	0.05	0.11	0.06	0.12	0.05	0.07	0.03
D. spicata	0.00	0.01	0.01	0.00	0.02	0.03	0.01	0.01	0.00	0.01	0.00	0.00
Benthic Diatoms	0.06	0.04	0.07	0.03	0.06	0.01	0.01	0.09	0.03	0.11	0.02	0.10
Ulva spp.	0.02	0.01	0.03	0.01	0.02	0.03	0.09	0.03	0.05	0.02	**0.24**	0.06
Ceramium sp.	0.12	0.01	0.09	0.02	0.08	0.01	0.05	0.12	0.04	0.14	0.09	0.03
Z. japonica	0.10	0.05	0.06	0.11	0.09	0.05	0.04	0.05	0.06	0.17	0.03	0.07
Z. marina	0.01	0.01	0.04	0.08	0.01	0.01	0.09	0.10	0.02	0.02	0.03	0.01
Phytoplankton	**0.42**	0.02	**0.50**	0.03	**0.49**	0.04	**0.39**	0.04	**0.39**	0.04	**0.40**	0.03
PORT SUSAN BAY												
D.spicata	0.06	0.03	0.11	0.04	0.04	0.03	0.02	0.05	0.00	0.00	0.00	0.00
Potentilla-Typha	0.04	0.04	0.02	0.01	0.03	0.04	0.00	0.00	0.01	0.00	0.01	0.02
Schoenoplectus	**0.33**	0.04	**0.41**	0.05	**0.33**	0.05	**0.39**	0.04	**0.42**	0.02	**0.39**	0.02
Triglochin-Carex	0.01	0.01	0.01	0.01	0.01	0.01	0.07	0.03	0.01	0.01	0.01	0.02
Benthic Diatoms	0.01	0.02	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Ulva spp.	0.27	0.07	0.17	0.05	0.14	0.05	**0.43**	0.02	**0.34**	0.05	0.01	0.02
Z.marina	0.01	0.09	0.00	0.05	0.01	0.06	0.00	0.01	0.01	0.04	**0.24**	0.02
Phytoplankton	**0.23**	0.04	**0.25**	0.05	**0.39**	0.06	0.07	0.02	**0.20**	0.04	**0.33**	0.03

Table 1.6. SIMPER results describing the pair-wise % dissimilarity between estuaries based on Bayesian mixing model diet estimations condensed into five OM source groups: marsh, benthic diatoms, macroalgae, eelgrass, and phytoplankton. The percent similarity within each estuary is also presented, describing food web consistency across space and time.

		% dissimilarity					
		Lopez	Padilla	Samish	Port Susan	Skagit	% similarity
Macoma	Lopez	-					83.19
	Padilla	26.91	-				88.15
	Samish	52.7	32.35	-			86.01
	Port Susan	67.9	43.59	38.37	-		73.50
	Skagit	23.99	39.04	60.45	79.64	-	82.16
Mytilus	Lopez	-					93.84
	Padilla	52.4	-				94.03
	Samish	37.77	16.93	-			90.13
	Port Susan	61.21	21.78	31.87	-		82.47
	Skagit	72.14	27.15	38.91	17.94	-	90.37

Table 1.7. PERMANOVA results describing the scale at which differences in food web connectivity were observed across five estuaries for *Macoma* spp. and *Mytilus* sp. (square-root transformed data, Bray-Curtis similarity).

		Macoma		*Mytilus*	
		Pseudo-F	P-value	Pseudo-F	P-value
Lopez Sound	Flow	313.67	**0.001**	179.72	**0.001**
	Transect	70.08	**0.001**	1.37	0.359
	Ecotone	2.38	0.103	0.62	0.626
	Location	0.901	0.553	0.16	0.996
Padilla Bay	Flow	1206.8	**0.001**	9477.7	**0.001**
	Transect	429.2	**0.001**	2631.6	**0.001**
	Ecotone	4.45	***0.005**	0.21	0.828
	Location	0.74	0.695	0.96	0.511
Samish Bay	Flow	178.1	**0.003**	452.57	**0.001**
	Transect	2.19	0.170	10.48	**0.005**
	Ecotone	1.91	0.199	0.98	0.443
	Location	0.7	0.714	0.27	0.963
Port Susan Bay	Flow	4528.6	**0.001**	1986	**0.001**
	Transect	759.19	**0.001**	2891.8	**0.001**
	Ecotone	2.76	***0.017**	4.27	***0.02**
	Location	0.55	0.961	0.35	0.991
Skagit Bay	Flow	500.84	**0.001**	1739.9	**0.001**
	Region	12.73	**0.001**	356.83	**0.001**
	Transect	1218.7	**0.001**	996.75	**0.001**
	Ecotone	1.55	0.160	0.57	0.600
	Location	0.93	0.589	0.35	0.980

Each successive factor is nested in the above factor, hence, there are no interaction effects.

* Pairwise post-hoc tests indicate no significant differences at the cross-ecotone scale ($P < 0.05$).

** Pairwise post-hoc tests indicate one significant cross-ecotone difference during high flows in transect 1.

Permutations of residuals under a reduced model

Type III sums of squares (partial), 999 permutations

Table 1.8. Distance-based linear regression model (DISTLM) results describing environmental drivers of food web support differences across estuaries for *Macoma* spp. and *Mytilus* sp., based on a stepwise regression procedure and AIC selection criterion. Pseudo-F = DISTLM test statistic, P= Permutated p-value, Prop = proportion of variation described by a specific variable, Cum= cumulative proportion of variation described by a variable and those above it.

Macoma	**AIC**	**Pseudo-F**	**P**	**Prop**	**Cum**
Net range of transport	6555.8	1026.2	0.001	0.489	0.489
Mean flow velocity	6305.7	283.41	0.001	0.107	0.596
Zostera spp. area	5988.2	370.67	0.001	0.104	0.7
Acoustic backscatter	5823.2	179.93	0.001	0.043	0.743
Transect	5657.5	180.44	0.001	0.037	0.78
Flow period	5640.9	18.64	0.001	0.004	0.784
Mytilus					
Max salinity	2610.4	778.6	0.001	0.612	0.612
Mean monthly flow	2369.1	312.5	0.001	0.151	0.763
Marsh area	2216.5	179.9	0.001	0.064	0.826
Transect	2085.1	151.6	0.001	0.041	0.867
Net range of transport	2070.5	16.7	0.001	0.004	0.872
Flow period	2061.1	11.4	0.001	0.003	0.874

Chapter 2. Organism movement or organic matter transport? Using stable isotopes to discern mechanisms of connectivity in estuarine detritus-based food webs

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Abstract

The transfer of materials, energy and organisms across ecosystem boundaries (ecotones) often controls population, community and food web structure, highlighting the importance of connectivity at the landscape level. However, landscape-scale changes that affect lateral transfer, such as disruptions to boundary permeability or habitat fragmentation, can alter or destabilize food web structure and function. Thus, understanding the nature of cross-ecotone transfer may be critical to maintaining ecosystem integrity. In this paper, we focus on two ways in which energy flows across ecotone boundaries in Puget Sound estuaries: passive transport of water-advected organic matter (OM) and active movement of organisms. We investigate whether differences in behavioral life history traits correspond to differences in the diets and isotopic signatures of two fish species, bay pipefish (*Syngnathus leptorhynchus*), which display site fidelity to eelgrass beds, and the more transitory juvenile English sole (*Parophrys vetulus*), which move throughout the estuarine delta during the early demersal growth stage. We use multiple stable isotopes and Bayesian mixing models to identify differences in the OM sources assimilated by each fish species, testing whether the more transitory species displays greater food web connectivity by assimilating OM originating from multiple ecosystems within the estuary. We also examine the variation in food web connectivity represented by the two fishes in two different estuaries, one displaying limited freshwater inputs (Padilla Bay, an embayment-type estuary), and the other the terminus of a major river system that exhibits frequent flooding and pulsed, seasonal outflow (Skagit Bay, a river delta-type estuary). Our results indicate that both OM transport and organism movement enhance connectivity in Pacific Northwest estuaries. In the estuary exhibiting high fluvial discharge, water-advection plays a dominant role in large-scale OM transport and delivery to adjoining ecosystems, while trophic relay by organisms may provide the more important vector of food web connectivity in the estuary exhibiting minor fluvial discharge. However, the two mechanisms certainly interact to enhance food web connectivity across estuarine ecotones.

Introduction

Inter-ecosystem transfer of trophic energy, which includes organic materials, nutrients, or organisms, often controls population, community, and food web structure, highlighting the importance of connectivity at the landscape scale (Polis *et al.* 1997, Huxel and McCann 1998, Marczak *et al.* 2007). Much attention has been devoted to the consequences of food web connectivity versus compartmentalization, where the majority of research implies that species populating food webs connected by low to moderate allochthonous resource subsidies exhibit enhanced stability (Polis *et al.* 1997, Huxel and McCann 1998, Marczak *et al.* 2007). Flow rates of trophic energy subsidies across ecosystem boundaries, *aka* ecotones (Holland 1990), are strongly influenced by landscape-scale factors, including ecosystem availability (Greenwood and McIntosh 2008), differential permeability of boundaries to energy flux and organism movement (Candesso *et al.* 2004, Garcia *et al.* 2011), boundary length relative to the area of the recipient ecosystem (Polis *et al.* 1997), seasonal differences in productivity between adjacent ecosystems (Nakano and Masashi 2001), and organism mobility (Polis *et al.* 1997). As such, landscape-scale changes that interrupt cross-ecotone energy transfer and organism movement, such as disruptions to ecotone permeability, introduced species, or ecosystem fragmentation, can destabilize population, community, and food web structure and function (Greenwood and McIntosh 2008). For example, the proliferation of commonly introduced coconut palms across tropical and subtropical shorelines has been shown to disrupt the flow of marine nutrient subsidies to terrestrial systems, as birds avoid nesting in the introduced trees (Young *et al.* 2010). Without the allochthonous nutrient subsidy provided by bird guano, leaf nutrient quality, leaf palatability, and rates of herbivory declined, resulting in an interruption in the flow of allochthonous marine subsidies due to plant community changes along the marine-terrestrial ecotone. Because resource subsidies are ubiquitous across ecosystems, with generally positive effects on broad taxonomic groups (Polis *et al.* 1997), understanding the mechanisms of and responses to cross-ecotone transfer is critical to maintaining ecosystem integrity.

However, the specific mechanisms that promote or disrupt food web connectivity across landscape ecotones and among different types of ecosystems are still relatively unidentified (Greenwood and McIntosh 2008, Sheaves 2009). For example, in fluvial and estuarine environments, water is considered the principle vector of connectivity, pushing nutrients across ecosystem boundaries to regulate metabolism in the adjacent ecosystem (Polis *et al.* 1997)[3]. Traditionally, this cross-ecotone connectivity

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[3] Here, we adopt a fine-scale resolution definition of "ecosystem", referring to different vegetative zones (i.e., marsh, mudflat and eelgrass) commonly found within the estuarine mosaic. We define "ecotone"as the boundary between adjacent "ecosystems".

was considered to be the unidirectional, downstream/net seaward movement of OM and nutrients, as described by Vannote *et al.*'s (1980) river continuum concept, and Odum's (1980) estuarine outwelling hypothesis. In both cases, the lateral distribution of OM production through river flow and tidal action has since been recognized as an extremely important landscape process that promotes cross- ecotone food web connectivity (Polis *et al.* 1997), and that landscape-scale processes, such as flooding and ecotone characteristics, can alter the direction, magnitude, and community response to trophic energy flows at the aquatic-terrestrial interface (Greenwood and McIntosh 2008). Further challenging the assumption of unidirectional flows of trophic energy through the aquatic environment, organism movement has been shown to transport nutrients across entire landscapes, even opposite the gravitational gradient, exemplified by anadromous salmonids subsidizing freshwater ecosystems with marine-derived nutrients (e.g.Schindler *et al.* 2003, Moore *et al.* 2008).

We focus on two pathways in which trophic energy flows across estuarine and nearshore marine intertidal ecotones: the passive transport of organic matter (OM) in the form of detritus, and the active movement of organisms among ecosystems. Over the past decade, cross-ecotone energy fluxes of both forms have been repeatedly shown to subsidize food webs in adjacent ecosystems (e.g., (Nakano and Masashi 2001, Connolly *et al.* 2005, Vizzini and Mazzola 2006). However, the spatial scale of cross-ecotone energy fluxes due to passive OM transport in estuarine systems is highly variable. In some cases, extensive mixing and large-scale transport of OM occurs across multiple fluvial-estuarine-coastal ecotones (Odum 1980, Gordon *et al.* 1985), promoting broad scale connectivity across the estuarine landscape. In other cases, strong gradients in the sources of OM assimilated by consumers occurs over more confined spatial scales, on the order of meters to a few kilometers (Deegan and Garritt 1997, Grenier 2004, Guest and Connolly 2004, Guest *et al.* 2004), suggesting that the discrete spatial assemblage structure of estuarine primary producers can lead to estuarine food web compartmentalization in different systems. Several studies indicate that estuarine circulation plays an important role in the movement and consequent assimilation of OM throughout an estuary and its associated food web (e.g. Odum *et al.* 1979, Nixon 1980, Connolly *et al.* 2005). For example, isotopically distinct food webs exhibited under low freshwater discharge conditions in the Tagus River estuary have been demonstrated to coalesce during high flow years or flood events, indicating that hydrological processes strongly influence food web connectivity in that estuarine environment (Vinagre *et al.* 2006).

In contrast to the passive movement of OM determined by estuarine circulation, Kneib (2000) suggested that marsh -produced OM energy can also cross ecosystem boundaries by active 'trophic relay'. In this case, organisms grow and obtain energy in one ecosystem, but then cross ecosystem boundaries to support food webs in the adjacent ecosystem either by becoming prey, or by depositing nutrients via metabolic wastes, death or decay. In estuarine marsh ecosystems, tidal inundation allows estuarine consumers to move from their deep subtidal habitats into dendritic tidal channels and the marsh plain for feeding on endemic prey, before moving back offshore during low tide, exporting marsh-produced trophic energy with them. At the same time, the chain of predator-prey interactions among different ontogenetic stages of resident and transient nekton species, which utilize different, but overlapping habitats within estuarine marsh complexes, creates a trophic relay that also moves marsh production offshore. Alternatively, consumption of subtidal prey, such as mysids, relays subtidal channel production into the intertidal marsh food web in a reciprocal subsidy (Dean *et al.* 2005). Thus, organism movement and trophic relays can transfer energy against the circulation gradient in estuarine systems, linking otherwise spatially compartmentalized food webs.

In this paper, we assess whether passive (hydrologic) and active (organismal) processes are the primary mechanism by which trophic energy flows across estuarine ecotones. We specifically compare passive OM transfer by estuarine circulation to the active trophic relay of OM via nekton movement by comparing isotopic and diet compositions of resident (bay pipefish, *Syngnathus leptorhynchus*) and transient (English sole, *Parophrys vetulus*) fishes in two estuaries with contrasting hydrologic regimes, i.e., freshwater inflows. We first investigate whether strong differences in life history traits correspond to differences in isotopic signatures and diet between the bay pipefish and English sole. We then use multiple stable isotopes in a Bayesian mixing model to infer cross-ecotone connectivity by identifying the OM sources supporting each fish species. Given the limited sample sizes in this study, our description of bay pipefish and English sole diets is not meant to provide a robust or detailed account of feeding preferences of these two species. Rather, we use this supplemental dataset to provide context for and deeper understanding of our isotope and mixing model results with respect to food web connectivity between consumer species, seasons, and estuarine contexts. We hypothesize that: (1) highly mobile, transitory fish will display greater food web connectivity by assimilating OM originating from more ecosystems within the estuarine environment, while; (2) less mobile, resident fish may draw on a more compartmentalized, or isolated, food web supported by a restricted suite of OM sources. In light of recent studies indicating the role that freshwater flow plays in regulating food web connectivity (Greenwood and McIntosh 2008, Mortillaro *et al.* 2011, Vinagre *et al.* 2011), we also examine seasonal variation in food web connectivity between two estuaries, one which has very limited freshwater inputs and one at the terminus of a major river system that exhibits frequent flooding and pulsed, seasonal outflow. Thus, we further hypothesize that: (3) increased fluvial influence will reduce food web compartmentalization by spatially integrating OM sources originating from discrete ecosystems across the estuarine landscape.

Methods

Study sites

Our study area was located in Padilla and Skagit bays, two estuaries located in Washington State, Pacific Northwest USA (48° 25’ N, 122° 29’W, Figure 1). Both estuaries are subject to mixed, semi-diurnal macrotidal regimes (> 3 m), with strong spring-neap tidal cycles. Surface water temperatures generally range between 10-17 °C in the summer, and 7-10 °C in the winter (Bulthuis 1993, Gustafson *et al*. 2000). Additionally, both sites are very shallow (< 5 m depth), and exhibit a mosaic of ecosystems, including emergent tidal marsh, sand or mudflats, and eelgrass (*Zostera marina*). Approximately 35% of Skagit Bay’s nearshore area is vegetated, largely by tidal wetlands (785 ha), and eelgrass meadows (2846 ha), as well as a small amount of saltmarsh (25 ha) (McBride *et al.* 2006). Sandflats comprise the majority of Skagit Bay’s nearshore area (58%, 7225 ha), with mud and mixed fines comprising less than 10% of that ecosystem. Padilla Bay exhibits a comparable area of eelgrass (3170 ha), and saltmarsh (57 ha), but much less bare substrate (1951 ha) (Bulthuis and Shull 2006). Close to 70% of nearshore areas are vegetated in Padilla Bay. Periodically, the bare mudflats and sandflats are covered by as much as 289 ha of macroalgae in Skagit Bay, and 350 ha in Padilla Bay (McBride *et al.* 2006, Bulthuis and Shull 2006).

These two estuaries exhibit profoundly different hydroperiods due to a large difference in the source and seasonality of their respective freshwater inputs. The Skagit River is the largest river draining into Puget Sound, providing 34 - 50% of the Sound’s freshwater inputs, depending on season (Babson *et al.* 2006). Much of the Skagit’s 8544 km^2 watershed includes a large portion of the North Cascade Mountains (peak elevation 3285 m), including glacial discharge during summer months. River flow peaks during heavy winter rains (November through January), and again during the late spring freshet snowmelt (Hood 2010). Winter storm and spring freshets are often associated with lowland flooding events, with maximum flows recorded as high as 5100 m^3 s^{-1}, while the lowest flows typically occur in September, with flow recordings as low as 78 m^3 s^{-1} (Wiggins *et al.* 1997). Mean discharge at the recording station nearest the estuary is 468 m^3 s^{-1} (USGS 2011). The Skagit River splits into the North and South Fork before entering Skagit Bay, with more than 80% of fresh water discharging through the South Fork and associated distributary channels. The South Fork river plume occupies a greater area of the tidal flats compared to the North Fork plume because the South Fork enters the bay through multiple tidal sloughs, while the North Fork enters through a single channel. The North Fork plume is further constrained by its proximity to the deep channel along the Whidbey Island coastline, which causes mixing and transport caused by strong cross flow associated with tidal currents (Yang and Khangaonkar 2009). Circulation in the estuary is strongly affected by the magnitude of freshwater inflow, coupled with the orientation of the river plume with respect to the deep channel running along the inside of Whidbey Island, parallel to the delta face. The deep channel constrains the spreading of the river plume, resulting in strong horizontal gradients in tidal currents and salinities (Yang and Khangaonkar 2009). Mean salinities in Skagit Bay range between 18 – 28 psu, but intertidal channel salinities are often < 0.5 psu (Howe, Dissertation 2012). During spring tides, maximum flow velocities over intertidal flats fall between 24 cm s^{-1} and 60 cm s^{-1} (Howe, Dissertation 2012). The Skagit River estuary exhibits strong stratification, but destratification can occur during the flood tide (Yang and Khangaonkar 2009).

In contrast, although Padilla Bay was historically part of the Skagit Bay delta's distributary channel network before extensive historic diking, it is now virtually isolated from significant freshwater inputs (Collins and Sheikh 2005). As an "orphaned" estuary, the largely agricultural 93 km^2 watershed of Padilla Bay is now directly receives fresh water from only three agricultural sloughs and one seasonal stream that reach peak precipitation-based flows during fall and winter (Nelson 1989, Bulthuis 1996). Freshwater flows are limited (mean flow = 0.2 m^3 s^{-1}), and connectivity is truncated by tide gates on each slough. Despite minimal freshwater and sediment inputs to Padilla Bay, both estuaries are characterized by extensive intertidal deltaic fans (<3 m depth), although the intertidal area of Skagit Bay (75 km^2) is larger than Padilla Bay (45km^2) (Nelson 1989, Grossman et al. 2011). Surface currents in Padilla Bay are driven by tidal action, as > 80% of the volume of Padilla Bay enters and exits the system within a tidal cycle. Wind events can affect the generally clockwise circulation pattern observed in the bay but, unlike Skagit Bay, density-driven circulation is not an important feature, largely due to minimal freshwater inflow. Current speeds, however, can reach high velocities over the intertidal flats during the flood tide, reaching speeds of 30 cm s^{-1} (Bulthuis and Conrad 1995).

Study organisms

Two estuarine-dependent fish, representative of contrasting life history strategies, were chosen for this study; bay pipefish because of their close association and assumed specific fidelity to eelgrass habitats (Wilson 2006, Shokri *et al.* 2009, Johnson *et al.* 2010), and juvenile (< 150 mm TL) English sole because they are thought to forage in a wide array of habitats across the estuarine intertidal area (Gunderson *et al.* 1990, Rooper *et al.* 2003, Chittaro *et al.* 2009). The range of *S. leptorhynchus* extends from Prince William Sound, Alaska to Baja, California, where the fish are common in eelgrass to depth of 30 m (Wilson 2006). Although research describing the life history patterns of bay pipefish is somewhat limited, they are thought to be requisite eelgrass residents (Hart 1973, Wilson 2006), usually spawning in late winter through September in Pacific coast estuaries (Bayer 1980), and using eelgrass for refuge from predators and foraging grounds (Hart 1973). Many temperate pipefish species undergo seasonal migrations between shallow, vegetated estuarine areas in the spring and summer, and deeper offshore areas during the winter (Lazzari and Able 1990), but this scale of migration has not been described for *S. leptorhynchus.* In contrast, bay pipefish undergo cross-estuary migrations, moving from lower to upper estuarine areas in the summer when salinities become favorable (Bayer 1980). Given that multiple size classes of *S. leptorhynchus* have been observed simultaneously within the same estuary, it is thought that they complete their entire lifecycle within the same estuary (Bayer 1980, Murphy et al. 2000).

While syngnathids are widely known for their strong association with preferred habitat, low mobility, and extremely restricted home ranges (Shokri et al. 2009), larval English sole in contrast are released in coastal waters and migrate to estuaries, where they reside through their first year (Gunderson *et al.* 1990). During the estuary-dependent life stage, young-of-the-year English sole extensively occupy intertidal flats and side channel habitats, generally following the tide across estuarine ecosystem components (Rooper *et al.* 2003). Juveniles emigrate from the estuary into deeper waters at about 75 mm (TL), although some individuals remain in shallow water ecosystems through their second year of life (Gunderson *et al.* 1990).

Previous studies of *S. leptorhynchus* and juvenile *P. vetulus* indicated both species prey predominantly on detritivorous epibenthic and benthic macroinvertebrates and large meiofauna such as *Harpacticus* spp. and other harpacticoid copepods, polychaetes, bivalve siphons, cumaceans, gammarid amphipods, tanaids, and isopods (Thayer *et al.* 1978, Toole 1980b, Ryer and Orth 1987, Simenstad *et al.* 1995, Vizzini and Mazzola 2004). The prey resources of both bay pipefish and juvenile English sole thus represent an important food web link between estuarine-derived detritus and higher trophic levels (Pennak 1953, Brown and Sibert 1977, Araya-Schmid and Schmid 2000).

Sample collection

We collected fish in the spring, after the winter period of high freshwater flows, and at the end of the low flow summer period between 2007 and 2009. Due to difficulty in consistently capturing fish, samples were only available from Skagit Bay in May and August 2007, but sample sizes (n=10) were sufficient from Padilla Bay in March and September 2008, and May 2009. English sole were collected in tidal channels in the mudflat ecosystem of each bay, while bay pipefish were collected within eelgrass ecosystems near the seaward edge of each delta. Most juvenile English sole and bay pipefish samples were collected using a 2.0-m pole seine with a 6-mm mesh bag during low spring tides. A 0.5-m diameter plankton net (273-um mesh) towed behind a boat was also used to capture bay pipefish in Skagit Bay. Additionally, the United States Geological Survey (USGS) and the Skagit River System Cooperative provided the study with some fish in Skagit Bay using a modified lampara net. Fish were placed on ice immediately following collection in order to slow digestion, and then frozen.

In order to characterize OM at the base of Skagit and Padilla bays' food webs, in June 2006 we collected from each site four replicate apical foliage samples of each dominant primary producer species in marsh and the other intertidal ecosystems. Plants and algae were frozen until preparation for lab analysis. While inter-annual variation in isotopic sources has been documented (Cloern *et al.* 2002), the magnitude of variation is usually between 2-4‰ for δ^{13}C and δ^{15}N, and 2-5 ‰ for δ^{34}S (Stribling et al. 1998), such that terrestrial and marsh-derived OM sources do not overlap with marine-derived phytoplankton, algae, and eelgrass. However, spatial variation in isotopic signatures for OM sources can be much greater, even within conspecifics (Stribling *et al.* 1998, Cloern *et al.* 2002, Fry 2006). Thus, for the purposes of our question, we assumed that inter-annual variation in OM sources was minimal enough to warrant building an isotopic mixing model for consumers based on sources from previous years, but we developed separate models for each estuary (Table 1).

Sample preparation

Fish Diet Composition

In the laboratory, we individually weighed (damped wet weight) and measured (TL) bay pipefish and juvenile English sole prior to stomach removal. Stomachs were preserved in 70% isopropanol for later laboratory processing. Upon examination, the state of the stomach fullness and contents digestion were characterized using standard processing methods (Terry 1977) where stomach fullness (1= empty to 7 = distended) and stage of digestion (1= all prey items unidentifiable to 6= no digestion) were rated for each fish. Prey items were sorted and identified to the lowest taxonomic resolution possible under a dissecting microscope, and assigned to functional habitat groups: (1) benthic infauna asssociated with the bottom substrate (e.g., bivalves and burrowing polychaetes); (2) epifaunal (epibenthic and epiphytic) organisms associated with eelgrass, algae, and near-bottom habitats (e.g., harpacticoid copepods, caprellid amphipods); and, (3) "pelagic" organisms occurring mainly in the water column (e.g., calanoid copepods, mysids, planktonic larvae). Prey items from each category were enumerated and weighed in order to quantify the numerical and gravimetric composition and frequency of occurrence of prey consumed by each fish. Due to its direct measure of biomass intake, we primarily used gravimetric data to indicate prey consumption, but validated with numerical and frequency of occurrence that prey were representative.

Isotopic Composition

We prepared fish for isotopic analysis by extracting dorsal muscle tissue from above the lateral line of individual juvenile English sole, whereas all available muscle tissue was extracted from individual bay pipefish due to their small size. Samples of primary producers were rinsed in 10% HCl to remove soil carbonates deposited on plant surfaces during high tide, then thrice rinsed in deionized water to neutral pH. Both fish muscle and plant tissues were freeze-dried for 48 h, ground to a fine powder with a Wig-L-Bug® amalgamator outfitted with a stainless steel vial and ball pestle. Samples were weighed on a microbalance into tin capsules for isotope processing by Washington State University's Stable Isotope Core Laboratory. The isotopic ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ and C:N ratio values were analyzed for all samples using a Delta PlusXP Thermofinnigan CF- IRMS coupled with a Costech Analytical elemental analyzer (ECS 4010). The isotopic 2-sigma uncertainty of carbon and nitrogen isotopic results was 0.5‰. We express isotopic ratios in δ notation, which describes the per mil (‰) enrichment (+) or depletion (-) of the heavy isotope to the light isotope of the targeted element relative to an international standard (δX (‰) = $[(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$), where $X = {}^{13}C$, ${}^{15}N$, or ${}^{34}S$, and $R = {}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$, or ${}^{34}S/{}^{32}S$).

Because the lipid content of skeletal muscle tissue can vary across space and time, and because lipids are approximately 6-7‰ depleted in ${}^{13}C$ relative to protein, it has been recommended to either perform an arithmetic lipid correction or chemical lipid extraction of fish muscle tissues before interpreting isotopic signatures (Post 2002, Sweeting *et al.* 2006). Accordingly, we extracted lipids from a subset of fish muscle samples using the methods of Sweeting *et al.* (2006). The lipid-free tissue was then weighed into tin capsules for the isotope processing described above. Results from the lipid-free tissues were regressed against non-extracted values in order to determine conversion equations for each species of fish (juvenile *P. vetulus*: lipid corrected $\delta^{13}C_{\text{sample}} = 0.9507 \times$ uncorrected $\delta^{13}C_{\text{sample}} - 1.2385$, $R^2 = 0.99$, *S. leptorynchus:* lipid corrected $\delta^{13}C_{\text{sample}} = 0.8731 \times$ uncorrected $\delta^{13}C_{\text{sample}} - 2.5567$, $R^2 = 0.98$). Lipid corrected values were used for mixing model analyses.

Organic matter source contributions

We estimated the relative percent contribution of primary producer groups to integrated fish consumer diets using a combination of SOURCE and the MixSIR model suite, two types of multiple source mixing models (Lubetkin and Simenstad 2004, Semmens *et al.* 2009, Ward *et al.* 2010). SOURCE uses linear programming techniques to estimate the central tendency of a consumer's direct and indirect uptake of autotrophic sources and was primarily used for the following two procedures: 1) to identify which primary producer groups exhibited isotopic signatures too similar to be considered distinct by the model's nearest neighbor distance requirements ($NND^2 < 0.10$); and, 2) to estimate the consumer's trophic position. While the SOURCE model is capable of estimating the relative percent contribution of primary producers to the base diet of individual consumers, we instead performed that analysis using the MixSIR model suite because of its superior treatment of uncertainty using a Bayesian framework (Layman et al. 2012). For example, SOURCE only allows for a single value input for each primary producer, while MixSIR allows a range of values to represent the isotopic composition of any particular autotrophic source. Similarly, MixSIR incorporates a range of values for the trophic fractionation of each isotope, and allows for priors to be included in the model. Most importantly, MixSIR estimates posterior probabilities of each primary producer's contribution to the base diet, thus providing a measure of confidence to the model output. In contrast, SOURCE simply provides the average of all possible combinations of source contributions, regardless of the probability associated with that value. The MixSIR suite of models can also be run at different levels of hierarchical structure, which consequently allows for model selection via the Deviance Information Criterion (DIC), a measure used to evaluate which models were best supported by the data (Semmens *et al.* 2009). However, MixSIR is limited in its assumption of direct uptake between the "prey" and the consumer. Because we were examining autotrophic contribution to secondary consumers (indirect uptake), we used the SOURCE model to estimate the trophic position of the fish consumers, and then altered the trophically-associated fractionation effect in the MixSIR-type models to accurately reflect the amount of isotopic fractionation associated with the appropriate number of trophic level shifts.

Data analysis

We employed multivariate data analyses (Primer® 6) to examine patterns associated with fish diets (gut contents), fish isotope signatures, and proportional contributions of OM sources supporting consumers. Gravimetric composition of prey to individual fish diets was analyzed according to two levels of resolution: a) the lowest taxonomic resolution possible; and, b) the prey habitat, including benthic, epifaunal, and pelagic. Only prey organisms comprising ≥ 10% of total gravimetric diets were included in statistical analyses. To compare differences in diets, isotope signatures, and proportional OM contributions across sites, species, and dates, we analyzed all data types using Primer's nonmetric multidimensional scaling (NMDS) ordination, permutational multivariate analysis of variance (PERMANOVA), and similarity percentage (SIMPER) analyses. Prior to the aforementioned analyses, all diet data and OM source estimates were square-root transformed to reduce the contribution of highly abundant species in comparison to rare ones (Clarke and Warwick 2001). For these two data types, we constructed similarity matrices using the Bray-Curtis similarity coefficient (Clarke and Gorley 2001). No transformation was required for isotope data, and we used the Euclidean distance coefficient to construct a similarity matrix prior to further analysis. PERMANOVA calculates a Pseudo-F value, similar to the F value of an ANOVA, as well as a permutational P-value, with an α level of 0.05. Due to uneven sample sizes, we conducted PERMANOVA analyses as both main and mixed effects models (depending on the site) using a Type III partial sums of squares with 9999 permutations. Permutations of residuals were run under a reduced model because it yields the best power and the most accurate Type I error estimate (Anderson *et al.* 2008). When testing for differences between bay pipefish and English sole diets, isotope signatures, and OM support estimates, we identified fish species and seasonal flow periods as main fixed effects, and year as a random effect.

In order to eliminate ontogenetic changes in fish diet composition as a confounding factor in isotopic and diet variation with flow regime, we utilized Primer's distance-based linear models (DISTLM) to examine the relationship between fish length and diet variation. In order to use DISTLM to analyze and model the relationship between a multivariate data cloud and one or more predictor variables, we performed a square-root data transformation prior to creating a Bray Curtis similarity matrix. DISTLM returns an R^2 value equal to the percent of variation explained by the predictor variable, as well as a P-value calculated by permutation that describes significance.

We performed Gibbs sampling (Plummer 2003) for each Bayesian mixing model using three parallel chains in JAGS and a burn-in phase of 70,000 vectors, followed by sampling 80,000 remaining vectors (retaining every 2nd sample). Convergence and diagnostic statistics were calculated using the CODA package in R (Plummer *et al.* 2006). We started with the following input parameters for trophic fractionation effects: $\delta^{13}C = 1.3 \pm 0.3‰$; $\delta^{15}N = 3.4 \pm 0.32‰$, and $\delta^{34}S = 0.5 \pm 0.56‰$, using values specific to white muscle tissue and aquatic species (Vander Zanden and Rasmussen 2001, McCutchan *et al.* 2003, Sweeting *et al.* 2007). In doubling the fractionation effect to capture two trophic level shifts, we summed the variances according to the methods of Vander Zanden and Rasmussen (2001). Final values used for the model were: $\delta^{13}C = 2.6 \pm 0.18‰$; $\delta^{15}N = 6.8 \pm 0.20‰$; and, $\delta^{34}S = 1.0 \pm 0.63‰$.

Results

Fish diets

We retained 93 fish for analysis, including 26 bay pipefish and 29 juvenile English sole from Padilla Bay, and 18 bay pipefish and 20 juvenile English sole from Skagit Bay (Table 2). With the exception of bay pipefish from Skagit Bay, we found a significant within-species difference in length between high and low flow sampling periods, with larger fish captured towards the end of the summer.

We were able to eliminate ontogenetic changes in diet composition as a confounding factor in our seasonal comparisons of diet, isotope, and OM support, because fish length generally did not explain appreciable variation in gravimetric diet composition for either fish species. Juvenile English sole length accounted for only 9.3% (P= 0.04) of the overall observed diet variation between flow sampling periods in Skagit Bay, and only 7.4% (P= 0.04) of overall diet variation observed in Padilla Bay. Similarly, ontogenetic shifts of bay pipefish did not strongly correspond to variation in diet composition; length explained only 14.9% (P= 0.08) of overall diet variation in Skagit Bay, and 16.0% (P= 0.006) of overall diet variation in Padilla Bay. For both species and sites, length explained considerably more variation in diet within a season, suggesting that ontogenetic shifts are less influential than seasonal shifts in determining the diets of bay pipefish and juvenile English sole.

Estuarine embayment fish diets (Padilla Bay)

Juvenile English sole consumed more diverse prey taxa in Padilla Bay, in terms of prey taxa richness, gravimetric diet composition, and diet similarity among individuals: 12 different prey taxa during high river flow conditions (2008: 6 taxa, 2009: 10 taxa), and nine prey taxa during low flow conditions (Figure 2, Table 3). Oligochaetes were the only unique prey taxa consumed in gravimetric composition during low flow conditions, replacing greater reliance on polychaetes, tanaids, and clam siphons during the spring high flow conditions (Table 4). Bay pipefish in Padilla Bay consumed five prey taxa during the high flow sampling period, and six prey taxa during the low flow sampling period (Figure 2). Notably, pipefish consumed more caprellid amphipods during low flow conditions, rather than the gammarid amphipods *Pontogeneia* rostrata, *Grandidierella japonica* and *Paracalliopiella pratti* and harpacticoid copepods consumed during high flow. SIMPER analyses substantiated that individual juvenile English sole diets varied more than individual bay pipefish diets, as the average similarities among the flatfish diet compositions were lower than those for bay pipefish (Table 5). These results suggest greater diet shifts between flow periods occurs among English sole as compared to bay pipefish, greater individual variation in diets among English sole as compared to pipefish, and stronger homogenization of diets during the summer flow period for both species.

We observed no differences in overall diet composition between English sole and bay pipefish when prey were categorized by prey species as opposed to prey habitat group, but we did observe significant differences in diets between flow periods and years (PERMANOVA: Flow: pseudo-F = 5.61, p = 0.0001, Year: pseudo-F = 3.34, p = 0.0001), as well as significant interaction effects for species x flow (pseudo-F = 7.32, p = 0.0001) and species x year (pseudo-F = 3.99, p= 0.0001). Gravimetric diet composition was significantly different between high and low flow periods (PERMANOVA post-hoc tests: juvenile English sole*:* t = 1.86, p = 0.0001, bay pipefish: t = 3.15, p = 0.0001) for both species. Additionally, we found a significant difference in the diets of the two fish during the high (PERMANOVA post-hoc test, t= 2.29, p= 0.001) and low flow (t = 3.35, p= 0.0002) periods when we excluded the 2009 high flow dataset. In summary, the two fish species fed differently from one another regardless of season in Padilla Bay, juvenile with English sole exhibiting a more variable diet than bay pipefish.

Estuarine river delta fish diets (Skagit Bay)

The pattern of juvenile English sole exhibiting a more varied diet than bay pipefish was more pronounced in Skagit Bay (Figure 2). Juvenile English sole consumed nine prey taxa during high flow conditions, and 11 prey taxa during low river flow conditions, notably shifting to juvenile bivalves and tanaids, rather than harpacticoid copepods and the gammarid amphipods*, Pontogeneia rostrata* and *Eogammarus confervicolus*, that were prominent prey during low flow*.* We found that bay pipefish in Skagit Bay consumed four taxa during high flow conditions, and six taxa during low flow conditions, shifting to a caprellid amphipod and two new juvenile shrimps (Hippolytidae, Pandalidae) between high and low flow periods (Table 4). Additionally, not only did juvenile English sole exhibit a more varied diet as a group compared to bay pipefish, individual sole diets in Skagit Bay varied more than did individual bay pipefish diets (Table 5). Results of SIMPER analyses indicated that the within group diet similarity of juvenile English sole increased between the high and low flow periods (Table 5). Juvenile English sole diets thus become more homogenized with decreasing flow. Average diet similarities calculated for bay pipefish indicated greater change in diet between flow regimes than seen for juvenile English sole, higher within group similarity in diets as compared to English sole, and increasing diet individualization with decreasing flow (Table 5).

We found significant diet differences between juvenile English sole and bay pipefish from Skagit Bay when prey taxa were described at the species level and between seasonal flow periods (PERMANOVA: Fish species: pseudo-F = 4.27, p = 0.0001, Flow: 3.03, p = 0.0003). Additionally, we observed a significant interaction effect between fish species and flow period (PERMANOVA: Flow x Species: pseudo-F = 2.03, p = 0.008). Pairwise tests revealed that diets of both juvenile English sole and bay pipefish were significantly different under both high (t=1.78, p=0.0007) and low (t = 1.78, p = 0.0005) river flow conditions. We also compared diets within a species under different flow conditions and found a significant shift in both English sole (t = 1.30, p=0.032) and pipefish diets (t = 1.87, p = 0.015) between flow regimes.

Comparison of fish prey by habitat classification

Despite significant differences in diet composition at the prey species level, when prey were classified by prey habitat, diet differences were not significant for either fish species in either bay (Figure 2). However, when we compared juvenile English sole to bay pipefish diets within each estuary at the prey habitat level, differences in the prey resources utilized by each species were significant during each flow period (PERMANOVA post-hoc tests: Skagit Bay high flow: t= 2.03, p= 0.004; Skagit Bay low flow: t= 3.49, p= 0.001; Padilla Bay high flow: t= 3.78, p= 0.001; Padilla Bay low flow: t= 2.28, p= 0.002). In both bays, juvenile English sole consistently relied upon benthic prey species for >80% of their gravimetric consumption. Epifaunal invertebrates made up the second most important prey habitat group for juvenile English sole (Padilla Bay: 5-8%, Skagit Bay: 14-15. In contrast, bay pipefish diets were composed predominantly of epifaunal invertebrates (84-96%), seconded by benthic infauna (3-19%).

In summary, juvenile English sole exhibited more diverse diets than bay pipefish. The greatest shift in diet composition between seasonal river flow regimes was observed among bay pipefish in Skagit Bay. Seasonal diet shifts of bay pipefish in Padilla Bay and among juvenile English sole in Skagit Bay were far less pronounced, indicating that only pipefish in Skagit Bay were significantly affected by seasonal fluctuations in freshwater flow regimes. Juvenile English sole diets were composed of benthic invertebrates, while bay pipefish consumed a mixture of epifaunal and benthic organisms.

Isotope delineation of organic matter food web sources

Estuarine embayment (Padilla Bay)

δ^{13}C and δ^{15}N isotope values revealed trophic separation both between species and between river flow regimes in Padilla Bay (Figure 3). The range in δ^{13}C and δ^{15}N values of juvenile English sole (δ^{13}C -15.6‰ to -10.6‰, range: 5.0‰; δ^{15}N 12.5‰ to 15.3‰, range: 2.8‰) was greater than those of bay pipefish (δ^{13}C -13.0‰ to -8.4‰, range: 4.6‰; δ^{15}N 13.1‰ to 14.6‰, range: 1.5‰). In contrast, the δ^{34}S values were very similar between species, although bay pipefish exhibited a larger, more enriched range due to one outlier individual (juvenile English sole: 13.2‰ to 15.5‰, range: 2.2‰; bay pipefish*:* 14.4‰ to 19.9‰, range: 5.5‰, outlier removed: max = 15.9‰, range : 1.5‰).

We observed a significant effect of species and flow, as well as a significant interaction effect between the two factors for juvenile English sole and bay pipefish in Padilla Bay (PERMANOVA: Species: Pseudo-F = 25.17, p = 0.0001, Flow: Pseudo-F = 9.86, p = 0.0001, SP x FL: Pseudo-F = 15.05, p = 0.0001). Pair-wise tests indicated a significant difference for juvenile English sole between high and low river flow periods (t = 6.84, p = 0.0001). This difference was largely due to more depleted δ^{13}C values after the high flow period as compared to after the low flow period (-13.88 ± 0.51 and -12.29 ± 0.17 respectively).

Additionally, mean δ^{15}N of juvenile English sole became more depleted (equal to three quarters of a full trophic level) as freshwater flow declined (high flow: 14.68 ± 0.16‰, low flow: 13.05 ±0.08 ‰). In contrast, bay pipefish values were not significantly different between flow periods (t = 1.11, p = 0.28). As a result, the difference in isotope signatures between species was significantly different during both high flow (pairwise test; t= 2.16, p= 0.0009) and low flow (t= 7.00, p= 0.0001).

Estuarine river delta (Skagit Bay)

Unlike several of the patterns observed for Padilla Bay, isotopic values both between fish species and between seasonal flow regimes were similar in Skagit Bay (Fig. 3). However, juvenile English sole in Skagit Bay displayed a wider range in δ^{13}C (-15.8‰ to -11.1‰, range: 4.7‰) and δ^{34}S (11.3‰ to 16.5‰, range: 5.2‰) than the δ^{13}C (-15.8‰ to -12.7‰, range: 3.1‰) and δ^{34}S (13.3‰ to 15.4‰, range: 2.0‰) of bay pipefish (Figure 3). The δ^{15}N signatures were remarkably similar between the two species (juvenile English sole: 11.8‰ to 14.7‰, range: 2.9‰; bay pipefish*:* 12.0‰ to 14.5‰, range: 2.5‰).

Isotope values between fish species were significantly different in Skagit Bay, (PERMANOVA: Pseudo-F = 4.96, p = 0.009), but no effect of flow regime. However, the species effect was only evident during low flow, when the isotope values of the two fish species diverged (PERMANOVA post-hoc tests, t = 2.30, p = 0.007). No significant difference in isotope values was detected between the two species during high flows (t = 1.05, p = 0.34), or within each species between seasonal low and high flow regimes.

Mixing model analysis

Estuarine embayment (Padilla Bay)

Bayesian mixing model results indicate that although juvenile English sole and bay pipefish in Padilla Bay assimilated a variety of OM sources, the similarity in these contributions varied systematically with the period and magnitude of river discharge. During the high flow season, the OM contributions to both fishes were similar, with marine macroalgae comprising the largest contribution to juvenile English sole (38.3%) and bay pipefish diets (35.4%) (Table 6). Marsh OM comprised a total of 18.9% to English sole diets, and 21.30% to pipefish diets, while eelgrass contributed 21.3% and 13.7%, respectively. The most notable difference in the OM base supporting the two fish species during the high flow season was reflected in the assimilation of marine phytoplankton: juvenile English sole assimilated only 6.6% (IQR= 4%) phytoplankton, while marine phytoplankton accounted for 19.5% (IQR = 5%) of the bay pipefish diet. In contrast, the sources of OM assimilated by the two fish species diverged during the low flow period (Table 6). Under reduced flow, juvenile English sole diets originated primarily from marine phytoplankton (30.5%, IQR = 3%), followed by marsh macrophytes OM (27.5%), eelgrass (15.8%), marine macroalgae (17.1%), and benthic diatoms (5.8%, IQR= 5%). In contrast, bay pipefish assimilated OM derived from marine macroalgae (32.89%), followed by eelgrass (22.4%), phytoplankton (20.8%, IQR = 4%), marsh macrophytes (13.6%), and benthic diatoms (6.5%, IQR = 4%).

As a result, the sources of OM assimilated by English sole were significantly different between seasonal flow periods (PERMANOVA post-hoc tests: t= 7.63, p = 0.001, Fig. 4). Additionally, OM support of juvenile English sole during the low flow period significantly differed from OM sources of bay pipefish assimilation during both flow periods (PERMANOVA post-hoc tests: High: t= 1.71, p = 0.041 Low: t= 6.08, p = 0.001, Figure 4). The OM contributions assimilated by pipefish between low and high flow seasons did not change significantly (t= 0.75, p = 0.524). Also of note, juvenile English sole consistently assimilated a greater diversity of OM sources compared to bay pipefish (Table 3).

Estuarine river delta (Skagit Bay)

In contrast to Padilla Bay, juvenile English sole and bay pipefish in Skagit Bay were both supported by similar food web linkages during the high flow season. The macroalgae *Ulva* spp. supported the majority of their diets (median: 66.5%, interquartile range (IQR) = 3% and 59.4%, IQR = 3% respectively) in this estuary, while additional OM derived from *Typha* sp. contributed 14.4% (IQR = 3%) to juvenile English sole diets, and 21.0% (IQR = 4%) to bay pipefish diets. Benthic diatoms comprised the remaining 15.7% (IQR= 2%) and 15.6% (IQR= 2%) of their OM support, respectively. Somewhat surprisingly, given the proximity to other OM source categories, river POM, scrub-shrub vegetation, C_3 marsh plants, *Zostera marina*, and phytoplankton were not substantially assimilated (each source <0.009%). While OM supporting bay pipefish was nearly identical between the high and low flow seasons (*Ulva* spp*.:* 60.3%, IQR= 3%, *Typha* spp.: 21.3%, IQR = 4%, benthic diatoms: 14.4%, IQR = 2%), OM source contributions to juvenile English sole differed slightly between seasonal flow periods: the contribution of *Typha* spp. diminished (8.8%, IQR = 3%), and *Ulva* spp. increased (71.9%, IQR = 3%).

Overall, the type and amount of OM assimilated by juvenile English sole and bay pipefish in Skagit Bay was significantly different (PERMANOVA: Pseudo-F= 12.16, p = 0.001, Fig. 4). However, OM support was only significantly different between the two fish species during the low flow period (PERMANOVA post-hoc tests, t = 3.45, p = 0.005, Fig. 4). Flow regime made no significant difference in the OM contributions for either species. Juvenile English sole assimilated a greater diversity of OM sources compared to bay pipefish, especially during the low flow period (Table 3).

Discussion

In some contrast to the findings of Polis *et al.* (1997), who asserted that water movement is the principle vector of food web connectivity in estuarine systems, and to the findings of Guest and Connolly (2004), who document that minimal OM transport and organism movement creates spatially compartmentalized food webs at the scale of meters in some estuarine settings, our results indicate that both OM movement and organism movement enhance connectivity among ecosystems in the more tidally and fluvially influenced Pacific Northwest estuaries. In estuaries exhibiting high fluvial discharge, water-advection is a major mechanism of large-scale OM transport and delivery to adjoining ecosystems, while trophic relay by organisms may provide the more important vector of food web connectivity in estuaries exhibiting little to no fluvial discharge. The two mechanisms, however, certainly work in tandem to enhance food web connectivity across estuarine ecotones. Support for these assertions is provided by comparing and contrasting the observed patterns of diet composition, isotopic signatures, and OM assimilation for juvenile English sole and bay pipefish in the two contrasting estuaries we examined.

Prey resources

The diets of juvenile English sole were markedly more diverse and variable compared to the diet composition of bay pipefish, a pattern attributable in part to differences in mobility between the two fish. Juvenile English sole consistently preyed upon more invertebrate taxa than bay pipefish, providing preliminary evidence that English sole may act as a stronger integrator of food webs than pipefish simply by incorporating more members of the invertebrate community, and potential food web linkages, into their diets. Furthermore, similarity analyses (SIMPER) based on the composition of prey consumed not only suggest that the diets of juvenile English sole and bay pipefish are extremely dissimilar (avg. dissimilarity: 89-96%) in both estuaries, but that the within-species diets of juvenile English sole are more dissimilar from one another than are the diets of bay pipefish. Therefore, the individual foraging efforts by juvenile English sole, resulting in a higher diversity of prey consumed, potentially provide greater food web connectivity than bay pipefish, which show less variation among individuals. Increased prey species richness or diet variability may indicate increased food web connectivity in two ways. Firstly, food web connectivity may be enhanced through the sampling effect (Hooper *et al.* 2005), in which increasing prey species diversity consequently increases the chance that a new prey organism will represent a new food web linkage from a different combination of OM sources. Secondly, food web connectivity associated with elevated prey diversity may increase as a result of expanded niche complementarity, wherein different prey species utilize different resources, or utilize the same resources at different times or points in space, thereby expanding foraging niches (Tilman 1999, Hooper *et al.* 2005, Stachowicz *et al.* 2007).

Differences in resource utilization and feeding niche breadth may be linked to differences in feeding locations between juvenile English sole and bay pipefish. Since bay pipefish are relatively confined to eelgrass patches, their diet is mostly linked to prey resident to those patches, with some supplementation from organisms advected through the patches by tidal currents. In comparison, juvenile English sole may cross combinations of eelgrass, mudflat, and marsh channel ecotones with every tidal excursion. This level of mobility allows them to integrate the diverse prey assemblages associated with each ecosystem through which they pass. As a result, juvenile English sole feed on diverse integrated (landscape mosaic) prey assemblages, while pipefish feed on a local (patch) prey assemblage. Since prey assemblages originate from different ecosystems within the estuarine landscape, differences in consumer mobility may ultimately affect the degree of food web connectivity reflected by each consumer species (Wiens 2002, Pittman et al. 2004).

Irrespective of differences in mobility on a daily temporal scale, however, feeding specialization and ontogenetic shifts in feeding location may also influence the degree of food web connectivity reflected by juvenile English sole and bay pipefish. Functional morphology of pipefish may restrict the types of prey they can consume, as pipefish snouts are specifically designed to consume epibenthic crustaceans (Leysen et al. 2011, Van Wassenbergh et al. 2011). Pipefish head morphology is characterized by a long tubular snout with minute jaws at the end. Because the jaws of pipefish are fused together until the mouth opening, pipefish are severely gape limited. This restricts the size of prey obtainable by pipefish, resulting in highly specialized diets. Thus, reduced diet variability in pipefish, as compared to juvenile English sole, may result from a combination of site fidelity (patch-specific feeding) and specialized feeding morphology, both of which reduce the assemblage of potential prey available for consumption. By comparison, juvenile English sole feeding is less restricted by morphology, such that English sole not only have access to prey across the mosaic of estuarine ecosystems, but they also have access to prey inhabiting different habitats (i.e., benthic infauna, epibenthic, epiphytic) within each ecosystem (Hurst *et al.* 2007).

While the elevated diet diversity and variability exhibited by juvenile English sole as compared to bay pipefish may be attributed to the wider range of ecosystems utilized by individual sole during each tidal cycle, previous work on juvenile English sole resource utilization suggests that diet variability among individuals may also relate to ontogenetic shifts. The ontogenetic stanzas of juvenile English sole are accompanied by a shift in feeding location as well as prey type, whereas ontogenetic shifts in pipefish are only accompanied by a shift in prey (Toole 1980a). Recently metamorphosed recruits of juvenile English sole settle and feed in intertidal and shallow sand, eelgrass, and mudflat areas, where they feed primarily on epibenthic copepods and cumaceans (Toole 1980a). At lengths between 50-65 mm, English sole begin feeding on benthic infauna, focusing primarily on polychaetes, but remain on intertidal flats until they grow to ~85 mm, when they move into subtidal channels to feed primarily on infaunal polychaetes and bivalves, as well as on a variety of other organisms such as amphipods (Toole 1980a). While we were able to exclude ontogenetic changes in diet composition as a confounding factor in our between- season comparisons, fish length explained a larger proportion of diet variation within season. This suggests season plays a stronger role than ontogeny in determining juvenile English sole diets, but ontogenetic stanzas still explain considerable variability in their diets within a season. Juvenile English sole sampled during the high flow period spanned all three estuarine-associated ontogenetic stanzas, ranging between 37-101 mm in Padilla Bay and 41-122mm in Skagit Bay. Thus, in addition to reflecting greater mobility than pipefish on a daily, tidally-associated scale, juvenile English sole diet variability also reflects a sequence of ontogenetic stanzas captured within the sampled individuals. This appears to have a considerable effect on diet variability, as length explained more diet variation during the high flow period (22.04%) in Skagit Bay when the sampled fish represented three ontogenetic stages, as compared to the low flow period (12.41%), when fish represented only two ontogenetic stages. As a group, diet composition of juvenile English sole also displayed increasing homogenization during the low flow period in Skagit Bay, indicating that diet variability among individuals may decrease when the fish represent fewer life stages. These stanzas, in turn, affect juvenile English sole ecosystem use on a longer temporal scale compared to their daily movements, as juvenile sole concentrate their feeding in specific estuarine ecosystems depending on their life stage, gradually moving across the estuarine mosaic before migrating offshore at the end of their first year (Toole 1980a, Gunderson et al. 1990, Rooper et al. 2003). With each successive ontogenetic shift, juvenile English sole gradually relay estuarine-produced trophic energy seaward in the form of their bodies and metabolic wastes (Kneib 2000).

Further examination of bay pipefish and juvenile English sole diets reveals that although the species composition and variability of direct prey organisms changes between seasons for both predators, the prey habitat contributions to their diets does not change. During both high and low river flow periods, juvenile English sole relied upon benthic organisms for > 90% of their diets in Padilla Bay and > 80% of their diets in Skagit Bay. In contrast, bay pipefish derive the majority of their diets from epifaunal organisms (76-96%). Therefore, the habitats and behaviors associated with prey (i.e., water column, benthos, macrophytes) do not change significantly across season, indicating that the mode of food acquisition by prey organisms may not change significantly either. Given the locations of prey acquisition by each species, patterns of diet data at the prey habitat level provide further evidence that the benthic and epibenthic-feeding juvenile English sole may be a more effective integrator, and therefore potential transporter, of estuarine OM as compared to the epifaunal-feeding bay pipefish. More importantly, because seasonal patterns of prey habitat groups do not shift strongly within a species for either bay, we can attribute any shifts in fish isotope signatures or patterns of OM assimilation to a change in food web linkages from divergent sources, as opposed to a marked shift in prey species composition or prey habitat group.

Cross- ecotone food web connectivity: evidence from isotope signatures and OM sources

Species effects

Juvenile English sole consistently displayed broader variation in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ as compared to bay pipefish. In other systems, such isotopic variation has signified broader dietary niche space (Newsome et al. 2012). This result is therefore consistent with our hypothesis that a more mobile species should display a broader dietary niche space as compared to a species inhabiting one specific habitat. In this case, the higher diversity of prey items identified in juvenile English sole diets translates to a broader isotopic niche space among individuals, suggesting that their assimilated trophic energy represents greater integration of available OM sources. The results of our mixing model analysis further support this contention, as the diversity of OM sources assimilated by juvenile English sole was consistently higher than for bay pipefish. However, both species exhibited trophic connectivity to all estuarine ecosystems available in Padilla and Skagit bays. This indicates that in the absence of organism movement, physical forces, such as tidal action and freshwater discharge, are strong enough to translocate OM across ecosystem boundaries in Pacific Northwest estuaries. Physically-mediated OM translocation thus creates a base-level of trophic connectivity across estuarine systems.

The strength of ecosystem trophic connections, however, differed between the juvenile English sole and bay pipefish. The proportions of OM sources supporting juvenile English sole were slightly more evenly distributed across sources than bay pipefish, suggesting that the sole consistently use a broader suite of ecosystems for trophic support. Pipefish tended to assimilate marine sources of OM more heavily, such as phytoplankton, macroalgae, and eelgrass. Juvenile English sole, on the other hand, assimilated more OM from marsh and mudflat ecosystems compared to marine sources. Thus, organism mobility appears to affect physically-mediated levels of food web connectivity in estuarine systems, with the more mobile species incorporating trophic energy more evenly from a wider mosaic of estuarine ecosystems. Connectivity within the coastal ecosystem mosaic is thus a multifaceted process that includes physical and biological translocation of trophic energy (Sheaves 2009).

Fluvial effects between estuaries

As described previously, we originally hypothesized that fluvial discharge in the Skagit River estuary would enhance OM movement, thereby spatially integrating the pool of OM sources from different ecosystems across the estuary. We therefore expected stronger food web connectivity in Skagit Bay as compared to Padilla Bay, which receives no fluvial input. Specifically, we expected Skagit Bay fish to reflect broader isotopic ranges and OM source assimilation compared to those captured in Padilla Bay. However, when we applied mixing models to fish isotope data, we observed greater evenness and diversity of OM source assimilation in Padilla Bay as compared to Skagit Bay for both species. This suggests that Padilla Bay fish display broader connectivity to the various ecosystems and characteristic OM sources as compared to Skagit Bay fish, disproving our initial hypothesis.

One possible explanation for decreased food web connectivity among ecosystems in Skagit Bay may relate to OM deposition and retention. Ecosystem-specific OM source availability depends on the extent of habitat for source-specific production, the proximity of different ecosystems to one another, and the transport, deposition, and retention of OM within the estuary. While both estuaries contain similar assemblages of primary producers (i.e., emergent marsh, benthic microalgae, eelgrass, macroalgae), Padilla Bay may more effectively retain deposited OM compared to Skagit Bay. Firstly, Padilla Bay is primarily composed of dense eelgrass beds which not only generate detritus, as well as benthic and epiphytic microalgae, but also facilitate the deposition and retention of all types of OM by muting hydrodynamic energy (Asmus and Asmus 2000, Chen et al. 2007). Secondly, Padilla Bay sediments are predominantly composed of fine particles (28 – 100 µm) and organic matter (Silver 2009), whereas the Skagit River delta is comprised of coarse sand and low OM, suggesting that OM is not as well retained in those intertidal flats (Webster et al. in press). Our evidence suggests that strong river discharge and tidal currents transport organic materials and fine sediments off the deltaic flats before depositing them in deeper, less hydraulically energetic waters (Yang and Khangaonkar 2009). These materials may therefore be unavailable to consumers foraging on the Skagit River delta's intertidal flats, despite the potential for fluvially-mediated transport of marsh and terrestrially-derived OM during pulsed flood events; a phenomenon absent in Padilla Bay. As compared to Padilla Bay, the expedient export of OM from the Skagit River estuary may decrease the temporal availability of these materials to consumers. As a result, juvenile English sole and bay pipefish from Skagit and Padilla Bays exhibit similar levels of connectivity to the marsh ecosystem, despite greater potential for fluvial transport and greater marsh ecosystem area in Skagit Bay.

Compared to our mixing model results, the isotopic data present a more complex suite of evidence with respect to our first flow-related hypothesis. While we expected Skagit Bay fish to exhibit a greater range in isotope values as compared to Padilla Bay, only the ranges of δ^{15}N and δ^{34}S were broader in Skagit Bay. Broader isotope ranges provide preliminary evidence that fish in Skagit Bay may be utilizing a broader suite of OM sources as a result of fluvial mixing. However, the isotopic range of consumer organisms is constrained by the range of isotope values encompassed by the primary producers in the system (Newsome et al. 2012). Thus, despite greater mixing in Skagit Bay, we may not have observed a broader range of fish δ^{13}C values in Skagit Bay because the range in δ^{13}C values of OM sources is similar between the two estuaries (PB: 22.3‰, SB 22.7‰). In contrast, the range in δ^{15}N signatures of OM sources was broader in Skagit Bay, perhaps allowing for a greater range of N^{15} to be reflected in consumers, regardless of fluvial mixing. Furthermore, because N^{15} fractionates considerably between trophic levels, wider N^{15} ranges may not necessarily indicate assimilation of a broader mixture of OM sources, but rather feeding at a number of different trophic levels. Padilla Bay primary producers exhibit a far greater range in δ^{34}S values (39.5‰) compared to those in Skagit Bay (28.6‰), yet Skagit Bay consumers reflect the broader δ^{34}S range. The sulfur data thus suggest that Skagit Bay consumers assimilate a broader assemblage of OM sources than Padilla Bay consumers.

Since we are using stable isotopes to infer greater food web connectivity among ecosystems, it is important to determine whether primary producers exhibit greater variability in isotope values within or among ecosystems. If variation in source signatures is relatively the same within an ecosystem as among ecosystems, it is difficult to infer whether broader consumer isotope values indicate feeding across a broader suite of ecosystems or assimilation of a broad mixture of OM sources originating in a single ecosystem, which is the case for S^{34} in Skagit and Padilla bays. Thus, it is difficult to ascertain whether expanded isotope values relate directly to expanded assimilation of OM sources or to expanded food web connectivity among estuarine ecosystems. As described by Newsome et al. (2012), only with the conversion of isotopic data to numerical estimates of resource use via mixing models can an organism's niche be evaluated with isotopic tools. We thus consider isotopic data to be but a preliminary step towards food web understanding.

Interaction effects of species and estuarine fluvial setting

We next examine the interaction of estuarine fluvial setting (embayment versus river delta estuary) and species mobility differences, expecting the degree of similarity in food web support between species to shift according to estuarine setting.

Embayment estuary (Padilla Bay)— In line with our hypothesis, we consistently found strong, significant differences in isotopic values and OM assimilation between juvenile English sole and bay pipefish in Padilla Bay. We attribute this pattern to the lack of fluvial influence in the estuary, which decreases the potential for OM transport and leads to the patchy distribution of ecosystem-specific OM sources across space. As a result, food web compartmentalization occurs among spatially restricted species, but not among highly mobile species. Differences in movement patterns between the two fish, coupled with the lack of strong freshwater discharge in Padilla Bay, may thus explain why juvenile English sole consistently exhibited broader isotope values than bay pipefish, and why the isotope values of bay pipefish remained relatively constant throughout the season while English sole values shifted.

Estuarine river delta (Skagit Bay)— In contrast with Padilla Bay, where food web support of juvenile English sole and bay pipefish was consistently different in both seasons, species comparisons were not consistent across seasons in Skagit Bay. Under high flow conditions, no difference in OM support was observed between bay pipefish and juvenile English sole, indicating that when freshwater discharge is high, the pool of OM sources within the Skagit estuary is integrated to such an extent that differences in organism movement and feeding locations are obscured. Under low flow conditions, however, we observed a significant difference in the isotope signatures and dietary sources of OM between the two species. OM transfer thus appears to become more compartmentalized under low discharge conditions, perhaps because the transport and deposition of detritus becomes less mixed and more zoned as fluvial forcing diminishes in the estuary.

Our observations of food web convergence between the two species under high flow conditions, but divergence under low flow conditions matches observations of seasonal river plume convergence in Skagit Bay; the North and South Fork river plumes coalesce across the delta under high river flow conditions, but remain separated during low flow conditions (Yang and Khangaonkar 2009). This pattern of food web convergence with increasing freshwater discharge has been described in the Tagus River estuary, although at a much larger spatial scale (Vinagre *et al.* 2010). The phenomenon of compartmentalization during low river flow conditions is further supported by the observed increase in the difference between the ranges of isotope values of juvenile English sole and bay pipefish during the low flow period in Skagit Bay. This perhaps indicates that when fluvial discharge diminishes, the range in bay pipefish isotope values decreases because the amount and types of OM delivered to *Z. marina* beds diminishes. Meanwhile, the continued movement of English sole throughout the estuary during periods of low freshwater discharge likely accounts for the broad range of their isotopic values.

As such, organism movement likely drives patterns of food web connectivity and support in the non-fluvial estuary, Padilla Bay. In contrast, OM transport likely drives patterns of food web connectivity during periods of high fluvial discharge in Skagit Bay. We thus show that estuarine trophic connectivity depends strongly on fluvial context, providing insight on the extreme diversity of spatial scales over which food web compartmentalization has been documented across the coastal ecosystem mosaic (Odum 1980, Gordon et al. 1985, Deegan and Garritt 1997, Guest et al. 2004).

Seasonal effects of fluvial discharge by estuary and species

In addition to examining the degree of food web compartmentalization between contrasting estuarine settings, we also examined seasonal, species-specific effects within each estuary. By contrasting species-specific shifts between an embayment estuary and river delta estuary, we can differentiate between food web shifts relating to season or species, and those relating to seasonal shifts in freshwater discharge. Firstly, we hypothesized that seasonal shifts in fish isotopic values and OM support would be stronger in the Skagit River estuary as compared to Padilla Bay because the Skagit River estuary experiences seasonal differences in fluvial discharge, while Padilla Bay does not. Secondly, we expected Skagit Bay pipefish to exhibit stronger seasonal shifts in isotope signatures and OM source contributions compared to juvenile English sole because their restricted mobility confines their assimilation of OM sources to those available in eelgrass ecosystems, which presumably shifts in accordance with changes in fluvial discharge.

English sole

Despite their mobility, juvenile English sole in both Padilla and Skagit Bays exhibited significant seasonal differences in food web support, indicating that seasonal food web shifts unrelated to fluvial discharge occur for this species (i.e., seasonal availability of OM sources that align with producer growing seasons). In contrast with our hypothesis, however, Padilla Bay juvenile English sole exhibited a stronger seasonal shift in isotope values compared to juvenile English sole in Skagit Bay, despite there being no accompanying shift in fluvial discharge at that location. We suggest that although strong seasonal shifts in freshwater discharge occur in Skagit Bay, summer river discharge likely provides a temporally continuous mechanism of OM integration throughout the estuary. As a result, the pool of OM sources available to juvenile English sole in Padilla Bay may be more spatially compartmentalized as compared to Skagit Bay. The patchier availability of ecosystem-specific OM sources in Padilla Bay may in turn allow ontogenetic shifts in juvenile English sole feeding location (Toole 1980a, Rooper et al. 2003) to reflect changes in isotopic values on a seasonal scale, whereas stronger OM spatial integration in Skagit Bay obscures seasonal ontogenetic shifts in feeding location.

Padilla Bay English sole $\delta^{15}N$ and $\delta^{13}C$ become significantly depleted from March to August, a pattern that either indicates a distinct shift in the OM sources supporting juvenile English sole, or a downshift in trophic level. We suspect that ontogenetic shifts in feeding location, as opposed to ontogenetic shifts in diet items, may explain this depletion. Firstly, most ontogenetic shifts in diet are usually associated with an increase in trophic level, as larger fish overcome gape and other morphological or bioenergetic limitations, thereby increasing the variety and size of available prey (e.g. Oliveira et al. 2007, Kolasinski et al. 2009, Romanuk et al. 2011). Secondly, the lengths of juvenile English sole from Skagit Bay increased by a greater degree (+ 44.8 mm) than seen in Padilla Bay (+ 26.0 mm) between seasons, yet their isotopic values did not change significantly, perhaps due to greater spatial integration of OM throughout the estuary. Finally, it has been shown that most juvenile English sole move to the outer edges of the estuarine deltas as they prepare for migration to deeper waters by the time they reach 75 mm (TL) (Gunderson *et al.* 1990, Rooper *et al.* 2003). The juvenile English sole we collected at Padilla Bay originated from the outer edge of the bay's delta at a mean TL of 86.6 +/- 13.3 mm, suggesting that these fish may have spent enough time on the outer margins of the estuary to exhibit a depleted carbon and nitrogen signature reflective of phytoplankton, which is more depleted in these isotopes as compared to marsh-derived OM. However, it is also possible that a shift in feeding location coincides with a shift in prey species to trigger the depletion of isotope values. SIMPER results for juvenile English sole diet composition indicated a decrease in the gravimetric contribution by polychaete worms (Nereidae) between the early and late seasons, a pattern observed previously in Padilla Bay English sole (Simenstad *et al.* 1995). Because polychaetes could not be identified to species due to stage of digestion, we cannot definitely comment on whether sole were primarily feeding on predatory or detritus-feeding polychaetes. Detrital feeding species, however, have been found to be the most common forms available in the bay (Jeffrey 1976). The juvenile English sole also switched to feeding more heavily on oligochaetes and clam siphons late in the season, neither of which are predatory organisms. Thus, while we expected to observe greater shifts in OM support among juvenile English sole in Skagit Bay, a combination of patchy OM distribution, ontogenetic shifts in feeding location, and diet shifts likely work in concert to produce greater seasonal shifts in food web connectivity among juvenile English sole Padilla Bay.

Bay pipefish

Despite dietary shifts observed through bay pipefish diet analysis, we found no change in the isotopic signatures or OM source contributions supporting the pipefish between the high and low river flow sampling periods in either Padilla or Skagit bays. Because bay pipefish show fidelity to eelgrass habitats, the OM available to their prey in eelgrass is more likely stable across variations in freshwater discharge

(Skagit Bay) or season (Padilla Bay). This pattern was unexpected in Skagit Bay, where we expected bay pipefish isotope signatures to reflect strong terrestrial or marsh influence early in the season, under high river discharge, but greater influences of OM sources of marine origin during periods of low freshwater discharge. Instead, marine macroalgae was consistently the dominant OM source assimilated by pipefish (~60%), followed by tidal marsh producers (~22%), and benthic diatoms (~15%). The lack of seasonal shifts in OM support among Skagit Bay pipefish suggests that the OM pool remains mixed in consistent proportions in eelgrass beds throughout the year, despite pulses of marsh-derived OM during periods of high freshwater discharge. As described earlier, these pulses of marsh-derived OM are expediently exported away from the delta (Yang and Khangaonkar 2009), leaving little time for consumer assimilation. Hence, marine-derived OM likely forms the consistent food web base for Skagit Bay eelgrass inhabitants, leading to consistent OM support across seasons.

There is also the possibility that the lack of seasonal shifts in isotope values or OM source contributions to pipefish indicates that pipefish growth rates are so low that tissue turn-over rates do not reflect a seasonal shift. Examination of liver tissue, for example, may be able to reflect isotope shifts at a finer temporal scale (Maier and Simenstad 2009, Suring and Wing 2009, Buchheister and Latour 2010). However, because collecting sufficient liver tissue for isotope analysis can be difficult and variable due to the minimal body mass of pipefish, we did not analyze liver tissue because we suspected that this would introduce more undesirable variability into our analysis. However, prior studies of pipefish growth rates indicated that they grow relatively rapidly until they reach maturity at ~ 200 mm, depending on the species and sex of the fish (Takahashi *et al.* 2003, Barrows *et al.* 2009). With the exception of one individual, all the pipefish we captured in Padilla Bay during the high flow period were < 150 mm TL, while fish caught during the low flow period were mostly between 180 – 250 mm. The pattern was similar in Skagit Bay, where the length of individual pipefish ranged between 130-215 mm TL during the high flow period, and between 154 – 282 mm TL during the low flow period. While not definitive, these data suggest these relatively non-transient, habitat-specific pipefish are growing over the sampling season. Because growth and metabolic rates can work in tandem to cause a shift in tissue isotope values over time (Nelson *et al.* 2011), it is likely that the separation in sampling periods (Skagit: 122 d, Padilla: 273 d) was sufficient to detect a seasonal shift in diet, especially given that other studies have reported fish muscle turnover rates between 49 and 231 days (Maier and Simenstad 2009, Buchheister and Latour 2010, Nelson *et al.* 2011). Thus, it appears that OM available in the pipefish eelgrass habitats does not change with the season, perhaps because the eelgrass itself promotes the deposition and retention of OM particles (Asmus and Asmus 2000, Bouillon *et al.* 2007, Chen *et al.*

2007). Through their detritus-feeding prey, pipefish appeared to have access to an integrated pool of OM sources that accumulates over long time periods.

OM source availability

In addition to the physical conditions associated with patterns of food web connectivity in Skagit and Padilla bays, several biological observations also enhance our understanding of food web support of nekton in these systems. Detritus feeders generally target microbially-conditioned material rather than feeding directly on refractory fragments (Kreeger and Newell 2000). Perhaps because Skagit Bay is hydraulically more energetic than Padilla Bay, higher level consumers may only assimilate OM that is easily consumed by first-order detritivores and grazers. Benthic diatoms and marine macroalgae (in this case *Ulva* spp.) are readily accessible fresh food sources to estuarine invertebrates, and are thus the primary sources of OM support to the Skagit Bay fish we sampled. In fact, much of the marine macroalgae available in the system consists of *Ulva intestinalis*, which accumulates in large, ropey masses on the flats beginning in early June (Personal observation). The mats tumble back and forth across the flats with the ebbing and flooding of the tide, accumulating a diverse and dense community of benthic, epibenthic, and epifaunal organisms within the algal strands (Personal observation). These algal mats thus likely become important food sources for a variety of estuarine consumers, playing a similar role to the floating *Sargassum* spp. communities described by Vandendriessche *et al.* (2007) in the North Sea. In addition to being readily available fresh sources of food, these mats may provide detritivores with a source of OM, as the mats tend to decompose from the inside out (Personal observation). The algal mats also create pools of anoxic, decomposing detritus when they either grow too large to move with the tide, or snag on buried branches, rocks, or other forms of relief. These pockets of algal detritus can be found in deltaic sediments at least a year after the initial growing season (Personal observation), likely providing a stable, slowly decomposing OM source to benthic detritivores in an otherwise unstable sandy environment. Additionally, *Typha* spp*.,* the third most prevalent source of OM supporting Skagit Bay juvenile English sole and bay pipefish, is pervasive in the lower Skagit River estuary (Greg Hood, SRSC unpublished data). *Typha* spp. grows along protected backwater sloughs from which it may require considerable time to flush out, especially compared to the marsh fringes, where the more saline-tolerant species of emergent marsh macrophytes reside. It is likely that *Typha* spp. detritus arrives at the sandflats and eelgrass beds already microbially conditioned, making for an easier transition into the food web compared to other rushes, sedges, and grasses that grow along the outer fringes of the marsh ecosystem. Thus, while physical forcing caused by fluvial discharge may spatially integrate OM throughout an estuarine system, unless detrital retention times are long enough, that material may not be assimilated into the estuarine food web. As a result, consumers inhabiting a large fluvial system such as the Skagit River estuary reflect less trophic connectivity to the suite of estuarine ecosystems than expected.

Conclusion

In conclusion, we found no seasonal shift in the prey habitat groups supporting either bay pipefish or English sole in Skagit and Padilla Bays, two estuaries exhibiting dramatic differences in fluvial discharge, and two fish species exhibiting strong differences in daily and seasonal mobility within estuarine ecosystems. Food web connectivity was strongly affected by differences in organism mobility. The more mobile juvenile English sole, *P. vetulus*, consistently displayed broader connectivity to estuarine ecosystems compared to bay pipefish, *S. leptorhynchus,* a pattern attributable to greater access to a variety of OM sources as well as access to a broader assemblage of prey types. As a result, we attribute observed shifts in the isotopic values of these fish to shifts in the OM sources that support their food web pathways among the estuarine ecosystems they can occupy.

Food web connectivity was also strongly influenced by differences in fluvial forcing at the estuary scale. In contrast to our assumption that fluvial forcing would more effectively integrate the OM pool of an estuary, creating strong trophic connections to a variety of OM sources in the estuary, fish in Padilla Bay unexpectantly exhibited broader trophic connectivity than fish in Skagit Bay. We attribute this pattern to differences in OM retention between the two systems, as OM is quickly exported from the Skagit River delta, making many sources of OM unavailable to estuarine consumers. Food web connectivity was also affected by seasonal shifts in fluvial discharge. Under high river flow conditions, fluvial forcing in the Skagit estuary spatially integrated the OM pool such that the daily movements of juvenile English sole did not result in a difference in the sources of OM supporting this fish as compared to bay pipefish, which exhibit site fidelity to eelgrass beds. Thus, fluvial forcing can enhance food web connectivity by transporting OM through estuarine systems. Under low flow conditions, however, we observed divergence in the sources of OM supporting juvenile English sole and bay pipefish in Skagit Bay, suggesting that diminished freshwater discharge into an estuary promotes a more compartmentalized food web with respect to the various estuarine ecosystems. Compartmentalization was also observed in Padilla Bay, where the lack of fluvial forcing likely creates spatial shifts in available OM pools, such that consumers feeding in different locations reflect different degrees of trophic connectivity to specific ecosystem components. However, our evidence from Padilla Bay suggests that organism movement can also continually relay OM throughout an estuarine ecosystem, regardless of flow dynamics, providing some measure of food web connectivity through this trophic relay. Coupled with evidence of fluvially-mediated OM transport from Skagit Bay, this study provides a more holistic understanding of trophic connectivity in the coastal ecosystem mosaic, suggesting that biological and physical mechanisms of trophic connectivity not only work in tandem, but that the importance of one mechanism versus another is strongly dependent on the fluvial context of the estuary.

Given the importance of trophic connectivity to the food web dynamics of a wide variety of systems (Polis et al. 1997), a detailed understanding of the links between physical ecological processes and biological patterns is essential if we are to accurately describe interdependent interactions among organisms and their habitats and adjoining ecosystems (Sheaves 2009). As described by Sheaves (2009), this complexity is difficult to study, yet its pervasive nature and likelihood of producing unexpected patterns implies that it needs to be recognized, embraced, and understood. In this study, we have begun to tease apart the conditions under which organism movement versus OM transport create important avenues of food web connectivity, uncovering, as Sheaves (2009) predicted, many unexpected patterns that contradicted our initial hypotheses. This observation alone suggests that patterns and processes describing the maintenance of ecosystem linkages are less intuitive or simple than previously considered.

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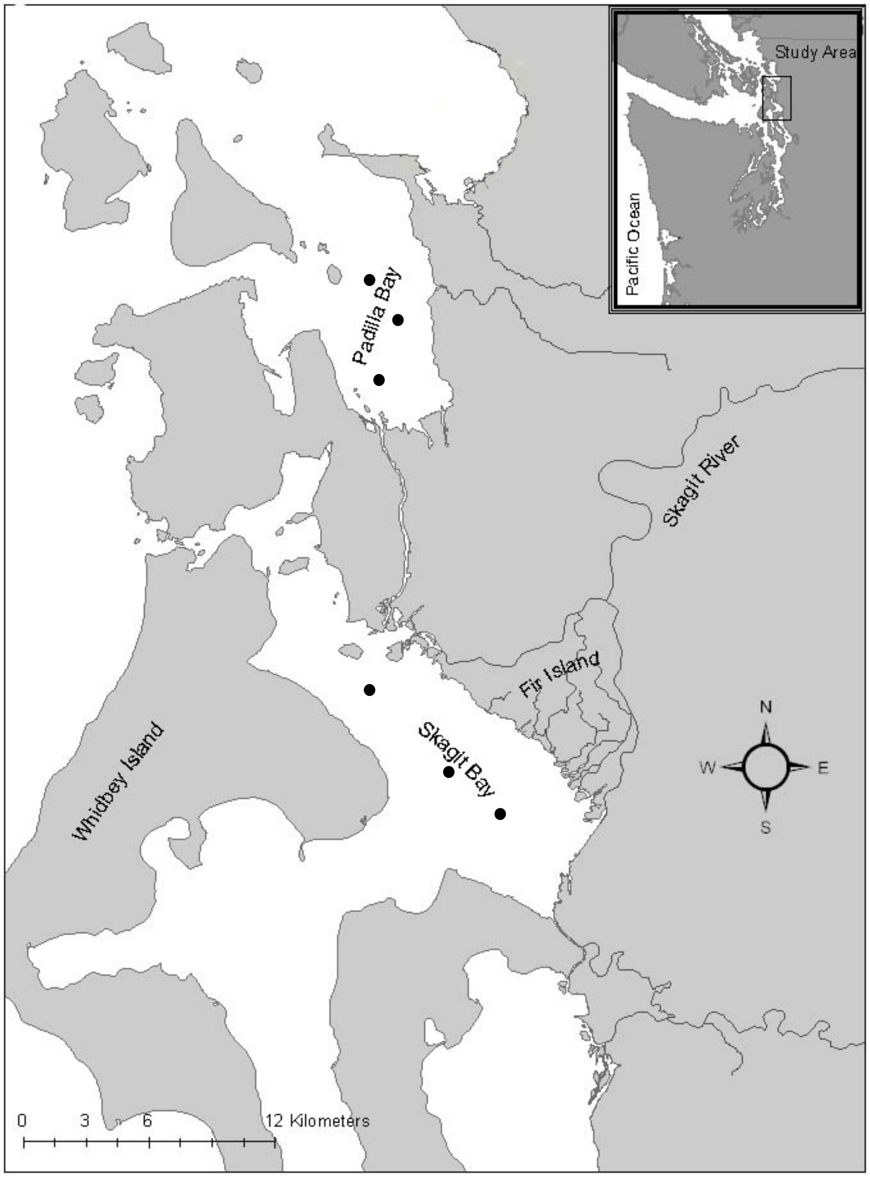

Figure 2.1. Map of the study area in northern Puget Sound, Washington, USA. Black filled circles identify sampling sites.

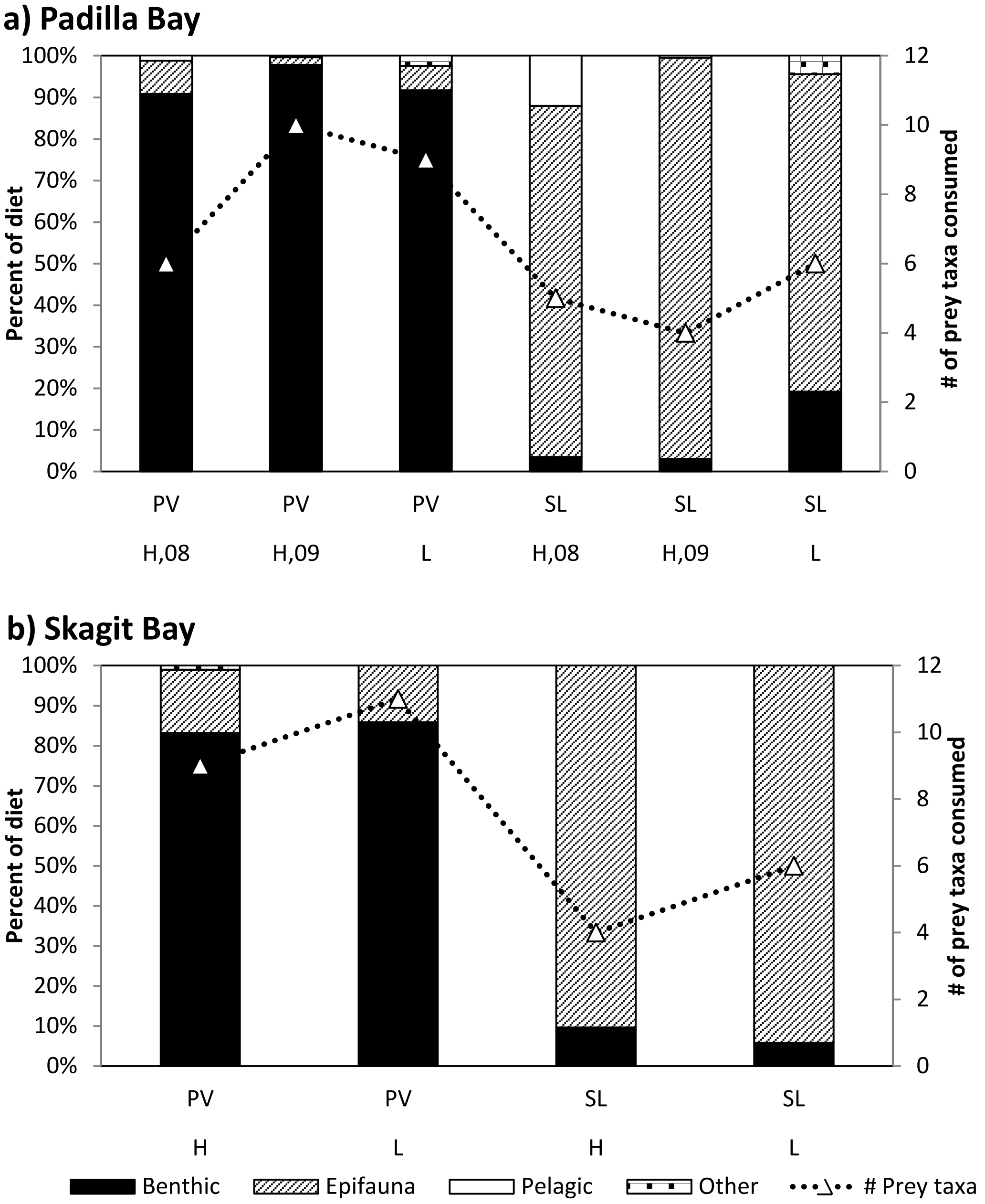

Figure 2.2. Gravimetric composition, designated by functional habitat, of juvenile English sole (*Parophrys vetulus*; PV) and bay pipefish (*Syngnathus leptorhynchus*; SL) in Padilla Bay (A) and Skagit Bay (B), northern Puget Sound, Washington, H = High flow period, L = Low flow period. For Padilla Bay, “H,08” refers to 2008 fish, while ”H,09” refers to 2009 fish. Additionally, the total # of prey taxa consumed by each group of fish, and comprising ≥10% of the group diet by weight, is displayed.

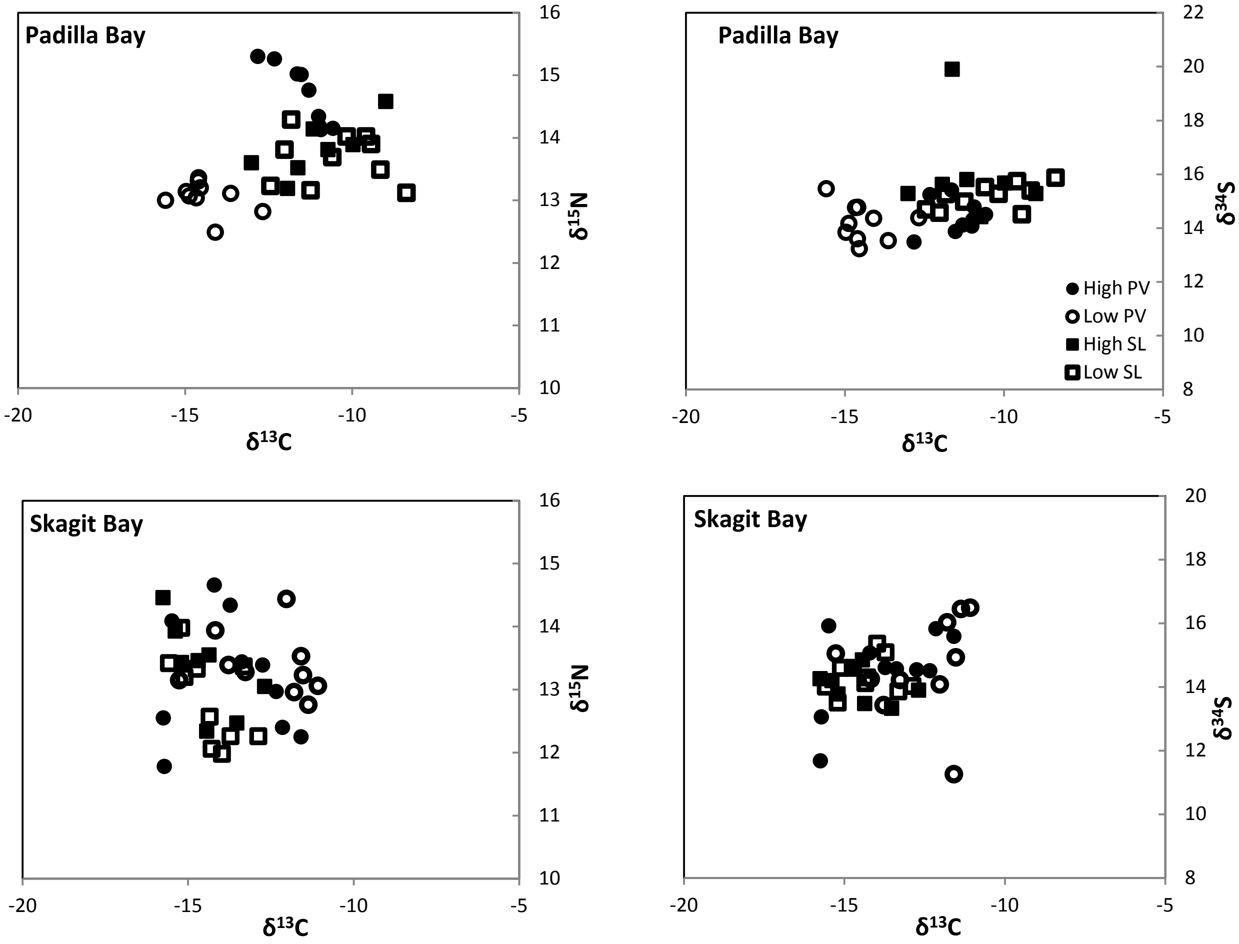

Figure 2.3. Dual isotope plots (left, $\delta^{13}C$:$\delta^{15}N$; right, $\delta^{13}C$:$\delta^{34}S$) of juvenile English sole (*P. vetulus*, PV*)* and bay pipefish (*S. leptorhynchus*, SL) in Padilla and Skagit bays during high and low river flow periods, 2008-2009, in Padilla and Skagit bays, Puget Sound, Washington.

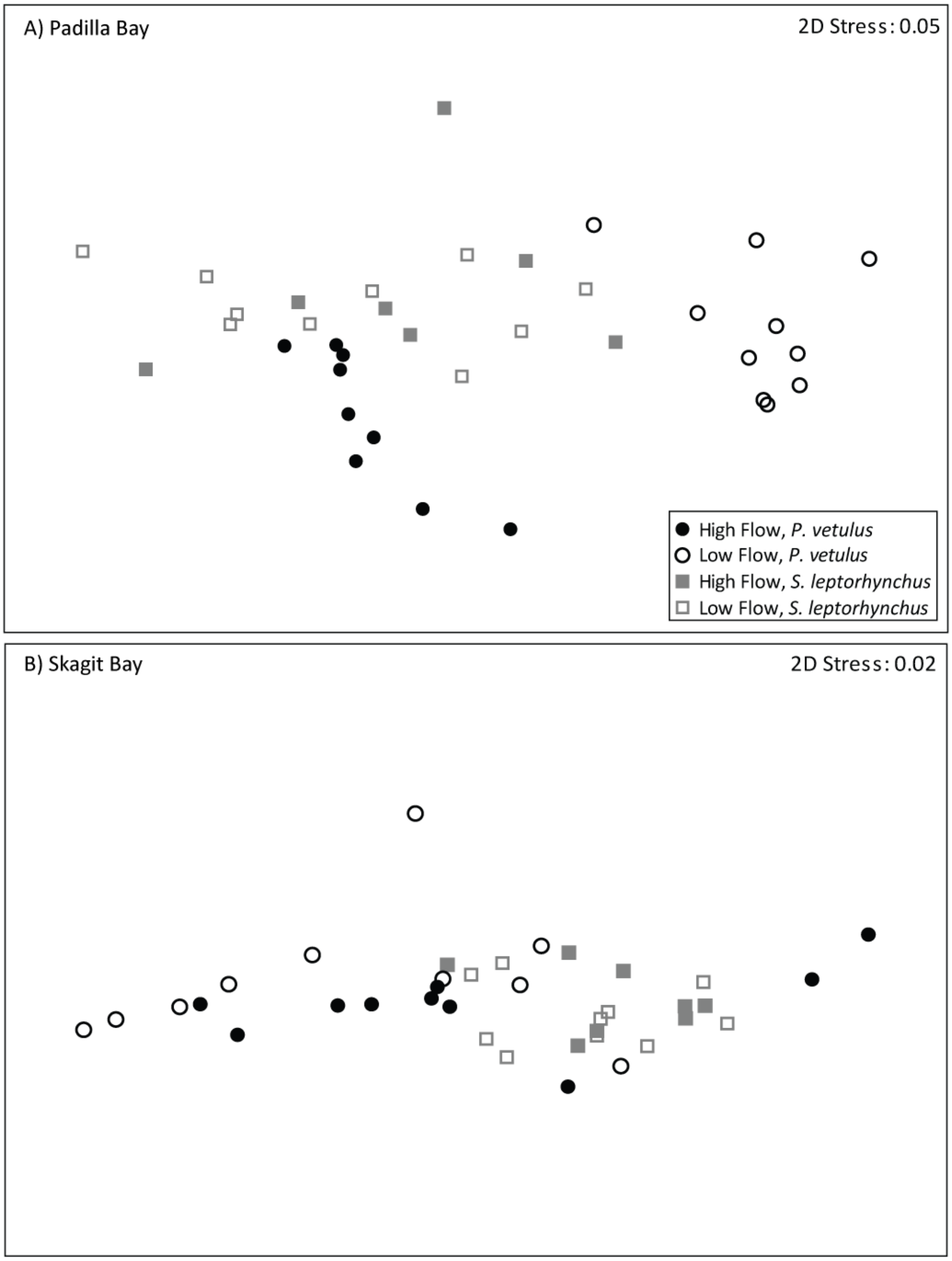

Figure 2.4. MDS ordination of organic matter contributions to juvenile English sole (*P. vetulus*) and bay pipefish (*S. leptorhynchus*) during the high and low river flow periods in A) Padilla and B) Skagit bays, Puget Sound, Washington.

Table 2.1. Average (± 1 standard deviation) $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of dominant organic matter sources in Skagit and Padilla bays, Puget Sound, Washington. Some plant species were combined into a single category because the similarity of their isotope signatures violated SOURCE's NND^2 minimum value of 0.1. Scrub -shrub includes *Salix* spp*., Lonicera involucrata, Myrica gale, Deschampsia caespitosa*, and *Potentilla anserina*. Marsh complex includes *Carex lyngbyei, Juncus balticus,* and *Schoenoplectus americanus*, *Schoenoplectus acutus, Schoenoplectus maritimus, Cotula coronopifolia,* and *Glaux maritima. Ulva spp.* includes *Ulva intestinalis* and *Ulva fenestrata.* ASG= *Atriplex patula, Salicornia virginica,* and *Glaux maritma.*

Skagit Bay Sources	**$\delta^{13}C$**	**(SD)**	**$\delta^{15}N$**	**(SD)**	**$\delta^{34}S$**	**(SD)**
River POM	-25.83	0.51	1.95	0.96	-2.58	1.74
Scrub-Shrub	-27.56	2.04	-0.81	1.57	16.83	1.33
Typha spp.	-27.29	0.68	4.80	0.88	13.69	2.22
Distichlis spicata	-16.62	4.27	3.21	0.80	9.62	1.42
Marsh complex	-27.78	2.01	3.09	1.71	9.33	6.35
Diatoms	-19.36	1.73	5.37	2.32	-11.11	6.64
Ulva spp.	-13.41	1.01	6.10	0.76	19.27	0.35
Zostera marina	-10.74	0.50	5.62	0.16	17.79	0.70
Phytoplankton	-19.52	1.41	3.37	0.63	21.70	0.77
Padilla Bay Sources						
ASG	-26.57	1.2	7.16	1.73	18.12	1.87
Ceramium sp.	-14.70	1.05	9.82	0.32	19.63	0.25
Diatoms	-19.36	1.73	5.37	2.32	-11.11	6.64
Distichlis spicata	-15.03	0.22	6.84	1.60	8.14	10.83
Triglochin maritima	-25.67	0.75	6.92	0.37	4.63	1.27
Ulva spp. & epiphytes	-10.35	3.62	8.44	0.81	19.53	0.68
Zostera japonica	-8.57	0.52	7.74	0.48	9.78	2.58
Zostera marina	-8.08	0.54	8.92	0.65	15.01	2.45
phytoplankton	-19.52	1.41	3.37	0.63	21.70	0.77

Table 2.2. Mean lengths and sample sizes of juvenile English sole (*P. vetulus*) and bay pipefish (*S. leptorhynchus)* during high and low river flow periods, 2008-2009, in Padilla and Skagit bays, Puget Sound, Washington. Variation is indicated as ± 1 standard deviation.

		High flow		**Low Flow**		***t*-stat**	***p*-value**
Padilla Bay		Mean length (mm)	*n*	Mean length (mm)	*n*		
	P. vetulus	57.6 ± 13.5	19	83.6 ± 13.3	10	-4.95	<0.0001
	S. leptorhynchus	131.6 ± 35.1	16	205.2 ± 21.7	10	-5.93	<0.0001
Skagit Bay							
	P. vetulus	69.2 ± 29.4	10	114.0 ± 14.2	10	-4.34	<0.001
	S. leptorhynchus	186.5 ± 34.1	8	217.7 ± 38.8	10	-1.78	0.09

Table 2.3. Species richness (S), Simpson's Diversity Index (D), and Shannon Evenness Index (E) of diets and organic matter (OM) source contributions of juvenile English sole (*P. vetulus*) and bay pipefish (*S. leptorhynchus*) during high and low flow periods, 2008-2009, in Padilla and Skagit bays, Puget Sound, Washington. Indices for diets were calculated on prey items composing > 10% of the diet based on gravimetric composition.

			Diet			OM Sources		
Estuary	Species	Flow	*S*	*D*	*E*	*S*	*D*	*E*
Padilla Bay	*P. vetulus*	High (2008)	5	2.63	0.70	9	5.64	0.84
		High (2009)	4	1.09	0.16	9		
		Low	6	1.86	0.48	9	5.11	0.82
	S. leptorhynchus	High (2008)	6	4.29	0.88	9	5.02	0.86
		High (2009)	10	3.51	0.64	9		
		Low	9	2.76	0.58	9	4.71	0.86
Skagit Bay	*P. vetulus*	High	9	4.57	0.80	11	1.66	1.32
		Low	11	5.73	0.77	11	2.81	0.48
	S. leptorhynchus	High	4	3.09	0.89	11	1.58	0.24
		Low	6	2.20	0.63	11	1.61	0.24

Table 2.4. Prey taxa that contributed >10% gravimetric contribution to the diets of juvenile English sole (*P. vetulus*) and bay pipefish (*S. leptorhynchus*) during different river flow conditions, 2008-2009, in Padilla and Skagit bays, Puget Sound, Washington. “High” and “Low” refer to seasonal river discharge periods.

	Padilla Bay						Skagit Bay			
	P. vetulus			*S. leptorhynchus*			*P. vetulus*		*S. leptorhynchus*	
	High		Low	High		Low	High	Low	High	Low
Prey	March 2008	May 2009	Sept 2008	March 2008	May 2009	Sept 2008	May 2007	August 2007	May 2007	August 2007
Amphipoda	5.15	2.23	1.96	1.46	1.17	14.70	-	1.95	-	4.90
Americorophium salmonis	-	2.36	13.84	-	-	0.64	4.92	15.37	-	-
Caprella laeviscula	-	0.39	-	-	-	70.61	-	-	-	3.45
Amphilochidae	19.07	-	-	-	-	-	-	-	-	-
Eogammarus confervicolus	-	0.13	-	-	-	-	20.77	-	-	-
Paracalliopiella pratti	-	-	-	17.52	-	-	-	-	-	-
Pontogeneia rostrata	-	1.18	2.46	52.55	95.54	0.32	2.30	0.35	-	-
Copepoda										
Harpacticoida	3.09	0.26	0.58	1.46	0.47	0.32	1.09	0.04	16.35	0.91
Malacostraca										
Decapoda	-	-	-	-	-	-	0.89	-	45.19	-
Crangon sp.	-	-	-	-	-	-	11.50	11.83	-	-
Hippolytidae	-	-	-	-	-	-	-	-	-	64.61
Mysida	-	-	-	-	-	-	-	-	28.85	12.52
Tanaidacea										
Leptochelia dubia	14.95	-	-	-	-	-	-	0.43	-	-
Sinelobus stanfordi	-	14.96	2.17	-	-	-	-	0.19	-	-
Bivalvia										
Macoma sp.	-	-	0.36	-	-	-	36.68	12.49	-	-
Clinocardium sp.	-	-	-	-	-	-	-	10.66	-	-
Clam siphons	-	35.04	-	-	-	-	-	-	-	-
Annelida										
Polychaeta	24.23	36.61	0.22	-	-	-	8.88	25.10	-	-
Oligochaeta	-	-	26.09	-	-	-	-	-	-	-
Other	33.51	6.82	52.32	27.01	2.82	13.42	12.97	21.60	9.62	13.61

Table 2.5. Percent similarity in the composition of diets for juvenile English sole (*P. vetulus*) and bay pipefish (*S. leptorhynchus*) during different river flow conditions (2008-2009), in Padilla and Skagit bays, Puget Sound, Washington (SIMPER analysis).

		High Flow	Low Flow
Embayment estuary- Padilla Bay			
P. vetulus	High flow	10.66	-
	Low flow	10.75	23.95
S. leptorhynchus	High flow	24.95	-
	Low flow	9.90	23.95
English sole x bay pipefish		3.15	4.37
River delta estuary- Skagit Bay			
P. vetulus	High flow	5.59	-
	Low flow	5.99	13.39
S. leptorhynchus	High flow	36.02	-
	Low flow	8.83	16.49
English sole x bay pipefish		3.07	1.71

Table 2.6. Bayesian mixing model median estimates and interquartile ranges (IRQ's) of proportional organic matter source contributions to the ultimate diet of juvenile English sole (*Parophrys vetulus*) and bay pipefish (*Syngnathus leptorhynchus*) based on their lipid-corrected isotope values during low and high river flow periods in Padilla and Skagit bays, Puget Sound, Washington.

	Parophrys vetulus				*Syngnathus leptorhynchus*			
	High flow		Low flow		High flow		Low flow	
PADILLA BAY								
Marsh complex	**0.02**	0.05	**0.02**	0.03	**0.02**	0.04	**0.02**	0.03
Triglochin maritima	**0.13**	0.11	**0.22**	0.07	**0.07**	0.06	**0.09**	0.06
Distichlis spicata	**0.03**	0.03	**0.03**	0.03	**0.12**	0.06	**0.03**	0.03
Benthic Diatoms	**0.06**	0.06	**0.06**	0.05	**0.04**	0.04	**0.07**	0.04
Zostera japonica	**0.12**	0.11	**0.11**	0.08	**0.08**	0.08	**0.17**	0.11
Zostera marina	**0.09**	0.16	**0.05**	0.06	**0.05**	0.08	**0.06**	0.08
Ulva spp.	**0.22**	0.13	**0.13**	0.06	**0.29**	0.09	**0.29**	0.08
Ceramium spp.	**0.16**	0.20	**0.04**	0.05	**0.06**	0.07	**0.04**	0.05
Phytoplankton	**0.07**	0.04	**0.31**	0.03	**0.20**	0.05	**0.21**	0.04
SKAGIT BAY	Median	IQR	Median	IQR	Median	IQR	Median	IQR
River POM	**0.00**	0.01	**0.00**	0.00	**0.00**	0.01	**0.00**	0.01
Scrub shrub	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00
Typha sp.	**0.14**	0.03	**0.09**	0.03	**0.21**	0.04	**0.21**	0.04
Distichlis spicata	**0.00**	0.01	**0.00**	0.00	**0.00**	0.01	**0.00**	0.01
Marsh complex	**0.01**	0.02	**0.01**	0.01	**0.01**	0.02	**0.01**	0.02
Benthic Diatoms	**0.16**	0.02	**0.16**	0.02	**0.16**	0.02	**0.14**	0.02
Macroalgae	**0.66**	0.03	**0.72**	0.03	**0.59**	0.03	**0.60**	0.03
Zostera marina	**0.01**	0.01	**0.01**	0.01	**0.01**	0.01	**0.01**	0.01
Phytoplankton	**0.00**	0.01	**0.00**	0.01	**0.00**	0.01	**0.00**	0.01

Chapter 3. Using isotopic measures of connectivity and ecosystem capacity to compare restoring and natural marshes in the Skokomish River estuary, WA, USA

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Abstract

Restoring food web functions for estuarine detritivores depends in part on system capacity and connectivity. More specifically, detritus-based food webs typically rely on diverse sources and timing of organic matter (OM) delivery to fuel secondary production, as well as adequate hydraulic connectivity enabling the migration of consumers into highly productive locations and the transfer of allochthonous detritus to consumer habitats. These processes are likely to be particularly important to the patterns and rates in the initial community development of restoring estuarine emergent marshes, where OM sources and connectivity might vary as a function of coastal landscape setting. The purpose of this study was to quantify functional trophic dynamics in restoring and natural marsh ecosystems in the Skokomish estuary, Washington, USA, using manipulation experiments with Pacific blue mussels (*Mytilus trossulus*) as an indicator of suspended food web sources. The restoring marshes represent different ages since restoration implementation—14 yrs and 3 yrs—as well as different restoration approaches—a levee breach and a full levee removal. We used stable isotopes in combination with a Bayesian mixing model to evaluate: (1) how the sources of food web support vary among restoring and reference marshes; (2) whether within-marsh food web connectivity differs between restoring marshes and natural marshes; (3) how food web support shifts seasonally; and, (4), whether patterns in consumer growth track patterns in OM support. Finally, we incorporated simulations of landscape change scenarios into a Bayesian mixing model to quantify the effects of marsh ecosystem loss or restoration on food web support. We found considerable temporal and spatial heterogeneity in seston food availability among the marshes. Variation in *chl α* and seston concentration and seston C:N ratios suggest sestonic OM was less available and of lower quality in the two restoring marshes than in the natural marsh site. The mixing model indicated that mussel diets tracked seasonal trends in OM availability: that phytoplankton consumption was highest in March and June; marsh detritus consumption was highest in the winter; and, macroalgae consumption was highest in September. During all seasons, however, marsh detritus comprised at least 30% of OM assimilated by the mussels, highlighting the trophic importance of restoring marsh ecosystems to estuarine detritivores and particularly the role of OM subsidies from adjacent natural ecosystems. Mussel OM source compositions were significantly different across dates, marshes, and locations within each marsh. Among-marsh differences signify that trophic equivalency has yet to be reached in either restoration site. Trophic equivalency for suspension feeding mussels, however, appears to be restoring more rapidly in the younger restoration site, perhaps because increased hydrological connectivity achieved through the full levee removal promotes maximal OM exchange than the single levee breach restoration approach. Despite differences in food web support among the three marshes, however, mussel growth rates did not differ significantly among marshes. We suggest that food availability, temperature, feeding behavior, and physiological processes work in conjunction to mediate growth rates throughout the year. When we modeled scenarios of landscape change by simulating the areal extent of OM sources available in the estuary, we found strong differences between the uninformed model and those using areal extents of OM categories as prior probabilities in the Bayesian mixing model, but few differences in estimated OM source contributions to mussel diets among the five informed models corresponding to different eras and landscape configurations within the Skokomish River estuary. Isotopic food web model utility may thus be limited in its ability to predict trophic response to any scenarios other than large-scale landscape change. We conclude that increasing ecosystem capacity for detritus production by restoring emergent marsh ecosystems can bolster support for detritus-based food webs, and suggest that restoration actions that enhance connectivity across estuarine ecotones may achieve functional equivalency more rapidly than restoration projects exhibiting limited connectivity to the surrounding landscape.

Key words: estuarine marsh restoration, stable isotopes, food web connectivity, landscape influence, *Mytilus trossulus*

Introduction

The goal of restoring estuarine ecosystems is usually to recover lost ecological functions (e.g., production, fish and wildlife habitat, sediment retention, nutrient cycling, trophic support), by focusing on structural replication, functional success, and the ability of a restoration site to be self-sustaining (Higgs 1997). Most restoration goals address the site itself, with restoration performance assessed by comparison to undisturbed, adjacent reference ecosystems. However, many important ecosystem functions are reliant on the broader landscape context, particularly the connectivity of the "physical and biological translocation of nutrients, ontogenetic, life history, spawning, and feeding migrations, food-web dynamics, predator-prey interactions and many more" (Sheaves 2009). Unfortunately, restoration designs rarely consider functional linkages among ecosystem units in the estuarine landscape, ignoring the importance of connectivity among system components (Weinstein et al. 2005). Without recognizing the importance of connectivity in mediating ecosystem processes and functions, restoration actions will likely fall short of achieving their full potential. Fortunately, amidst the growing and dynamic literature on restoration, coastal wetland restoration planning has recently moved beyond individual restoration actions, considering watershed setting and landscape function in order to more adequately address the appropriate scale at which ecosystem interactions occur (Simenstad *et al.* 2006).

In the Pacific Northwest, declining salmon (*Oncorhynchus* spp.) populations have spawned renewed interest in the restoration of estuarine ecosystems because they are critically important nursery grounds for juveniles of many species migrating through the continuum of aquatic habitats between watershed and marine ecosystems (Healey 1982). Due to the importance of estuarine marshes to juvenile salmonid growth and survival, and emerging evidence of the benefits of estuarine restoration, there has been increasing support for restoration of estuarine wetlands at multiple scales (Simenstad and Thom 1996, Simenstad *et al.* 2002, Bottom *et al.* 2005). Examples from the Pacific Northwest include large-scale levee removal and levee breach projects which have returned tidal inundation to historical marsh lands in the Nisqually, Skagit, Stillaguamish, Duwamish, and Skokomish river estuaries; all systems representing important salmon habitat, but often suffering from extensively altered estuarine deltas. While many of these projects were initially driven by salmon enhancement objectives, restoration of estuarine marsh ecosystems typically supports a wealth of other organisms as well, including migratory shorebirds, waterfowl, and forage fish (Warren *et al.* 2002, O'Connell and Nyman 2011). Most of these organisms are supported by detritus-based food webs which translate terrestrial, riverine, and estuarine-produced organic matter (OM) to higher trophic levels (Simenstad and Wissmar 1985, Romanuk and Levings 2005, Akin and Winemiller 2006).

The restoration of estuarine marsh ecosystems has particular importance to detritus-based food webs because these food webs rely on both adequate production of allochthonous and autochthonous OM matter to fuel secondary production, and adequate hydraulic connectivity. Hydraulic connectivity enables the migration of consumers into highly productive habitats and promotes the translocation of detritus to subsidize foodwebs to consumers within those habitats. The restoration of functional food web support for estuarine detritivores thus depends on both system capacity and connectivity (aka *capacity* and *opportunity* of Simenstad and Cordell 2000). Ecosystem capacity refers to the ability of the system to support population growth and survival, and is determined, in part, by resource abundance and availability. Ecosystem connectivity refers to the ability of organisms or materials to move or flow among ecosystem components, linking habitats or ecosystems in space and time (Sheaves 2009). The importance of pairing these concepts in restoration planning was highlighted in the Skagit River estuary, where Greene and Beamer (2011) found that both diminished system capacity and connectivity limited Chinook salmon populations; fish exhibited distinct density dependence due to the reduced amount of estuarine marsh channel habitat available to them, as well as reduced ability to fully utilize the available habitat due to structural and salinity barriers which disrupted connectivity between ecosystem components.

Estuarine restoration can increase capacity of detritus-based food webs by increasing the overall area suitable for estuarine primary production in the estuary, thereby increasing the biomass of available OM to detritivores, and potentially leading to an increase in secondary production. Detritus-based food webs may also benefit from the restoration of estuarine ecosystems because these systems increase the diversity and temporal availability of detrital food sources, largely by supporting a community mosaic of OM sources that vary in their spatial distribution, their rates of decomposition, and their rates of delivery to the detrital pool. Levee breach projects are a typical example of restoring for capacity, where one or more channels are excavated through levee walls to restore tidal inundation to a historical wetland. Evidence shows that the resulting ecosystem may eventually achieve similar structure and function to undisturbed reference ecosystems, but does so with reduced connectivity with the surrounding landscape because the remaining levee does not allow sheet flow or over-bank circulation (citation). Given growing evidence outlining critical ecosystem functions reliant on connectivity, more recent restoration efforts have undertaken full levee removal projects which more completely address both system capacity and connectivity. In contrast with levee breach designs, complete levee removals are thought to enhance cross-ecosystem connectivity between the river, marsh, and nearshore marine ecosystems by allowing over-bank inundation from both the upstream (fluvial flooding) and downstream (tidal) directions.   Because circulation is the principle vector for OM transport in estuarine systems, increased hydraulic connectivity likely supports cross-boundary food web subsidies of OM, a phenomenon shown to increase secondary production in a variety of ecosystems (Polis *et al.* 1997). Although estuarine restoration is no longer in its infancy, questions still remain regarding how ecosystem functions, such as food web connectivity and production, respond both over time, and to alternate restoration designs. For example, rigorous testing of the effectiveness of complete levee removal restoration designs in achieving enhanced connectivity have yet to be explored.

Despite large-scale, estuarine mixing, there is strong evidence for spatial compartmentalization in which detritus-based food webs mirror local differences in available primary producers along the estuarine gradient. For instance, application of stable isotopes has revealed strong gradients in the sources of OM supporting estuarine consumers at the scale of meters to tens of kilometers (Gordon *et al.* 1985, Deegan and Garritt 1997, Gordon and Goni 2003, Guest *et al.* 2004), for both motile fish and sessile invertebrates (Ruckelshaus *et al.* 1993, Hill *et al.* 2006, Vinagre *et al.* 2011, Green *et al.* 2012). The sensitivity of stable isotope geochemistry to track changes in detritus-based food web sources and organization with tidal wetland restoration has shown increasing promise and refinement (Kwak and Zedler 1997, Weinstein *et al.* 2000, Currin *et al.* 2003, Moseman *et al.* 2004, Wozniak *et al.* 2006). In this study, we take advantage of this phenomenon to investigate food web support and connectivity in restoring and natural marsh ecosystems in the Skokomish River estuary, Washington, USA, using Pacific blue mussels (*Mytilus trossulus*) as an indicator organism. Specifically, we use stable isotopes in combination with Bayesian mixing models to evaluate: (1) how food web support shifts seasonally; (2) how the sources of food web support vary among restoring and reference marshes; and, (3) whether within-marsh food web connectivity differs between restoring marshes and natural marshes, quantifying OM support subsidies with increasing distance from marsh channel outlets into interior marsh ecosystems and intertidal flats. We then examine whether patterns in mussel growth track patterns in OM support. Finally, we model scenarios of landscape change, quantifying the effects of marsh ecosystem loss or restoration on detritus-based food web support.

Methods

Study Area

Located in southern Hood Canal, WA, USA (47° 20’N, 123° 7’W), the Skokomish River estuary drains a 588 km^2 watershed, much of which is located within the boundaries of Olympic National Park (Fig. 1). The upper watershed is characterized by steep gradients and high-energy streams, while the lower ten miles of the river flow through a broad floodplain (Batts 2005). Like many Pacific coast estuaries, the Skokomish estuary was converted to agricultural land in the early 1900’s. As a result, connectivity between the river, marine ecosystem, and marsh plain was obstructed in two major portions of the estuarine delta; Nalley Farm, a marsh island between the main Skokomish River mouth and Nalley slough, and the area of marsh located just west of Nalley slough (Fig. 1).

Two restoration events have occurred in the estuary: a natural levee breach and full levee removal, slightly over a decade apart. The natural levee breach at Nalley Farm (NF) occurred during a large flood event in the winter of 1996. The breach is located on the seaward margin of the island, restoring access to one of the historical tidal channels penetrating the island's interior marsh. Ownership and management of the estuarine delta was subsequently transferred from the City of Tacoma to the Skokomish Tribal Nation in 2009. Given their historical and cultural dependence on the river for sustenance, the Skokomish Tribal Nation immediately implemented a large-scale habitat restoration effort aimed at recovering salmon populations in the watershed. A dedicated restoration action in 2007 involved removal (to the level of the marsh plain) of the levee encircling 43 ha of abandoned agricultural lands west of the NF site (Fig. 1). Tidal access was further enhanced by the excavation of three deep cuts where historical tidal distributary channels were located. The levee removal (LR) site is located directly west of NF across the shallow Nalley Slough. In both areas, tidal access has been restored to historical marsh ecosystems once used for agriculture at the mouth of the Skokomish River, and more recently used for access to power-line towers. This project was initiated in 2009 and completed in 2010. A second, 86 ha levee removal and drainage ditch filling has since been completed (2010 after completion of this study) at NF such that levees no longer restrict tidal inundation to or fluvial flooding of the entire island.

Study Design

We sampled three discrete areas of the estuarine emergent tidal marsh ecosystem (Fig. 1). The Natural Marsh (NM) site was located furthest to the west, receiving the least amount of freshwater discharge from the Skokomish River, but connected to small amounts of direct freshwater inputs via several small creeks. The two year old levee-removal (LR) site, where the levee was reduced to the elevation of the marsh plain and historic channel connections were breached across the levee footprint, is adjacent to the NM site but separated hydraulically under low freshwater and normal tidal conditions. The 13-year old restoration site, Nalley Farm (NF) island, is located east of the LR site across Nalley Slough.

At each site, there were one or two narrow openings or channel outlets between the marsh and marine ecosystems. We conducted our sampling along each of the three outlets (one per marsh) Four sampling locations were placed in each marsh: (1) in the interior of the marsh (IMS); (2) inside the channel mouth (IMO); (3) outside the channel mouth (OMO); and, (4) in the intertidal (INT) ecosystem directly downstream of the other sampling sites (Figure 1).

Five main sampling periods were conducted: September and December 2009, and March, June, and September 2010. Due to high flows during December 2009, we were unable to access all but the intertidal location at NF. High river discharge later in the winter washed out the sampling cages at this site as well. As a result, growth and isotope data are not available for NF during December and March, except for intertidal (INT) mussels during December.

Mussel translocation, collection, and growth

Mytilus trossulus was used as a representative consumer organism because of its ability to adapt to a variety of environmental conditions (Elliott *et al.* 2008). Although the exact species we used is uncertain, as *M. trossulus* , *M. galloprovincialis,* and *M. edulis* <40 mm long are only distinguishable via genetic techniques (Moreau *et al.* 2005, Elliott *et al.* 2008), *M. trossulus* is the dominant species on the Pacific coast of North America (Riginos and Cunningham 2005). *M. trossulus* are suspension-feeding generalists that selectively assimilate the organic fraction of available seston (Bayne 1976). Thus, we expected their isotopic signatures to closely reflect the composition of POM in the seston, rather than a strongly selected POM component. However, when environmental concentrations and quality of seston are low, *M. trossulus* will ingest both the inorganic and organic fractions together (Arifin and Bendell-Young 1997).

At each of the locations within the three marshes (i.e., INT, OMO, IMO, IMS), we collected for stable isotope analysis five naturally occurring individual mussels during each sampling period. Collected mussels were bagged by location, and kept cool on ice in the field. Additionally, we placed five cages, with each cage containing five individual juvenile mussels at each study location. Cages were constructed of 1-cm plastic mesh and anchored 10 cm above the substrate on rebar stakes. Caged mussels 1-3 cm in total valve length were collected from one location 3 km north of the study sites to minimize differences in environmental histories and genetic composition, which are known to influence growth rates (Dickie *et al.* 1984). Prior to translocation, individual mussels were tagged with a bee tag secured with marine Splash Zone® epoxy putty, each tag bearing an identifying number and color. At the initiation of the experiment and subsequently during each sampling trip, we used calipers to measure the lengths of each individual along the long axis of their valves to quantify growth.

Mussel growth rates were standardized by inundation time between sampling dates. Exposure-inundation times and elevation of the sites were determined by surveying cage locations in reference to local tidal charts. Cage elevations were lowest at the intertidal sites (= 0.4 ± 0.27 m relative to MLLW), and highest at the inner marsh sites (= 1.13 ± 1.06 m relative to MLLW). Cage elevations were higher on average in the natural marsh site (= 1.13 ± 0.62 m) and lowest in the NF site (= 0.15 ± 0.26 m). Based on location specific elevations, the range of submergence times for cages was estimated per day as follows: 15-25hr INT, 15-24hr OMO, 15-26 hr IMO, and 11-26 hr IMS, depending on the marsh and date.

Primary producer collection

Displaying one of the last remnant examples of the natural vegetation continuum across estuarine ecotones in Puget Sound, the Skokomish River estuary contains a mosaic of ecosystem types, including forested tidal wetlands, scrub-shrub tidal wetlands, oligohaline and euryhaline marsh, mudflats, and *Z. marina* beds. Dominant sources of OM from each ecosystem were collected to provide isotopic baselines of primary production available to the natural and caged mussels—including (1) marine POM (phytoplankton proxy), (2) benthic microalgae, (3) macroalgae, (3) eelgrass, *Z. marina*, (4) scrub-shrub vegetation, and (5) emergent marsh and adjoining wetland vascular plants—were collected during June 2009 (Table 1). Marine POM was collected with a 0.5-m, 30-µm plankton net towed behind a small boat in Hood Canal. Samples of benthic microalgae (diatoms) inhabiting the intertidal and shallow subtidal sediments were collected using a method adapted from Cloern *et al.* (2002). Triangular 0.25-m^2, 20-µm mesh Nitex screens were distributed haphazardly on exposed mudflats in areas of observed high benthic diatom cover. Screens were pulled and rinsed clean after 2-4 hr exposure, depending on ambient light levels and visual assessments of diatom migration into the screens. Samples of benthic microalgae and phytoplankton were filtered through 100-µm sieves in order to remove larger detritus fragments and organisms, examined under a dissecting scope to ensure that the majority of the sample was composed of live algal cells, before vacuum filtering onto pre-combusted (500°C, 4 hr) 0.2-µm Whatman GF/F glass fiber filters and freeze-dried for 24 hr. Four replicate samples from the apical foliage of vascular plants and macroalgae were collected from each of the dominant species in the marshes and peripheral wetlands around the estuary.

Seston collection

To quantify food availability and quality, three replicate seston samples for bulk POM concentration and chlorophyll *α* (chl *α*) concentration were collected from a small boat during high slack tide at each marsh sampling station and date. To determine bulk POM concentration, we vacuum filtered 100 ml of seston water samples through pre-weighed 0.2-µm Whatman GF/F glass fiber filters, dried the filters at 60°C, and weighed them on a microbalance. We characterized seston quality by quantifying chl *α* concentration and C:N ratio. Chl *α* was collected from seston samples filtered onto 0.2-µm Whatman GF/F glass fiber filters. Chl *α* was then extracted for 24hr using 90% acetone, then measured using a fluorometer (Holm-Hansen and Rieman 1978). C:N ratios of seston were quantified from bulk seston samples collected using a 0.25-m diameter, 20-µm mesh Nitex plankton net towed against the current. POM seston samples were passed through a 100-µm sieve to remove coarse particulate matter, vacuum filtered onto pre-combusted (500°C, 4 hr) 0.2-µm Whatman GF/F glass fiber filters, and frozen prior to freeze-drying and transfer to tin capsules for elemental and stable isotope analysis. Due to high inorganic content of bulk seston samples, C:N data was only obtainable using this method during September 2010, as we simply did not manage to collect enough POM for successful analysis during the other sampling periods.

Site metrics

Three replicate measurements of temperature and salinity were obtained at the water surface and the sediment interface from each marsh location during each sampling period using a YSI probe. As described above, location elevations within each marsh were determined by surveying cage locations in reference to local tidal charts and time of sampling using a surveying rod. We took three depth measurements at each location, using the average depth to calculate inundation times.

Tissue preparation and isotope analysis

Collected mussels and OM sources were frozen until processing in the lab. Foliage of OM sources was rinsed in dilute 10% HCl to remove soil carbonates, then rinsed in deionized water until neutral pH. Mussel specimens were thoroughly rinsed in deionized water to remove sediments, then muscle tissue was removed for isotope analysis. Mussels were processed individually. We followed the methods of Arrington and Winemiller (2002) to prepare mussel tissues and OM sources for stable isotope analysis. Samples were freeze-dried for 48 hr and ground to a fine powder using a Wig-L-Bug® dental mill and a stainless steel vial and ball pestle. Samples were weighed into tin capsules for isotope processing of δ^{13}C, δ^{15}N, and δ^{34}S. Isotope analyses were performed by Washington State University's Stable Isotope Core lab using a Costech Analytical ECS 4010 elemental analyzer connected via a gas dilution to a Thermofinnigan Delta PlusXP mass spectrometer.

Isotopic ratios are expressed in δ notation, which indicates the enrichment (+) or depletion (-) of the heavy isotope relative to the light isotope of an element compared with the standard substance:

δX(‰) = [$R_{sample}/R_{standard}$)-1] x 10^3

where X = ^{13}C, ^{15}N, or ^{34}S, and R = ^{13}C/^{12}C, ^{15}N/^{14}N, or ^{34}S/^{32}S. Sulfur isotopic ratios are relative to the VCDT (Vienna Canon Diablo Troilite) standard; the standard used for carbon was VDDB (Vienna Peedee belemnite), and atmospheric nitrogen was used as the standard for δ^{15}N.

Mixing model diet estimations

We used Semmens *et al.'s* (2009) hierarchical Bayesian stable isotope mixing model to estimate the proportional contributions (assimilation strength) of OM sources to mussel diets. The model is an extension of the mixing model MixSir (Moore and Semmens 2008), and allows for the estimation of individual diet heterogeneity in addition to estimating the proportional contributions of OM sources assimilated by mussels at the group or population level. The model addresses uncertainty in data sources by incorporating the means and variances of OM source isotope signatures as well as fractionation rates of each isotope. *Mytilus* sp. fractionation values of 2.17 0.09‰ for δ^{13}C and 3.78 0.10 for δ^{15}N were obtained from Dubois *et al.* (2007). Because we could find no bivalve-specific sulfur fractionation rates in the published literature, we adopted a more generic fractionation value of 0.5 0.31‰ for δ^{34}S (McCutchan *et al.* 2003).

While the model can incorporate a wide number of discrete OM sources, we found it difficult to achieve model convergence using the full suite of OM sources available in the estuary. This likely occurred because the model requires the isotopic value of each source to be distinguishable in order to be considered a separate source. We therefore coalesced OM sources with similar isotope values (Table 1) based in results of the SOURCE model (Lubetkin and Simenstad 2004), which designates an acceptable separation value of 0.1 NND^2 using a nearest neighbor distance test.

For the descriptive portion of this study, non-informative priors were used for each OM source, indicating no *a priori* preferences or differences in availability among OM sources (Moore and Semmens 2008). For the modeling portion of this study (see below), we assigned each OM source category a prior equal to its relative areal abundance in the estuary under different landscape conditions (i.e., historical, altered for farmland, restored, etc.). In all cases, Gibbs sampling was performed for each model using two parallel chains in JAGS (Plummer 2003). Following a burn-in phase of 19000 vectors, 20000 remaining vectors were sampled (retaining every other sample). Convergence and diagnostic statistics were performed using the CODA package (Best *et al.* 1995).

Modeling scenarios of landscape change

One of the advantages of Bayesian modeling is the ability to incorporate prior information (priors) describing observed relationships among model components, thus allowing for more informed model output evaluation (Hilborn and Mangel 1997). Priors in of stable isotope mixing models are usually implemented to describe the proportional contributions of specific prey categories derived from gut content analysis data. This approach helps to guide model selection towards the more likely scenarios, given the data. When attempting to estimate the proportional contribution of OM sources to a consumer organism in detritus-based food webs, it is impractical, if not impossible, to obtain such prior information— hence the use of stable isotopes to elucidate food web pathways. However, data describing the relative proportions of available OM sources present in an ecosystem may exist, especially in systems that have been extensively mapped. In our case, we used the areal extent of major OM source categories within the Skokomish River estuary as priors, assuming that areal extent relates to the proportional contribution of each source category to seston composition. The specific model we used, however, assumes uniform availability of all "prey" sources in the environment (Eric Ward, pers. comm.), which, in our case, are the sources of OM. Thus, the way in which we designed our modeling exercise violates this assumption. However, we feel the violation is justified as it is well known that potential prey items are not uniformly available in the environment either. We thus contend that using detrital availability proportions as priors is little different from using priors derived from gut content analysis, which, especially in the case of non-selective generalist feeding, also reflects prey availability in the environment.

Within the Bayesian framework discussed above, we modeled scenarios of landscape change, quantifying the effects of marsh ecosystem loss or restoration on food web support. Landscape scenarios were based on five eras in the estuary: (1) historical conditions prior to human development circa 1884; (2) "altered" post-levee construction circa 1958; (3) "natural restoration" circa 1996 when the levee breached at NF; (4) "mid-restoration" circa 2007 after full levee removal at the LR site; and, (5) "post-restoration" circa 2012 following the full levee removal and reconnection of tidal channels at NF. Because detailed vegetation maps of the Skokomish estuary are non-existent for many of the time periods, we simplified the types of OM sources to five categories: tidal fresh/oligohaline marsh, euryhaline marsh, benthic diatoms, *Zostera marina*, marine macroalgae, and phytoplankton.

Each category was assigned a prior in the model based on that source's approximate proportional areal extent in the estuary during each time period (Table 2). We focused on temporal shifts in the extent of marsh and *Z. marina* ecosystems, largely holding the other categories constant because there is little documentation of historical shifts in marine macroalgae and mudflat ecosystem extents. Using the recent ecosystem change analyses for the estuary (Collins and Sheikh 2005, Simenstad et al. 2011), we associated benthic diatoms with tidal flat area (570.95 ha), marine algae with lagoon areas (1.83 ha prior to 2007, 11.89 ha after 2007), and estuarine mixing zone marsh vegetation with the "marsh and channel" categorization (226.07 ha in 1884, 673 ha under 2012 conditions). Interim time periods of marsh ecosystem extent were achieved by adding or subtracting the known area of the NF (86 ha) and LR (43 ha) sites to the 2011 estimates as appropriate. The difference between our "mid-restoration" scenario and our "post-restoration" scenario is that the natural breach at NF did not result in full inundation of the island. Using aerial photographs of the island's vegetation, we determined a difference of approximately 40 ha between the "natural restoration" (1996) conditions and the "post-restoration" (2012) conditions, in which nearly the entire island is inundated during high tide. *Z. marina* area was determined based on recent mapping efforts (2010) for the area (121.75 ha) by the Department of Natural Resources (Jeff Gaeckle, unpublished data), as well as pre-disturbance conditions (146.7 ha) as estimated by Jay and Simenstad (1996). We considered phytoplankton to be available wherever inundation occurs, which is equal to the total area of the previous categories. We therefore assigned this category a prior of 0.5. The model scenarios were compared to a final scenario with uninformed priors, allowing us to determine whether the method of using priors to describe ecosystem configuration can be used to observe and predict shifts in food web response to large-scale changes in estuarine ecosystems.

We chose to use the isotope signatures of mussels inhabiting the intertidal (INT) locations in order to examine whether increasing marsh area via restoration efforts affects the export and consequent assimilation of marsh detritus by organisms outside the marsh ecosystem. This allowed us to assess whether restoration efforts increase food web connectivity between the marsh and intertidal flat ecosystems.

Data analysis and statistics

Data were analyzed using Microsoft Excel®, SPSS (univariate statistics), R (mixing model), and Primer 6 with PERMANOVA+ (multivariate statistics) software. We performed ANOVAs to test for differences in seston concentration, chl α concentrations, and C:N ratios across dates, marshes, and locations. Bonferroni *post hoc* tests were performed to identify significant differences within factors.

Multivariate analyses were used to compare consumer isotope signatures and estimated OM source assimilation strengths derived from the Bayesian mixing models. The mixing model produces posterior probability distributions describing the proportional contribution of each OM source to individual consumers. We extracted the median value from these distributions for use in statistical analyses after conferring with model authors regarding the most appropriate summary metric (Eric Ward; pers. comm.). All mixing model output was square-root transformed prior to further analyses, as the data are proportional (Schafer *et al.* 2002). Similarity matrices for mixing model output were constructed using the Bray-Curtis similarity coefficient, while those created for isotope data used Euclidean distance (Clarke and Warwick 2001). We used nonmetric multidimensional scaling (NMDS) in combination with permutational multivariate analysis of variance (PERMANOVA) to visualize and test for differences among factors. PERMANOVA was used to examine the effects of sampling date, marsh, and locations within marshes on isotope signatures, OM source assimilation strengths, and mussel growth. PERMANOVA tests were performed using Type III partial sums of squares and 999 permutations. Each factor was nested in the previous factor, such that no interaction effects were examined.

We performed SIMPER analysis to estimate the average Euclidean distance of mussel isotope signatures between locations within a particular marsh and date. We performed the same procedure on estimated diet proportions, but because diet proportions were based on the Bray-Curtis similarity index, we computed the average percent similarity in diets between locations within a particular marsh and date. We then used these distance and percent similarity values to assess the degree of food web connectivity within the marsh as a whole. Smaller distances indicate more similar isotope signatures, while greater distances indicate stronger differences in isotope signatures. In contrast, higher percent similarity indicates more similar OM source composition, while lower percent similarity indicates less similar sources. We performed a fixed effects linear model to test for differences in Euclidean distances and percent similarities (connectivity indicators) among marshes and dates. Model terms included date, marsh, and marsh site nested within date, allowing us to examine overall date and marsh effects, as well as date-specific differences in the similarity of isotope signatures and diets among locations within each marsh.

We performed a mixed-effects model in PERMANOVA on square-root transformed growth data using the Bray-Curtis similarity index. Sampling date, marsh, and cage locations were treated as fixed factors, with locations nested in marsh, and marsh nested in sampling date. We treated cage number as a random factor, nested in cage location. Permutations of residuals were run under a reduced model using Type III (partial) sums of squares and 999 permutations. We used Primer's DISTLM (distance-based linear model) and distance-based redundancy analysis (dbRDA) (Anderson *et al.* 2008) to quantify the relative importance of specific environmental variables as predictors of mussel growth rates. Explanatory variables included seston concentration, chl α concentration, season, marsh, location, and the assimilation strengths of the ten OM sources estimated by the Bayesian mixing model. Redundancy analysis was performed on growth rate and environmental data averaged by date, marsh, and location, as this was the level at which seston and chl α metrics were measured. DISTLM modeling was conducted using a step-wise selection procedure. The most parsimonious model was chosen using a distance-based multivariate analogue to the univariate AIC selection criterion.

Results

Organic matter sources

The OM sources largely partitioned in isotope space according to location of origin, with the exception of the C_4 marsh grass, *D. spicata,* which had a similar signature to marine algae. Due to overlapping isotopic signatures, the 15 OM sources (Fig. 2) collected in the Skokomish River estuary grouped into ten categories for use with the mixing model (Table 1). Combined sources included the terrestrial/facultative trees *A. circinatum* and *A. rubra,* low marsh herbaceous vegetation *C. lyngbyei, J. balticus,* and *T. maritima,* low marsh succulents *S. virginia* and *G. maritima,* and the marine algae *Ulva intestinalis* and *Fucus distichus.* Uncombined sources included the bulrush *S. maritimus,* the gumweed *G. stricta,* a saltmarsh grass *D. spicata,* the native eelgrass *Z. marina,* benthic diatoms, and phytoplankton. *Z. marina* was the most δ^{13}C-depleted source (-9.11 ± 0.72‰), followed by marine macroalgae (-16.63 ± 0.82‰), benthic diatoms (-19.36 ± 1.73‰), and phytoplankton (-19.53 ± 1.73‰) (Fig. 2). Marsh primary producers were more depleted in δ^{13}C (range of mean δ^{13}C: -29.14 to -14.23‰) compared to terrestrial/facultative trees and the marine OM components, including benthic diatoms, *Z. marina*, marine algae, and phytoplankton. δ^{15}N values were similar among marsh primary producers and the marine components (δ^{15}N range: 2.04 to 8.43‰), but δ^{15}N values of terrestrial trees were much more depleted (δ^{15}N range: -0.18 to -2.9‰). Benthic diatoms were most depleted in δ^{34}S (-11.11‰), followed by *J. balthicus* (-7.59‰). Other primary producers with depleted δ^{34}S values included *C. lyngbyei, T.*

maritime, A. circinatum, and *A. rubra* (δ^{34}S < 5‰). Phytoplankton and marine macroalgae were most enriched in δ^{34}S (δ^{34}S > 19‰).

Seston concentration, chl α, and C:N ratio

Seston concentrations were significantly different among sampling dates, marshes, and locations (ANOVA, F = 86.54, p < 0.001, F = 19.73, p < 0.001,F =2.88, p = 0.039, respectively). Seston concentration was highest in September 2009 (0.23 ± 0.05 mg-ml^{-1}) and lowest in December 2009 (0.07 ± 0.03 mg-ml^{-}1) (Figure 3b). The natural marsh, and the OMO location exhibited higher average seston concentrations compared to the other marshes and locations, while the LR marsh and IMO location exhibited lower concentrations (Figure 3a,c). Bonferroni *post hoc* tests show that seston concentrations do not differ between the two restoration sites, but that the natural marsh has significantly higher concentrations of seston as compared to either of the restoration sites (NM:LR, p < 0.001, NM:NF, p < 0.001).

Chl *α* concentrations were significantly different across dates, marshes, and locations (ANOVA, F = 154.32, p < 0.001, F = 34.43, p < 0.001, F = 6.00, p = 0.001, respectively). Chl *α* concentration was highest in the natural marsh, in September 2009, and in the INT location (Figure 3 a-c). The lowest chl *α* concentration was observed in March 2010, the NF marsh, and the IMS location. Significant differences in chl *α* concentration were observed between the natural marsh and the two restoration sites (Bonferroni *post hoc* test, NM:LR, p < 0.001, NM:NF, p < 0.001), but not between restoration sites.

As described earlier, C:N data for seston was only obtainable in September 2010 from a limited number of locations such that we could only test for differences across marshes. Seston C:N ratios were not significantly different among marshes, although lower average C:N seston ratios were noted at the natural marsh (Figure 3h).

Isotopic signatures of mussels

Mussel δ^{13}C values ranged between -22.15‰ to -16.87‰, δ^{15}N values between 7.40‰ and 11.23‰, and δ^{34}S values between 11.00‰ and 20.91‰ (Table 3). Isotopic signatures of mussels were significantly different across sampling dates, marshes, and locations (PERMANOVA, date: F = 42.67, p < 0.001, marsh (date): F = 22.46, p < 0.001, location (marsh(date)): F = 3.21, p < 0.001). Isotope signatures were significantly different across sampling dates, for every pair-wise test (all p < 0.01 except December 2009 x March 2010, p = 0.047). Likewise, mussel signatures were consistently different among marshes within each sampling date ($p < 0.05$), with the exception of one instance (March 2010, NF and LR, p = 0.097). While mussel isotope signatures were significantly different among locations (within a marsh and sampling date), there was no consistent pattern of which location pairs were different. However, mussels inhabiting the INT location often exhibited significantly different isotope signatures compared to the other locations. Likewise, mussels inhabiting the IMS location furthest into the marsh often exhibited significantly different isotope signatures compared to the other locations. The two intermediate locations (OMO and IMO), were not often significantly different from one another.

We observed significant seasonal differences in the average Euclidean distance between pairs of locations within a marsh (Table 4), indicating that the degree of food web connectivity within a marsh is influenced by season. Average Euclidean distance was greatest in March and least in September 2009, indicating greater connectivity in September compared to March. Average Euclidean distances in mussel isotope signatures were also significantly different among the three marshes when the marsh variable was not nested in date (Table 4). Average distances were higher in the restoration sites compared to the natural site (LR: = 1.43 ± 0.60, NF: = 1.52 ± 0.67, NM: = 1.23 ± 0.52), indicating less connectivity in the restoring marshes. However, when marshes were nested within sampling date, no significant marsh effects in the average Euclidean distance were evident between pairs of locations within a marsh.

Estimated proportional contributions of OM sources to mussels

The composition of OM sources assimilated by the mussels significantly differed by date, marsh, and location (PERMANOVA: date: F = 877.71, p = 0.0001, marsh (date): F = 527.76, p = 0.001, location (marsh(date)): F = 7.67, p = 0.001, Fig. 4). Mussels assimilated marsh vegetation detritus most strongly in December 2009 (= 62.7 ± 0.09%) and in the LR site (= 46.6 ± 0.14%, Fig. 5). Generally, marsh detritus comprised 30 to 60% of mussel diets (Table 5). Macroalgae comprised a larger component of mussel diets in September (2009: = 28.4 ± 0.13%, 2010: = 29.4 ± 0.10%) as compared to the other sampling periods (< 17%), but very little was assimilated by mussels in the LR marsh (= 12.7 ± 0.09%) compared to the NM (= 27.5 ± 0.08%) and NF (= 32.8 ± 0.11%) sites. Phytoplankton was most prevalent in mussel diets in March (= 36.4 ± 0.09%) and June 2010 (= 35.0 ± 0.09%), and least prevalent in December (= 0.10 ± 0.01%). In most cases, trees, benthic diatoms and *Z. marina* were minimally assimilated (< 2%) by mussels across sites and dates, although *Z. marina* contributed > 10% to LR mussel diets in September and December 2009.

As measured by % similarity, OM source compositions were slightly more similar between the young restoration marsh (LR), and the natural marsh (NM) than between the older restoration marsh (NF) and the natural marsh (Table 4), but this difference was not significant. Mussel diets in the youngest marsh were significantly less similar ($t = 2.4$, $p = 0.04$) to those in the older restoration marsh (Table 6), than they were to those in the natural marsh. Diets in NF were not significantly more similar to natural marsh diets than they were diets in the young restoration site.

We observed significant seasonal differences in the average percent similarities between pairs of locations within a marsh ($F = 9.62$, $p < 0.001$), indicating the degree of food web connectivity within a marsh varies by season. Diet similarities were highest in December 2009, when detritus dominated mussel diets, and lowest in March 2010, when phytoplankton dominated. Average percent similarity in mussel isotope signatures was also significantly different among the three marshes (Table 4, nested and not nested in date). Diet similarities were highest in the LR restoration marsh, followed by the NM, and NF (Table 4).

Mussel growth rates

Mussel growth rates differed significantly across dates, locations, and individual transplant cages, but not among marshes (PERMANOVA, date: $F= 32.95$, $p = 0.001$, marsh (date): $F = 1.66$, $p < 0.09$, location (marsh(date)): $F = 1.85$, $p = 0.002$, cage (location (marsh(date)): $F = 1.40$, $p = 0.001$). The highest growth rates were observed between March and June 2010, where mussels located at IMO in NF reached growth rates > 0.05 mm- d^{-1}, followed by the growth period between June and September 2009 (Fig. 6). Growth rates remained below 0.02 mm d^{-1} between September 2009 and December 2009, December through March 2010, and June through September 2010.

Sequential tests of the explanatory contribution of OM sources to growth rate indicated that the assimilation of *S. virginia* and *G. maritima* explained the most variation (20.2%), followed by chl α concentration (8.9%), *Z. marina* (8.9%), *C. lyngbyei, J. balticus, T. maritimus* (7.4%), marine algae (7.2%), *G. stricta* (2.4%), and seston concentration (2.3%). The only variables without a significant relationship with the growth data were *G. stricta* and seston concentration; all other variables were significant at the $p < 0.05$ level. Together, the predictor variables in the best model explain 57.2% of the total variation present in the mussel growth data (Figure 7).

Modeling scenarios

Results of the different Bayesian mixing modeling scenarios indicated that estimated mussel OM source assimilation varied significantly according to marsh extent and connectivity (PERMANOVA, F = 10112, p = 0.004). The model based on uninformed priors produced diet estimates more similar to contemporary conditions (post 1990's) as compared to historical (1888) or strongly altered (~1938) conditions (Table 7). Compared to more historical conditions, the estimated assimilation of phytoplankton and euryhaline marsh detritus was lower for mussels under the more contemporary model scenarios, while the assimilation of macroalgae was higher (Fig. 8). Estimated mussel diet similarities among interim restoration scenarios (i.e., 1884, 1958, 1996, and 2007) ranged between 76% and 99%. Among the restoration scenarios using informative priors, we observed the strongest response for marine algae, which contributed and average of 27% to mussel diets in the post-restoration scenario, but less than 1% in the other scenarios. However, shifts in the estimated proportions of OM sources among restoration scenarios were minimal (< 1% among scenarios).

Discussion

Our findings show significant temporal and spatial heterogeneity in food availability, food quality, and OM assimilation by mussels inhabiting two restoring marshes and one natural emergent marsh within the Skokomish River estuary. Significant differences in mussel diets suggest trophic equivalency among restoring and reference marsh ecosystems has yet to be achieved, an expected result given the relatively short time since restoration implementation. However, the trajectory of trophic equivalency within the estuary does not appear to follow the expected course of progression. In line with current scientific understanding regarding functional equivalency trajectories in restoring estuarine systems (Morgan and Short 2002, Simenstad *et al.* 2006, Borja *et al.* 2010), our results suggest that landscape-scale processes and configurations, as well as site-level characteristics and restoration approaches combine to determine the character of a particular location's path towards recovery. We contend that these considerations are especially pertinent to the restoration of estuarine ecosystems, in which ecosystem capacity for OM support of food webs is equally important as ecosystem connectivity to the surrounding landscape. We find support for these assertions by comparison of trophic support, diet similarity, and mussel growth rates among two restoring and one reference marsh ecosystem.

Seston quantity and quality

Bulk concentration, chl α concentration, and the C:N ratio of seston provide preliminary evidence of small-scale spatial and temporal heterogeneity in seston quality and quantity among study marshes (<2 km apart), as well as among sampling locations within a marsh (< 0.25 km apart). While materials and energy are rarely distributed homogeneously across the landscape, instead usually concentrated in patches (Puth and Wilson 2001), such small scale heterogeneity is notable, but not uncommon (Ruckelshaus *et al.* 1993, Guest *et al.* 2004), within estuarine ecosystems where tidal fluxes and fluvial forcing coincide to transport detrital particles across ecosystem transitions, aka ecotones (Polis et al. 1997).

While small-scale spatial heterogeneity in seston food availability has been described in other estuarine systems (Ruckelshaus *et al.* 1993), the consistency with which we observed within and between-marsh differences suggests landscape features can be a pervasive influence the quality and availability of seston in the Skokomish River estuary. Of particular interest, seston appears less available and of lower quality in the two restoration marshes as compared to the natural marsh site. The natural site was characterized by higher seston concentration, higher chl α concentration, and lower C:N ratios, indicating less refractory materials in the seston pool. Differences in seston quality and availability can be caused by differences in sediment composition among the three marshes. Previous studies have documented consistent coupling between seston and sediment composition in tidal marsh ecosystems (Carmichael and Valiela 2005). Seston within estuarine marsh channels may therefore track restoration trajectories described for sediments. Given that reference marshes have exhibited lower sediment C:N ratios and higher OM content in comparison to restoring coastal marshes elsewhere (Morgan and Short 2002, Able *et al.* 2008), our data further suggest that undisturbed marshes often offer higher quality food resources compared to newly restoring areas. Seston concentration and C:N ratios were second highest at NF, the older of the two restoration sites (13 yrs), further indicating that seston quantity and quality may increase with increasing restoration status (age). However, observed seston C:N ratios were less than 10 at all three marsh sites (measured in September 2010), indicating that seston was of sufficient nutritional value for assimilation by mussels regardless of restoration status. C:N ratios greater than 17 generally indicate inadequate nutritional value for estuarine invertebrates (Russel-Hunter 1970).

As with C:N ratio and seston concentration, we observed higher chl α concentrations in the natural marsh site as compared to the two restoration sites, providing a third indication of higher food quality and quantity in the undisturbed system. Elevated chl α concentrations at the natural marsh site may result from three separate mechanisms. Firstly, the natural marsh site receives less fluvial influence than the two restoring marshes, which are more directly connected to the mainstem Skokomish River through Nalley Slough. If chl α is representative of phytoplankton biomass advecting into marsh ecosystems from Hood Canal, then it is possible that seaward physical forcing from river outflow reduces phytoplankton advection into the two restoration sites. However, salinities measured during high slack tide did not vary dramatically across the three marshes, suggesting that this mechanism is less likely. Alternatively, chl α concentrations may be higher in the natural marsh location because the opening between the marsh lagoon and the open water marine environment in southern Hood Canal is much larger at the natural marsh location as compared to the narrow channels leading into the two restoration sites. Given that suspension-feeding organisms have the ability to drawn down phytoplankton populations, an observation noted at the scale of meters as well as kilometers *(*Noren *et al.* 1999, Banas *et al.* 2007), it is possible that chl α concentrations diminished more strongly in the narrow-mouthed restoration sites where consumers and water flow were concentrated in a smaller area. A third possibility is that the natural marsh location produces higher quality seston compared to the restoration sites, as chl α in the seston can originate from sources other than phytoplankton, including resuspended benthic microalgae, vascular plant detritus, and macroalgae detritus. This explanation falls in line with past studies quantifying functional equivalency of detritus-based trophic support among restoring and reference marsh ecosystems; near-equivalency was observed in restoration sites older than eight years, and strong differences were observed between reference ecosystems and restoring sites less than five years old (Howe and Simenstad 2007, Llewellyn and La Peyre 2011). However, the observed decrease in chl α concentration from the INT locations to the IMS locations suggests that the majority of available chl α fluxes into the marsh ecosystem where it is subsequently consumed by marsh consumers. Thus, we surmise that elevated seston quantity and quality within the natural marsh site most likely results from greater connectivity to Hood Canal's marine ecosystem as compared to the restoration marshes, which may be more influenced by the freshwater plume emanating from the Skokomish River.

Mussel isotope signatures and estimated diets

The diets of *M. trossulus* appear to track seasonal trends in OM availability. Phytoplankton consumption was highest in March and June, coinciding with the typical timing of spring blooms in Puget Sound and Hood Canal (Khangaonkar *et al.* 2011). While it has long been suggested that estuarine and marine bivalves assimilate most of their dietary OM through the uptake of phytoplankton (Widdows *et al.* 1979, Asmus and Asmus 1991, Dame and Prins 1998), mussel diets in the Skokomish River estuary never reflected phytoplankton contributions greater than 50%, even during peak bloom conditions. Increasingly, the application of stable isotopes has revealed that mussels and other suspension feeders often assimilate macroalgae detritus for large portions of their diets (Duggins *et al*. 1989, Bustamante and Branch 1996, Hill *et al.* 2006). However, we have found that the proportion of marsh detritus often exceeded that of macroalgae and phytoplankton, depending on the season, in the Skokomish River estuary. Even during peak macroalgae senescence in September, mussels assimilated similar proportions of marsh- and macroalgae-derived OM. Macroalgae does not exhibit high biomass in the estuary compared to vascular marsh producers (personal observation), so mussels may indeed select for nutritionally valuable and bioavailable algal detritus when it becomes available. Nevertheless, our results suggest that marsh detritus is palatable enough to comprise a major portion of mussel diets year round, likely due to microbial and fungal protein enrichment (Torzilli *et al.* 2006). Phytoplankton consumption was largely replaced by the assimilation of marsh detritus during the winter, when phytoplankton concentrations are typically at their lowest. That mussels assimilated 30% to 60% of their diet from marsh detritus was unexpected given previous discussions of the refractory nature of this food source in comparison to algal sources (Mueller-Solger *et al.* 2002, Sobczak *et al.* 2002).

Mussel diets were significantly different across dates, marshes, and among locations within each marsh. These differences signify trophic equivalency has yet to be reached in either restoration site in the estuary. Given the relatively young age of the restoring marshes (LR = 2yrs, NF = 13 yrs), this result is not surprising. Trophic equivalency exhibits one of the slower recovery trajectories, lagging behind physical and chemical marsh characteristics, plant assemblages, and invertebrate and nekton community abundance and assemblage. A twenty year study of coastal restoration sites in Delaware Bay, for example, showed the achievement of sediment grain size and total organic carbon equivalency within one year, benthic macroinvertebrate abundance equivalency within two years, vegetation structural equivalency within five years, and intertidal creek fish abundance within four to five years, depending on the species (Able *et al.* 2008). While gut content analysis suggests that trophic linkages between fish and invertebrate prey can gain equivalency within three years (Able *et al.* 2008), other studies based on stable isotopes indicate that comparable trophic diversity and depth does not become apparent until at least eight years post-restoration (Llewellyn and La Peyre 2011).

Because previous work describing restoration trajectories implies that trophic equivalency can be achieved if given enough time, we expected diets in the older restoration site (NF) to be more similar to the natural marsh (NM) as compared to the difference in mussel diet composition between the younger restoration marsh (LR) and the natural marsh. Instead, mussel diets in restoration sites were statistically similar to the natural marsh. Mean similarity was even slightly higher between the youngest restoration marsh and the natural marsh. Thus, the trajectory of trophic equivalency within the Skokomish River estuary does not appear to follow the expected course of progression. Functional equivalency trajectories, however, are influenced by more than age, and are notorious for being difficult to predict (Simenstad *et al.* 2006). Landscape-scale processes and setting, as well as site-level characteristics and restoration approach, combine to determine the pattern and rate of a particular location's path towards recovery (Simenstad *et al.* 2006). These considerations are especially pertinent to the restoration of estuarine ecosystems because estuaries are inherently open systems, incapable of functioning indefinitely on their own in isolation from the surrounding landscape, and necessarily influenced at multiple scales by their level of connectivity to the land and water masses in the vicinity (Elliott and Whitfield 2011).

That both restoring sites in the Skokomish River estuary demonstrated similar levels of mussel diet equivalency (~80%) with the natural marsh suggests that trophic functionality for suspension feeders in the younger site is approaching equivalency more rapidly than the older restoration site. Given the close proximity of the marshes to one another, landscape-scale factors were likely very similar among the three marshes. Therefore, differences in the pace of trophic development between the two restoration sites were unlikely the result of landscape setting. Rather, site-scale marsh characteristics may have driven food web trajectories among marshes, the most prominent being the restoration action. The older marsh was achieved passively by a storm event that breached the levee surrounding the abandoned farmland on the island. By retaining the remainder of the levee, hydrologic connectivity between the marsh and the surrounding landscape remained severely altered. Given the importance of hydrologic connectivity in estuarine systems, especially with respect to OM transport and assimilation into food webs, the levee surrounding NF may have prevented the marsh from achieving trophic equivalency with the natural marsh system, even after 13 years.

In contrast, the younger restoration marsh reflects extensive planning and engineering. Levees were completely removed, the relict pasture and agricultural fields were tilled, and the footprints of historical marsh channels were reconnected. In comparison to the passive approach, these actions can enhance the initial level of connectivity between restoring marshes and surrounding ecosystems, creating more permeable "boundaries" for OM transport and exchange. As a result, suspension feeding mussels in the younger restoration marsh were likely more able to take advantage of allochthonous subsidies of detritus, leading to more natural diets at an earlier stage in the trajectory of marsh function recovery. To be clear, trophic equivalency for mussels inhabiting the LR marsh was likely achieved by the immigration of allochthonous materials originating from nearby marsh ecosystems, rather than from equivalency in internal production. Clearly, the internal production of the LR marsh was not yet equivalent to the NM or NF marshes— the site is still characterized by broad mudflats with sparse emergent vegetation.

On the other hand, mussel diets across the three sites are remarkably similar (between 70 and 90%) given the disparity in ages and structure among them, and it may actually be illogical to ever expect complete equivalency among them. This may signify that landscape level processes governing detritus-based food web support, such as OM production and exchange, are sufficiently intact at the spatial scale associated with inter-marsh connectivity. Furthermore, the level of diet similarity among marshes is only slightly less than the mean percent similarity (94%) of mussel diets within each marsh. Since estuarine functions are extremely dependent on scale and spatial positioning within the landscape (Simenstad *et al.* 2006, Elliott and Whitfield 2011), perhaps greater diet similarity is not possible to achieve, especially if diet similarity scales with distance between sites. While both restoration sites share similar historical timelines and antecedent land use patterns— factors known to influence functional equivalency trajectories (Simenstad *et al.* 2006)— the island (NF) site will always experience stronger fluvial influences compared to the natural or levee removal marshes due to its position in the Skokomish delta landscape. As salinity strongly affects vegetation community assemblage structure within estuaries (Bertness and Pennings 2000, Tuxen *et al.* 2011), differences in fluvial influence will have ramifications for the types and availability of OM at the base of the food web. Thus, for suspension feeding organisms, the homogenizing effects of cross-ecotone OM exchange may be countered by spatially–explicit heterogeneity in the landscape, such that 100% equivalency would not be expected. Furthermore, organisms whose trophic linkages are less related to the homogenizing effects of OM transport may exhibit even lower rates of diet similarity between restoring sites and reference ecosystems. For example, during their estuarine residence, juvenile Chinook salmon (O. *tshawytsacha*) feed heavily on insects, (Levings *et al.* 1991, Gray *et al.* 2002), the availability, assemblage composition and abundance of which is strongly related to wetland community structure (Jimenez *et al.* 2008, Wu *et al.* 2009, Reynolds and Boyer 2010, Rickert 2011). Thus, for these types of organisms, dissimilarity in diets may not necessarily indicate a failure in restoring trophic function, but rather spatially-explicit relationships between ecosystem characteristics and the organisms that inhabit them.

Food web connectivity among marsh locations in restoring versus reference marshes

Within-marsh food web connectivity was significantly affected by season. Mussel diets were most similar across locations within a marsh during the winter, coinciding with the time period when mussels assimilated the most marsh detritus. Detritus resuspension and transport are typically enhanced during the winter as a result of increased freshwater discharge and wave disturbance associated with storm events (de Jonge 2000), providing a possible explanation for increased diet similarity among locations within marshes during the winter. The lowest measures of food web connectivity were observed in March, when mussels assimilated higher proportions of phytoplankton. As discussed earlier, chl α concentrations decreased along the gradient from the intertidal flats outside the marsh ecosystems to the interior blind channels within them, indicating strong spatial gradients in the availability of OM sources for suspension-feeding mussels. Stronger spatial variations in diets have been described during the summer for mussels elsewhere in Puget Sound (Padilla Bay; (Ruckelshaus *et al.* 1993) and for a variety of estuarine organisms in the San Francisco estuary (Howe and Simenstad 2007, Howe and Simenstad 2011). Increasing spatial variation in juvenile sole isotope signatures has also been described during periods of low river flow in the Tagus River estuary, Portugal (Vinagre *et al.* 2011), indicating that estuarine food web connectivity can be directly related to hydrological transport processes.

When we assessed marsh connectivity based on the multiple isotope values of the mussels, connectivity appeared to be significantly reduced in the restoring marshes compared to the natural reference marsh. However, this pattern was not mirrored in patterns of connectivity measured by diet OM composition. Using diet as a metric, the LR marsh exhibited the strongest degree of connectivity among locations. In both cases, however, the levee breach site (NF) consistently exhibited the least trophic connectivity along the gradient from the open intertidal flats to the interior marsh locations. Trophic connectivity may therefore be less a function of marsh age, and more a function of landscape structure. Further examination of this relationship is needed, as site age and structure are confounded and unreplicated in the present study. Nonetheless, a single levee breach provides fewer connecting corridors as compared to a full levee removal with multiple channels cutting between the marsh, river, and marine ecosystems. If chl α concentrations mostly represent phytoplankton concentrations (see above discussion), then the reduced concentration of chl α observed at NF supports the contention that the single levee breach at NF has reduced the flux of OM exchange at this marsh in comparison to the other two marshes, suggesting greater food web compartmentalization at this site.

Mussel growth rates relative to season, location and diet

Growth rates, standardized by inundation time, differed most significantly with season. Seasonal patterns in mean growth rates matched previous descriptions for Puget Sound mussels (Ruckelshaus *et al.* 1993). We observed the highest growth rates between March and June, which coincides with optimal temperatures for growth and the spring phytoplankton bloom (Khangaonkar *et al.* 2011, Rensel *et al.* 2011). During other periods of the year, growth rates were lower despite fluctuations in food availability and quality. While the availability of organic food particles has been mentioned as the single most important factor determining growth rates in mussels (Seed and Suchanek 1992), these data suggest that food availability and quality, temperature and physiological processes work in conjunction to mediate growth rates throughout the year. For example, gonadal maturation in the early spring may contribute to low growth rates despite high quality seston availability (Kreeger 1993). Furthermore, in the Pacific Northwest, summer low tides occur in the middle of the day, subjecting exposed intertidal mussels to a wide range of temperatures. While warmer temperatures usually elevate growth rates, high temperatures (25 to 35°C) can induce heat shock (Hoffman and Somer 1995). This condition is characterized by protein denaturation, the repair of which is energetically costly (Hoffman and Somer 1995). Heat shock may explain low growth rates between June and September in the Skokomish River estuary. In contrast, low tides during the winter occur during the middle of the night, often exposing intertidal mussels to temperatures below freezing. Combined with lower quality and availability of food, low winter temperatures likely influenced growth rates between September and December.

In addition to seasonal differences in growth rates, growth rates among locations within marshes were significantly different, but inconsistent. In some cases, mussels grew most rapidly in the INT location, while in other cases the most rapid growth was observed in the IMO location. Thus, unlike for chl α concentrations, growth rates do not directly relate to position along the gradient from the interior of a marsh to the intertidal flats. Inconsistent growth rates among marsh locations likely result from the intersection of multiple factors, including, but not limited to diet, exposure, salinity, and seston availability. Current velocities may also play a role in regulating growth rates at the various locations within each marsh. Velocity has a significant, inverted-U shaped effect on the growth of mussels, indicating that *M. trossulus* grow best under intermediate flows, and less quickly in stagnant areas or under high flow conditions (Ackerman and Nishizaki 2004). We did not consider the effect of flow velocity when choosing the locations of transplanted mussels within each marsh, nor did we measure current velocities during the ebb or flood tides. However, the INT, OMO, and IMO locations certainly experienced higher flow velocities than the IMS sites, located in the upper reaches of blind marsh channels (personal observation). As such, inconsistencies in growth rates among marsh locations may reflect a velocity growth response in conjunction with other controlling factors.

Although intra- and inter-marsh differences in OM support and seston quality and seston quantity were statistically significant, they did not translate into differences in mussel growth among the three marshes. Thus, despite significant differences in OM support across the three marshes, our data suggest that diet has minimal functional effects on mussel growth, and that other controlling factors, as described above, play a larger role in determining mussel growth rates. One reason that differences in seston quality and quantity may not translate into differences in growth rates among marshes is that mussels are able to feed in a compensatory manner, changing their filtration rate, assimilation efficiency, and degree of particle selectivity in order to satisfy metabolic demands under shifting conditions (Arifin and Bendell-Young 1997). Another reason that inter-marsh differences in seston quality and quantity may not have resulted in differences in growth rates is that our measurements reflect snapshots in time that may not fully represent the quality and availability of food, especially considering that spatial heterogeneity in seston composition and concentration has been observed over the course of a single tidal cycle (Ruckelshaus *et al.* 1993). Additionally, inter-marsh exchange of detrital matter can link spatially distinct marshes within estuarine systems (Howe and Simenstad 2011). Given the close proximity of these marshes to one another, exchange is probably high in this estuary. The elevated assimilation of marsh detritus in the LR marsh exemplifies strong detrital transport across ecotones within the study area, as this very young restoring marsh produces little vascular plant-based detritus of its own. Finally, although mussel diets were significantly different among the three marshes, the magnitude of those differences was relatively small. It is likely that growth rates would be more responsive to stronger shifts in diet composition.

While much attention focuses on phytoplankton availability in relation to the growth of estuarine suspension-feeders (Cloern 1982, Jassby *et al.* 2003, Banas *et al.* 2007), more variation in mussel growth rates was explained by the assimilation strength of marsh detritus from *S. virginica* and *G. maritima* (20.2%) as compared to indicators of phytoplankton availability, including chl α concentration (8.9%), seston concentration (2.3%), and estimated phytoplankton assimilation strength (<1%). Descriptions of primary producer nutritional content are limited (Kreeger 1993), and the mechanisms of caloric bioavailability are complex and interactive (Tenore 1983, Kreeger 1993, Torzilli *et al.* 2006), making it difficult to ascertain why this type of detritus leads to the higher mussel growth rates we documented in the Skokomish River estuary. However, it is possible that detritus from *S. virginica* and *G. maritima* provides an important source of dietary protein for *M. trossulus*, which elevate their assimilation rate of bioavailable protein during gametogenesis in late winter and early spring (Kreeger 1993). Firstly, salt marsh succulents, such as *Salicornia bigelovii,* are often used to supplement grass-based diets of grazing ruminants, the major advantage of succulent additions being in the form of high crude protein intake and digestibility (Bohra *et al.* 2009). Secondly, succulents such as *S. virginia* and *G. maritima* are distinctly different in structure from other marsh plant genera such as *Distichlis, Spartina, Carex,* and *Juncus.* Plant structure affects microbial and fungal conditioning, as plant type has been shown to be the primary factor responsible for the composition of microbial and fungal communities on dead-standing plants, with very distinct fungal communities observed between grass-type plants and *Sarcocornia* (previously *Salicornia)* (Torzilli *et al.* 2006). If differences in microbial community assemblages affect the timing and bioavailability of detritus, factors associated with the structure of *S. virginica* and *G. maritima* may partially explain why mussel growth rates respond more sensitively to availability of detritus derived from marsh succulents as opposed to other marsh plants.

Observed mean growth rates, ranging between 0.005 to 0.06 mm d^{-1}, were low in comparison to previously observed rates for *M. trossulus,* suggesting conditions within the Skokomish River estuary's marsh channels are limiting to growth. Growth rates of up to 0.2 mm d^{-1} have been reported in experimental flow-through systems, and up to 0.3 mm d^{-1} under docks on the outer coast of Vancouver Island, British Columbia (Ackerman and Nishizaki 2004). Ruckelshaus *et al.* (1993) reported growth rates ranging between 0.03-0.17 mm d^{-1} in Padilla Bay, located in northern Puget Sound. The lower growth rates were observed in the slough habitats, as opposed to mudflat, eelgrass, and neritic habitats, where seston characterization indicated that available POC was largely composed of refractory materials emanating from marsh ecosystems, rather than from $\delta^{13}C$ enriched materials, such as eelgrass DOC, benthic microalgae, or phytoplankton, all of which are thought to be more nutritious food sources (Mueller-Solger *et al.* 2002). Marsh channels in the Skokomish River estuary resemble the slough habitats in Padilla Bay. As such, low overall growth rates in the Skokomish estuary marshes may similarly result from lower quality food resources.

In addition to the possibility of lower quality food sources, our documented mussel growth may be affected by low salinities and long exposure periods during the study period. Low salinities (<15‰) and air exposure impose constraints on the physiological performance of mussels, as mussels respond to both conditions by isolating themselves from ambient conditions and undergoing anaerobic metabolism (Aunaas *et al.* 1988, Sukhotin and Portner 1999). Anaerobic metabolism depletes energy reserves, a phenomenon known as "oxygen debt", leaving less energy available for growth (Sukhotin and Portner 1999). While air exposure induces further stress due to fluctuating temperatures and desiccation, behavioral isolation from low salinity water reduces feeding time beyond the period of inundation. Thus, correcting growth rates for inundation time may not have been sufficient to account for sources of energy depletion beyond feeding time. Finally, turbid conditions characterized by high suspended sediment loads in the Skokomish River estuary are not likely responsible for low mean overall growth rates, as behavioral responses in filtration rate couple with flexible absorption efficiencies to achieve net energy balance despite decreasing organic content of particles during turbid periods (Hawkins *et al.* 1996, Arifin and Bendell-Young 1997).

Modeling restoration scenarios

As described earlier, we tested several scenarios, varying the areal extent of OM sources available to the estuary's food web, as a way to test whether this type of prior information could be used to predict or hindcast shifts in trophic support resulting from changes to landscape configuration (i.e., marsh ecosystem loss or gain via restoration actions). We observed strong differences between the uninformed model and those using areal extents of OM categories as prior probabilities, as well as strong differences between historical conditions (1888 & 1938) compared to the more contemporary scenarios. However, we found few differences in source contributions to mussel assimilation among the three informed models corresponding to different restoration eras (1996- 2012) and landscape configurations within the estuary. Shifting the priors to match marsh areal extent among the three restoration scenarios did little to alter the estimated diets of mussels, indicating that the model is sensitive to large-scale shifts in landscape configuration (hundreds of hectares), but not the meso-scale shifts (tens of hectares) reflective of many real-world restoration scenarios.

The lack of model response to recent shifts in landscape-configuration may result from the use of OM areal extent, which may not be the most appropriate metric to estimate OM availability, and certainly not quality. Annual biomass production, for example, may more accurately reflect the proportion of available OM sources contributing to the detrital pool. However, detailed vegetation mapping of the Skokomish River delta marsh and intertidal flats was not available for the suite of eras examined in this exercise, making it difficult to apply species-specific biomass data in appropriate proportions to each

OM source category. Additionally, the absence of detailed vegetation maps necessitated the collapse of more than 10 isotopically-distinct vegetation categories down to five. As a result, variability associated with category isotope signatures increased, leading to greater uncertainty in model output and reducing the resolution at which diet shifts can be observed.

Conclusions and implications for restoration

Our analysis demonstrates that access to marsh detritus positively affects the growth rates of estuarine detritivores, despite a strong body of literature describing the nutritional paucity of this resource in comparison to algal sources (Mueller-Solger *et al.* 2002, Sobczak *et al.* 2002, Sobczak *et al.* 2005, Brett *et al.* 2009). Although speculative, microbial and fungal conditioning of marsh detritus may provide estuarine consumers with specific dietary requirements, such as protein, during periods of the year when physiological demands are high, but the availability of high quality algal food sources are limited. We therefore conclude that increasing ecosystem capacity for detritus production by restoring emergent marsh ecosystems could bolster detritus-based food web support in estuaries. However, we were unable to confirm trophic responses to changes in landscape capacity for OM production through our modeling exercise, in which we used the areal extent of OM sources available in the landscape as informative priors in the Bayesian stable isotope mixing model. Nonetheless, compared to phytoplankton, conditioned marsh detritus likely provides an alternative and more temporally stable source of energy for estuarine consumers. In addition to ecosystem capacity for OM production, we also suggest that detritus-based food webs are enhanced by ecosystem connectivity. Differences in the structure of the two restoration marshes provide evidence for this assertion, as equivalent growth rates, higher assimilation strengths of marsh detritus, and increased similarity to natural marsh consumer diets were observed at the full levee removal site even though restoration implementation was much more recent. This suggests that restoration actions that enhance connectivity across estuarine ecotones may achieve functional equivalency more rapidly than restoration projects exhibiting limited connectivity to the surrounding landscape.

Functional equivalency trajectories for coastal marsh ecosystem processes are extremely variable and strongly dependent on a wide number of factors, including, but not limited to, the degree of site degradation, the number and type of ecological stressors, the degree of degradation in the surrounding ecosystem, the extent to which ecosystem processes still exist in the surrounding landscape, and the landscape setting of the site (Simenstad *et al.* 2006, Borja *et al.* 2010). In some cases, recovery of lost ecological functions can take less than five years, while in other situations, full recovery can take a minimum of 15-25 years, if attainment of complete functioning is acheived at all (Borja et al. 2010). Regardless of whether the original historical state is achieved through restoration actions, ecological status of estuarine coastal marshes generally improves when natural processes are recovered, making consideration of the landscape context an indispensible component of restoration planning.

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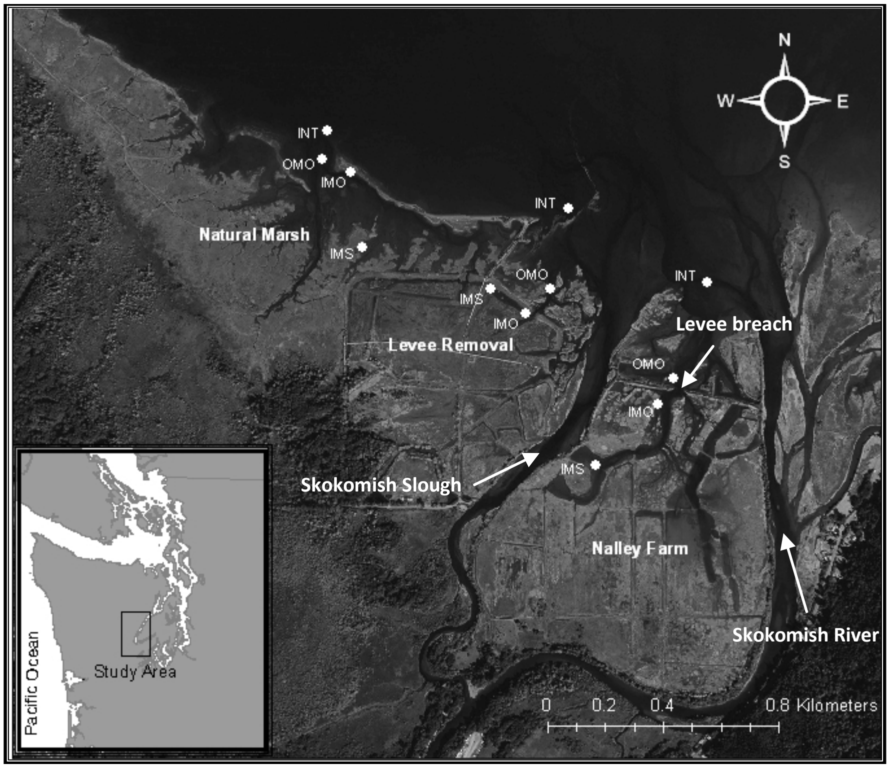

Figure3.1. Study site locations in southern Hood Canal, Washington, USA at the mouth of the Skokomish River. Mussel transplants and collections occurred at each white filled circle, with two points inside the main inlet channel to the marsh, and two points seaward of the channel outlet. Sampling points are coded as follows: IMS = inner marsh, IMO= inner marsh, near the mouth of the marsh channel outlet, OMO= outer side of the marsh channel mouth, and INT= intertidal flats beyond the channel outlet.

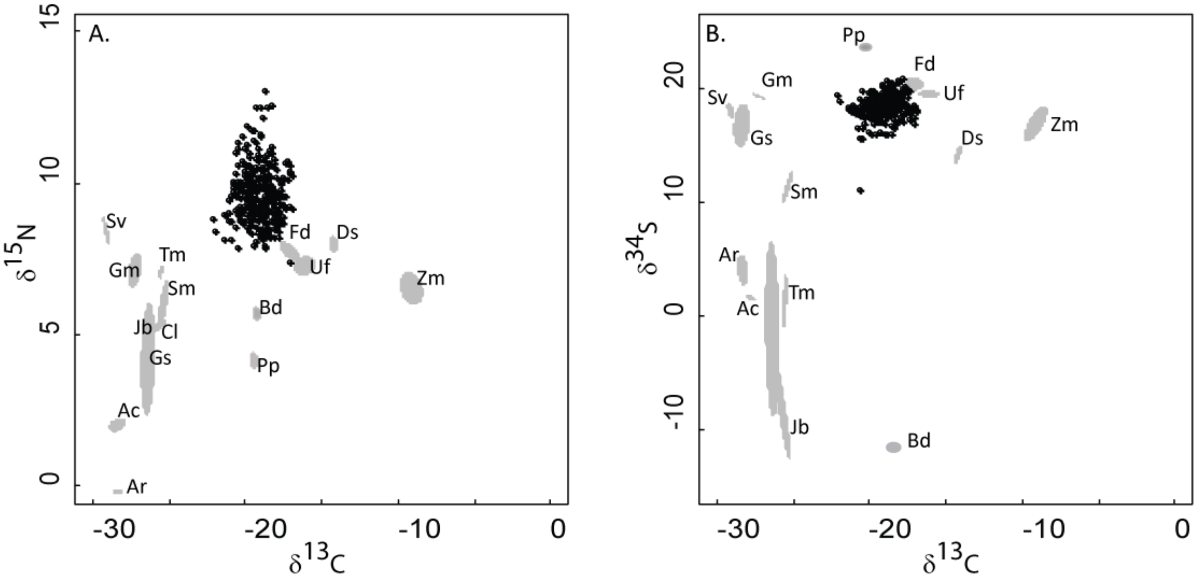

Figure 3.2. Isotopic signatures (A: $\delta^{13}C$ x $\delta^{15}N$, B: of $\delta^{13}C$ x $\delta^{34}S$) of organic matter (OM) sources and mussels collected in the Skokomish River estuary. Grey ellipses demarcate the 95% confidence intervals around the mean OM source values. Species of OM sources are indicated as follows: Ac= *Acer circinatum,* Ar= *Alnus rubra,* Bd= benthic diatoms, Cl= *Carex lyngbyei,* Ds= *Distichlis spicata,* Fd= *Fucus distichus,* Gm= *Glaux* maritima, Gs= *Grindelia stricta,* Jb= *Juncus balthicus,* Pp= phytoplankton, Sv= *Salicornia virginica,* Tm= *Triglochin maritima,* Uf= *Ulva fenestrata,* Zm= *Zostera marina.*

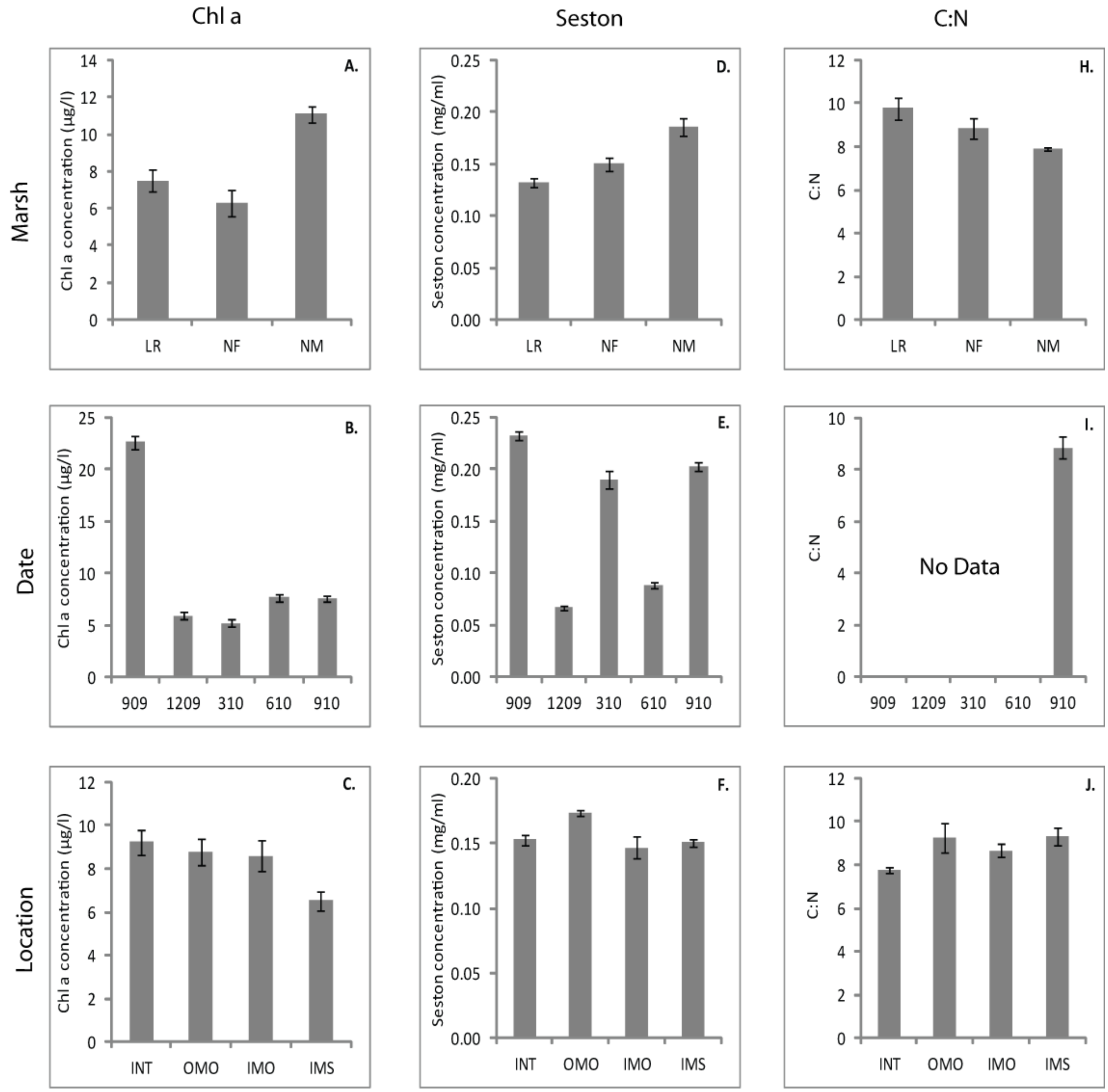

Figure 3.3. Concentrations of chlorophyll α and bulk seston, and the C:N ratio of bulk seston by marsh location (LR= levee removal, NF= Nalley Farm, NM = natural marsh), sampling date (090 = Sept 2009, 1209 = Dec 2009, 310 = Mar 2010, 610 = Jun 2010, 910 = Sept 2010), and sampling locations across the marsh-marine ecotone (INT= intertidal, OMO= outer marsh outlet, IMO= inner marsh outlet, IMS= inner marsh site).

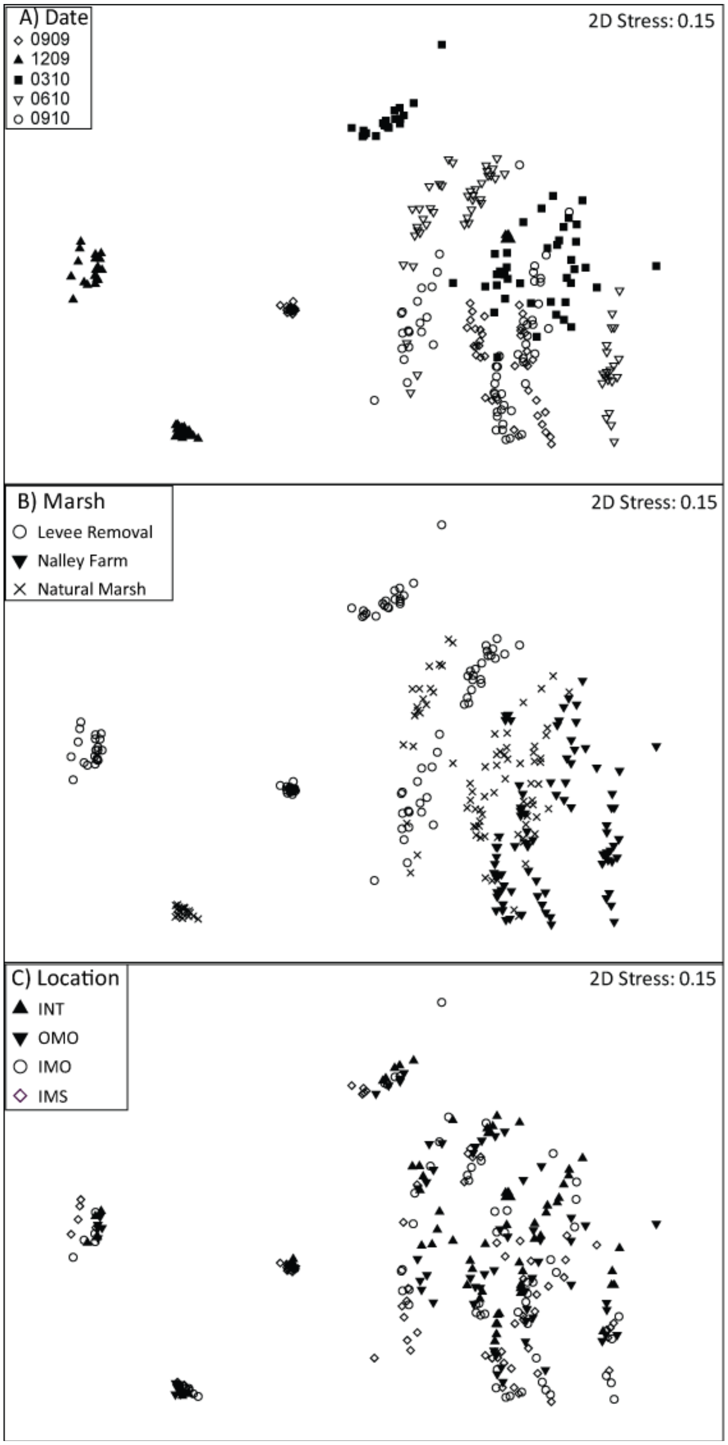

Figure 3.4. MDS ordination of OM source contributions assimilated by mussels in the Skokomish River estuary by date (A), marsh (B), and site (C). Ordination is based on square-root transformed data using a Bray-Curtis similarity index.

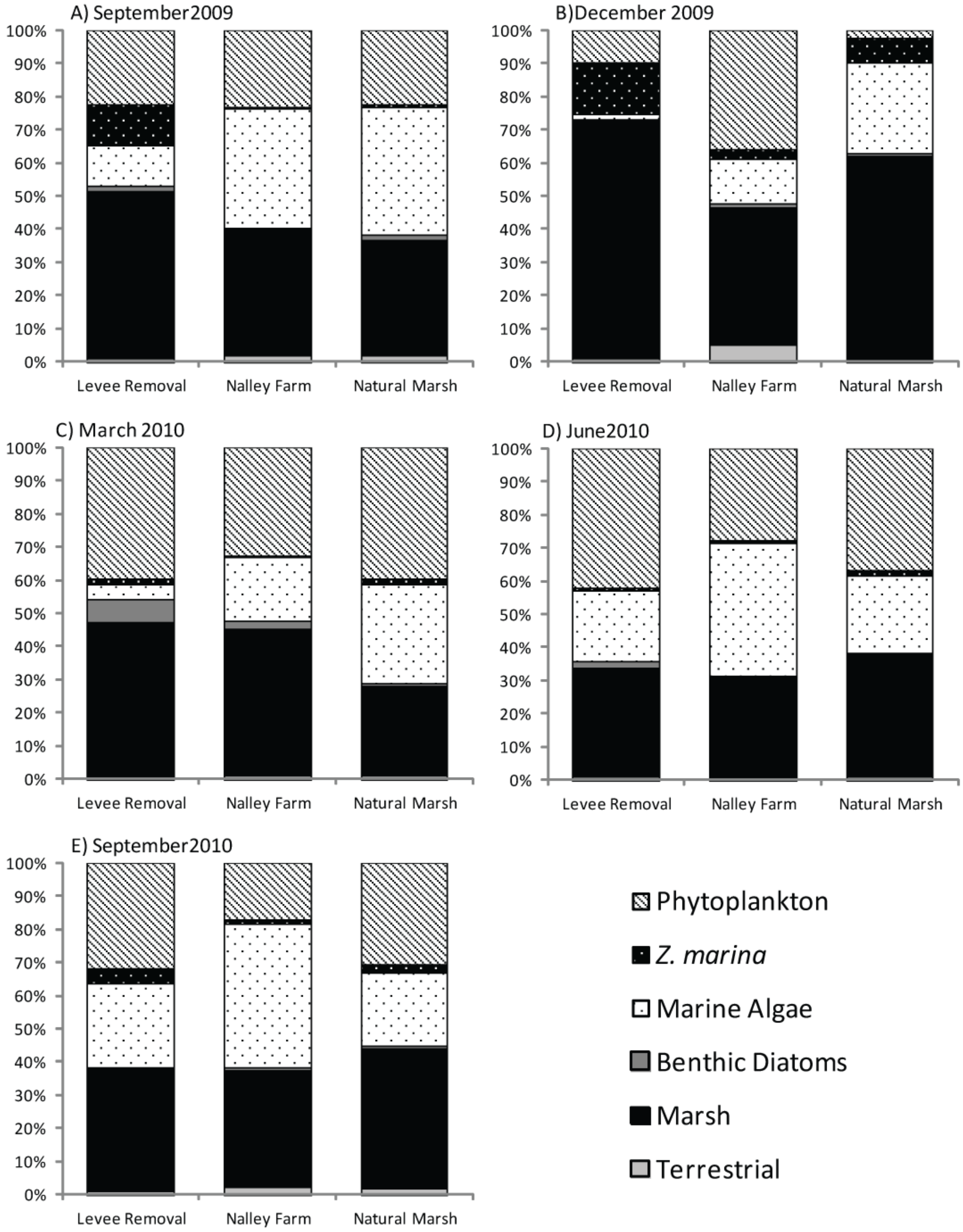

Figure 3.5. Average percent seasonal OM source contributions to mussels by marsh site. Marsh-derived OM sources have been condensed into one source category; see text and Table 1 for species composition.

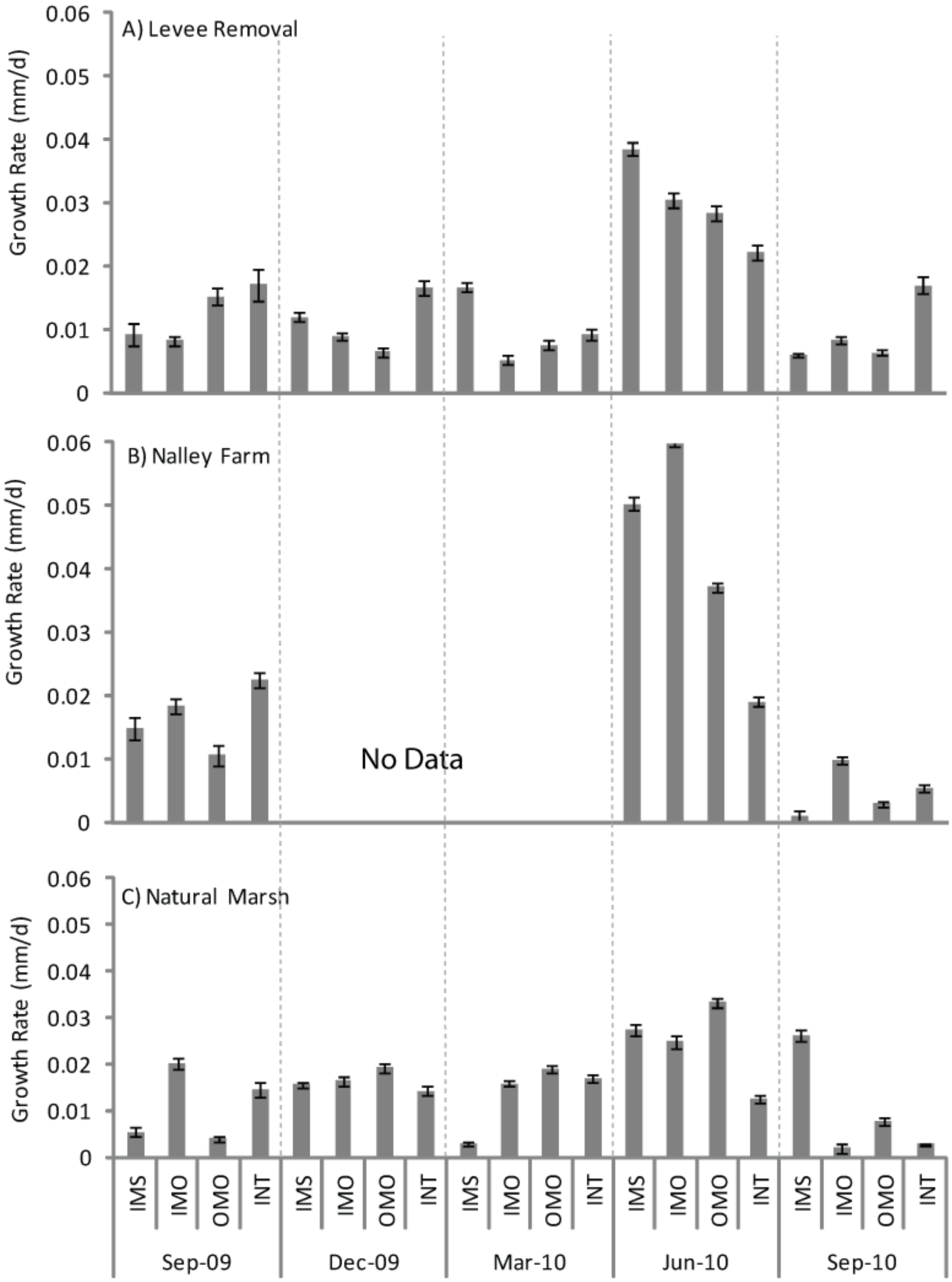

Figure 3.6. Growth rates (mm d^{-1}) of transplanted mussel at the levee removal restoration site (A), the Nalley Farm natural levee breach restoration site (B), and the natural marsh site (C) across the five sampling dates and four transplant locations within each marsh in the Skokmish River estuary. Growth rates were standardized to inundation time for the elevation of each site location and time period. No data were available at the Nalley Farm levee breach site in December 2009 and March 2010 because winter flooding prevented sampling efforts in the inner marsh sites, and destroyed sample sites outside the levee.

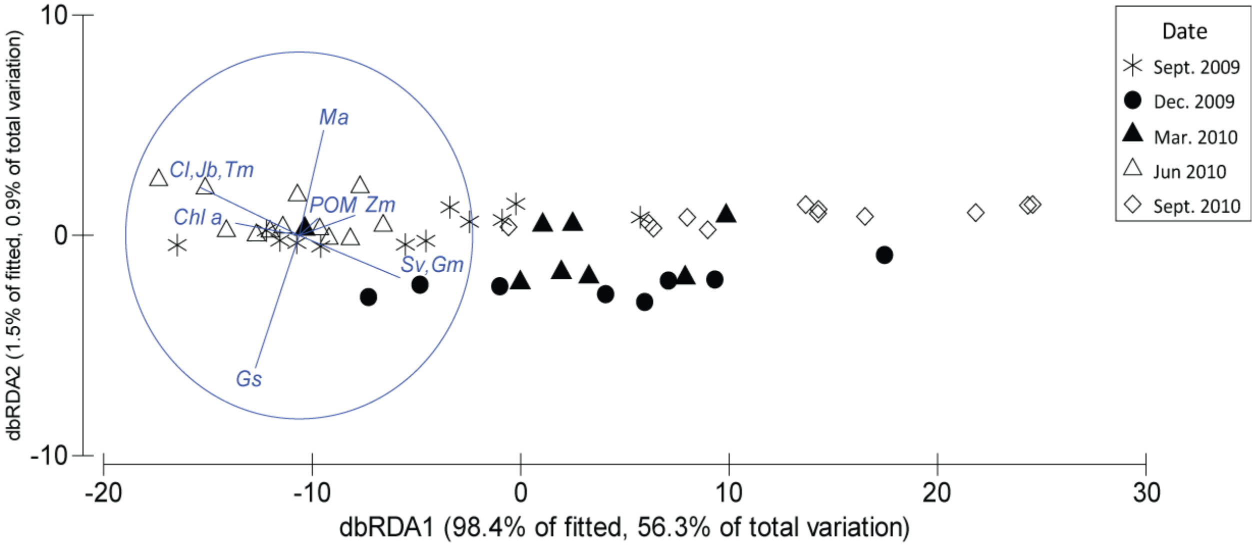

Figure 3.7. Distance-based RDA (dbRDA) ordination for the fitted model of mussel growth during each sampling period (based on Bray-Curtis distance measure after square-root transformation). The estimated proportional contributions of OM sources were used as explanatory variables to describe variation in growth. *Cl,Jb,Tm* = *C. lyngbyei, J. balthicus,* and *T. maritimum, Chl a* = chlorophyll α concentration, *Gs*= *G. stricta, Ma* = Marine algae, *Zm* = *Z. marina, Sv,Gm* = *S. virginica* and *G. maritima, POM* = seston concentration.

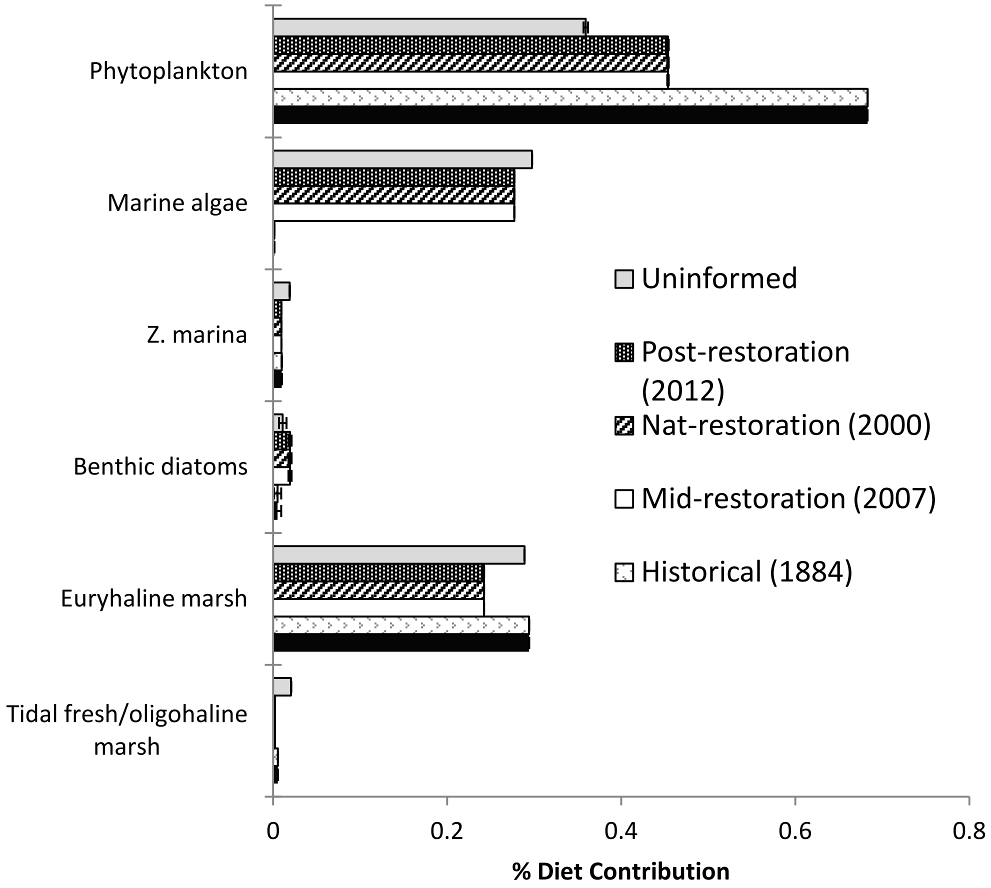

Figure 3.8. Percent contributions of OM sources to mussel diets under the six Bayesian mixing model scenarios. Diets based on the measured isotopic values of mussels collected in December and June in the INT locations of each marsh in the Skokomish estuary.

Table 3. 1. Average (± 1 standard deviation) $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of the dominant sources of organic matter in the Skokomish River estuary. Multiple vascular plant species were combined into a single category because the similarity of their isotope signatures violated SOURCE's NND^2 minimum value of 0.1.

	$\delta^{13}C$	(SD)	$\delta^{15}N$	(SD	$\delta^{34}S$	(SD)
Alnus rubra, Acer circinatum	-28.07	0.39	-1.54	1.46	2.83	1.57
Carex lyngbyei, Juncus balthicus, Triglochin maritima	-25.92	0.54	5.52	1.57	-2.45	6.29
Distichlis spicata	-14.23	0.26	8.00	0.25	14.15	0.76
Grindelia stricta	-28.41	0.50	2.04	0.20	16.78	1.83
Schoenoplectus maritimus	-25.38	0.32	6.03	0.75	11.34	1.36
Salicornia virginica, Glaux maritima	-28.20	1.05	7.78	0.83	18.71	0.77
benthic diatoms	-19.36	1.73	5.37	2.32	-11.11	6.64
marine algae	-16.63	0.82	7.53	0.37	19.98	0.59
Zostera marina	-9.11	0.72	6.54	0.50	16.94	1.50
phytoplankton	-19.53	1.73	3.27	0.57	21.73	0.77

Table 3.2. Priors estimating the proportion of each organic matter source available to mussels under different restoration scenarios in the Skokomish River estuary.

Scenario	Oligohaline/ freshwater marsh	Euryhaline marsh	Benthic diatoms	*Z. marina*	Marine algae	Phytoplankton
Uninformed	1	1	1	1	1	1
Historical (1884)	0.05	0.11	0.27	0.07	0.00	0.50
Altered (1938)	0.02	0.19	0.24	0.05	0.00	0.50
Natural Restoration (1996)	0.02	0.22	0.21	0.05	0.00	0.50
Mid –Restoration (2007)	0.02	0.23	0.21	0.04	0.00	0.50
Post –Restoration (2012)	0.02	0.24	0.20	0.04	0.00	0.50

Table 3.3. Average $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope values (and standard deviations) of mussels by date, marsh, and cage location in Skokomish River estuary.

Date	Marsh	Location	$\delta^{13}C$	(SD)	$\delta^{15}N$	(SD)	$\delta^{34}S$	(SD)
Sep-09			-19.24	0.65	9.57	0.64	18.60	0.73
	Levee Removal		-19.54	0.57	9.22	0.29	18.21	0.86
		IMO	-19.54	0.35	9.26	0.21	18.30	0.26
		IMS	-19.15	0.55	9.43	0.41	17.03	0.63
		INT	-19.69	0.72	9.07	0.17	18.85	0.28
		OMO	-19.77	0.53	9.14	0.25	18.70	0.50
	Nalley Farm		-19.37	0.40	9.96	0.75	19.01	0.69
		IMO	-19.39	0.38	10.61	0.50	19.22	0.39
		IMS	-19.45	0.32	10.46	0.54	18.35	1.27
		INT	-19.24	0.21	9.04	0.23	18.97	0.06
		OMO	-19.41	0.64	9.82	0.41	19.39	0.31
	Natural Marsh		-18.84	0.72	9.53	0.59	18.58	0.36
		IMO	-18.96	0.19	9.64	0.11	18.77	0.24
		IMS	-17.82	0.53	10.34	0.17	18.15	0.35
		INT	-19.35	0.49	9.00	0.36	18.65	0.23
		OMO	-19.21	0.31	9.12	0.24	18.73	0.25
Dec-09			-19.89	0.75	9.52	0.44	18.00	0.81
	Levee Removal		-20.18	0.91	9.39	0.36	17.62	0.97
		IMO	-20.55	0.29	9.27	0.33	17.05	0.99
		IMS	-19.05	0.66	9.69	0.40	16.70	0.66
		INT	-21.14	0.57	9.37	0.35	18.56	0.19
		OMO	-19.99	0.42	9.22	0.20	18.17	0.24
	Nalley Farm		-20.09	0.36	9.15	0.44	18.36	0.20
		INT	-20.09	0.36	9.15	0.44	18.36	0.20
	Natural Marsh		-19.55	0.49	9.75	0.42	18.28	0.56
		IMO	-19.28	0.45	10.14	0.30	18.30	0.22
		IMS	-19.52	0.39	10.01	0.21	17.56	0.41
		INT	-19.32	0.35	9.51	0.32	18.37	0.31
		OMO	-20.09	0.34	9.32	0.18	18.90	0.22
Mar-10			-19.60	1.17	9.26	0.65	18.33	1.61
	Levee Removal		-20.22	1.00	9.00	0.64	17.55	1.04
		IMO	-19.40	1.34	8.58	0.67	17.60	0.63
		IMS	-20.09	0.39	9.64	0.23	16.14	0.50
		INT	-21.35	0.49	8.55	0.39	18.32	0.69
		OMO	-20.05	0.30	9.21	0.47	18.14	0.55

	Nalley Farm		-20.16	0.61	9.64	0.65	17.72	1.85
		IMO	-20.32	1.00	9.67	0.50	18.21	0.20
		IMS	-20.24	0.19	10.16	0.24	17.58	1.34
		INT	-19.59	0.07	8.80	0.34	18.98	0.35
		OMO	-20.50	0.28	10.02	0.43	16.08	2.88
	Natural Marsh		-18.44	0.82	9.18	0.52	19.69	0.77
		IMO	-18.30	0.27	8.77	0.37	19.74	0.56
		IMS	-17.64	0.17	9.53	0.30	19.48	0.52
		INT	-19.03	1.28	9.41	0.45	19.46	1.32
		OMO	-18.80	0.14	9.00	0.64	20.07	0.43
Jun-10			-18.12	0.49	9.25	0.64	18.59	0.91
	Levee Removal		-18.10	0.45	8.91	0.40	18.55	0.86
		IMO	-18.27	0.13	9.13	0.49	18.43	0.41
		IMS	-17.59	0.40	9.20	0.28	17.53	0.39
		INT	-18.51	0.24	8.49	0.18	19.31	0.30
		OMO	-18.04	0.39	8.81	0.07	18.92	0.94
	Nalley Farm		-18.48	0.28	9.73	0.63	18.20	1.05
		IMO	-18.41	0.31	10.28	0.67	16.97	0.77
		IMS	-18.24	0.18	9.92	0.35	18.92	0.58
		INT	-18.66	0.19	9.07	0.50	19.07	0.54
		OMO	-18.60	0.29	9.64	0.28	17.85	0.61
	Natural Marsh		-17.76	0.44	9.11	0.59	19.01	0.61
		IMO	-17.77	0.16	8.90	0.50	19.19	0.47
		IMS	-17.16	0.22	9.90	0.47	18.18	0.21
		INT	-18.20	0.27	8.77	0.26	19.23	0.49
		OMO	-17.94	0.20	8.86	0.26	19.43	0.27
Sep-10			-18.91	0.85	9.88	0.66	18.99	0.88
	Levee Removal		-18.17	0.53	9.74	0.51	19.20	0.97
		IMO	-18.34	0.05	9.82	0.28	18.57	0.36
		IMS	-17.47	0.40	10.22	0.29	18.27	0.72
		INT	-18.75	0.13	9.12	0.48	20.33	0.49
		OMO	-18.13	0.23	9.81	0.23	19.62	0.39
	Nalley Farm		-18.65	0.29	10.28	0.64	19.22	0.65
		IMO	-18.35	0.22	10.91	0.29	19.17	1.02
		IMS	-18.56	0.32	10.33	0.29	19.49	0.76
		INT	-18.82	0.22	9.37	0.28	19.31	0.17
		OMO	-18.85	0.09	10.51	0.31	18.91	0.33

Natural Marsh	-19.90	0.47	9.60	0.65	18.54	0.87
IMO	-19.55	0.39	9.97	0.20	18.62	0.70
IMS	-20.11	0.43	10.00	0.28	17.60	0.48
INT	-19.89	0.60	8.79	0.57	19.35	0.64
OMO	-20.04	0.32	9.66	0.58	18.59	0.74

Table 3.4. Mean Euclidean distance and percent similarity values for mussel isotope and diet composition across locations (i.e., INT, OMO, IMO, IMS) by marsh (LR, NF, NM). Distances and similarities were first calculated across all locations for each marsh and date, then merged to examine overall differences among marshes. A fixed-effect GLM tests for significant differences in isotope and diet data among dates, marshes, and date-specific marshes. Bonferroni *post hoc* tests describing pair-wise comparisons between marshes were performed on factor-specific ANOVA's for marsh and date.

		Isotope Data		Estimated diet data		
		Mean Euclidean Distance	(SD)	Mean % similarity	(SD)	n
Date						
	Sept 2009	1.23	0.53	96.88	2.24	30
	Dec 2009	1.30	0.57	97.86	1.18	20
	Mar 2010	1.80	0.71	95.11	1.83	30
	June 2010	1.26	0.51	96.47	1.55	30
	Sept 2010	1.31	0.49	96.10	1.99	30
Marsh						
	LR	1.43	0.60	96.94	1.78	50
	NF	1.52	0.67	95.87	2.03	40
	NM	1.23	0.52	96.24	2.08	50

PERMANOVA factors: Date, Marsh, Marsh(Date)

		Isotope Data	Estimated diet data
	Intercept	F = 859.724, p < 0.001	F = 535348.2, p < 0.0001
	Date	F = 5.365, p = 0.001	F = 9.62, p < 0.001
	Marsh	F = 3.125, p = 0.047	F = 3.42, p = 0.036
	Marsh(Date)	F = 1.277, p = 0.267	F = 8.30, p < 0.001
post hoc	tests	*p*	*p*
Marsh	LR x NF	1	0.035
	LR x NM	0.263	0.244
	NM x NF	0.065	1.000
Date	Sept 2009 x Dec 2009	1.000	0.653
	Sept 2009 x Mar 2010	0.001	0.003
	Sept 2009 x June 2010	1.000	1.000
	Sept 2009 x Sept 2010	1.000	1.000
	Dec 2009 x Mar 2010	0.025	< 0.001
	Dec 2009 x June 2010	1.000	0.095
	Dec 2009 x Sept 2010	1.000	0.012
	Mar 2010 x June 2010	0.003	0.049
	Mar 2010 x Sept 2010	0.010	0.381
	June 2010 x Sept 2010	1.000	1.000

Table 3.5. Estimated medians and interquartile ranges (IQR) of the percent contribution of OM sources to mussel diets by date, marsh, and site.

Date	*Marsh*	*Site*	*Acer/Alnus*	*IQR*	*G. stricta*	*IQR*	*D. spicatus*	*IQR*	*S. maritimus*	*IQR*	*Cl_Jb_Tm*	*IQR*	*Sv_Gm*	*IQR*	*Benthic diatoms*	*IQR*	*Marine algae*	*IQR*	*Z. marina*	*IQR*	*Phytoplankton*	*IQR*
Sep-09																						
	LR																					
		IMS	**0.02**	0.03	**0.09**	0.05	**0.07**	0.11	**0.04**	0.04	**0.05**	0.04	**0.22**	0.06	**0.01**	0.01	**0.17**	0.11	**0.07**	0.08	**0.23**	0.08
		IMO	**0.02**	0.02	**0.10**	0.06	**0.05**	0.07	**0.03**	0.03	**0.03**	0.02	**0.23**	0.06	**0.00**	0.01	**0.17**	0.09	**0.05**	0.05	**0.28**	0.08
		OMO	**0.01**	0.02	**0.09**	0.06	**0.05**	0.06	**0.03**	0.03	**0.03**	0.02	**0.24**	0.06	**0.00**	0.01	**0.14**	0.09	**0.05**	0.05	**0.33**	0.08
		INT	**0.01**	0.02	**0.09**	0.05	**0.04**	0.06	**0.03**	0.03	**0.02**	0.02	**0.24**	0.06	**0.00**	0.01	**0.14**	0.08	**0.04**	0.05	**0.35**	0.07
	NF																					
		IMS	**0.00**	0.01	**0.06**	0.05	**0.02**	0.03	**0.01**	0.02	**0.01**	0.01	**0.44**	0.12	**0.00**	0.01	**0.15**	0.22	**0.18**	0.17	**0.07**	0.06
		IMO	**0.00**	0.01	**0.05**	0.04	**0.01**	0.03	**0.01**	0.02	**0.01**	0.01	**0.38**	0.08	**0.00**	0.00	**0.35**	0.17	**0.07**	0.09	**0.09**	0.07
		OMO	**0.00**	0.01	**0.07**	0.08	**0.01**	0.03	**0.01**	0.02	**0.01**	0.01	**0.31**	0.09	**0.00**	0.00	**0.26**	0.18	**0.06**	0.08	**0.22**	0.11
		INT	**0.00**	0.01	**0.20**	0.12	**0.02**	0.04	**0.01**	0.02	**0.01**	0.01	**0.18**	0.09	**0.00**	0.01	**0.24**	0.17	**0.07**	0.08	**0.23**	0.14
	NM																					
		IMS	**0.01**	0.01	**0.07**	0.05	**0.06**	0.11	**0.04**	0.09	**0.00**	0.01	**0.16**	0.08	**0.01**	0.01	**0.45**	0.17	**0.03**	0.05	**0.10**	0.05
		IMO	**0.01**	0.01	**0.12**	0.08	**0.04**	0.06	**0.03**	0.07	**0.00**	0.01	**0.19**	0.07	**0.01**	0.01	**0.33**	0.13	**0.03**	0.04	**0.19**	0.09
		OMO	**0.01**	0.02	**0.15**	0.11	**0.04**	0.06	**0.03**	0.07	**0.00**	0.01	**0.16**	0.07	**0.01**	0.01	**0.26**	0.13	**0.03**	0.04	**0.25**	0.11
		INT	**0.02**	0.02	**0.15**	0.10	**0.04**	0.06	**0.04**	0.08	**0.00**	0.01	**0.16**	0.07	**0.01**	0.01	**0.23**	0.13	**0.03**	0.04	**0.28**	0.10
Dec-09																						
	LR																					
		IMS	**0.01**	0.01	**0.23**	0.03	**0.12**	0.10	**0.06**	0.01	**0.00**	0.00	**0.30**	0.06	**0.00**	0.00	**0.02**	0.02	**0.16**	0.11	**0.10**	0.05
		IMO	**0.01**	0.01	**0.23**	0.03	**0.12**	0.10	**0.06**	0.01	**0.00**	0.00	**0.30**	0.06	**0.00**	0.00	**0.02**	0.02	**0.16**	0.11	**0.10**	0.05
		OMO	**0.01**	0.01	**0.23**	0.03	**0.12**	0.10	**0.06**	0.01	**0.00**	0.00	**0.30**	0.06	**0.00**	0.00	**0.02**	0.02	**0.16**	0.11	**0.10**	0.05
		INT	**0.01**	0.01	**0.23**	0.03	**0.12**	0.10	**0.06**	0.01	**0.00**	0.00	**0.30**	0.06	**0.00**	0.00	**0.02**	0.02	**0.16**	0.11	**0.10**	0.05
	NF																					
		INT	**0.02**	0.05	**0.06**	0.12	**0.03**	0.07	**0.02**	0.07	**0.01**	0.02	**0.29**	0.08	**0.00**	0.01	**0.10**	0.18	**0.03**	0.07	**0.31**	0.13
	NM																					
		IMS	**0.02**	0.02	**0.11**	0.06	**0.19**	0.09	**0.02**	0.04	**0.00**	0.01	**0.27**	0.05	**0.00**	0.01	**0.20**	0.11	**0.01**	0.01	**0.13**	0.06
		IMO	**0.02**	0.01	**0.09**	0.05	**0.15**	0.09	**0.02**	0.03	**0.00**	0.01	**0.28**	0.05	**0.00**	0.01	**0.26**	0.13	**0.01**	0.01	**0.15**	0.06
		OMO	**0.02**	0.01	**0.14**	0.08	**0.09**	0.05	**0.02**	0.03	**0.00**	0.01	**0.26**	0.06	**0.00**	0.01	**0.19**	0.10	**0.01**	0.01	**0.24**	0.09
		INT	**0.02**	0.02	**0.12**	0.07	**0.13**	0.08	**0.02**	0.03	**0.00**	0.01	**0.23**	0.05	**0.00**	0.01	**0.22**	0.12	**0.01**	0.01	**0.21**	0.08

Date	Marsh	Site	Acer/Alnus	IQR	*G. stricta*	IQR	*D. spicatus*	IQR	*S. maritimus*	IQR	Cl_Jb_Tm	IQR	Sv_Gm	IQR	Benthic diatoms	IQR	Marine algae	IQR	*Z. marina*	IQR	Phytoplankton	IQR
Mar-10																						
	LR																					
		IMS	**0.02**	0.04	**0.10**	0.08	**0.16**	0.15	**0.08**	0.12	**0.04**	0.06	**0.25**	0.08	**0.00**	0.02	**0.09**	0.11	**0.02**	0.04	**0.16**	0.09
		IMO	**0.02**	0.03	**0.05**	0.05	**0.06**	0.06	**0.05**	0.11	**0.04**	0.07	**0.14**	0.08	**0.00**	0.02	**0.05**	0.05	**0.01**	0.02	**0.48**	0.07
		OMO	**0.02**	0.02	**0.08**	0.07	**0.07**	0.08	**0.04**	0.06	**0.02**	0.03	**0.26**	0.07	**0.00**	0.01	**0.09**	0.11	**0.01**	0.04	**0.32**	0.09
		INT	**0.02**	0.03	**0.18**	0.11	**0.04**	0.04	**0.04**	0.05	**0.02**	0.03	**0.25**	0.07	**0.00**	0.01	**0.05**	0.06	**0.01**	0.02	**0.34**	0.10
	NF																					
		IMS	**0.01**	0.01	**0.03**	0.05	**0.04**	0.05	**0.01**	0.01	**0.09**	0.03	**0.35**	0.07	**0.00**	0.01	**0.23**	0.14	**0.02**	0.04	**0.18**	0.10
		IMO	**0.01**	0.01	**0.03**	0.04	**0.04**	0.05	**0.01**	0.02	**0.06**	0.03	**0.36**	0.06	**0.00**	0.01	**0.12**	0.10	**0.02**	0.04	**0.31**	0.08
		OMO	**0.01**	0.01	**0.03**	0.04	**0.03**	0.04	**0.01**	0.01	**0.20**	0.04	**0.29**	0.06	**0.00**	0.01	**0.21**	0.11	**0.02**	0.03	**0.17**	0.09
		INT	**0.01**	0.01	**0.06**	0.12	**0.03**	0.04	**0.01**	0.01	**0.04**	0.03	**0.22**	0.07	**0.00**	0.01	**0.13**	0.11	**0.02**	0.03	**0.44**	0.13
	NM																					
		IMS	**0.00**	0.00	**0.05**	0.05	**0.02**	0.03	**0.02**	0.02	**0.00**	0.01	**0.22**	0.08	**0.00**	0.01	**0.19**	0.16	**0.14**	0.13	**0.32**	0.07
		IMO	**0.00**	0.01	**0.06**	0.06	**0.02**	0.03	**0.02**	0.02	**0.00**	0.01	**0.19**	0.06	**0.00**	0.01	**0.12**	0.08	**0.10**	0.09	**0.45**	0.08
		OMO	**0.00**	0.00	**0.04**	0.04	**0.02**	0.02	**0.02**	0.02	**0.00**	0.01	**0.24**	0.06	**0.00**	0.00	**0.12**	0.08	**0.08**	0.07	**0.45**	0.07
		INT	**0.00**	0.00	**0.04**	0.05	**0.02**	0.03	**0.02**	0.02	**0.00**	0.01	**0.29**	0.06	**0.00**	0.01	**0.10**	0.08	**0.10**	0.09	**0.38**	0.08
Jun-10																						
	LR																					
		IMS	**0.04**	0.04	**0.14**	0.07	**0.03**	0.05	**0.00**	0.02	**0.02**	0.03	**0.09**	0.09	**0.01**	0.01	**0.32**	0.19	**0.11**	0.14	**0.18**	0.08
		IMO	**0.03**	0.03	**0.16**	0.08	**0.02**	0.03	**0.00**	0.02	**0.02**	0.02	**0.11**	0.10	**0.01**	0.01	**0.32**	0.19	**0.07**	0.10	**0.22**	0.10
		OMO	**0.02**	0.02	**0.18**	0.09	**0.02**	0.03	**0.00**	0.01	**0.01**	0.02	**0.07**	0.08	**0.01**	0.01	**0.30**	0.15	**0.08**	0.09	**0.27**	0.10
		INT	**0.02**	0.02	**0.18**	0.09	**0.02**	0.02	**0.00**	0.01	**0.01**	0.01	**0.08**	0.07	**0.01**	0.01	**0.24**	0.13	**0.06**	0.07	**0.36**	0.10
	NF																					
		IMS	**0.01**	0.01	**0.08**	0.05	**0.01**	0.05	**0.01**	0.01	**0.02**	0.03	**0.18**	0.07	**0.01**	0.01	**0.43**	0.14	**0.04**	0.04	**0.17**	0.10
		IMO	**0.01**	0.01	**0.05**	0.04	**0.02**	0.05	**0.02**	0.02	**0.06**	0.03	**0.21**	0.06	**0.01**	0.01	**0.42**	0.10	**0.05**	0.04	**0.09**	0.08
		OMO	**0.02**	0.01	**0.10**	0.04	**0.02**	0.04	**0.02**	0.01	**0.03**	0.04	**0.18**	0.06	**0.01**	0.01	**0.34**	0.11	**0.06**	0.03	**0.16**	0.09
		INT	**0.01**	0.01	**0.08**	0.12	**0.01**	0.04	**0.01**	0.01	**0.02**	0.03	**0.16**	0.07	**0.01**	0.01	**0.26**	0.11	**0.04**	0.03	**0.35**	0.13
	NM																					
		IMS	**0.02**	0.00	**0.08**	0.00	**0.03**	0.00	**0.04**	0.00	**0.00**	0.00	**0.08**	0.00	**0.01**	0.00	**0.45**	0.00	**0.06**	0.00	**0.15**	0.00
		IMO	**0.02**	0.00	**0.10**	0.00	**0.02**	0.00	**0.03**	0.00	**0.00**	0.00	**0.06**	0.00	**0.01**	0.00	**0.30**	0.00	**0.05**	0.00	**0.36**	0.00
		OMO	**0.02**	0.00	**0.12**	0.00	**0.02**	0.00	**0.03**	0.00	**0.00**	0.00	**0.06**	0.00	**0.00**	0.00	**0.31**	0.00	**0.04**	0.00	**0.35**	0.00
		INT	**0.02**	0.00	**0.14**	0.00	**0.02**	0.00	**0.03**	0.00	**0.00**	0.00	**0.06**	0.00	**0.00**	0.00	**0.29**	0.00	**0.05**	0.00	**0.34**	0.00

Date	Marsh	Site	Acer/Alnus	IQR	*G. stricta*	IQR	*D. spicatus*	IQR	*S. maritimus*	IQR	Cl_Jb_Tm	IQR	Sv_Gm	IQR	Benthic diatoms	IQR	Marine algae	IQR	*Z. marina*	IQR	Phytoplankton	IQR
Sep-10																						
	LR																					
		IMS	**0.01**	0.02	**0.06**	0.05	**0.03**	0.06	**0.04**	0.08	**0.01**	0.02	**0.14**	0.11	**0.00**	0.01	**0.47**	0.22	**0.04**	0.12	**0.11**	0.07
		IMO	**0.01**	0.02	**0.09**	0.07	**0.03**	0.05	**0.04**	0.07	**0.01**	0.02	**0.18**	0.08	**0.00**	0.01	**0.37**	0.16	**0.04**	0.08	**0.17**	0.08
		OMO	**0.01**	0.01	**0.06**	0.06	**0.02**	0.03	**0.02**	0.03	**0.00**	0.01	**0.18**	0.07	**0.00**	0.01	**0.41**	0.11	**0.02**	0.05	**0.24**	0.08
		INT	**0.00**	0.01	**0.04**	0.04	**0.01**	0.02	**0.02**	0.02	**0.00**	0.01	**0.20**	0.06	**0.00**	0.00	**0.23**	0.10	**0.02**	0.03	**0.44**	0.06
	NF																					
		IMS	**0.00**	0.01	**0.07**	0.06	**0.03**	0.03	**0.01**	0.02	**0.01**	0.01	**0.25**	0.06	**0.00**	0.00	**0.46**	0.10	**0.03**	0.03	**0.12**	0.07
		IMO	**0.00**	0.01	**0.04**	0.03	**0.03**	0.03	**0.01**	0.02	**0.01**	0.01	**0.28**	0.06	**0.00**	0.00	**0.52**	0.09	**0.02**	0.04	**0.06**	0.04
		OMO	**0.01**	0.01	**0.06**	0.04	**0.04**	0.05	**0.01**	0.02	**0.01**	0.01	**0.29**	0.06	**0.00**	0.01	**0.41**	0.12	**0.03**	0.05	**0.09**	0.05
		INT	**0.01**	0.01	**0.11**	0.09	**0.04**	0.04	**0.01**	0.02	**0.01**	0.01	**0.20**	0.07	**0.00**	0.01	**0.29**	0.12	**0.04**	0.04	**0.26**	0.10
	NM																					
		IMS	**0.01**	0.02	**0.07**	0.06	**0.06**	0.09	**0.05**	0.08	**0.00**	0.02	**0.36**	0.08	**0.02**	0.02	**0.13**	0.15	**0.06**	0.09	**0.16**	0.09
		IMO	**0.01**	0.01	**0.06**	0.06	**0.05**	0.07	**0.03**	0.04	**0.00**	0.01	**0.33**	0.09	**0.01**	0.01	**0.20**	0.21	**0.05**	0.09	**0.21**	0.11
		OMO	**0.01**	0.01	**0.06**	0.07	**0.04**	0.06	**0.03**	0.04	**0.00**	0.02	**0.36**	0.08	**0.01**	0.01	**0.10**	0.13	**0.05**	0.10	**0.26**	0.10
		INT	**0.01**	0.01	**0.06**	0.07	**0.03**	0.04	**0.03**	0.04	**0.00**	0.01	**0.26**	0.07	**0.01**	0.01	**0.07**	0.08	**0.03**	0.05	**0.45**	0.08

Table 3.6. Average percent similarity (and standard deviation) in mussel diets among marshes and among locations within each marsh. Marsh sites: LR = Levee Removal, NM = Natural Marsh, NF = Nalley Farm.

Marsh Comparison	% Diet Similarity
LR x NM	84.0 ± 4.6%
NF x NM	81.7 ± 7.9%
LR x NF	78.4 ± 5.8%

Table 3.7. Average similarity of estimated mussel diet compositions between/within Bayesian mixing model scenarios of estuarine OM sources.

	Uninformed	Historical (1884)	Altered (1958)	Natural restoration (1996)	Mid-restoration (2007)	Post-restoration (2012)
Uninformed	99.755					
Historical (1884)	76.222	99.763				
Altered (1958)	76.222	99.771	99.763			
Natural restoration (1996)	92.331	78.181	78.181	99.897		
Mid-restoration (2007)	92.331	78.181	78.181	99.901	99.897	
Post-restoration (2012)	92.331	78.181	78.181	99.901	99.901	99.897

Conclusion: Challenges, lessons, and restoration strategies for estuarine detritus-based food webs

Challenges

Using stable isotopes and mixing models to discern detritus-based food webs

Unlike many food web linkages that can be identified through gut content analysis or observations of herbivory or predation events, detritus-feeding organisms keep their diets a muddy secret. In some cases, diatom consumption can be identified by the presence of frustules in the stomachs of detritus-feeding consumers (Kasim and Mukai 2006), but without chemical or molecular tools, the identification of remaining stomach contents is nearly impossible. By the time detritus is consumed, especially by estuarine benthic-deposit or suspension feeders, it has decomposed to such an extent that it no longer maintains physical traits identifiable with the original source. Even if detritus fragments did resemble the plants and algae prior to consumption, the radular action of many consumers would grind these fragments into an unidentifiable slurry. As a result, discerning the specific sources of organic matter (OM) that support estuarine food webs requires tools beyond the microscope.

Multiple stable isotopes (δ^{13}c, δ^{15}N, δ^{34}S) in combination with stable isotope mixing models are one of the most direct and widely used methods of tracking energy between primary producers and estuarine consumers. The technique has been used in a variety of ecosystems, including estuaries where food web ecologists have successfully employed them to identify food web subsides between discretely defined ecotones (Deegan and Garritt 1997, Hsieh et al. 2002, Guest et al. 2004). Because stable isotope distributions often vary among different ecosystems or among different plants, it is possible to describe the relative contribution of various ecosystems and sources to the structure and function of food webs, as I have done in the preceding chapters. In complex ecosystems with many contributing sources, multiple source mixing models provide the essential link between raw isotope signatures and the percent contribution of specific sources to consumer diets.

Rapid progress in the development and sophistication of multiple source mixing models has occurred over the past decade, moving beyond the initial simplistic models that largely ignored variability in model parameters and outputs (Lubetkin and Simenstad 2004), and those simply addressing output uncertainty (Phillips and Gregg 2001). Current models use Bayesian frameworks that allow for the use of prior information to inform model calculations, while also addressing uncertainty in trophic fractionation rates, source and consumer signatures, and model output (Moore and Semmens 2008, Semmens et al. 2009, Ward et al. 2010). While current models greatly outperform earlier versions, they are not to be used without caution. Mathematically, you can only calculate with certainty the percent contribution of *n+1* sources to a consumer's diet, where *n* = the number of stable isotopes used to describe the system. As such, using the three stable isotopes of ^{13}C, ^{15}N, and ^{34}S, it is only possible to identify the diet contribution of four food sources without estimation. This is problematic in an estuary, where there can be up to 20 or more sources contributing to the detritus pool. While the statistical methods of the aforementioned mixing models can be used to estimate more sources, it is prudent to remember that they are estimates which are sensitive to how well the source signatures and fractionation rates have been characterized (Bond and Diamon 2011), as well as the separation of each food source in isotopic space. As such, small shifts in the percent contributions of particular sources across space, time, or types of consumers may not reflect true biological shifts. However, if strong patterns in the percent contributions of OM sources align with patterns in the raw stable isotope signatures of consumers, it is likely that a true biological relationship underlies model outputs.

One of the major limitations of Bayesian mixing model output is the ability to incorporate the uncertainty associated with model results into statistical comparison models (i.e. ANOVAs). For example, MixSIR (Moore and Semmens 2008) and SIAR (Parnell et al. 2010), two Bayesian mixing models, output posterior probability distributions describing the percent contribution of a particular source to a consumer's diet. Little statistical support exists for the average mixing model user to then statistically determine whether several posterior probability distributions are significantly different from one another. At most, the ability to discern statistical differences between *two* distributions exists in the associated literature using Bhattacharyya distance for Dirichlet distributions (Rauber et al. 2008). This is not so useful when comparing multiple populations at multiple points in space and time with multiple potential diet items. Thus, in order to move beyond simply stating the output diet distributions from the models, simplified data must be extracted from model output for use with traditional statistical methods. In my case, I chose to use the median value of the posterior probability histograms to represent the proportion of a particular diet source. The method works well for use with multivariate statistics, but ignores the level of uncertainty associated with model estimates. I was fortunate, however, that the distributions of posterior probabilities calculated for my data were narrow, providing a higher level of confidence that the median value represents the true proportion of a particular OM source assimilated by a consumer. While I feel confident that my methods reflect the scale at which food web connections occur in Pacific Northwest estuaries, and the relative proportions of marsh-derived versus marine-derived OM contributing to consumer diets, I agree with critics of mixing models that better statistical methods are needed to compare model output such that uncertainty associated with model results is not lost.

I also contend that the use of only three stable isotopes limits our ability to confidently describe the proportional contributions of OM to estuarine consumers. Other methods to discern food web linkages among primary producers and invertebrate consumers include using molecular tools, such as matching fatty acid biomarker profiles among consumers and producers (Cifuentes and Salata 2001, Dalsgaard et al. 2003, Xu and Jaffe 2007, Descroix et al. 2010) , or fingerprinting the DNA mixtures of consumer feces and comparing them to known DNA profiles of potential food sources (O'Rorke et al. 2012). One strength of Bayesian mixing models is that they are not limited in the number of descriptor variables available for input. This flexibility means that stable isotope methodology can be integrated with the fatty acid methodology to increase the number of descriptors used in mixing models, and thereby the accuracy of estimated diet proportions. Scientists have long called for the integration of stomach content analysis, stable isotopes, and mixing models in order to avoid misinterpretation of consumer diets. As mentioned earlier, stomach content analysis is impractical for tracing detritus through estuarine food webs. Thus, the integration of stable isotopes with other types of biomarkers may help elucidate food web relationships more clearly. For complex food web systems, I think this is the new direction we need to follow.

The problem of scale in a world without boundaries

In his seminal MacArthur Award lecture, *The problem of pattern and scale in ecology*, Simon Levin (1992) maintains that “understanding patterns in terms of the processes that produce them is the essence of science”. The problem, he contends, is that process mechanisms operate at different scales than those on which the patterns are observed. We therefore have difficulty identifying the appropriate scales at which mechanistic functions create observable patterns in ecology. In some cases, observable patterns arise from the collective behaviors of large ensembles of smaller scale units, while in other cases, larger scale constraints impose pattern on the landscape in a type of top-down effect. Because the patterns of ecological systems are generated by a range of internal and external mechanisms, operating at a variety of spatial and temporal scales, it is critical to identify and address the scales at which each process operates if we are to understand the relationship between the patterns we observe and the mechanisms that produce them (Levin 1992).

In my examination of estuarine food web connectivity, I designed the project to accommodate several scale considerations. Temporal scales were considered with respect to several biological processes, including tissue-turnover rates, ontogenetic-diet shifts, and macrophyte cycles of growth and senescence. With respect to chemical processes, I considered the appropriate spatial scale at which to combine or divide primary producers, as environmental conditions can affect species-specific stable isotope ratios across the landscape (Tieszen 1991). Finally, I designed the project to the best of my ability to address scales appropriate to physical processes, capturing seasonal and spatial variability in freshwater discharge, while minimizing variation in tidal amplitude among estuaries and estuarine geomorphology (only broad, littoral flat systems were included).

One of the major challenges I encountered in this study, however, was how to appropriately scale the influence of fluvial discharge to the size of the estuary across which it flows, thereby providing a metric by which to compare food web connections across estuaries of different spatial extents. Estuarine size varies dramatically among the chosen study sites such that the areal coverage of specific ecosystems is different. For example, the mudflat ecosystem of Skagit Bay runs perpendicularly between the North and South forks of the river in a roughly 4 km wide band before reaching eelgrass beds. In contrast, the band of mudflats that fringe the marsh in Padilla Bay are closer to 200 m wide. Given the differences in estuary size, and given Levin's contentions (1992), it is important that the lengths and strengths of food web connections be scaled accordingly. The question is how?

The problem inherent in estuarine ecosystems is that they lack distinct boundaries. In a world without boundaries, where do you begin measuring? Consider, for a moment, where an estuary "starts". Is it the head of tide? If so, how do you incorporate an estuary where tide gates have been installed on inflowing sources of freshwater? Is it the upstream point at which the salt wedge penetrates? If so, the "start" of the estuary strongly depends on season and tidal cycle. Does an estuary start at the upstream end of where the "marsh" starts? If so, which type of marsh? Freshwater wetland, scrub-shrub, Sitka spruce, saltmarsh? Furthermore, what do you do when the beginning of a marsh is lined with a levee, or has been converted to some other landuse? Surely that is not a natural line from which to begin measurements. Similarly, where does an estuary "end"? The edge of the deltaic fan? Maybe. But what defines that “edge”? You can choose a break in the slope angle, but some estuaries don’t exhibit steep slope faces. Or maybe an estuary’s end is defined when adjacent waters reach seawater salinity. In this case, the “end” of the estuary pulses in space with the tide, season, wind, and currents. In the case of a fjord like Puget Sound, where multiple estuaries feed into one confined inland water-body, salinities may never reach that of oceanic seawater. The challenge is to then define an acceptable level of salinity that represents the “edge” of an estuary. Otherwise, individual estuaries all merge into one. Thus, the task of defining an estuary’s spatial area, it turns out, is not straightforward.

One of my original intentions was to ascertain whether increased fluvial discharge results in greater spatial transport and mixing of OM. From the Skagit River results in Chapter 1, I learned that fluvial discharge alone may not explain patterns in food web connectivity. Rather, the effect of fluvial discharge on OM transport potential is related to the power with which it moves through a landscape, and thereby the spatial area to which is confined. Rivers confined to single channels, for example, exhibit jet-like plumes that expediently export sediments away from estuarine deltas, while rivers with intact distributary networks display diffuse plumes that retain sediments in nearshore areas (Syvitski et al. 2005). However, defining the power to space relationship, and hence “the conveyance of water, sediment, [and OM] from river to the sea is deceptively complex” (Phillips and Slattery 2007). The task quickly turns into an exercise for fluvial-estuarine geomorphologists, who unfortunately have yet to characterize the issue at hand. In fact, the current understanding is that no consistent downstream pattern of increases or decreases in the discharge, stream power, or water surface slope have been identified for coastal plain rivers, largely because backwater sloughs and distributary channels dissipate the power of fluvial discharge as it makes its way to the sea (Phillips and Slattery 2007). Estuaries generally exhibit more complexity than the lower reaches of coastal plain rivers, including such features as channel distributaries, daily overbank flow, off-channel meanders, tidal action, and wind-driven events that push seawater onshore. As a result, potentially attractive metrics such as stream power, become less effective, if not impossible to use, in estuaries.

Stream power is the rate of energy dissipation against the bed and banks of a river or stream per unit downstream length, and is effective at describing the force that a river or stream exerts on its physical environment (Rhoads 1987). It is therefore an attractive metric for describing a river’s potential for transporting OM across space, yet due to the elements required for its’ calculation, it is difficult to consistently employ in estuarine systems. The formula for calculating stream power includes; 1), the density of water, 2) channel slope, 3) acceleration due to gravity, and 4) discharge (Rhoads 1987, Phillips and Slattery 2007). The most obvious issue with the calculation of estuarine stream power is that the density of water depends on salinity, which varies in space and time in estuaries. Another problem is that discharge gauging stations are typically located at considerable distance upstream from the coast, and therefore do not accurately reflect discharge in specific distributary channels in estuarine deltas (Phillips and Slattery 2007). Discharge per distributary channel could of course be calculated if channel cross-sectional areas were known. However, here we run into another area where the lack of clear boundaries makes this a difficult proposition. Where would you measure those channel cross-sections? What counts as "bank-full" when tidal channels are consistently overrun during high tides? At what elevation would you choose to make channel-width or depth measurements? Furthermore, where does the "bay" begin and the "river" end?— an important question when determining the "unit per downstream length" for which stream power is calculated. And finally, channel width is only helpful if you know channel depth. Bathymetry data is typically limited in shallow, nearshore areas. It can be obtained using LiDAR during low tide, however, since most available LiDAR does not penetrate water (but see EAARL instrumentation, new as of 2010 which uses green lasers to penetrate water up to 20 m), accurate bathymetry has been difficult to obtain for turbid distributary channels that never dewater, even on the lowest of tides (Gilvear et al. 2004). Thus, for the myriad reasons outlined above, the data needed to scale fluvial discharge to landscape area in terms of *stream power* is deceptively complicated.

Describing the relationship between fluvial discharge and landscape area is not the only physical metric that presents a challenge in estuarine ecosystems. We also searched, and failed, to find a suitable physical metric defining "estuarine position" of translocated organisms that could be standardized across multiple estuaries differing in size and shape. Salinity is often used to describe position along the estuarine gradient, but upon closer examination, appears to be most useful at large spatial scales in long estuaries, such as Chesapeake Bay. In short, shallow Pacific Northwest estuaries, nearshore salinity shifts dramatically with tidal cycles and seasonal shifts in fluvial discharge. Thinking that pore water salinities may be more stable than water column salinity, I performed a pilot study of pore water salinities near my translocation study sites. I determined that hyporheic flow creates high variability in pore water salinity at the sub-meter scale, excluding this variable as a possible indicator for a transect's estuarine "position".

A third, and final physical challenge I encountered during the course of this study is the fact that the movement of detritus is not unidirectional in estuarine systems. Tidal action allows exported materials to reappear on the scene, in combination with new marine sources. So, it is pertinent to determine the degree of mixing or export off the delta shelf in order to ascertain whether marsh or terrestrial detritus has a second chance at contributing to the diets of detritivore. But how can we determine mixing? Again, salinity is one idea, but the nuts and bolts of when and where to sample quickly become complicated. Salinity at what depth? Less dense than seawater, fresh river water produces a lense atop seawater that can extend for kilometers and vary in depth depending on such factors as fluvial discharge, basin shape, wind, and wave heights. Salinity at what time? Seasonal shifts in freshwater discharge change the salinity of offshore waters, as do periods in daily tidal cycles. Furthermore, there is the spatial problem of where to sample. Salinity is strongly dependent on whether you sample inside, or outside the river plume. Tidal currents, wind, and discharge all coalesce to determine the exact location of the plume at a given point in time.

The problem of creating scale in a world without definable boundaries is not insignificant. Yet, finding ways in which to scale observable patterns to one another across estuaries has the potential to unearth important mechanistic relationships between observable patterns in estuarine food webs and the processes that create those patterns. Using fluvial discharge may not be perfect, but given the variability and immensity of the data needs required to accurately characterize the physical aspects of multiple estuaries at appropriate temporal and spatial scales, this metric may be sufficient for describing the OM transport potential of an estuary. This is not to say, however, that the problem does not warrant further exploration. It would be a boon to estuarine ecologists working to link biological patterns to the landscapes in which they occur if we were to uncover reliable, scalable metrics in complex estuarine deltas.

Lessons and implications for restoration strategies for estuarine detritus-based food webs

Food web resilience in a temporally and spatially patchy world

One of the major lessons emanating from this dissertation is that estuarine organisms, from detritus-feeders to fish, rely on a suite of OM sources for food web support, assimilating terrestrial, marine, vascular plant, and algal sources alike. Patterns of consumer OM source assimilation are driven by a combination of physical and biological forces, including spatial and temporal heterogeneity in resource availability, the feeding physiology, trophic level, and mobility of the consumer itself, and on the physical landscape dynamics in which a consumer lives. As a result, OM food web support is highly variable among estuaries and consumers; some detritus-feeders track OM source availability through time, while others exhibit stable diets throughout the year. Consumers in some estuaries exhibit distinct spatial shifts in OM support, while others show relatively little spatial heterogeneity. The basic rule is: "it depends".

While differential organism responses to temporal and spatial heterogeneity in resource availability is not a new or forgotten concept in ecology, (Huffaker 1958, Armstrong et al. 2010), diverse responses make management decisions more difficult. While the diversity we observed among Pacific Northwest estuarine food webs limits our ability to pinpoint the spatial extent and strength of food web connectivity in generalized estuarine ecosystems, we can confidently say that estuarine food webs rely on OM sources emanating from the full suite of ecosystems within the estuarine mosaic. Therefore, estuarine detritus-based food webs are likely enhanced when all ecosystem components are present in the landscape, and when physical and biological barriers to connectivity among estuarine ecosystem components are minimized such that estuarine consumers have access to a diverse array of OM types contributing to the detritus pool.

We thus borrow a page from financial planners, advocating that managers employ the portfolio theory (Figge 2004) when considering how to ensure strong food web support from the bottom up. In financial terms, the diversification of securities within an investment portfolio minimizes risk of economic losses, while generally providing stable returns. Large returns, of course, are generally associated with larger risk. Thus, the job of the portfolio manager is to optimize the risk-return ratio. When applied to ecology, portfolio theory suggests that increased biodiversity will guard against risk, such as the risk of population destabilization or decreased productivity. As pointed out by Figge (2004), however, ever increasing biodiversity may not continually result in better ecological returns (i.e. ecosystem resilience, population productivity). As we face reductions, extinctions, or local extirpation of species, ecosystems and landscape heterogeneity, the job of the natural resource manager is to identify which species or ecosystems to include in the conservation or preservation portfolio in order to optimize the risk-return ratio.

When applied to ecological resilience, the portfolio effect operates at multiple, hierarchical scales. As clearly exemplified in the Alaskan salmon fishery, a mixture of salmon populations emanating from different genetic stocks, stream reaches, lake regions, watersheds, and even landscape regions (i.e. Bristol Bay, Alaskan Peninsula, Gulf of Alaska) combine to form a robust whole that weathers shifts in environmental conditions throughout time (Schindler et al. 2010, Rogers and Schindler 2011). In years where one population blinks off, the population of another blinks on, thereby providing a system of checks and balances across the landscape that sustains the Alaskan meta-population. Preservation of heterogeneity within and across salmon habitats is therefore critical to the continued resilience and productivity of Alaskan salmon.

Similarly in estuarine detritus-based food webs, we can imagine the portfolio being composed of all the different sources of OM available in the system, as well as the ecosystems in which those sources are produced. Readily bioavailable sources of OM support, such as phytoplankton or benthic microalgae, are widely accepted as the sources of food web support that generate the most return (i.e. organism growth) (Mueller-Solger et al. 2002, Sobczak et al. 2002, Jassby et al. 2003, Ruesink et al. 2003, Banas et al. 2007, Brett et al. 2009). Phytoplankton production, however, is not only ephemeral in terms of its temporal availability, but is strongly dependent upon a myriad of factors including freshwater flow, water residence time, nutrient loading, turbidity, seasonal light levels, and grazing pressure (Jassby et al. 2003). As a result of these dependencies, large swings in phytoplankton production have been documented in estuarine ecosystems, often falling below the threshold of growth limitation for zooplankton (Jassby et al. 2003). Sole dependence on phytoplankton to fuel estuarine food webs is therefore risky from an organism's perspective, as the temporal dynamics of phytoplankton production are wrought with periods of feast and famine.

One of the main advantages of a detritus-based system occurs as a result of its temporal stability on a seasonal scale (Nakano and Masashi 2001, Takimoto et al. 2002). Detritus contributes material to the food web on a continual basis throughout the year, providing sustenance to estuarine consumers between the pulsed blooms of phytoplankton. Temporal consistency is achieved in three ways: 1) the differential timing of vascular plant and algal senescence in terrestrial, aquatic, and marine ecosystems, 2) the timing of physical forces (e.g. winter and spring freshets, spring tidal series) to transport detritus, and 3) the lability of plant material and the consequent relationship to decomposition timing within the microbial loop. In much the same way that temporal stability of Alaskan salmon meta-populations is achieved by variable population responses across a diverse landscape, diversity among sources of OM contributing to the detritus pool creates temporal consistency in OM availability in estuarine ecosystems. As my results show, estuarine consumers— detritivores and predators alike— capitalize on the steady availability of marsh-produced, eelgrass-produced, and marine macroalgae-produced detritus for year-round support. The stable availability of these combined sources, however, is balanced against lower nutritional returns compared phytoplankton (Mueller-Solger et al. 2002, Sobczak et al. 2002, Brett et al. 2009). When assessing how best to support estuarine food web productivity, the natural resource portfolio manager must thus balance the importance of temporally pulsed periods of high phytoplankton productivity with the benefits of longer-term availability associated with lower quality detritus.

In addition to managing for temporal stability in food web support, it is important to also manage for diversity in types of food web support. For example, phytoplankton provides only one type of food source which could theoretically be deficient in certain nutritional aspects important to the growth and survival of estuarine consumers. Microbial and fungal conditioning of marsh detritus may provide estuarine consumers with specific dietary requirements, such as protein, during periods of the year when physiological demands are high, but high quality algal sources are limited. As discussed in Chapter 3, descriptions of primary producer nutritional content are limited (Kreeger 1993), and the mechanisms of caloric bioavailability are complex and interactive (Tenore 1983, Kreeger 1993, Torzilli et al. 2006), making it difficult at this point to ascertain which specific types of detritus provide what types of nutritional support. What is known, however, is that the physiological requirements of organisms shift seasonally (Kreeger 1993), and that estuarine organisms have physiological demands that do not necessarily coincide with phytoplankton bloom timing. Given that plant structure strongly affects microbial and fungal conditioning, a process that in turn affects the nutritional content of conditioned detritus (Torzilli et al. 2006), it would be prudent to maintain a high diversity of OM sources in estuarine ecosystems to ensure that all physiological stages of organism growth and maintenance are supported.

My results, coupled with portfolio theory, suggest that in light of temporal and spatial heterogeneity in the availability and quality of food resources, the resilience of estuarine detritus-based food webs depends upon the conservation of the full portfolio of ecosystem components within the estuarine mosaic.

Landscape setting influences food web function and connectivity

In addition to the importance of obtaining a diverse portfolio of OM sources, my results suggest that the resilience of estuarine detritus-based food webs may also depend upon whether the mechanisms by which organisms access detritus produced within estuarine landscapes are intact. As identified in this dissertation, landscape configuration controls organism access to detritus by determining the system's capacity for OM production, as well as the availability, transport, and retention of OM sources across space and time. In addition to the physical transport of OM by water advection, the ability for mobile organisms to move across estuarine ecotones presents a second, critical mechanism for creating food web connectivity at the landscape scale, especially in embayment-type estuaries where fluvial forcing is limited. While organism access to the estuarine portfolio of OM sources clearly depends on a suite of interacting factors, I would like to reiterate my contention that landscape alterations that reduce distributary channels (in both length and number) in estuarine deltas have far reaching implications for food web support in estuarine ecosystems, and are therefore an important area on which to focus restoration efforts.

As discussed extensively in Chapter 1, distributary loss can result in the "firehose effect", whereby levees confining the full discharge of a river to one or a few outlet channels results in high velocity seaward plumes that expediently export sediments and OM away from estuarine deltas (Syvitski et al. 2005). Our results from the Skagit River estuary imply that high flow velocity can produce disjointed connectivity by exporting OM beyond the estuarine delta, instead depositing fine particulate detritus in deeper subtidal troughs, coastal basins, or nearby pocket bays. The "firehose"- type export of estuarine-produced OM may therefore render shallow estuarine deltas less productive as nursery habitats for commercially and culturally important species of juvenile fish and invertebrates, such as Chinook salmon (*O. tshawytscha*) and Dungeness crab (*Metacarcinus magister*). The observed "firehose effect" in the Skagit estuary, however, is largely without physical characterization, and thus begs for a resolution to the problem of determining appropriate physical metrics in a system without spatial and temporal consistency in boundary location.

A second ramification of distributary channel loss is the reduction of interaction potential between the location where OM is produced, and the vectors by which it is transported across ecotone boundaries (i.e. water-advected transport or organism movement). Not only does distributary channel loss reduce the interior/edge ratio of the fluvial-terrestrial interface, but if distributary channels are confined between levee walls, cross-ecotone flux of materials may be entirely blocked. Further work is required to determine the degree to which historical reduction of marsh area, distributary channel number, and ecotone permeability via levee construction have altered food web connectivity across Puget Sound deltas. The recent change analysis for Puget Sound shorelines (Simenstad et al. 2011), however, provides an excellent base from which to begin the process of identifying how upstream alterations have affected the spatial scales and strengths of food web connections in estuarine food webs. Coupling the change analysis with a characterization of the spatial extent and composition of historical detrital shadows (perhaps obtained through fatty acid biomarker characterization of OM sources in deep sediment cores) may unlock a portal through which we can identify how recent landscape alterations have affected food webs in the estuarine environment. From there, we can begin the process of identifying restoration strategies appropriate for specific estuarine landscapes and goals.

The final word: using science to inform restoration strategies

While the field of restoration ecology may be relatively young and therefore evolving, several recurring themes create a strong, scientifically-sound foundation upon which to base restoration and conservation strategies. First, restoring natural processes is paramount if the restoration or rehabilitation of ecosystem function is to be achieved, as natural process restoration at the landscape scale promotes long-term, self-sustaining mechanisms for the maintenance of ecosystem functions (Simenstad et al. 2006). Second, the inherent permeability and connectivity between estuarine ecosystems and adjacent marine, terrestrial, and aquatic systems infers that incorporating landscape context into restoration designs is paramount. Ecosystem components do not function in isolation from the surrounding landscape. Rather ecosystems are tightly bound to one another through a myriad of biological and physical mechanisms (Polis et al. 1997). Finally, ecosystem resilience may be best achieved by managing for greater diversity, as spatial and temporal heterogeneity is the rule for almost any natural process or pattern. As a result, most organisms, especially those in estuarine ecosystems, are poorly adapted to homogenous environments. While admittedly broad and open-ended, these foundations are fundamental to restoration success, and should therefore be incorporated to strategically restore and conserve estuarine ecosystems.

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Appendix 1

1. Isotope signatures of consumer indicators from Chapter 1.

Indicator Species	Estuary	Flow season	Ecotone Location	$\delta^{13}C$ (mean)	stdev	$\delta^{15}N$ (mean)	stdev	$\delta^{34}S$ (mean)	stdev
Macoma nasuta				-13.07	2.01	11.34	1.55	10.82	2.64
	Lopez			-12.06	1.14	11.23	1.12	9.27	2.46
		High		-11.45	1.17	11.04	0.98	11.20	1.52
			1	-12.09	0.55	11.41	0.51	10.53	1.15
			2	-11.00	1.29	10.77	1.15	11.68	1.60
		Low		-12.57	0.84	11.39	1.22	7.65	1.86
			1	-13.21	0.69	12.18	0.66	8.85	1.82
			2	-12.05	0.54	10.77	1.20	6.71	1.27
	Padilla Bay			-12.23	1.05	12.07	0.51	9.52	2.22
		High		-12.71	1.08	11.92	0.36	9.80	2.51
			1	-13.74	1.04	11.78	0.29	6.40	1.57
			2	-12.71	1.07	11.98	0.38	10.40	2.39
			3	-12.55	1.02	11.89	0.34	9.87	2.30
		Low		-11.83	0.85	12.20	0.57	9.29	1.93
			1	-12.27	0.70	12.46	0.72	9.82	1.39
			2	-12.02	0.90	12.26	0.56	9.32	1.90
			3	-11.50	0.70	12.06	0.50	9.10	2.07
	Port Susan Bay			-14.22	1.24	9.61	1.89	13.91	1.52
		High		-14.55	0.92	8.39	1.09	14.18	1.12
			1	-14.71	0.51	7.90	0.30	14.03	0.92
			2	-14.26	0.61	8.44	0.39	14.59	0.90
			3	-14.70	1.44	8.93	1.81	13.87	1.45
		Low		-13.95	1.40	10.57	1.84	13.70	1.74
			1	-15.26	1.05	8.62	1.36	15.04	1.76
			2	-13.60	1.72	10.78	1.34	13.46	1.22
			3	-13.25	0.31	11.83	1.12	12.89	1.47
	Samish Bay			-12.55	0.79	12.30	0.52	10.63	2.73
		High		-12.12	0.29	12.00	0.28	11.62	1.63
			1	-11.55	0.33	12.20	0.06	10.78	0.83
			2	-12.15	0.25	11.99	0.28	11.67	1.66
		Low		-12.85	0.89	12.51	0.56	9.95	3.11
			1	-12.57	0.73	12.91	0.61	9.74	2.42
			2	-12.95	0.93	12.36	0.47	10.03	3.35
	Skagit Bay			-14.29	2.92	10.76	1.79	11.79	1.85
		High		-13.98	3.03	10.35	1.72	11.62	1.85
			1	-18.28	1.79	8.38	1.22	9.76	1.86

			2	-12.26	1.11	11.14	1.17	12.36	1.23
		Low		-14.62	2.76	11.20	1.76	11.97	1.83
			1	-18.86	2.74	8.56	0.83	11.39	1.85
			2	-13.30	0.58	12.01	1.03	12.07	1.80
			3	-13.36	0.21	12.13	0.08	13.71	0.88
Mytilus trossulus				-17.17	1.56	9.05	0.91	17.34	1.15
	Lopez			-17.60	0.70	7.28	0.73	15.44	1.96
		High		-17.36	0.70	7.27	0.53	16.72	0.55
			1	-17.18	0.31	7.57	0.52	16.42	0.34
			2	-17.46	0.85	7.10	0.48	16.88	0.59
		Low		-17.73	0.68	7.29	0.83	14.73	2.10
			1	-17.83	0.31	7.73	0.71	15.42	0.73
			2	-17.67	0.82	7.04	0.81	14.34	2.52
	Padilla Bay			-15.71	0.90	9.68	0.44	17.56	0.82
		High		-15.87	0.61	9.46	0.40	17.29	0.65
			1	-15.77	0.67	9.87	0.34	16.96	0.74
			2	-15.94	0.49	9.37	0.36	17.40	0.75
			3	-15.82	0.73	9.41	0.38	17.30	0.39
		Low		-15.52	1.11	9.93	0.34	17.86	0.88
			1	-15.74	0.37	10.03	0.33	18.22	0.97
			2	-16.05	0.82	9.92	0.34	17.78	1.06
			3	-14.66	1.17	9.90	0.36	17.86	0.46
	Port Susan Bay			-18.08	0.86	9.16	0.65	17.64	0.94
		High		-18.22	1.02	8.89	0.55	17.62	1.05
			1	-17.66	0.66	9.12	0.47	17.50	0.96
			2	-18.73	1.41	8.81	0.49	17.34	1.41
			3	-18.81	0.43	8.61	0.42	18.10	0.69
		Low		-17.90	0.58	9.47	0.61	17.66	0.81
			1	-17.60	0.51	10.04	0.25	16.98	0.29
			2	-17.95	0.33	9.46	0.34	18.27	0.84
			3	-18.10	0.71	9.05	0.63	17.72	0.67
	Samish Bay			-15.83	0.40	9.38	0.58	16.91	1.00
		High		-15.75	0.49	9.09	0.50	16.80	1.00
			1	-15.85	0.55	9.21	0.38	16.10	1.04
			2	-15.69	0.46	9.02	0.56	17.20	0.75
		Low		-15.94	0.17	9.81	0.41	17.07	1.02
			1	-15.96	0.19	9.95	0.43	17.24	1.19
			2	-15.92	0.13	9.58	0.29	16.79	0.72
	Skagit Bay			-18.55	0.96	8.62	0.72	17.47	0.85
		High		-18.87	1.12	8.52	0.77	17.40	0.75
			1	-18.89	1.60	9.22	0.96	16.88	1.01
			2	-18.87	1.04	8.40	0.67	17.48	0.68
		Low		-18.23	0.61	8.73	0.65	17.54	0.94

1	-18.10	0.91	9.37	0.67	16.71	1.23
2	-18.27	0.47	8.52	0.49	17.81	0.62

Dedication

To my parents, John and Cynthia Howe

Vita

Emily Russell Howe grew up on Mercer Island, Washington, and graduated from Mercer Island High School with an interest in natural sciences and exploring the wild places of the world. In 2001, she received a B.S. from Middlebury College with a joint major in Environmental Studies and Biology. As an undergraduate, Emily focused on marine and aquatic ecology, studying coral reef communities in the San Blas Islands of Panama and Zanzibar, Tanzania. She also spent a field season documenting aquatic macrophyte communities in Vermont lakes before returning to Washington State, where she completed a two year internship with the Nature Conservancy. Emily earned both an M.S. (2006) and Ph.D. (2012) from the School of Aquatic and Fishery Sciences at the University of Washington. In her dissertation research, Emily focused on the estuaries of home- northern Puget Sound and Hood Canal. She was the recipient of a Graduate Research Fellowship from the National Estuarine Research Reserve program (2007-2009), the Claire L. and Evelyn S. Egtvedt Fellowship (2006), a scholarship from Anchor Environmental, Ltd. (2006) and the Garden Club of America (2006). She also wrote, and received a grant from the National Science Foundation (2007-2012), which largely funded this dissertation.

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