

Using stable isotopes to discern mechanisms of connectivity in estuarine detritus-based food webs

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ABSTRACT: In this paper, we focus on 2 mechanisms of cross-boundary food web connectivity in Puget Sound estuaries: passive transport of water-advected organic matter (OM) and active movement of organisms. Both mechanisms serve as potential vectors of food web connectivity, but little research has investigated whether landscape setting changes the dominance of one mechanism over another, or whether the influence of organism movement on food web connectivity can be detected in estuarine systems. We use fish diets, stable isotopes and Bayesian mixing models to identify differences in OM sources assimilated by estuarine fishes, testing whether increased organism mobility or increased fluvial influence results in greater food web connectivity. We compare food web connectivity in 2 different estuaries, one displaying limited freshwater inputs, and the other the terminus of a major river system. Within each estuary, we investigate whether differences in behavioral life history traits correspond to differences in the diets, isotopic signatures and OM assimilation of 2 fish species: bay pipefish *Syngnathus leptorhynchus*, which displays site fidelity to eelgrass beds, and the more transitory juvenile English sole *Parophrys vetulus*, which moves throughout estuarine deltas during the early demersal growth stage. Our results show water advection plays a dominant role in large-scale OM transport and delivery to adjoining ecosystems in the fluvial estuary, while organism movement provides the more important mechanism of food web connectivity in the estuary exhibiting minor fluvial discharge. However, the 2 mechanisms certainly interact to enhance food web connectivity across estuarine ecotones.

KEY WORDS: Stable isotopes · Estuarine ecology · Food web connectivity

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INTRODUCTION

Cross-ecosystem transport of organic matter (OM), nutrients, and organisms provides important subsidies of trophic energy to spatially disparate communities. These resource subsidies are not only ubiquitous across ecosystems, with generally positive effects on broad taxonomic groups, but often control population, community, and food web structure (Polis et al. 1997, Huxel & McCann 1998, Marczak et al. 2007). Energy subsidies across ecotones (i.e. ecosystem boundaries; Holland et al. 1990), thus highlight the importance of food web connectivity at the landscape scale.

Trophic energy subsidies are strongly influenced by landscape-scale factors, such as ecosystem availability and productivity, or boundary permeability and area-to-perimeter ratios (Polis et al. 1997, Cadenasso et al. 2004, Greenwood & McIntosh 2008, Garcia et al. 2011). Thus, landscape 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 & McIntosh 2008, Young et al. 2010). Therefore, it is critical to understand the mechanisms of, and responses to, cross-ecotone transfer, if our aim is to maintain ecosystem integrity.

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Specific mechanisms promoting or disrupting food web connectivity across landscape ecotones are still relatively unidentified for many ecosystems (Greenwood & McIntosh 2008, Sheaves 2009). In fluvial and estuarine ecosystems, water has long been considered the principle mechanism of connectivity, pushing nutrients across ecosystem boundaries to regulate metabolism in adjacent ecosystems (Odum 1980, Vannote et al. 1980, Polis et al. 1997)¹. More recently, however, active 'trophic relay', or organism movement (Kneib 2000), has been identified as an important mechanism of cross-ecotone energy transfer. 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. Energy transfer via trophic relay has the capacity to transport nutrients across entire landscapes, even against the gravitational gradient in a sort of reciprocal subsidy (Nakano & Masashi 2001), as exemplified by anadromous salmonids subsidizing freshwater ecosystems with marine-derived nutrients (e.g. Schindler et al. 2003, Moore et al. 2008).

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 & Masashi 2001, Connolly et al. 2005, Vinagre et al. 2006, Vizzini & Mazzola 2006). However, the relative importance of energy transferred via passive OM transport, as opposed to organism movement, is likely extremely variable and dependent upon specific ecosystem characteristics.

In this study, we focused on 2 pathways of trophic energy flows across estuarine ecotones: the passive (water-advected) transport of detrital OM, and the active movement of nekton among ecosystems. We assessed which process comprises the primary mechanism through which trophic energy flows across estuarine ecotones under estuarine settings with different amounts of fluvial influence. We specifically compared passive OM transfer by estuarine circulation to active OM transfer via nekton movement by comparing isotopic and diet compositions of resident fishes (bay pipefish *Syngnathus leptorhynchus*) and highly mobile, transient fishes (English sole *Parophrys vetulus*) in 2 estuaries with contrasting hydrologic regimes. We investigated whether strong differ-

ences in life history traits correspond to differences in isotopic signatures and diet² between the bay pipefish and English sole. We then used multiple stable isotopes in a Bayesian mixing model to infer cross-ecotone connectivity by identifying the OM sources supporting each fish species. Finally, we considered whether the relationship between the 2 fish species changes according to estuarine setting.

We hypothesized that (1) highly mobile, transitory fish will display greater food web connectivity by assimilating OM originating from more ecosystems within the estuarine environment, while 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 & McIntosh 2008, Mortillaro et al. 2011, Vinagre et al. 2011), we also examined variation in food web connectivity between 2 estuaries: an estuarine embayment with limited freshwater inputs, and an estuarine river delta at the terminus of a major river system that exhibits frequent flooding and pulsed, seasonal outflow. Thus, we further hypothesized that (2) increased fluvial influence will reduce food web compartmentalization by spatially integrating OM sources originating from discrete ecosystems across the estuarine landscape. We therefore expected the isotope signatures of the 'stationary' bay pipefish and mobile juvenile English sole to converge under high fluvial conditions, and diverge under low flow/non-fluvial conditions.

MATERIALS AND METHODS

Study sites

The study area was located in Padilla and Skagit bays, 2 estuaries located in Washington State, Pacific Northwest USA (48° 25' N, 122° 29' W, Fig. 1). Both estuaries exhibit mixed, semi-diurnal macrotidal regimes (>3 m tidal range), with strong spring-neap tidal cycles. Surface water temperatures range between 10 and 17°C in summer, and between 7 and 10°C in winter (Bulthuis 1993, Gustafson et al. 2000).

¹Here, 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

²Given 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 2 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

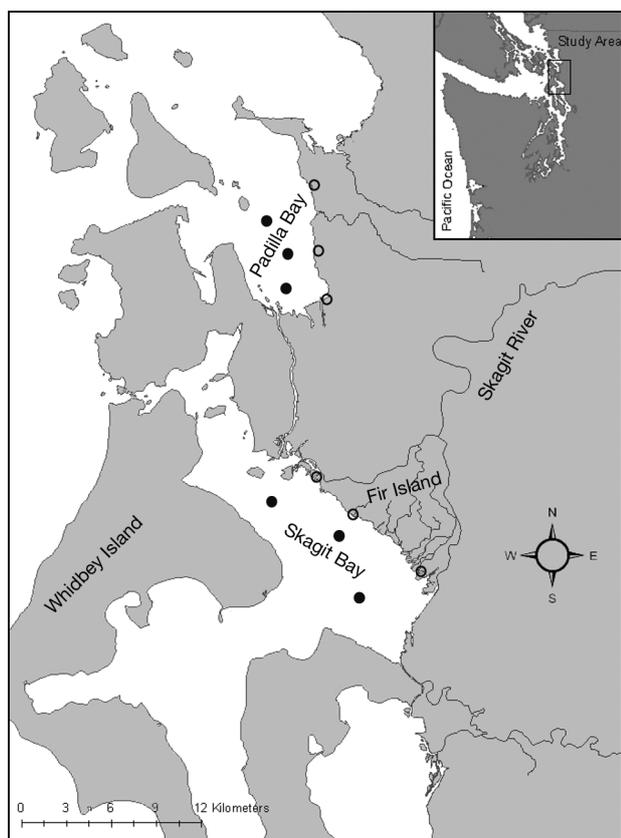


Fig. 1. Study area in northern Puget Sound, Washington, USA. (●) Fish, eelgrass, and particulate organic matter (POM) sampling sites, all located in eelgrass (*Zostera marina*) beds on the outer margin of the estuarine delta. (○) Collection sites for organic matter (OM) sources originating in emergent marsh ecosystems and adjacent mudflat ecosystems

Although the intertidal area of Skagit Bay (75 km²) is larger than Padilla Bay (45 km²) (Nelson 1989, Grossman et al. 2011), both sites are characterized by extensive deltaic fans (<5 m depth) and exhibit a mosaic of ecosystems, including emergent tidal marsh, sand or mudflats, and eelgrass (*Zostera marina*) (Bulthuis & Shull 2006, McBride et al. 2006). Eelgrass areas in Skagit and Padilla Bays are comparable in size (2846 and 3170 ha, respectively). However, in Skagit Bay, most of the nearshore (58%) is comprised of sandflats, while eelgrass forms a fringe at the delta's outer margin. In contrast, in Padilla Bay, nearly 70% of the nearshore is vegetated, mainly by extensive eelgrass meadows.

The 2 estuaries exhibit profoundly different hydroperiods. With a watershed covering 8544 km², the Skagit River is the largest river draining into Puget Sound, accounting for between 34 and 50% of the sound's freshwater inputs, depending on season

(Babson et al. 2006). River flow peaks (with maxima of up to 5100 m³ s⁻¹) during heavy winter rains (November to January), and again during the late spring due to snowmelt from the surrounding mountains (Hood 2010). The smallest flows (78 m³ s⁻¹) typically occur in September (Wiggins et al. 1997). Mean discharge near the estuary is 468 m³ s⁻¹ (USGS 2011). The Skagit River splits into north and south forks before entering Skagit Bay, with more than 80% of fresh water discharging through the South Fork distributary channels. Circulation in the estuary is strongly affected by the magnitude of freshwater inflow and strong tidal currents, as >90% of the volume of Skagit Bay enters and exits within a tidal cycle (Yang & Khangaonkar 2009). Mean salinities in Skagit Bay range between 18 and 28 psu, but intertidal channel salinities are often <0.5 psu (E. R. Howe unpubl. dissertation). During spring tides, maximum flow velocities over intertidal flats fall to between 24 and 60 cm s⁻¹ (E. R. Howe unpubl. dissertation). The Skagit River estuary exhibits strong stratification, but de-stratification can occur during the flood tide (Yang & Khangaonkar 2009).

In contrast, Padilla Bay is now virtually isolated from significant freshwater inputs, although historically it was part of the distributary channel network of the Skagit River delta, before extensive diking (Collins & Sheikh 2005). As an 'orphaned' estuarine embayment, the largely agricultural 93 km² watershed receives fresh water from 3 agricultural sloughs and 1 seasonal stream that reach peak precipitation-based flows during winter (Nelson 1989, Bulthuis 1996). Freshwater flows are limited (0.2 m³ s⁻¹ mean flow), and connectivity is truncated by tide gates on each slough. 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 (Bulthuis & Conrad 1995). 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 (30 cm s⁻¹) (Bulthuis & Conrad 1995).

Study organisms

Two estuarine-dependent fish, representative of contrasting life history strategies, were chosen for this study: bay pipefish *Syngnathus leptorhynchus*, because of its close association and assumed specific fidelity to eelgrass habitats (Wilson 2006, Shokri et al. 2009, Johnson et al. 2010); and juvenile (<150 mm

total length [TL]) English sole *Parophrys vetulus*, because it is 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). In general, syngnathids (pipefish) are widely known for their strong association with preferred habitat, low mobility, and extremely restricted home ranges (Shokri et al. 2009). 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), using eelgrass as refuge from predators, spawning habitat, and foraging grounds (Hart 1973, Bayer 1980). Many temperate pipefish species make seasonal migrations between shallow, vegetated estuarine areas in the spring and summer and deeper offshore areas during the winter (Lazzari & Able 1990), but this scale of migration has not been described for *S. leptorhynchus*. In contrast, bay pipefish undergo within-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).

In contrast, larval English sole hatch 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, following the tide across estuarine ecosystem components such as eelgrass beds, tidal channels, and sand flats (Rooper et al. 2003). Juveniles emigrate from the estuary into deeper waters at ~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 that 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 1980, Ryer & Orth 1987, Simenstad et al. 1995, Vizzini & 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 & Sibert 1977, Schmid-Araya & Schmid 2000).

Sample collection

Fish

We collected fish from Skagit and Padilla bays in the spring and late summer in order to capture seasonal food web differences associated with high and low freshwater flow periods. Specimens were collected using a combination of pole seines (2 m tall, 6 mm mesh bag), plankton nets (0.5 m diameter, 273 μm mesh), and modified lampara nets (operated by the US Geological Survey [USGS] and the Skagit River System Cooperative). Fish were exclusively collected from eelgrass (*Zostera marina*) beds positioned on the seaward edge of each estuarine delta (~1 m below mean lower low water [MLLW]) to ensure that both species were sampled in the same spatial context with regards to the sources of OM available in each estuary. In both estuaries, fish collection points were ~4 km seaward of the marsh ecosystem. Three sampling points were implemented across the face of each estuary in order to capture any variation in fish diets across space within the eelgrass beds. Due to difficulty capturing fish, sufficiently large samples ($n = 10$) were only available from Skagit Bay in May and August 2007, and from Padilla Bay in March and September 2008 and May 2009. Collected fish were placed on ice immediately to slow digestion; then frozen.

Organic matter sources

In June 2006, we collected 4 replicate samples of dominant primary producers from each of the major ecosystems found in each estuary (e.g. marsh, mudflat, and eelgrass ecosystems). Plants (apical foliage) and algae were frozen until preparation for lab analysis. Phytoplankton and particulate organic matter (POM) samples were collected using a 20 μm plankton net. Phytoplankton signatures were calculated from phytoplankton 'rich' samples, where the C:N ratio fell to between 5 and 9. Carbon and nitrogen signatures were extrapolated from a regression line fitting the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, or $\delta^{34}\text{S}$ versus the C:N ratio, with 'pure' phytoplankton assumed to correspond to a C:N ratio of 6.6, matching the Redfield ratio. While inter-annual variation in isotopic sources has been documented (Cloern et al. 2002), the magnitude of variation is usually between 2 and 4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and between 2 and 5‰ for $\delta^{34}\text{S}$ (Stribling et al. 1998). Temporal variation in isotope values is thus small enough to ensure that terrestrial and marsh-

Table 1. Average (± 1 SD) $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values of dominant organic matter (OM) 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 NND² 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*, *Schoenoplectus americanus*, *S. acutus*, *S. maritimus*, *Cotula coronopifolia*, and *Glaux maritima*. *Ulva* spp. includes *U. intestinalis* and *U. fenestrata*. ASG = *Atriplex patula*, *Salicornia virginica*, and *Glaux maritima*

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

derived OM sources rarely overlap with marine-derived phytoplankton, algae, and eelgrass³. 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 our purposes, we assumed that inter-annual variation in OM sources was minimal enough to warrant building an isotopic mixing model for consumers based on source signatures from previous years, but spatial variation was great enough to warrant developing separate OM baselines for each estuary (Table 1).

³In a tandem study, we observed no major shift across years in clam and mussel signatures collected from Padilla and Skagit bays, suggesting inter-annual variation in OM source signatures was minimal across the time period spanning OM source and fish collections (E. R. Howe unpubl. dissertation). We thus feel confident in using 2006 isotope data from OM sources, and 2007, 2008, and 2009 isotope data from fish

Sample preparation

Fish diet composition

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. 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 associated 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. Since it provides a direct measure of biomass intake, we primarily used gravimetric data to indicate prey consumption, but also considered the numerical abundance and frequency of occurrence of prey items.

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. Primary producers were rinsed in 10% HCl to remove soil carbonates, then rinsed 3 times in deionized water to neutral pH. All samples were freeze-dried for 48 h and 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 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ and C:N ratio values were analyzed using a Finnigan DELTAplusXP continuous flow Isotope Ratio Mass Spectrometer (IRMS) (Thermo Fisher) coupled with an ECS 4010 elemental analyzer (Costech). The isotopic 2-sigma

uncertainty of isotopic results was 0.5‰. We express isotopic ratios in δ notation, which describes the per mille (‰) enrichment (+) or depletion (–) of the heavy isotope to the light isotope of the targeted element relative to an international standard, i.e. δX (‰) = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $X = {}^{13}\text{C}$, ${}^{15}\text{N}$, or ${}^{34}\text{S}$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$, ${}^{15}\text{N}/{}^{14}\text{N}$, or ${}^{34}\text{S}/{}^{32}\text{S}$. Because lipid content of skeletal muscle tissue varies across space and time, and because lipids are approximately 6 to 7‰ depleted in ${}^{13}\text{C}$ relative to protein, we performed an arithmetic lipid correction before interpreting our fish isotope signatures (Post 2002, Sweeting et al. 2006). Arithmetic corrections were based on chemical lipid extractions performed on a subset of fish muscle samples using the methods of Sweeting et al. (2006). Isotopic results from 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 $\delta^{13}\text{C} = 0.9507 \times$ uncorrected $\delta^{13}\text{C} - 1.2385$, $R^2 = 0.99$; *S. lepto-*
rynchus: lipid corrected $\delta^{13}\text{C} = 0.8731 \times$ uncorrected $\delta^{13}\text{C} - 2.5567$, $R^2 = 0.98$). Lipid corrected values were used for mixing model analyses.

Estimating organic matter source contributions

We estimated the relative percent contribution of primary producer groups to the 'base' diet of fish using 2 multiple source mixing models: SOURCE and MixSIR (Lubetkin & Simenstad 2004, Semmens et al. 2009, Ward et al. 2010). SOURCE identifies isotopically distinct primary producer groups using a nearest neighbor distance, and estimates consumer trophic position. We used trophic position to calculate appropriate isotopic fractionation effects for each isotope for use with the MixSIR model. Based on values presented by Vander Zanden & Rasmussen (2001), McCutchan et al. (2003), and Sweeting et al. (2007), we started with the following input parameters for trophic fractionation effects: $\delta^{13}\text{C} = 1.3 \pm 0.3\%$ (SD), $\delta^{15}\text{N} = 3.2 \pm 0.43\%$, and $\delta^{34}\text{S} = 0.5 \pm 0.56\%$. Where appropriate, fractionation values account for variation associated with lipid extraction, aquatic species, and white muscle tissue. In doubling the fractionation effect to capture 2 trophic level shifts, we summed the variances according to the methods of Vander Zanden & Rasmussen (2001). Final fractionation values used for the model were: $\delta^{13}\text{C} = 2.6 \pm 0.18\%$, $\delta^{15}\text{N} = 6.4 \pm 0.20\%$, and $\delta^{34}\text{S} = 1.0 \pm 0.63\%$. While SOURCE can be used to estimate consumer diets, we used MixSIR because of its superior treatment of uncertainty using a Bayesian framework to

incorporate variation in source isotope signatures and fractionation rates (Layman et al. 2012). Additionally, MixSIR estimates posterior probability distributions describing the percent contribution of each primary producer's contribution to the base diet of individual consumers, thus providing a measure of confidence to the model output.

We performed Gibbs sampling (Plummer 2003) for each Bayesian mixing model using 3 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). Separate mixing models were used for each estuary.

Data analysis

Gravimetric composition of prey in individual fish diets was analyzed according to 2 levels of resolution: (1) the lowest taxonomic resolution possible and (2) the prey habitat (e.g. benthic, epifaunal, and pelagic). Only prey organisms comprising $\geq 10\%$ of total gravimetric diets were included in statistical analyses.

We employed multivariate data analyses (Primer® 6) to examine patterns associated with fish diets, isotope signatures, and proportional OM contributions supporting fish diets across sites, species, and dates. We analyzed all data types using Primer's non-metric multidimensional scaling (NMDS) ordination, permutational multivariate analysis of variance (PERMANOVA), and similarity percentage (SIMPER) analyses. All diet data and OM source estimates were square-root transformed and analyzed using a Bray-Curtis similarity matrix (Clarke & Gorley 2001, Clarke & Warwick 2001). For isotope data (untransformed), we used the Euclidean distance coefficient to construct a similarity matrix prior to further analyses. 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.

To eliminate ontogenetic changes in the gravimetric composition of prey in fish diets 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 (predictor variable) and diet variation (square-root transformed prey data, 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 permutational p -value describing significance.

RESULTS

Fish diets

We retained 93 fish for analysis (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 eliminated ontogenetic changes in diet composition as a confounding factor in our seasonal food web comparisons because fish length generally did not explain appreciable variation in diet composition for either fish species. For juvenile English sole, length respectively accounted for 9.3% ($p = 0.04$) and 7.5% ($p = 0.04$) of the seasonal gravimetric diet variation observed in Skagit and Padilla bays. For bay pipefish, length respectively explained 14.9% ($p = 0.08$) and 16.0% ($p = 0.006$) of diet variation in Skagit and Padilla bays. For both species and sites, length explained considerably more variation in diet within a season, suggesting that growth is less influential than seasonal shifts in determining the diets of bay pipefish and juvenile English sole.

Juvenile English sole consumed a higher diversity of prey compared to bay pipefish in both Padilla and Skagit bays (Fig. 2, Table 3). When prey were

Table 2. Mean lengths (± 1 SD) and sample sizes of juvenile English sole *Parophrys vetulus* and bay pipefish *Syngnathus leptorhynchus* during high and low river flow periods, 2008 to 2009, in Padilla and Skagit bays, Puget Sound, Washington. A two sample t -test was performed to examine length differences between flow periods within each species for each estuary

	— High flow	— Low flow			t	p
	Length (mm)	n	Length (mm)	n		
Padilla Bay						
<i>P. vetulus</i>	57.6 \pm 13.5	19	83.6 \pm 13.3	10	-4.95	<0.0001
<i>S. leptorhynchus</i>	131.6 \pm 35.1	16	205.2 \pm 21.7	10	-5.93	<0.0001
Skagit Bay						
<i>P. vetulus</i>	69.2 \pm 29.4	10	114.0 \pm 14.2	10	-4.34	<0.001
<i>S. leptorhynchus</i>	186.5 \pm 34.1	8	217.7 \pm 38.8	10	-1.78	0.09

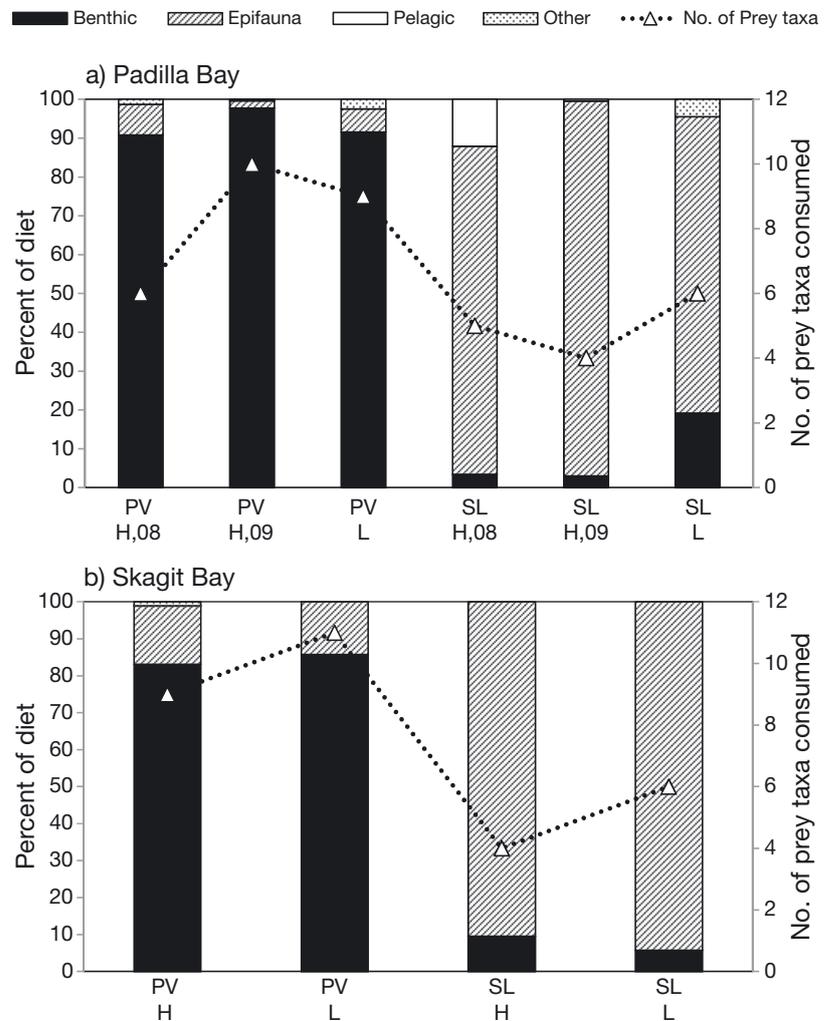


Fig. 2. *Parophrys vetulus* and *Syngnathus leptorhynchus*. Gravimetric composition of prey, designated by functional habitat (bar chart), and (Δ) total number of prey taxa consumed, for juvenile English sole (PV) and bay pipefish (SL) in (a) Padilla Bay and (b) Skagit Bay, Puget Sound, Washington. H: High flow period; L: low flow period. For Padilla Bay, 'H,08' and 'H,09' show results for fish collected in 2008 and 2009, respectively. Numbers of prey taxa shown include taxa comprising $\geq 10\%$ of the group diet by weight

classified by species, we consistently observed significant diet differences between fish species within a flow period, and between flow periods within a fish species (Table 4). Juvenile English sole notably consumed more polychaetes, bivalves and tanaids during

high flow conditions, and more harpacticoid copepods, oligochaetes, and gammarid amphipods during low flow conditions (Table 5). Pipefish consumed more caprellid amphipods and juvenile shrimp (Hippolytidae, Pandalidae) during low flow conditions, and more gammarid amphipods and harpacticoid copepods during high flow conditions (Table 5).

Table 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 *Parophrys vetulus* and bay pipefish *Syngnathus leptorhynchus* during high and low flow periods, 2008 and 2009, in Padilla and Skagit bays, Puget Sound, Washington. Indices for diets were calculated from prey items composing >10% of the diet based on gravimetric composition

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

When prey were classified by habitat group, seasonal diet differences within a species were not significant, indicating that each fish species continued to feed from the same functional habitats (i.e. benthic, epibenthic, water-column) year-round (Fig. 2, Table 4). We thus attributed 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. Significant differences in prey composition were observed between fish species within a flow period when prey were classified at the habitat level (Table 4). Over 80% of juvenile

Table 4. *Parophrys vetulus* and *Syngnathus leptorhynchus*. Significance tests (PERMANOVA analysis) comparing the diets of juvenile English sole (PV) and bay pipefish (SL) during high and low river flow conditions in Padilla Bay (embayment estuary) and Skagit Bay (river delta estuary), Puget Sound, Washington. Diets for significance testing were defined by species, habitat groups, isotope signatures and the organic matter (OM) source contributions, calculated by the MixSIR Bayesian stable isotope mixing model. NSD: no significant difference

Main effects	Prey species		Prey habitat		Isotope signatures		OM source	
	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>
Padilla Bay								
Flow period	5.61	0.0001			9.86	0.0001		
Species	NSD	NSD			25.17	0.0001		
Species × Flow	7.32	0.0001			15.05	0.0001		
Post-hoc tests	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
PV High × Low	1.86	0.0001	NSD	NSD	6.84	0.0001	7.63	0.001
SL High × Low	3.15	0.0001	NSD	NSD	NSD	NSD	NSD	NSD
High PV × SL	2.29	0.001	3.78	0.001	2.16	0.0009	1.71	0.041
Low PV × SL	3.35	0.0002	2.28	0.001	7.00	0.0001	6.08	0.001
Main effects	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>	Pseudo- <i>F</i>	<i>p</i>
Skagit Bay								
Flow period	3.03	0.0003			NSD	NSD	NSD	NSD
Species	4.27	0.0001			4.96	0.009	12.16	0.001
Species × Flow	2.03	0.008						
Post-hoc tests	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
PV High × Low	1.30	0.032	NSD	NSD	NSD	NSD	NSD	NSD
SL High × Low	1.87	0.015	NSD	NSD	NSD	NSD	NSD	NSD
High PV × SL	1.78	0.0007	2.03	0.004	NSD	NSD	NSD	NSD
Low PV × SL	1.78	0.0005	3.49	0.001	2.30	0.007	3.45	0.005

Table 5. Prey taxa that contributed >10% gravimetric contribution to the diets of juvenile English sole *Parophrys vetulus* and bay pipefish *Syngnathus leptorhynchus* during seasonal high and low river flow conditions, 2008 and 2009, in Padilla and Skagit bays, Puget Sound, Washington

Prey taxa	Padilla Bay						Skagit Bay					
	<i>P. vetulus</i>			<i>S. leptorhynchus</i>			<i>P. vetulus</i>			<i>S. leptorhynchus</i>		
	High	Low	March 2008	High	Low	Sept 2008	High	Low	August 2007	High	Low	August 2007
Amphipoda	5.15	2.23	1.96	1.46	1.17	14.70	-	1.95	-	-	-	4.90
<i>Americorophium salmonis</i>	-	2.36	13.84	-	-	0.64	4.92	15.37	-	-	-	-
<i>Caprella laeviscula</i>	-	0.39	-	-	-	70.61	-	-	-	-	-	3.45
Amphilocheidae	19.07	-	-	-	-	-	-	-	-	-	-	-
<i>Eogammarus confervicolus</i>	-	0.13	-	-	-	-	20.77	-	-	-	-	-
<i>Paracalliopiella pratti</i>	-	-	-	17.52	-	-	-	-	-	-	-	-
<i>Pontogeneia rostrata</i>	-	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	-	-	-
<i>Crangon</i> sp.	-	-	-	-	-	-	11.50	11.83	-	-	-	-
Hippolytidae	-	-	-	-	-	-	-	-	-	-	64.61	-
Mysida	-	-	-	-	-	-	-	-	28.85	12.52		
Tanaidacea												
<i>Leptochelia dubia</i>	14.95	-	-	-	-	-	-	0.43	-	-	-	-
<i>Sinelobus stanfordi</i>	-	14.96	2.17	-	-	-	-	0.19	-	-	-	-
Bivalvia												
<i>Macoma</i> sp.	-	-	0.36	-	-	-	36.68	12.49	-	-	-	-
<i>Clinocardium</i> 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		

English sole diets were composed of benthic infauna, while bay pipefish consumed a mixture of epifaunal (84 to 96%) and benthic (3 to 19%) organisms (Table 5).

Table 6. Percent similarity (SIMPER analysis) in the composition of diets for juvenile English sole *Parophrys vetulus* and bay pipefish *Syngnathus leptorhynchus* during high and low river flow conditions (2008 to 2009), in Padilla Bay (embayment estuary) and Skagit Bay (river delta estuary), Puget Sound, Washington. Within-species diet similarity is presented first for each flow period, followed by between-species similarity for each flow period

		High Flow	Low Flow
Padilla Bay			
<i>P. vetulus</i>	High flow	10.66	-
	Low flow	10.75	23.95
<i>S. leptorhynchus</i>	High flow	24.95	-
	Low flow	9.90	23.95
<i>P. vetulus</i> × <i>S. leptorhynchus</i>		3.15	4.37
Skagit Bay			
<i>P. vetulus</i>	High flow	5.59	-
	Low flow	5.99	13.39
<i>S. leptorhynchus</i>	High flow	36.02	-
	Low flow	8.83	16.49
<i>P. vetulus</i> × <i>S. leptorhynchus</i>		3.07	1.71

In both Skagit and Padilla bays, individual juvenile English sole diets varied more than individual bay pipefish diets, as mean similarities among flatfish diet compositions were lower than those for bay pipefish (Table 6). In Skagit Bay, within-group diet similarity of juvenile English sole increased between the high and low flow periods, indicating that juvenile English sole diets become more homogenized with decreasing flow (Table 6). In contrast, diets became more individualized among Skagit Bay pipefish with decreasing flow (Table 6). Results from Padilla Bay suggest greater seasonal diet shifts among English sole as compared to bay pipefish, with juvenile sole diets becoming more homogenized during the summer sampling period.

The greatest shift in diet composition between seasonal freshwater flow regimes was observed among bay pipefish in Skagit Bay, where mean diet similarities indicated greater change in diet between flow regimes than seen for juvenile English sole (Table 6). 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 strongly affected by seasonal fluctuations in freshwater flow regimes (Table 6).

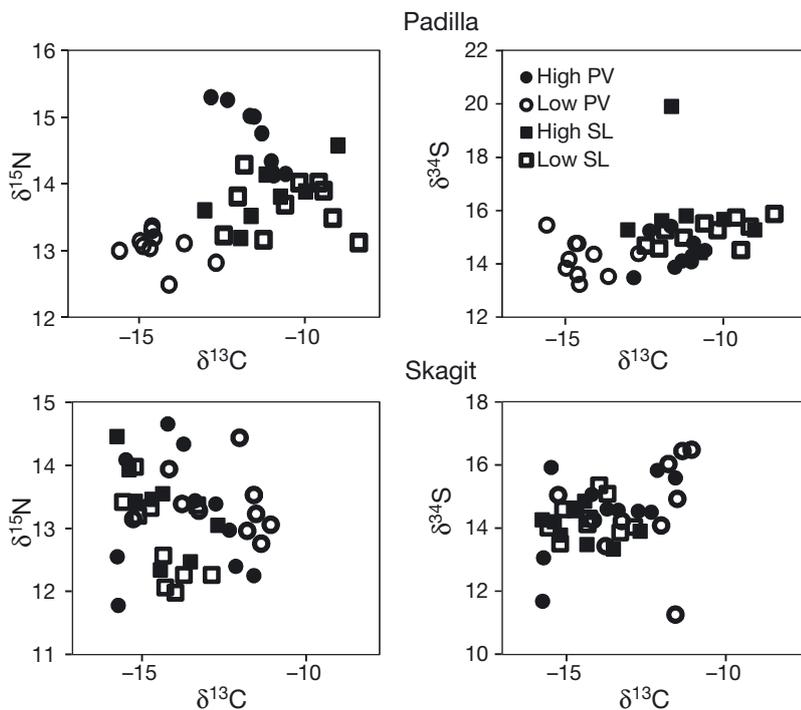


Fig. 3. *Parophrys vetulus* and *Syngnathus leptorhynchus*. Dual isotope plots (left: $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$; right: $\delta^{13}\text{C}$ vs. $\delta^{34}\text{S}$) of juvenile English sole (PV) and bay pipefish (SL) in (upper panels) Padilla and (lower panels) Skagit bays during high and low river flow periods, 2008 to 2009. (●) PV, high flow; (○) PV, low flow; (■) SL, high flow; (□) SL, low flow

Isotope delineation of organic matter food web sources

Despite overlapping $\delta^{34}\text{S}$ signatures, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values revealed consistently strong trophic separation between *Parophrys vetulus* and *Syngnathus leptorhynchus* in Padilla Bay (Table 4, Fig. 3). By contrast, the species effect in Skagit Bay was only evident during low flow conditions, when the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ isotope values of *P. vetulus* and *S. leptorhynchus* diverged (Table 4, Fig. 3). In Padilla Bay, we observed a significant seasonal depletion in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of juvenile English sole, but no seasonal shift in isotope signatures for bay pipefish (Table 4). The $\delta^{15}\text{N}$ depletion of juvenile English sole was especially notable, dropping by nearly three-quarters of a trophic level between flow periods. In Skagit Bay, no seasonal differences in isotope signatures were observed for either species.

Mixing model analysis

The contributions of OM sources assimilated by juvenile English sole and bay pipefish varied systematically with the river discharge period and estuarine setting. In Padilla Bay, OM assimilation was significantly different between species during both flow periods, but more pronounced in the summer (Table 4). We observed little seasonal change in OM support among bay pipefish in Padilla Bay. Bay pipefish consistently derived about one third of their dietary support from marine macroalgae (*Ulva* spp. and *Ceramium* spp.), followed by eelgrass (~15 to 20%), phytoplankton (~20%), marsh macrophytes (~15 to 20%), and benthic diatoms (~5%) (Table 7). In contrast, the OM assimilated by juvenile English sole changed significantly with season (Table 4, Fig. 4). During the high flow season, the OM contributions were similar to bay pipefish, with marine macroalgae comprising the largest contribution (~40%) to juvenile English sole diets, followed by marsh macrophytes (~20%), eelgrass (~20%), and phytoplankton (~6%). In the summer, juvenile English sole shifted to a diet primarily originating from marine phytoplankton (~30%), followed by marsh macrophytes (~30%), eelgrass (~15%), marine macroalgae (~15%), and benthic diatoms (5%) (Table 7). Also of note, Padilla Bay juvenile English sole consistently assimilated a greater diversity of OM sources compared to bay pipefish (Table 3).

In contrast to Padilla Bay, Skagit Bay fish were supported by statistically similar OM sources during the high flow season, but not under low flow conditions (Table 4, Fig. 4). Significant differences in the types and proportions of assimilated OM between the 2 fish species indicate divergence in food web support with decreasing freshwater flow. In general, the macroalgae *Ulva* spp. supported the majority of Skagit fish diets (~60 to 65%), followed by *Typha* sp. (~15 to 20%), and benthic diatoms (~15%) (Table 7). Somewhat surprisingly, river POM, scrub/shrub vegetation, C3 marsh plants, *Zostera marina*, and phytoplankton were not substantially assimilated. Although flow regime made no significant difference in the OM contributions assimilated by either species, OM source contributions to juvenile English sole differed slightly between seasonal flow periods: the contribution of *Typha* spp. diminished, while *Ulva* spp. contributions increased (Table 7). Additionally, juvenile English sole assimilated a slightly greater diversity of OM sources compared to bay pipefish, especially during the low flow period (Table 3).

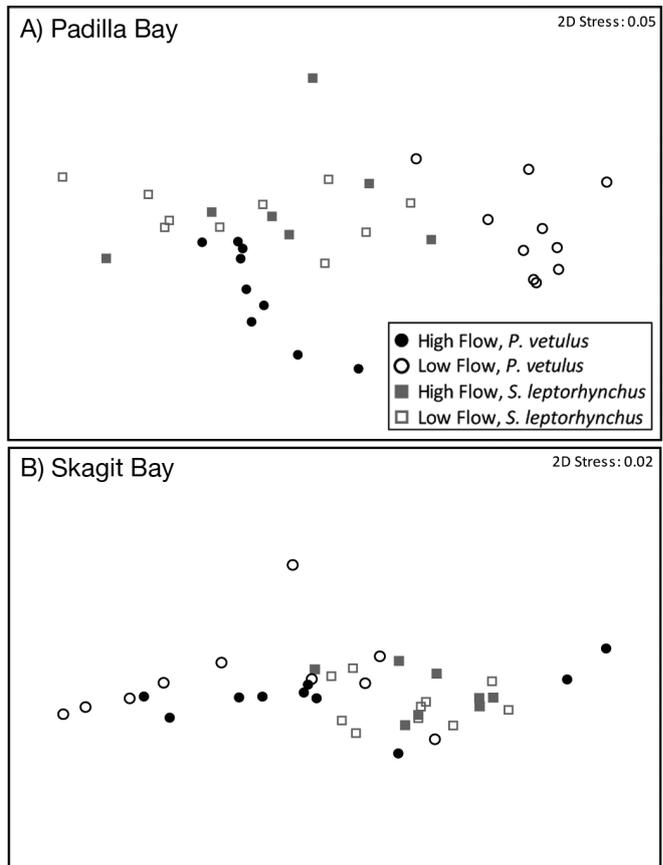


Fig. 4. *Parophrys vetulus* and *Syngnathus leptorhynchus*. Non-metric multidimensional scaling (NMDS) ordination of organic matter contributions to juvenile English sole (PV) and bay pipefish (SL) during the high and low river flow periods in (a) Padilla Bay and (b) Skagit Bay, Puget Sound, Washington. (●) PV, high flow; (○) PV, low flow; (■) SL, high flow; (□) SL, low flow

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 & 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 both OM transport and organism movement enhance connectivity among ecosystems in the more tidally and fluviually 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

Table 7. Bayesian mixing model median estimates and interquartile range (IQR) (i.e. between the 25th and 75th percentiles around the median) of proportional organic matter (OM) 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. See Table 1 legend for information on species composition of OM sources

OM source	<i>P. vetulus</i>				<i>S. leptorhynchus</i>			
	High flow		Low flow		High flow		Low flow	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Padilla Bay								
Marsh complex	0.02	0.05	0.02	0.03	0.02	0.04	0.02	0.03
<i>Triglochin maritima</i>	0.13	0.11	0.22	0.07	0.07	0.06	0.09	0.06
<i>Distichlis spicata</i>	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
<i>Zostera japonica</i>	0.12	0.11	0.11	0.08	0.08	0.08	0.17	0.11
<i>Z. marina</i>	0.09	0.16	0.05	0.06	0.05	0.08	0.06	0.08
<i>Ulva</i> spp.	0.22	0.13	0.13	0.06	0.29	0.09	0.29	0.08
<i>Ceramium</i> 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								
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
<i>Typha</i> sp.	0.14	0.03	0.09	0.03	0.21	0.04	0.21	0.04
<i>Distichlis spicata</i>	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 (<i>Ulva</i> spp.)	0.66	0.03	0.72	0.03	0.59	0.03	0.60	0.03
<i>Z. marina</i>	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

to no fluvial discharge. The 2 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 2 estuaries with contrasting freshwater influence.

Organism movement

Differences in prey diversity and OM assimilation between juvenile English sole and bay pipefish are likely attributable to differences in mobility, indicating that organism life histories can strongly influence food web connectivity at landscape scales. 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. Additionally, ontogenetic stanzas for maturing juvenile English sole are associated with spatial shifts in preferred

feeding locations; juveniles progressively move seaward across estuarine deltas before migrating to subtidal channels at the end of their first year at 85 mm TL (Toole 1980, Gunderson et al. 1990, Rooper et al. 2003). As a result, juvenile English sole feed on diversely integrated (landscape mosaic) prey assemblages associated with each ecosystem through which they pass, while pipefish feed on a local (patch) prey assemblage. Since prey assemblages originate from different ecosystems within the estuarine landscape (Wiens 2002, Pittman et al. 2004), differences in consumer mobility may explain observed differences in the degree of food web connectivity reflected by our 2 consumer species.

As a group and as individuals, the diets of highly mobile juvenile English sole were more diverse and variable compared to the more stationary bay pipefish. Our data suggest the higher diversity of prey items identified in juvenile English sole diets translates to a broader isotopic niche space among individuals and a higher diversity of assimilated OM types compared to pipefish. Further, higher diet diversity translates to greater food web connectivity across estuarine landscapes, as juvenile English sole more evenly integrated OM originating from spatially distinct estuarine ecosystems compared to bay pipefish.

Both species, however, assimilated OM from similar estuarine ecosystems (i.e. marsh, mudflat, and eelgrass) in Skagit and Padilla bays. Therefore, in the absence of organism movement, physical forces, such as tidal action or freshwater discharge, are strong enough to transport OM across ecosystem boundaries in Pacific Northwest estuaries, creating a base-level of trophic connectivity upon which motile and non-motile species are able to capitalize. The strength of ecosystem trophic connections, however, differed between juvenile English sole and bay pipefish. Juvenile English sole assimilated a more even distribution of OM sources compared to bay pipefish, suggesting that sole consistently use a broader suite of ecosystems for trophic support, including OM originating from marsh, mudflat, and marine ecosystems. In contrast, pipefish heavily assimilated marine sources of OM, such as phytoplankton, macroalgae, and eelgrass. Thus, organism movement appears to enhance physically mediated levels of food web connectivity, enabling more mobile species to incorporate trophic energy 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).

We should note, however, that feeding specialization may also influence the degree of food web connectivity reflected by juvenile English sole and bay pipefish. Pipefish are severely gape limited by their head morphology, restricting the types and sizes of prey they consume, as pipefish snouts are specifically designed to consume epibenthic crustaceans (Leysen et al. 2011, Van Wassenbergh et al. 2011). 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).

Estuarine fluvial setting

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. However, when we applied mixing models to fish isotope data, we observed greater evenness and diversity of OM source assimilation in Padilla Bay for both species. This suggests Padilla Bay fish display broader connectivity to the mosaic of estuarine ecosystems as compared to Skagit Bay fish, refuting 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, Padilla Bay's extensive eelgrass beds (and limited freshwater influx) effectively facilitate OM deposition and retention by muting hydrodynamic energy (Asmus & Asmus 2000, Chen et al. 2007). Accordingly, Padilla Bay sediments are predominantly composed of fine particles (28 to 100 μm) and OM (Silver 2009). In contrast, the Skagit River delta is comprised of coarse sand and low OM, indicating that OM is not as well retained (Webster et al. 2012). Although fine-grained sediments and OM are delivered to the Skagit's tidal flats, little of that material deposits on tidal-flat surfaces, and that which does settle is reworked and expediently transported off the deltaic flats by river and tidal currents (Webster et al. 2012) before depositing in deeper, less hydraulically energetic waters (Yang & Khangaonkar 2009). Materials emanating from terrestrial and marsh ecosystems are therefore unavailable to consumers foraging on the Skagit River delta's intertidal flats, despite the potential for fluvially mediated transport.

Interaction effects of organism movement and estuarine fluvial setting

Between-species comparisons

The isotope values and OM assimilation of Padilla Bay juvenile English sole and bay pipefish were consistently different from one another in both sampling seasons. In contrast, between-species comparisons were seasonally inconsistent in Skagit Bay. Consistent differences between species in Padilla

Bay likely arise from the lack of fluvial influence in the estuary which results in a patchy spatial distribution of ecosystem-specific OM, and reduces seasonal variation in this distribution. As a result, food web compartmentalization is stronger among spatially restricted species, but less evident among highly mobile species. Consistently patchy OM spatial distributions in Padilla Bay may also explain why the isotope values of 'stationary' bay pipefish remained relatively constant throughout the season while English sole values shifted dramatically with their ontogenetic migration towards the outer estuarine margins.

In contrast with Padilla Bay, between-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 divergence in the isotope signatures and assimilated sources of OM between the 2 species. OM distribution thus appears to become more compartmentalized as fluvial forcing diminishes in the estuary.

Our observations of food web convergence between the 2 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 & Khangaonkar 2009). The pattern of food web convergence with increasing freshwater discharge has also been described in the Tagus River estuary, although at a much larger spatial scale (Vinagre et al. 2011).

Seasonal effects within species

We also contrasted species-specific seasonal shifts between the embayment estuary (Padilla Bay) and river delta estuary (Skagit Bay) in order to differentiate between food web shifts relating to season or species, and those relating to seasonal shifts in freshwater discharge. 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.

Surprisingly, we observed no seasonal shift in the isotope values or OM support of bay pipefish in either estuary, indicating that seasonal changes in fluvial discharge do not change OM composition or availability in eelgrass beds. This suggests estuarine setting is the more important driver of food web connectivity for eelgrass-associated organisms with limited mobility⁴. Also surprising was that, 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 and/or ontogenetic shifts in preferred feeding location).

In further contrast with our hypothesis, 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 Skagit Bay is likely more homogenized compared to Padilla Bay. As described earlier, spatial compartmentalization of ecosystem-specific OM sources in Padilla Bay may allow juvenile English sole isotope values to reflect ontogenetic shifts in feeding location (Toole 1980, Rooper et al. 2003) on a seasonal scale, whereas stronger OM spatial integration in Skagit Bay obscures seasonal ontogenetic shifts in feeding location.

CONCLUSIONS

This study contributes to a 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

⁴Insufficient timing between sampling events can also result in isotope values that imply a lack of seasonal diet shifts, as tissue turnover rates must be rapid enough to isotopically reflect seasonal changes. In this case, however, 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 d (Maier & Simenstad 2009, Buchheister & Latour 2010, Nelson et al. 2011), and given that most sampled pipefish were <200 mm TL, and therefore still growing (Takahashi et al. 2003, Barrows et al. 2009)

that the importance of one mechanism versus another is strongly dependent on the fluvial context of the estuary. For example, organism movement likely drives patterns of food web connectivity and support in the non-fluvial estuary, Padilla Bay, where a lack of physical forcing results in patchy spatial distributions of ecosystem-specific OM. In contrast, OM transport likely drives patterns of food web connectivity during periods of high fluvial discharge in Skagit Bay, where strong physical forces spatially integrate different OM sources across space. In large fluvial systems, it appears organism movement plays a secondary role to water-advected OM transport, largely by enhancing connectivity under low flow conditions. 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 & Garritt 1997, Guest et al. 2004).

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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