



Variability in Juvenile English Sole Condition Relative to Temperature and Trophic Dynamics Along an Oregon Estuarine Gradient

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Abstract

Many benthic fish and invertebrates, including the flatfish English sole (*Parophrys vetulus*), utilize estuaries as nursery habitat during their juvenile life stage. Regions within an estuary differ with respect to salinity, temperature, oxygen, and organic inputs, highlighting the complexity of nursery habitat and the need to assess multiple habitats within a single estuary. To determine the effect of variable estuarine habitat on juvenile English sole, we examined morphometric and energetic (lipid) condition, fatty acids, and stable isotopes (¹³C and ¹⁵N) of wild-caught juveniles from upriver and downriver habitats within the Yaquina Bay, Oregon estuary. Downriver-caught fish exhibited higher energetic condition, which may be attributed to cooler temperatures and the marine-sourced carbon that typified that region's food web. Conversely, individuals from the upriver habitat exhibited higher morphometric condition. This may be due in part to the warmer temperatures upriver that have been observed to promote growth and suppress lipid storage in other marine species. Our findings highlight the important but variable contribution of both upriver and downriver habitats to English sole early life history.

Keywords Juvenile English sole · Lipids · Fatty acids · Stable isotopes · Nursery · Temperature

Introduction

Estuaries are valuable nursery grounds for many fish species, providing abundant prey, refuge from predation, and warm temperatures that enhance growth (Sogard 1997; Beck et al. 2001; Ryer et al. 2010). Since habitats within an estuary may serve different nursery functions, a comprehensive approach incorporating physical oceanography and food web dynamics is necessary to understand an estuary's overall nursery role (Sheaves et al. 2015).

In riverine estuaries, tidal and fluvial influences contribute to a seasonally changing gradient of both physical and biological parameters (Brown and Power 2011; Howe and Simenstad

2015; Bosley et al. 2017). Yaquina Bay, located in Newport, Oregon, USA (Fig. 1) is such a system, located at the interface of the Yaquina River and a productive coastal upwelling ecosystem (Huyer et al. 2007). During the summer season, influx of upwelled coastal waters to the estuary constructs a gradient from the saline, cold downriver region to the fresher, warmer upriver region (Brown and Power 2011). Organic inputs also vary along this gradient, with carbon sources of marine origin dominating downriver sites and those of terrestrial and freshwater origin influencing upriver sites (Vinagre et al. 2008; Shilla 2014; Bosley et al. 2017). Thus, variation in both temperature and food web dynamics can affect the growth and condition of juvenile fish during their estuarine residency (Meng et al. 2000; Islam and Tanaka 2006; Escalas et al. 2015).

One of the ecologically important species utilizing Oregon's estuaries early in life is English sole (*Parophrys vetulus*). After winter spawning, pelagic onshore transport, and settlement to the benthos (Budd 1940; Barss 1976), most English sole juveniles spend their first summer in an estuary (Krygier and Pearcy 1986; Boehlert and Mundy 1987; Gunderson et al. 1990). Juveniles aggregate in shallow areas surrounded by tidal flats, leading to higher densities in the lower estuary (Rooper et al. 2003). However, juveniles have been found to inhabit upriver sites as well (De Ben et al. 1990; Rooper et al. 2003). Therefore, the quality of habitats used by

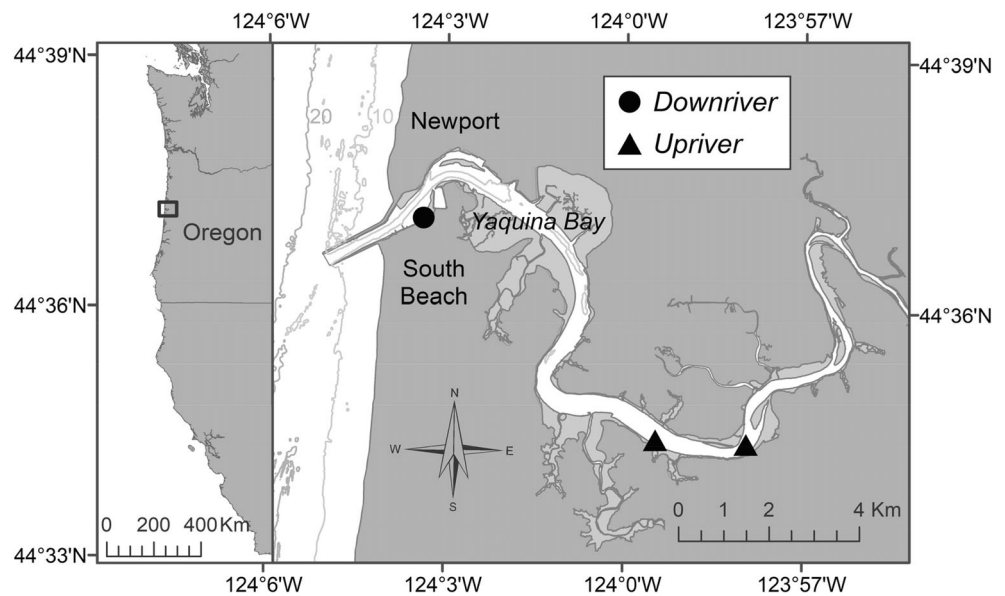
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Fig. 1 Yaquina Bay estuary, the habitat area of interest. Samples were collected downriver (44.6184° N, 124.0564° W; circle) and upriver (44.5744° N, 123.9911° W and 44.5737° N, 123.9658° W; triangles) in Yaquina Bay during summer of 2014



English sole juveniles along the estuarine gradient is variable, and fish condition along this gradient can be compared.

Variable environments can impact the growth and condition of resident juvenile fish, and this can be assessed morphometrically using length and weight or energetically using lipids. Juvenile fish with relatively elevated storage lipids (i.e., triacylglycerols (TAG)) are generally considered to be in greater energetic condition than those with low TAG storage (Copeman et al. 2015), because TAG can be mobilized easily to prevent starvation during periods of food scarcity. A ratio of dynamic levels of storage lipids (TAG) to stable size-scaling neutral structural lipids (sterols (ST)) may thus represent a juvenile's energetic condition (TAG/ST, Fraser 1989). Lipid storage has been found to be a successful strategy for juvenile flatfish and has been examined relative to anthropogenic disturbance, food limitation, and other environmental variables (Amara et al. 2007; Kerambrun et al. 2014).

To further investigate how different nursery habitats can drive trends in fish condition, one can examine the fatty acid (FA) composition of individual juveniles. FAs are components of acyl lipid classes (e.g., TAG and phospholipid (PL)) and can be used to understand trophic relationships (Budge et al. 2006). When consumed in the diet, FAs are transferred from prey to predator in a somewhat conservative manner, and because some FAs are more commonly synthesized by specific producers, their presence at higher trophic levels may indicate particular organisms at the base of a food web (Dalsgaard et al. 2003; Budge et al. 2006; Parrish 2013). In addition to clarifying food web dynamics, FAs can also provide insight into fish nutrition. Fish cannot synthesize all FAs in quantities required for normal physiological function; those that must be acquired through diet are referred to

as essential FAs (EFAs, Parrish 2013). Changes in physical oceanography and biological productivity along the estuarine gradient alter the contribution of various primary producers to the food web, and thus the availability of their associated EFAs (Mayzaud et al. 1989; Litz et al. 2010; Bergamino and Dalu 2014). Over the summer season, these changes may differentially affect the physiology and condition of juvenile English sole residing in upriver and downriver habitats (Litzow et al. 2006).

Analyses of FAs can be combined with stable isotope (SI) ratios to help clarify food web dynamics in complex systems with varying carbon sources, such as benthic systems and estuaries (Kelly and Scheibling 2012). Carbon isotope ratios ($\delta^{13}\text{C}$) provide additional information on dietary carbon sources, while nitrogen isotope ratios ($\delta^{15}\text{N}$) increase with trophic level (Fry and Sherr 1984; Peterson and Fry 1987).

In this study, we examined juvenile English sole condition at the ends of an estuarine gradient and explored how differences in temperature and food quality may explain observed spatial and temporal trends. To this end, we addressed three specific objectives: (1) to determine the morphometric and energetic (TAG/ST) condition of juveniles collected at upriver and downriver sites within Yaquina Bay, (2) to use dietary trophic biomarkers (FAs, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of fish) to assess variation in food quality along the estuarine gradient, and (3) to assess how these dietary trophic markers vary with the progression of the summer season. We expected to find faster growth and lower lipid storage in juveniles from the warmer upriver habitat, enrichment of downriver fish tissues with marine-associated carbon, and greater prevalence of marine-associated carbon sources with the progression of the summer season. Our findings will improve understanding of variable nursery habitat function within an estuary.

Methods

Field Sampling

Wild English sole juveniles were collected from downriver and upriver sites in Yaquina Bay near Newport, Oregon in June, July, and August of 2014. Downriver collections were made approximately 1 km from the mouth of the estuary (Fig. 1, *circle*) with a beach seine (15.25 m × 1 m, 0.635 cm mesh) at low tide (water depth approximately 1 m, Table 1). Upriver collections were made approximately 11.5 and 13.5 km from the mouth (Fig. 1, *triangles*) via otter trawl (4.75 m × 1.25 m mouth, 0.32 cm mesh) towed at approximately 3 to 4 m deep behind a 6-m whaler traveling at a target speed of 0.5 m s⁻¹ over ground. Upriver sampling began at the location furthest upriver at high tide and progressed downriver until either the sampling quota or the end of the cruise time window was reached. Individuals from the two upriver sites were grouped for statistical analyses due to similar oceanographic conditions.

At the time and location of each fish collection, temperature and salinity conditions were measured using a hand-cast YSI CastAway CTD which was lowered into the water until touching bottom (indicated by slack in the line), at which point measurements were recorded.

Samples were stored on dry ice for the duration of each sampling event and then moved to a -80 °C freezer for long-term storage. Only English sole smaller than 80 mm standard length (SL) were kept in order to focus on age-0 juveniles (Rosenberg 1982). However, collected individuals ($n = 110$) ranged from 22 to 82 mm SL, with downriver-caught fish generally smaller in size than upriver-caught fish (Fig. 2a). To remove possible ontogenetic effects from the examination of spatial and temporal effects, only fish within the overlapping size range (46.43 mm ≤ SL ≤ 71.56 mm, Fig. 2a, $n = 81$, Table 1) were used in statistical analyses. This subsample resulted in an average SL of 56.7 ± 1.1 mm for downriver fish and 63.0 ± 1.0 mm for upriver fish, a difference

of only 6–7 mm in average fish size. The particular morphometric and energetic condition metrics used in this study also control for size, by relating fish weight to length and storage lipids (TAG) to structural lipids (ST). The sample size for each of the six sampling groups (two sites over 3 months) varied widely after subsampling, from $n = 4$ in August upriver fish to $n = 25$ in June downriver fish (Table 1).

Morphometric Data and Dissection of Samples

Each frozen fish was rinsed with deionized water and patted dry. Digital calipers were used to take measurements of SL (mm ± 0.01) and a digital balance was used to measure wet weight (g, ± 0.001). Linear regression (Sokal and Rohlf 1969) was utilized to relate log-transformed wet weight to log-transformed SL. Residuals from this regression provide an index of fish morphometric condition, based on the assumption that fish with greater weight per length are in better condition (Copeman et al. 2015; Koenker et al. 2018). This condition index was examined with respect to sampling location and month using two-way analysis of variance (ANOVA, fixed effect model, $\alpha = 0.05$, Sokal and Rohlf 1969). All samples were dissected on ice in order to prevent excess warming of the fish tissue, as warming could result in lipid oxidation and hydrolysis. A triangle of dorsal muscle tissue for SI analysis was removed and stored at -20 °C. Then the stomach and intestines were removed and the remainder of the fish body (the lipid sample) was weighed and stored in 2 mL of chloroform, topped with nitrogen, and sealed with Teflon tape, at -20 °C for less than 1 year prior to lipid extraction.

Lipid Extraction and Analyses

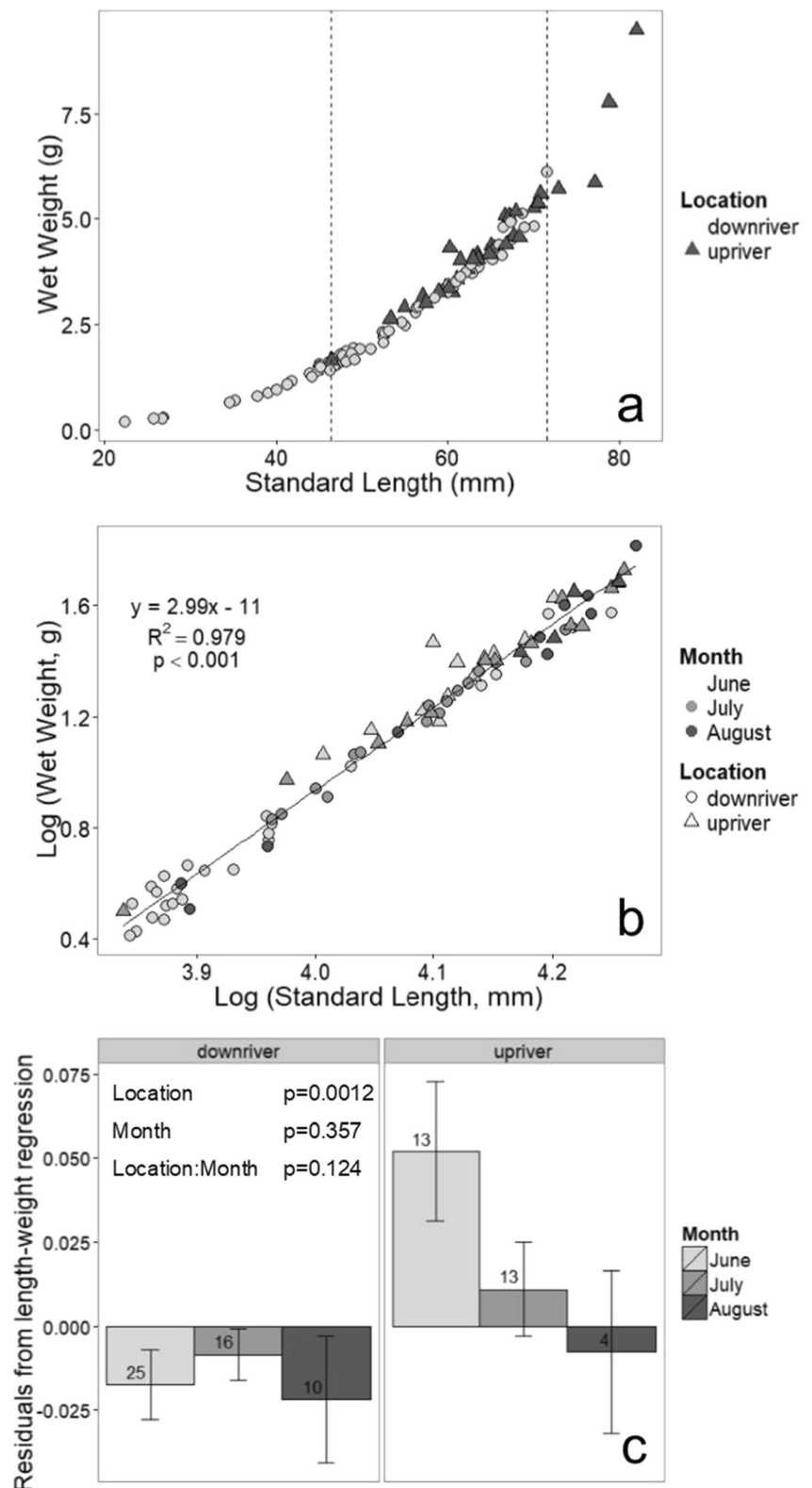
Lipids were extracted from each sample ($n = 81$) using a modified Folch procedure (Folch et al. 1957; Parrish 1987). Each sample was homogenized in a 2:1 proportion of chloroform

Table 1 Summary table of oceanographic data collected from Yaquina Bay in the summer of 2014 and number of juvenile English sole included in each step of analysis

| | Downriver | | | Upriver | | |
|-------------------------------|-----------------|-----------------|-------------------|-----------------|-----------------|-------------------|
| | June $n = 2$ | July $n = 2$ | August $n = 5$ | June $n = 2$ | July $n = 6$ | August $n = 6$ |
| Depth (m) | 0.86 (0.02) | 1.0 (0.05) | 1.0 (0.05) | 2.3 (0.2) | 3.9 (0.2) | 3.6 (0.2) |
| Salinity | 31.1 (0.4) | 33.0 (0.01) | 32.7 (0.2) | 26.2 (1.2) | 29.7 (1.0) | 30.3 (0.2) |
| Temperature (°C) | 12.6 (0.02) | 12.3 (0.09) | 11.0 (0.06) | 18.4 (0.6) | 16.9 (1.1) | 18.6 (0.2) |
| Fish caught/processed | 41 | 21 | 14 | 13 | 14 | 7 |
| Fish in lipid data analyses | 25 | 16 | 10 | 13 | 13 | 4 |
| Fish in isotope data analyses | 13 | 14 | 10 | 13 | 13 | 4 |

Oceanographic data were collected at the same time and location as fish and are averaged for each of six sampling groups (means with standard errors in brackets, $n =$ number of CTD casts)

Fig. 2 **a** Relationship between standard length (SL, mm) and wet weight (WWT, g) of juvenile English sole (*Parophrys vetulus*) collected from downriver and upriver Yaquina Bay sites during summer of 2014. To remove potential ontogenetic effects resulting from the wide fish size range in the full data set ($n = 110$), this study only examined fish in the size range overlapping between sampling sites ($n = 81$, between the dashed lines). **b** Linear relationship between log-transformed SL and log-transformed WWT. $\text{Log(WWT, g)} = -11 + 2.99 \times (\text{Log(SL, mm)})$, $R^2 = 0.979$, $p < 0.001$. **c** Residuals of linear log-length and log-weight relationship for English sole in different sampling groups. Differences in length-weight residuals between groups were analyzed using a fixed effect two-way ANOVA, $\alpha = 0.05$



and methanol, using a Euro-Turrax T20b automated homogenizer (27,000 RPM, IKA Works, Inc.). After homogenization, samples were vortexed, sonicated for 4 min, and centrifuged (1500 RPM) for 3 min. The lower, lipid-containing

layer of solvent was removed from the test tube using a double-pipetting technique, and transferred to a vial to be blown down under nitrogen. The procedure was repeated from the vortex step three more times, for a total of four washes.

Analysis of lipid classes was performed using thin-layer chromatography with flame ionization detection (TLC-FID) as described by Lu et al. (2008) and as modified by Copeman et al. (2015), on a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan). Extracts were spotted onto duplicate silica-coated glass rods (Chromarods, Iatron Laboratories), which were developed in a series of three mobile phases in order to separate lipid classes. Chromarods were first developed in a chloroform/methanol/water mobile phase (5:4:1 by volume) until the leading edge of the solvent system was 1 cm above the spotting origin. The second and third developments took place in hexane/diethyl ether/formic acid (99:1:0.05) for 48 min and hexane/diethyl ether/formic acid (80:20:0.1) for 38 min, respectively. Before each development, chromarods were allowed to dry for 5 min and were then conditioned for 5 min in a constant humidity chamber (33% humidity). After development in all three phases, chromarods were scanned (Iatroscan) to obtain a signal in millivolts. The resulting chromatograms were integrated using PeakSimple software (ver. 3.67, SRI Inc.), and absolute amounts of four lipid classes (triacylglycerols, free fatty acids, sterols, and polar lipids) were quantified using lipid standards as detailed in Copeman et al. (2015). TAG/ST, the ratio of storage to structural neutral lipid classes (Fraser 1989), was used as a measure of energetic condition, which was examined with respect to sampling location and month using two-way ANOVA.

Lipids were then derivatized through acid transesterification using heat and sulfuric acid in methanol and were isolated in hexane following a procedure based on that recommended by Budge et al. (2006). Prior to derivatization, 23:0 (methyl tricosanoate) was added to the lipid extract at approximately 10% of the estimated total FAs to allow FA quantification. Fatty acid methyl esters (FAMES) resulting from derivatization were analyzed via gas chromatography, on an HP 7890 GC-FID equipped with an autosampler and a DB wax+ column 30 m in length, with an internal diameter of 0.25 mm and a 0.25- μm film (Agilent Technologies, Inc.). Hydrogen was used as the carrier gas, with a flow rate of 2 mL min^{-1} . The temperature of the GC column began at 65 °C for 0.5 min, was increased (40 °C min^{-1}) to 195 °C and held for 15 min, and finally was increased (2 °C min^{-1}) to 220 °C and held for 1 min. The temperature of both the injector and the detector was 250 °C. Chromatograms were integrated using Chem Station software (ver. A.01.02, Agilent Technologies, Inc.) in conjunction with several standards. Chromatogram peaks were identified from retention times based on four Supelco standards (37-component FAME, BAME, PUFA 1, PUFA 3), while correction factors for individual FAs were obtained using Nu-Check Prep GLC 487 quantitative FA-mixed standard.

Percentages of individual FAs present at >0.75% in all samples, lipid class percentages of TAG and PL (the acyl lipid classes containing FA side chains), and total lipid density

(mg g^{-1}) were included in multivariate analyses using PRIMER v7 (Primer-E Ltd). Together, TAG and PL accounted for ~80–90% of the total lipid classes in juvenile English sole. We were not able to perform tissue-specific lipid class analyses due to the small size of these fish. However, the inclusion of TAG (neutral lipid storage) and PL (membrane structures) in multivariate analyses allowed us to determine FAs that were associated with trophic accumulation and storage of neutral lipids versus those in polar lipids that are largely related to species-specific molecular PL FA composition (Copeman and Parrish 2003; Copeman et al. 2018). We also included lipid density in our multivariate analyses of English sole lipids in order to show FAs that were associated not only with TAG but also with total lipid density (mg g^{-1}). Qualitative data (% total FA, % lipid classes, lipid density) were square-root-transformed prior to analyses and were then used to calculate a triangular matrix of similarities (Bray-Curtis similarity) between each pair of samples. Non-metric multidimensional scaling (nMDS), an iterative graphical technique that uses ranked similarities to arrange multivariate data in two dimensions, was used to visualize data. To determine the specific lipid variables responsible for dissimilarity between fish from different sampling locations and months, individual parameters were correlated to nMDS axes, and vectors with $R^2 > 0.7$ were included on the plot. English sole lipid profile data (square-root-transformed resemblances) were statistically examined for differences between sampling locations and months using a permutational multivariate ANOVA (perMANOVA) in PRIMER v7. PerMANOVA uses distance matrices to partition distances among sources of variation and fits linear models. Significance tests are done using F tests based on sequential sums of squares from permutations of the data. To determine the specific lipid parameters responsible for most of the variance between sampling locations and months, similarity percentage routines (SIMPER) were also performed on square-root-transformed resemblances in PRIMER v7.

Stable Isotope Analyses

SI analyses (^{13}C and ^{15}N) were performed on a subset ($n = 67$) of fish analyzed for lipid composition. As much muscle tissue as possible was obtained from frozen dorsal samples and shipped overnight on dry ice to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University (CPSIL-NAU, Flagstaff, AZ) for sample preparation and SI analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, % C, % N, and C:N). Frozen tissue samples were dried at 50 °C to constant weight (72 h), ground into a fine powder with a cleaned ball-mill grinder (Retsch MM200), and weighed (0.600 to 1.200 mg) into tin capsules for analysis. Grinding was not performed on small samples that were close to the lower weight limit for analysis, in order to avoid sample loss. Samples were analyzed using a Thermo-Electron Delta V

Advantage isotope-ratio mass spectrometer (IRMS) configured through a Finnigan CONFLO III for automated continuous-flow analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and coupled with a Carlo Erba NC2100 elemental analyzer for combustion and separation of carbon and nitrogen.

Isotopic ratios in this study are reported in δ notation, relative to Vienna PeeDee belemnite for carbon and atmospheric N_2 for nitrogen, according to the formula:

$$\delta X (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3$$

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$ and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The analytical precision, based on the standard deviation of replicate analyses of a standard (peach leaves, NIST 1547), was 0.04‰ for $\delta^{13}\text{C}$ and 0.03‰ for $\delta^{15}\text{N}$. Lipid content of muscle tissue varies, with lipids 6–8‰ lower in $\delta^{13}\text{C}$ than protein or carbohydrates, so $\delta^{13}\text{C}$ values were arithmetically normalized using the following formula (Post et al. 2007):

$$\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C} : \text{N}$$

The average adjustment resulting from this correction was -0.068‰ . Corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were examined with respect to sampling location and month using two-way ANOVA.

Results

Water Conditions at Sample Sites

Bottom salinity was lower upriver (~ 29) than downriver (~ 32) and increased at both locations with the progression of the summer season (Table 1). As expected, bottom temperature was higher upriver ($\sim 18\text{ }^\circ\text{C}$) than downriver ($\sim 12\text{ }^\circ\text{C}$) and decreased over the summer at the downriver site, likely due to increased coastal upwelling.

Morphometric Condition

Log-transformed length and weight of individual fish exhibited a linear relationship well described ($R^2 = 0.979$, $p < 0.001$, Fig. 2b) by the regression equation $\log(\text{weight, g}) = -11 + 2.99 \times (\log(\text{length, mm}))$. Residuals from this regression compared across sampling groups indicated significantly lower morphometric condition at the downriver habitat (two-way ANOVA, $F_{[1, 75]} = 11.29$, $p = 0.0012$, Fig. 2c). While there was no significant effect of sampling month, upriver-collected fish showed a graphical trend of decreasing condition over the season.

Energetic Condition

We detected four lipid classes in all individuals. Polar lipids (PL, $\sim 90\%$ phospholipids and a small amount of undetermined polar lipids) were present in the greatest abundance ($56.2 \pm 1.35\%$), followed by TAG ($30.7 \pm 1.79\%$), sterols (ST, $9.9\% \pm 0.39\%$), and free fatty acids (FFA, $3.3 \pm 0.21\%$). The neutral storage lipid class, TAG (Fig. 3a), varied more among sampling groups than the structural lipid classes, ST and PL (Fig. 3b, c). Patterns exhibited by total lipid content (Fig. 3d) were similar to those exhibited by TAG, indicating that variation in lipid content was primarily driven by variation in TAG.

Comparison of average TAG/ST across sampling groups (Fig. 3e) revealed higher energetic condition in fish downriver (two-way ANOVA, $F_{[1, 75]} = 22.10$, $p < 0.001$), which contrasted with their lower morphometric condition (see above). Energetic condition also exhibited significant, though less consistent, seasonal differences (two-way ANOVA, $F_{[2, 75]} = 4.00$, $p = 0.022$), with an increase in TAG/ST visibly evident at the downriver site only. Examination of TAG/ST with respect to field-measured temperature revealed a weak negative relationship (linear regression, $R^2 = 0.219$, $p < 0.001$, TAG/ST = $10.2 - 0.44 \times \text{temperature (}^\circ\text{C)}$).

Fatty Acid Composition

FA analysis detected ten FAs at $> 0.75\%$ in all samples (Table 2). Multivariate statistical analysis of major lipid classes (% TAG and PL), total lipid density (mg g^{-1}), and FA profiles among fish revealed significant differences across sampling locations (perMANOVA, $F_{[1, 74]} = 50.0$, $p < 0.001$ on 9999 permutations) and months (perMANOVA, $F_{[2, 74]} = 5.5$, $p = 0.003$ on 9999 permutations). The interaction term was not significant (perMANOVA, $F_{[2, 74]} = 1.9$, $p = 0.145$ on 9999 permutations), indicating that at each location, the lipid class and FA composition varied similarly with time. SIMPER analyses revealed that the top six lipid parameters explaining spatial differences were TAG (26.3%), PL (11.4%), 16:1n-7 (11.4%), 20:4n-6 (7.9%), total lipid density (7.9%), and 20:5n-3 (7.8%). Cumulatively, these lipid parameters explained 72.6% of the dissimilarity between downriver and upriver habitats. As seen in Table 2, downriver fish were characterized by higher TAG, 16:1n-7, total lipid density, and 20:5n-3 (eicosapentaenoic acid (EPA)), while upriver fish exhibited higher PL and 20:4n-6 (arachidonic acid (ARA)). Pairwise comparisons of sampling months (SIMPER) revealed that the four most important factors driving seasonal differences were TAG, PL, total lipid density, and 22:6n-3 (docosahexaenoic acid (DHA)). These four factors varied in order of importance but cumulatively explained 65.2–68.8% (depending on the pairwise comparison) of the dissimilarity between sampling months. While seasonal differences in

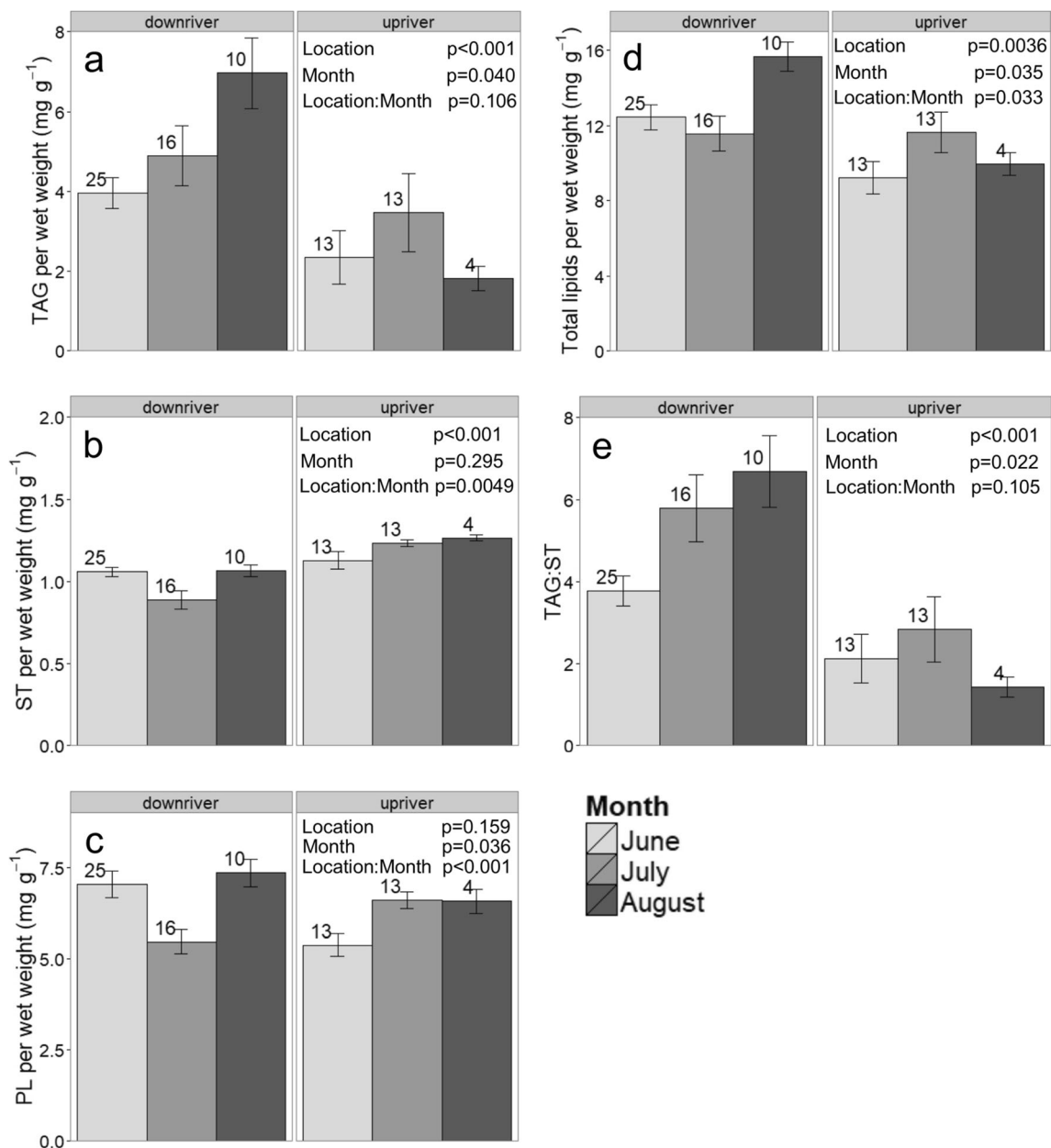


Fig. 3 Triacylglycerol (TAG, **a**), sterol (ST, **b**), polar lipids (PL, **c**), and total lipids (**d**) per wet weight (mg g^{-1}), as well as TAG:ST (ratio of storage to structural lipid classes, **e**) of juvenile English sole (*Parophrys*

vetulus, $n = 81$) collected from downriver and upriver Yaquina Bay sites during summer of 2014. Differences in lipid metrics between sampling groups were analyzed using a fixed effect two-way ANOVA, $\alpha = 0.05$

TAG, PL, and lipid density did not exhibit consistent directional trends, DHA clearly decreased over the summer season in both habitats (Table 2).

Multivariate graphical analyses also showed that patterns in FA composition varied spatially and, to a lesser degree, seasonally (nMDS, Fig. 4). Downriver- and upriver-collected fish exhibited clear dissimilarity, appearing in two distinct, separate groups on the nMDS plot. Some individual lipid parameters correlated to the nMDS axes better than others; vectors with $R^2 > 0.7$ are shown on the nMDS plot (2D stress = 0.08, Fig. 4). Downriver fish were strongly associated with EPA (20:5n-3) and 16:1n-7 (FAs indicative

of diatoms, Fig. 4; Table 2). Conversely, upriver fish were strongly associated with ARA (20:4n-6) and 18:1n-9 (Fig. 4; Table 2). Weaker seasonal trends were evident for some FAs, particularly in the downriver habitat. Juveniles caught earlier in the summer were associated with lower total lipid density (mg g^{-1}) and higher proportions of DHA (22:6n-3, Fig. 4; Table 2). Examining the vectors for TAG, PL, and lipid density (mg g^{-1} , Fig. 4), it is likely that seasonal changes in FAs within the downriver site are due to changes in lipid class composition (greater TAG relative to PL) and total lipid storage, rather than differences in dietary carbon sources alone.

Table 2 Summary of lipid classes, fatty acids (FAs), and stable isotopes (SIs) of juvenile English sole (*Parophrys vetulus*) collected from the Yaquina Bay estuary in June, July, and August of 2014, as well as burrowing shrimp (*Neotrypaea californiensis*) and sediment particulate organic matter (SPOM) surface scrapes collected in August 2012 by Bosley et al. (2017)

| | Downriver | | | | | Upriver | | | | |
|---|---------------------------|---------------------------|----------------------------|--|--------------------------------------|---------------------------|---------------------------|---------------------------|--|--------------------------------------|
| | June <i>n</i> = 25, 13 | July <i>n</i> = 16, 14 | August <i>n</i> = 9, 10 | Shrimp ^d <i>n</i> = 10, 13 | SPOM ^e <i>n</i> = 2, 3 | June <i>n</i> = 13, 13 | July <i>n</i> = 13, 13 | August <i>n</i> = 4, 4 | Shrimp ^d <i>n</i> = 10, 11 | SPOM ^e <i>n</i> = 2, 3 |
| Total lipid density (mg g ⁻¹) | 12.4 (0.7) | 11.6 (0.9) | 15.2 (0.7) | – | – | 9.2 (0.9) | 11.6 (1.1) | 10.0 (0.6) | – | – |
| Lipid classes (% of total lipids) | | | | | | | | | | |
| Triacylglycerols (TAG) | 30.4 (2.1) | 39.4 (4.2) | 41.6 (4.2) | – | – | 21.2 (4.4) | 24.3 (5.1) | 17.8 (2.2) | – | – |
| Sterols (ST) | 9.0 (0.5) | 8.1 (0.7) | 7.1 (0.4) | – | – | 13.2 (1.1) | 11.6 (1.0) | 12.8 (0.8) | – | – |
| Polar lipids (PL) | 57.2 (1.7) | 49.5 (3.3) | 49.3 (3.6) | – | – | 61.0 (3.1) | 60.7 (3.9) | 66.0 (1.7) | – | – |
| Total fatty acids (mg g ⁻¹) | 7.7 (0.4) | 7.3 (0.6) | 8.8 (0.4) | 82.2 (16.8) | – | 5.7 (0.6) | 6.2 (0.7) | 5.8 (0.5) | 34.5 (15.6) | – |
| Fatty acids (% of total FA) | | | | | | | | | | |
| 14:0 | 1.7 (0.1) | 1.8 (0.1) | 2.2 (0.1) | 4.6 (0.4) | 4.5 (0.1) | 1.5 (0.1) | 2.0 (0.2) | 1.6 (0.1) | 3.6 (0.2) | 4.4 (0.1) |
| 16:0 | 16.1 (0.2) | 15.1 (0.2) | 15.0 (0.3) | 15.5 (0.8) | 17.2 (0.4) | 16.1 (0.2) | 16.3 (0.2) | 16.7 (0.2) | 17.4 (0.9) | 16.2 (0.2) |
| 18:0 | 6.0 (0.1) | 5.8 (0.1) | 5.9 (0.2) | 2.1 (0.7) | 3.5 (0.8) | 6.6 (0.1) | 7.0 (0.1) | 7.1 (0.2) | 3.0 (0.2) | 3.4 (0.1) |
| Σ SFA ^a | 26.1 (0.2) | 25.7 (0.2) | 25.6 (0.3) | 22.7 (1.0) | 29.4 (1.5) | 27.5 (0.3) | 29.1 (0.4) | 29.4 (0.5) | 24.8 (1.0) | 34.2 (1.4) |
| 16:1n-7 | 6.7 (0.3) | 6.3 (0.5) | 5.1 (0.3) | 13.9 (0.8) | 20.2 (2.6) | 2.3 (0.1) | 2.8 (0.2) | 2.3 (0.1) | 8.8 (0.9) | 15.5 (0.7) |
| 18:1n-9 | 4.9 (0.1) | 5.1 (0.1) | 6.0 (0.1) | 7.7 (2.9) | 2.4 (0.3) | 8.2 (0.2) | 8.0 (0.1) | 7.6 (0.2) | 8.3 (1.3) | 3.4 (0.1) |
| 18:1n-7 | 4.2 (0.1) | 5.6 (0.2) | 4.8 (0.2) | 4.2 (0.2) | 9.5 (0.6) | 2.9 (0.0) | 3.3 (0.1) | 3.5 (0.1) | 3.5 (0.3) | 7.7 (0.2) |
| Σ MUFA ^b | 19.3 (0.3) | 23.6 (0.7) | 24.3 (0.7) | 30.5 (2.5) | 38.3 (1.9) | 17.8 (0.3) | 19.5 (0.7) | 19.0 (0.5) | 25.0 (1.8) | 35.2 (1.2) |
| 20:4n-6 (ARA) | 1.6 (0.0) | 1.6 (0.1) | 1.3 (0.1) | 0.8 (0.1) | 1.0 (1.5) | 4.2 (0.2) | 3.1 (0.2) | 2.9 (0.2) | 0.8 (0.1) | 1.2 (0.1) |
| 20:5n-3 (EPA) | 18.9 (0.3) | 21.7 (0.1) | 23.2 (0.3) | 25.3 (1.0) | 13.3 (1.6) | 14.6 (0.4) | 15.0 (0.3) | 14.9 (0.5) | 12.6 (1.1) | 7.1 (2.0) |
| 22:5n-3 | 3.9 (0.1) | 4.3 (0.1) | 4.4 (0.2) | – | – | 4.2 (0.5) | 4.3 (0.4) | 5.4 (0.6) | – | – |
| 22:6n-3 (DHA) | 23.3 (0.6) | 16.2 (0.9) | 14.4 (0.8) | 4.5 (0.3) | 1.5 (2.1) | 21.9 (0.6) | 20.6 (1.2) | 19.5 (0.6) | 10.2 (0.8) | 2.9 (0.7) |
| Σ PUFA ^c | 53.8 (0.4) | 49.7 (0.7) | 49.3 (0.6) | 45.3 (1.9) | 9.1 (2.0) | 53.3 (0.5) | 49.9 (0.9) | 50.2 (0.8) | 48.8 (2.4) | 13.2 (0.6) |
| Stable isotopes | | | | | | | | | | |
| δ ¹³ C | – 11.8 (0.1) | – 13.1 (0.1) | – 14.1 (0.1) | – 16.9 (0.3) | – 21.6 (0.2) | – 19.7 (0.3) | – 19.1 (0.3) | – 17.3 (0.4) | – 22.0 (0.1) | – 25.2 (0.1) |
| δ ¹⁵ N | 11.6 (0.1) | 11.7 (0.1) | 11.6 (0.1) | 9.9 (0.2) | 5.5 (0.2) | 12.1 (0.1) | 12.7 (0.1) | 12.4 (0.3) | 9.9 (0.3) | 4.0 (0.2) |
| % C | 45.3 (0.3) | 46.2 (0.2) | 46.5 (0.2) | 44.0 (1.3) | 0.3 (0.2) | 45.4 (0.2) | 45.7 (0.2) | 45.3 (0.4) | 40.3 (1.1) | 1.3 (0.4) |
| % N | 13.7 (0.1) | 13.9 (0.1) | 13.8 (0.1) | 10.3 (0.6) | 0.1 (0.0) | 14.2 (0.1) | 14.3 (0.1) | 14.3 (0.2) | 8.6 (0.5) | 0.1 (0.0) |
| C:N | 3.3 (0.0) | 3.3 (0.0) | 3.4 (0.0) | 4.3 (0.3) | 7.2 (0.9) | 3.2 (0.0) | 3.2 (0.0) | 3.2 (0.0) | 4.7 (0.3) | 11.8 (1.2) |

Means are shown for each sampling group, with standard errors in brackets. Sample sizes for each sampling group indicate *n* = FA samples, SI samples

^a 14:0, 15:0, 16:0, 17:0, 17:0, 17:0, 17:0, 17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 23:0, 24:0

^b 14:1, 15:1, 16:1n-11, 16:1n-9, 16:1n-7, 16:1n-5, 17:1, 18:1n-11, 18:1n-9, 18:1n-7, 18:1n-6, 18:1n-5, 20:1n-11, 20:1n-9, 20:1n-7, 22:1n-11, 22:1n-9, 22:1n-7, 24:1n-9

^c 16:2n-4, 16:3n-4, 16:4n-3, 16:4n-1, 18:2, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:4n-3, 18:4n-1, 18:5n-3, 20:2, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:2, 21:5n-3, 22:4n-6, 22:5n-6, 22:4n-3, 22:5n-3, 22:6n-3

^d Burrowing shrimp (*Neotrypaea californiensis*) data are from downriver site Seawall Island (SWI) and upriver site Tongue Island (TI) in Yaquina Bay (Bosley et al. 2017)

^e Sediment particulate organic matter (SPOM) surface scrape data are from downriver site Idaho Flats (IF) and upriver site Upriver (UPR) in Yaquina Bay (Bosley et al. 2017)

Stable Isotopes

Comparison of the six sampling groups revealed that δ¹³C and δ¹⁵N both exhibited significant spatial, temporal, and interaction effects (Fig. 5a, b; Table 2). Different primary producers fix carbon in different proportions of ¹³C and ¹²C that are

conserved from prey to predator, while δ¹⁵N increases in a predictable manner with each trophic level (Δδ¹⁵N ~ 2.9‰ for whole fish, Sweeting et al. 2007). Examining spatial trends, δ¹³C of downriver fish was on average 6.2‰ higher than that of upriver fish (two-way ANOVA, $F_{[1, 61]} = 986.9$, $p < 0.001$), while δ¹⁵N of downriver fish was on average

0.8‰ lower than that of upriver fish (two-way ANOVA, $F_{[1, 61]} = 115.1$, $p < 0.001$). Examining temporal trends, $\delta^{13}\text{C}$ decreased through time at the downriver site and increased at the upriver site (two-way ANOVA, $F_{[2, 61]} = 6.82$, $p = 0.0021$), becoming more similar between sites over the summer season. Temporal trends in $\delta^{15}\text{N}$ were significant (two-way ANOVA, $F_{[2, 61]} = 10.8$, $p < 0.001$) but less clear; fish collected in July exhibited the greatest $\delta^{15}\text{N}$. Variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ within sampling groups was low, as observed in Fig. 5c, d.

Discussion

The nursery function of estuaries for juvenile fish and invertebrates is complex, due to spatial and seasonal heterogeneity of physical and biological processes. We aimed to characterize juvenile flatfish nursery habitat along an estuarine gradient over a summer season in Yaquina Bay, Oregon. Examination of fish condition, dietary trophic markers, and trophic position of juvenile English sole revealed: (1) higher morphometric condition in juveniles from the warmer upriver habitat and higher energetic condition in juveniles from the colder downriver habitat, (2) enrichment in marine diatom-sourced trophic markers and slightly lower trophic position in downriver fish, and (3) a trend towards accumulation of marine-sourced lipids in downriver fish with the progression of summer. Our data indicate that both temperature and food quality play important roles in determining observed morphometric and energetic (lipid-based) condition metrics in wild juvenile English sole.

Effect of Habitat on Fish Condition

Energetic condition (i.e., TAG/ST) was higher in downriver-collected fish; however, morphometric condition (i.e., length–weight residuals) was higher in fish sampled upriver. Morphometric condition increases with the deposition of both protein and lipids, and therefore higher morphometric condition without concurrent elevation in energetic condition could be a function of increased protein-based structures (i.e., muscle tissue, increased % N indicates higher protein) or increased water content. The contrasting trends for morphometric versus lipid-based condition metrics underscore the importance of examining multiple condition indices when assessing the quality of fish habitat. A number of studies have found differing trends for growth and various condition indices within the same fish (Suthers et al. 1992; Amara and Galois 2004; Walther et al. 2010). This disparity may result from different scales of temporal integration and differing physiological processes underlying each condition index.

Differing growth and lipid storage strategies between upriver and downriver English sole were likely driven in part by temperature. In laboratory-reared individuals, growth has been observed to increase with temperature (Ryer et al. 2012; Koenker et al. 2018), while lipid storage has been observed to decrease or exhibit a dome-shaped relationship with temperature (Copeman et al. 2017; Koenker et al. 2018), suggesting an optimal temperature for lipid storage that may be lower than that for growth. Koenker et al. (2018) found that in Arctic cod and Walleye pollock larvae, morphometric

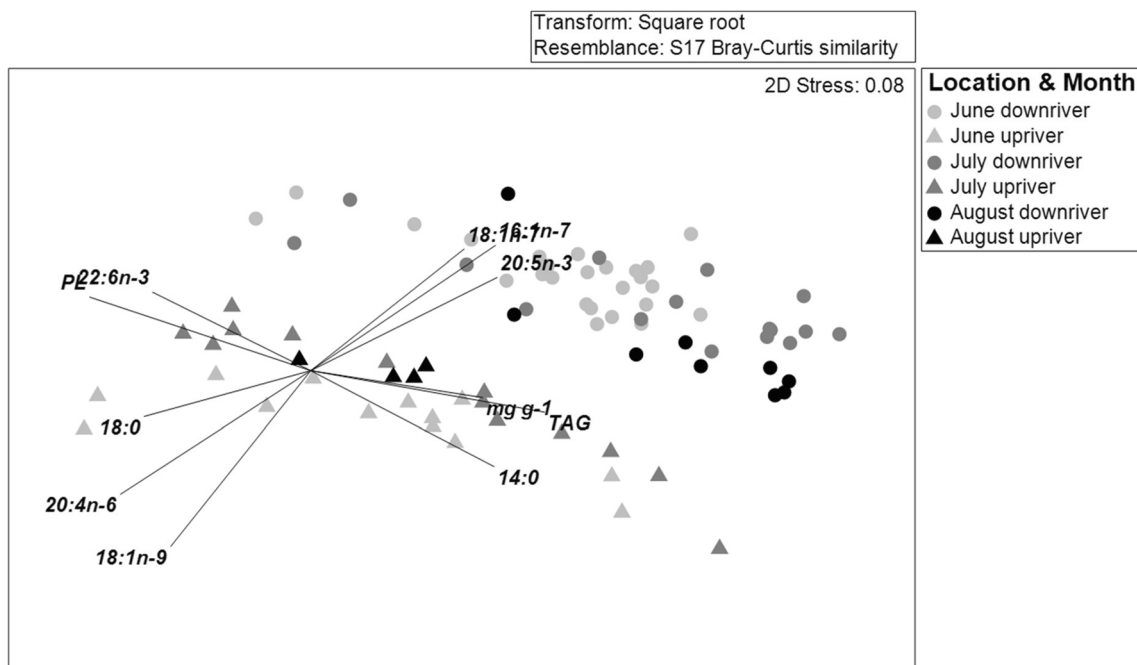


Fig. 4 Results from non-metric multidimensional scaling analysis (nMDS) showing the level of similarity in major fatty acid proportions, lipid class proportions, and lipid density per wet weight (mg g^{-1}) among

individual fish ($n = 80$) collected from Yaquina Bay in summer 2014. Overlays show vectors with $R^2 > 0.7$. Abbreviations: TAG, percentage triacylglycerols; PL, percentage phospholipids

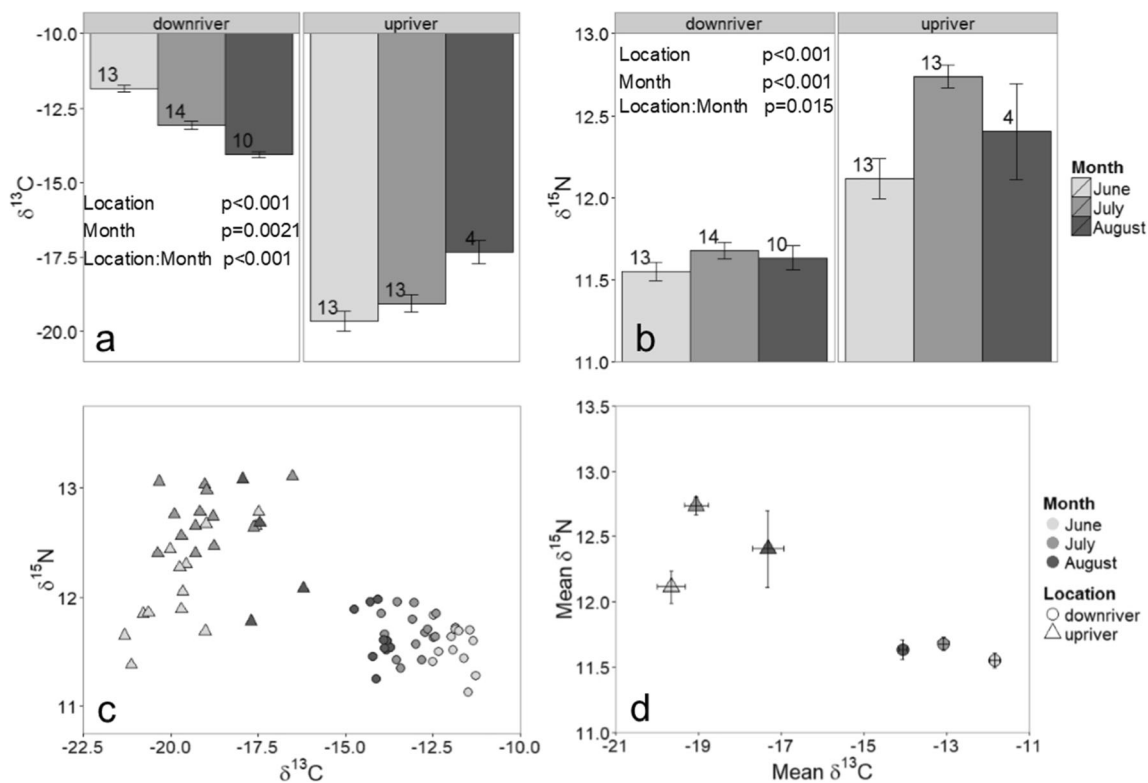


Fig. 5 Sampling group mean $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) for juvenile English sole (*Parophrys vetulus*, $n = 67$) collected from Yaquina Bay estuary during summer of 2014. Differences between groups were analyzed

using a fixed-effect two-way ANOVA, $\alpha = 0.05$. Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (c) and group mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (d). Error bars indicate standard error

condition was optimized at a higher temperature than energetic (lipid) condition. Both increased growth and lipid storage strategies could contribute to successful overwintering and recruitment strategies (Sogard 1997; Siddon et al. 2013); however, little is known about the complex mosaic of density-dependent and density-independent factors that affect survival from juvenile to adult stages in English sole.

Spatial Variation in Dietary Carbon Sources and Trophic Position

To more completely explain observed variation in English sole condition indices, we examined dietary trophic markers and found distinct dissimilarity between the two habitats. Fish from the downriver habitat were characterized by higher EPA and 16:1n-7, FAs which are commonly associated with marine diatom production (St. John and Lund 1996; Kelly and Scheibling 2012). ^{13}C enrichment in downriver-collected fish indicated a marine-sourced diet (Fry and Sherr 1984), corroborating high dietary diatom contribution. Juvenile English sole are benthophagous generalists, and within our size range, they feed on juvenile bivalves, harpacticoid copepods, amphipods, cumaceans, and polychaetes (Hogue and Carey Jr. 1982). In turn, these prey deposit feed or filter feed on microorganisms at the sediment–water interface. Although we did not directly quantify FAs and SIs in prey items for juvenile English sole,

Bosley et al. (2017) performed a similar study along the Yaquina Bay estuarine gradient in August 2012, by examining FAs and SIs of burrowing shrimp and their potential food items. Burrowing shrimp utilize feeding strategies and carbon sources that are similar to the prey of juvenile English sole (Bosley et al. 2017). Comparing FA profiles of sediment particulate organic matter (SPOM, Bosley et al. 2017) with burrowing shrimp (*Neotrypaea californiensis*, Bosley et al. 2017) with juvenile English sole (the current study) consistently shows the pattern of higher diatom-associated FAs (16:1n-7 and EPA) downriver compared with upriver sites (Table 2).

English sole collected from the upriver habitat were associated with higher relative levels of ARA (20:4n-6), which can be indicative of macroalgae (Kelly and Scheibling 2012). Bosley et al. (2017) also observed elevated proportions of ARA, as well as indicators of terrigenous detrital material (low $\delta^{13}\text{C}$ and high SFA/MUFA, 18:2n-6, and 18:3n-3) at the same upriver sampling sites as our current study. Contribution of terrestrial plant material to the upriver food web was supported by ^{13}C depletion of juvenile English sole in this habitat (Fry and Sherr 1984). Additionally, ^{15}N enrichment of upriver juveniles gives evidence that they may be feeding at a slightly higher trophic level than those downriver (Peterson and Fry 1987), which may have been due to the greater involvement of detritus and bacteria in the upriver food

web (Pepin and Dower 2007; Bosley et al. 2017). It should be noted that, while the higher $\delta^{15}\text{N}$ of upriver fish suggests minor differences in trophic dynamics, these fish are unlikely to be feeding at a full trophic level above the downriver fish, because the 0.8‰ difference is less than half of that expected for a full trophic level ($\Delta\delta^{15}\text{N} \sim 2.9\text{‰}$ for whole fish, Sweeting et al. 2007). Additionally, a clear ontogenetic shift in prey items for juvenile English sole has been observed around 35 mm SL (Hogue and Carey Jr. 1982), which is smaller than all fish included in our statistical analyses. Thus, the observed SI and FA trends in each habitat, combined with the more detailed findings of Bosley et al. (2017), generally support our initial expectation of terrestrially sourced and marine-sourced carbon being more predominant in upriver and downriver habitats, respectively. It should be noted that lipid class composition and total lipid density (TAG, PL, and mg g^{-1} vectors in Fig. 4) were not associated with either habitat, indicating that differences in downriver and upriver carbon sources are related to spatial variation in trophic dynamics, and are not simply due to differences in lipid class composition or total lipid density of the fish at these sites.

Observed spatial variation in dietary carbon sources can help to explain the higher energetic condition of fish downriver. These fish were associated with diatom-sourced diets, which have been found to contribute to increased condition in larval and juvenile fish (St. John and Lund 1996; Copeman et al. 2015). Furthermore, the essential fatty acids of greatest importance to juvenile fish (i.e., EPA, DHA, and ARA) are usually synthesized by marine producers (Sargent et al. 2002), which are more clearly associated with the downriver habitat. Long-chain polyunsaturated FAs (PUFAs) like these have higher physiological value than the short-chain PUFAs associated with terrestrial/freshwater producers, because marine fish are largely incapable of desaturating and elongating short-chain C_{18} PUFAs into long-chain C_{20+22} PUFAs (Cowey et al. 1976; Twining et al. 2016). The higher nutritional value of the marine-sourced carbon downriver was also evident in the higher condition (percent organic material and total FA per weight) of burrowing shrimp collected downriver (Table 2, Bosley et al. 2017).

Temporal Variation in Dietary Carbon Sources and Trophic Position

Though statistically significant, temporal trends in carbon sources were not as clear as spatial trends. DHA (and thus DHA/EPA, which relates dinoflagellate and diatom production) decreased with progression of the summer season, particularly at the downriver site. This trend may reflect the coastal transition from relaxed, stratified downwelling conditions to turbulent, mixed upwelling conditions (Cushing 1989; Dalsgaard et al. 2003) and the resulting seasonal transition from dinoflagellate to diatom production along the coast.

Miller et al. (2017) observed this seasonal transition concurrently in phytoplankton species composition and copepod FA composition, demonstrating the relationship between seasonally changing carbon sources and FAs observed at higher trophic levels.

However, since FA analyses were performed on total lipids rather than storage lipids, an alternative explanation for decreasing DHA/EPA must be considered. DHA is typically membrane-associated in fish, and is thus incorporated into structural lipids (PL) rather than storage lipids (TAG, Bell and Dick 1991; MacPherson et al. 1998). While TAG reflects diet, the PL class is more selective for specific species of FA, and reflects dietary inputs to a lesser extent. Therefore, a decreasing proportion of DHA in the total lipid pool could simply represent a decreasing proportion of PL or rather, an increasing proportion of TAG as fish accumulate storage lipid over the summer season. Examining the lipid class and lipid density vectors in Fig. 4 (TAG, PL, and mg g^{-1}), there does appear to be an association between lipid class composition and seasonal FA changes at the downriver site. Greater diatom biomarker proportions in late-summer, downriver-collected fish may thus be magnified by seasonal TAG accumulation, rather than simply reflecting increased diatom dietary contribution to the food web.

Additional support for a seasonal transition from fluvial to marine influence on carbon sources was offered by a convergence in carbon SI ratios over time. Over the summer, $\delta^{13}\text{C}$ increased upriver and decreased downriver, becoming more similar. This convergence could reflect decreased fluvial input, particularly supported by the $\delta^{13}\text{C}$ increase upriver. Howe and Simenstad (2015) similarly observed homogenization of English sole dietary sources with reduced river flow over the summer season. It should be noted that temporal changes may result not only from food web transitions within estuarine habitats, but also from movement of juvenile English sole, which migrate from the nearshore region into the estuarine nursery, and then leave the estuary by the end of summer (Gunderson et al. 1990). This possibility may be addressed by considering the time period integrated by SIs (~ 1 month) and the low variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ within sampling groups (Fig. 5c, d). If juveniles were moving along the estuarine gradient, we would expect to see less separation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between downriver and upriver groups, and more variability within groups. The clear separation and low variability observed suggests that juveniles spent most of the previous month within their site of capture.

Though seasonal trends in carbon sources appeared largely driven by changing lipid class composition, SI support of a fluvial-to-marine transition suggests a possible explanation for the increasing energetic condition of downriver fish over time. Marine-sourced diets contain important long-chain PUFAs,

which have higher physiological value to fish than the short-chain PUFAs associated with terrestrially sourced diets. The greater prevalence of marine carbon sources later in the season, and the resulting increase in nutritional value of prey for English sole, may help to explain the temporal increase in energetic condition of downriver fish. A larger sample size of late-summer English sole could provide stronger support for these seasonal trends in dietary carbon sources. The downriver site's decreasing temperature could also support increasing energetic condition in fish residing there, as discussed earlier.

Conclusions

In the Yaquina Bay estuary, the spatial and temporal variation observed in juvenile English sole energetic (lipid) and morphometric (growth) condition is likely explained by differences in temperature and food quality. The downriver habitat appears conducive to greater lipid storage, due in part to lower temperatures and the availability of marine-sourced prey. Conversely, the upriver habitat's warmer temperatures appear to be conducive to faster growth in juveniles residing there. This difference in nursery function at ends of the estuarine gradient highlights the necessity to examine habitats within a single estuary for better understanding of the estuary's nursery role. Estuaries and other coastal wetlands are extremely valuable ecosystems, but are rapidly degrading and fragmenting as the result of human activity. Detailed knowledge of processes underlying estuary function is imperative for effective management and protection of these ecosystems.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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