SUPPLEMENT 1

Gut contents analysis of English sole

Collection Methods

In a concurrent but separate study by our research group, we examined the gut contents of English sole, and determined that there was no proportional change over time. We used 68 UWFC English sole specimens collected from 1930–2015 (SL = 141.553 ± 38.257 mm). Twenty-three of these specimens were also used for our CSSIA-N analyses. In 2018, a UW research trawl collected an additional 76 English sole, and these specimens were preserved following UWFC protocol (SL = 155.066 ± 40.306 mm). Of these 76, nine were randomly sampled to increase replication in the decade 2010-2020 (see *Statistical analysis* below) in the gut content analysis. Across UWFC stomachs and UW research trawl stomachs, we selected stomachs to sample by choosing those that appeared to be full. The contents were classified into 19 categories (amphipods, brachiopods, brittlestars, clams, copepods, corophid amphipods, crabs, *Crangon* spp., cumacea, isopods, mysids, polychaetes, scallops, snails, tubeworms, worms, unidentified digested material, empty). We recorded the mass of the categorized food items and total mass of the food items per fish in grams. None of the UW research trawl fish were used for CSSIAA-N. See Table S2 for UWFC accession numbers of fishes.

Statistical Analysis

For each food item detected from our gut contents analysis, we conducted a beta regression. We selected this approach because response variables were a proportion of the total stomach content. Using this dataset (n = 75, 1930-2018) we examined proportional change in each prey item over time. To account for differences in fish size, we offset our response variable by fish standard length. We chose to offset our data by fish size as there is a known positive relationship between fish gape size and fish length, and gape is correlated with the size and type of prey a fish can consume ((Karpouzi & Stergiou 2003, Barnes et al. 2010). We also accounted for site differences by including a random effect of site. We did not include an effect of season because English sole diet, which predominantly consists of polychaetes and bivalves, is consistent year-round (Reum & Essington 2008). Because our response variable was a proportion, we implemented a betatrans function (Smithson & Verkuilen 2005) on the response variable, and used a beta distribution in our model. The models were implemented using the glmmADMB() function in the R package glmmADMB (Fournier et al. 2012, Skaug et al. 2016) [Eq 4] for all prey items except brittle stars. The brittlestar model was implemented with the glmmTMB() function in the R package glmmTMB, year was scaled to our response variable and the model was fit with a beta family and link logit function. This function was used because the brittlestar dataset contained all zeros except for the year 2018. Because many statistical tests were conducted, we applied a False Discovery Correction to all p-values to reduce type I error (Benjamini & Hochberg 1995). This correction was implemented using the *p.adjust()* function in base R.

Prey Proportional Mass ~ Year Collected + fish standard length + (1 | site) [Eq. S1]

Results

There was no change in the proportion of any prey item found in English sole (See Table S1 and Figure S1 Below).

Table S1. Results of the generalized lin	ear mixed models for	or each prey item	classified.
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Food Item	Predictor	estimate	std.error	statistic	p.value	p.bh
Amphipod	Year	-0.003	0.005	-0.552	0.581	1.000
Amphipod	SL	0.001	0.004	0.417	0.677	1.000
Algae	Year	0.002	0.005	0.530	0.596	1.000
Algae	SL	0.000	0.003	-0.065	0.948	1.000
Brachiopod	Year	0.000	0.002	0.000	1.000	1.000
Brachiopod	SL	0.000	0.002	0.000	1.000	1.000
Brittlestar	Year	0.007	0.004	1.768	0.077	1.000
Brittlestar	SL	-0.001	0.003	-0.516	0.606	1.000
Clam	Year	0.007	0.005	1.504	0.133	1.000
Clam	SL	0.001	0.003	0.267	0.790	1.000
Copepod	Year	0.000	0.002	0.000	1.000	1.000
Copepod	SL	0.000	0.002	0.000	1.000	1.000
Corophid Amphipod	Year	0.000	0.002	0.000	1.000	1.000
Corophid Amphipod	SL	0.000	0.002	0.000	1.000	1.000

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Crab	Year	0.003	0.004	0.682	0.495	1.000
Crab	SL	-0.001	0.003	-0.296	0.767	1.000
Crangon spp.	Year	-0.005	0.004	-1.097	0.273	1.000
Crangon spp.	SL	-0.002	0.003	-0.507	0.612	1.000
Cumacea	Year	-0.003	0.004	-0.716	0.474	1.000
Cumacea	SL	-0.002	0.003	-0.757	0.449	1.000
Isopod	Year	-0.002	0.003	-0 569	0.570	1.000
		0.002	0.005	0.007	0.570	1.000
Isopod	SL	0.000	0.002	-0.107	0.914	1.000
Mysid	Year	-0.003	0.004	-0.813	0.416	1.000
Mysid	SL	-0.001	0.003	-0.347	0.728	1.000
Polychaete	Year	0.000	0.004	-0.055	0.956	1.000
Polychaete	SL	0.004	0.003	1.151	0.250	1.000
Scallop	Year	0.002	0.005	0.530	0.596	1.000
Scallop	SL	0.000	0.003	-0.065	0.948	1.000
Snail	Year	0.000	0.002	0.000	1.000	1.000
Snail	SL	0.000	0.002	0.000	1.000	1.000
Tubeworm	Year	0.001	0.005	0.308	0.758	1.000
Tubeworm	SL	0.003	0.003	0.810	0.418	1.000

Worm	Year	-0.002	0.003	-0.488	0.626	1.000
Worm	SL	0.002	0.003	0.708	0.479	1.000



Figure S1. The mean proportion of each type of English sole stomach content identified by decade.

Table S2. List of UWFC specimens used in analyses.				
Gut Content Analysis	CSIAA-N	Both		
UW 048204 HSP 026	UW 026114	UW 10394_02		
UW 048204 HSP 033	UW 026115_02	UW 10394_03		
UW 048204 HSP 034	UW 026118_01	UW 110373_02		
UW 048204 HSP 036	UW 026121	UW 110373_05		
UW 048204 HSP 028	UW 048602_01	UW 110373_07		
UW 048204 HSP 029	UW 10394_01	UW 111168_01		
UW 18222_01	UW 111197	UW 112218		
UW 5815	UW 112208_01	UW 1177_01		
UW 4495_01	UW 119958	UW 151702_06		
UW 48351_01	UW 119986_01	UW 151702_07		
UW 48351_03	UW 152939_01	UW 151702_08		
UW 4885_01	UW 155836_01	UW 155822		
UW 5468_01	UW 158625_01	UW 16616_02		
UW 112224	UW 158631_01	UW 17077_01		
UW 026122	UW 16925	UW 17077_02		
UW 17077_02	UW 5441_01	UW 2269_02		

UW 110373_01	UW 5523_01	UW 40664_01
UW 110373_03	UW 5523_02	UW 4491_01
UW 110373_04	UW 5829	UW 4579_01
UW 110373_06	UW 789_01	UW 48351_02
UW 5441_01		UW 48351_04
UW 16925		UW 4925_02
UW 4614_01		UW 6013_01
UW 4794_01		
UW 4925_01		
UW 16589		
UW 2269_01		
UW 026120		
UW 110086_01		
UW 026114		
UW 026131_01		
UW 026131_02		
UW 026131_03		
UW 026115_03		

UW 026115_02	
UW 026115_01	
UW 026121	
UW 110080	
UW 16616_01	
UW 4885_02	
UW 10394_01	
UW 17851_01	
UW 17851_02	
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SUPPLEMENT 2

Fish collection sites

Whenever possible, fish collection sites were reported as the latitude and longitude given from the UWFC record cards. If site names but no latitude and longitude were reported on a record card, we assigned a latitude and longitude coordinate to each unique site. If sites had similar names and were obviously identical in general locality, they were assigned the same latitude and longitude. We restricted the region in which we sampled to ensure our specimens were collected from Puget Sound proper and not the coast. Thus, we sampled specimens that were collected no further west than Port Angeles, WA and no further north than Bellingham, Bay, WA.

Compound-specific stable isotope analysis of nitrogen amino acids (CSSIA-N)

Stepwise derivatization, analysis, and drift correction procedures are fully detailed in Welicky et al. (2021) and were based on Metges et al. (1996), Popp et al. (2007), and Chikaraishi et al. (2009). Briefly, we conducted CSSIA-N using a Trace 1310 GC in combination with a TriPlus RSH autosampler. All eluting compounds (H₂O, CO₂, and N₂) off the column were oxidized inside a GC Isolink II combustion interface. Water was removed through a Nafion membrane downstream of the reactor while CO₂ was cryogenically trapped in tubing submerged in liquid nitrogen before transfer to the IRMS (DELTA V) through a Conflo IV universal interface. All equipment was manufactured by Thermo Scientific, USA. High purity N₂ (>99.9997% N₂, Airgas) was used as reference gas to initially calculate the isotopic composition. Raw data were drift-corrected. See Welicky et al. (2021) for drift corrections procedures. Partially-automated drift correction procedures in R are available in a GitHub repository (https://github.com/rlwelicky/CSIA_Allfish).

Statistical Analysis

- *Testing for spatial autocorrelation* Because some of the sites from which fish had been collected were close in space (geographically), we examined whether our data were spatially autocorrelated. To test for spatial autocorrelation, we first conducted generalized linear models with a Gaussian distribution, where the dependent variable was trophic position, glutamic acid, or phenylalanine and the independent variable was collection year. This was implemented using the 'glm' function in the "MASS" package (Venables & Ripley 2020). Residuals of these models were extracted and then tested for spatial autocorrelation using Moran's I test for spatial autocorrelation using the function "testSpatialAutocorrelation" via the packages "DHARMa" (Hartig) and "spdep" (Bivand et al. 2013). We detected no spatial autocorrelation.
- *Testing for temporal autocorrelation* Since our response variables may be influenced by factors that change in relation to time (i.e. season or annual patterns), we also examined whether our

data were temporally autocorrelated. We conducted a Durbin-Watson test to test for temporal autocorrelation for each of our response variables: trophic position, glutamic acid, and phenylalanine. We specified the order as a collection year and implemented this test using the 'dwtest' function from the package 'lmtest' (Zeileis & Hortorn 2002). We detected no temporal autocorrelation. We could not assess seasonal autocorrelation as we did not have data from multiple seasons or even the transition of one season in a single year in our dataset. Nonetheless, we were curious if we could capture any seasonal autocorrelation. We could not test this continuously, because this would incorrectly sequence seasons, as 1 is January, and furthest numerically is 12, December, yet seasonally these months and numbers are most similar. Therefore, we conducted a simple categorical regression analysis to test whether year was a significant predictor of season, and we determined there was no significant effect of year on month (est = 0.009, se = 0.007, t = 1.289, p = 0.199). Testing for collinearity between fish size and year

- Verifying if there was correlation between year and fish size This was implemented using the 'cor.test' function in base R, where the response variable was fish standard length and the independent variable was year. We determined that for Pacific Herring there was significant correlation between year and standard length, and there was no temporal trend in size for the other species sampled. Therefore after conducting our herring models, we verified that the variance inflation factors of the models were under 5. We tested for collinearity using the check collinearity function in the R package performance (Hebbali 2020). Acceptable value thresholds for the variance inflation factor (VIF) have been suggested to be 5 and 10 (reviewed in Dormann et al. 2013) and the VIF value for our models were 1.2 and under. Therefore, we did not detect collinearity in our models and no statistical corrections for collinearity were made. In a post hoc analysis, we verified that there was no significant interaction effect between size and year on rockfish trophic position (p = 0.276) using the following model: Trophic position_{*iik*} ~ Year collected_{*i*} + fish standard length_{*iik*} + $(1 | site_k)$ + Year collected, * fish standard length_{*iik*}, where the response variable_{*iik*} represents the trophic position value of the *i*th fish collected from the *k*th site in the *j*th year. Year collected and fish standard length were centered and scaled.
- *Testing for discrete time points when rapid trophic change occurred* We chose the recursive residual options because this option is better at detecting regime shifts than ordinary least squares residuals (e.g. Brown et al. 1975, Galpin & Hawkins 1984). We used cumulative sums, rather than moving sums, because cumulative sums have been used in a number of disciplines to track and monitor total changes over time (e.g., Zeileis 2005, 2006). Cumulative sums also have been reported to perform better than moving sums (e.g., Brown et al. 2002), because moving sums can be sensitive to stochastic events (e.g., Zeileis & Hortorn 2002).



Supplemental Figures

Figure S2. Glutamic acid values across time by fish species. A = Pacific herring, B = English sole, C = walleye pollock and <math>D = Pacific hake.



Year collected

Figure S3. Phenylalanine values across time by fish species. A = Pacific herring, B = English sole, C = walleye pollock and <math>D = Pacific hake.

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