**Supplementary Information Text**

**1. Methods for compound specific stable isotope analysis**

Collagen samples have been analyzed for both CSSIA and bulk δ15N which require 10 mg of purified collagen (100 mg of bone). Preliminary analyses were conducted to determine the highest rate of collagen return from bone sampled from different parts of the skull to minimize destruction. Samples were taken from the internal occipital shelf to maintain external integrity. Bone was decalcified using 0.2 M HCl for 24-72 hours depending on bone thickness, followed by centrifugation and nanopure water rinse. Removal of humic acids was conducted using 0.125 M NaOH for 20 hours. Samples were washed to a neutral pH, then solubilized in 0.01N HCl. Once solubilized samples were blown down under N2 to prevent isotopic fractionation, and freeze dried. Freeze dried collagen was analyzed for bulk isotopic composition of nitrogen by the UW IsoLab (isolab.ess.washington.edu) using a coupled elemental analyzer-isotope ratio mass spectrometer following the standard protocols of the laboratory. C:N ratios were calculated from this data, which is a measure of the quality for carbon and nitrogen analyses of bone collagen for isotopic analysis. Only three observations were outside of the acceptable rang of 2.7-3.6; indicating there was no substantial loss of glycine or addition of nitrogen due to microbial processing from mortality, decay, curation, and analysis.

δ15N of eleven amino acids[[1]](#footnote-1) were measured in the UW Facility for Compound-Specific Isotope Analysis of Environmental Samples. Samples were prepared following the procedures developed by Chikaraishi et al. (2007) and protocols by Rachel Jeffrey’s lab at University of Liverpool UK which are modifications of that published by Metges et al. (1996) and Popp et al. (2007). Briefly, proteins were hydrolyzed in 6N HCl and purified using a cation exchange column. 20 μL or norleucine was added as an internal standard. Amino acids were esterified using isopropanol acetyl chloride, and derivatized via acylation with 4:1 toluene: pivaloyl chloride. Samples were brought up in ethyl acetate and analyzed using a coupled gas chromatography-combustion-isotope ratio mass spectrometer system (GC-C-irMA; Thermo Scientific Trace GC + GC IsoLink coupled to a Delta V irMS) in continuous flow mode monitoring masses (m/z) 28 and 29. A 30 m x 0.32 mm x 0.50 μm Agilent Technologies DB-35 capillary column with 35% Phenyl and 65% polysiloxane stationary phase and moderate polarity was used (Chikaraishi et al. 2010) with an inlet temperature of 260 C, column flow of 2 ml/min and oven ramp of 9 ˚C min-1. For each run a 12 amino acid external standard with known isotopic composition was injected four times followed by sample injections. Samples were injected in triplicate, with the 12 amino acid standard mixture injected every two samples (or six injections). A two-hour column oxidation was performed after 6 samples (25 injections) followed by a 30 minute backflush and conditioning using 4 standard injections.

For each machine run, a linear model was fit for each individual amino acid using the following equation:

Where *m* represents the slope of the precision drift, *t* represents the injection number since last column oxidation, and *Std* represents the δ15N of an individual amino acid for a standard observation. The data was then corrected using the following equations:

Where *Daa,t* is the difference between an observed standard δ15N (*Stdaa,t*) for a given amino acid (*aa*) at a given injection number (*t*) and the true δ15N for that standard. Then:

Where the drift value, Daa,t, is subtracted from the sample value for a given amino acid and a given injection to correct the observed sample values for precision drift since last column oxidation. Mean sample corrected values for the triplicate injections were used for all analyses and trophic position calculations. Norleucine had lower precision in standards (mean = 0.4) compared to phenyalanine, therefore no correction using the internal standard was applied. Mean precision for a given AA standard was calculated using the standard deviation of the external standard injections for a given run after drift correction and taking a mean of each run's standard deviation. Conditioning injections were omitted from this calculation.

**2. Approaches for variations in Trophic Enrichment Factors**

Trophic discrimination factors are variable based on animal diet (omnivory/carnivory verse herbivory), pathways of nitrogen excretion, and trophic level (Nielsen et al. 2015, McMahon) with ominvory/carnivory and higher trophic levels demonstrating thew lowest trophic enrichment for most amino acids. Trophic discrimination has ultimately been attributed to diet quality (similarity in tissues between consumer and prey) and mode of nitrogen excretions, although the relative impacts of each is diffeicult to discern, especially considering most controlled feeding studies include low-trophic level ammonia excretors but not high (i.e., adult hake or salmon). In coastal Washington, most trophic transfers are between high diet quality, piscivorous fish (ammonia excretors) with a high-quality transfer between fish and harbor seal (urea excretor). Studies exploring using multiple trophic discrimination factors based on the food web structure and consumption type produce more accurate trophic position estimations especially for higher level consumers (McMahon et al 2015a, McMahon et a. 2015b, McMahon et al. 2019).

We applied multiple trophic position calculation frameworks for harbor seals to determine the best approach (Table 1 & 2). We also applied these approaches to herring, a known harbor seal prey species, with data from Germain et al. Based on known foraging patterns, we anticipate harbor seals have an average trophic position of 4 to 5 and herring will have a average trophic position of 2.5-2.9. Equation 2 produced the most accurate herring trophic position estimates for most amino acids (however valine was impossibly low). In contrast equation 3 produced the most accurate results for most amino acids compared to harbor seals, but these estimates were still unrealistically low for some amino acids (proline, valine), which is common for CSIA-AA (McMahon et al.). Additionally, this is not the most ecologically accurate parameterization, as it assumes all trophic transfers are of high prey quality, where there must be at least one herbivorous-low quality trophic transfer in the food web from phytoplankton to zooplankton (parameterization of equation 4). It also assumes prey quality (carnivorous) and trophic level of the consumer is more important than nitrogen excretion pathway (urea verse ammonia) for some amino acids but not others. Seemingly, these assumptions also impact trophic position estimates using different trophic amino acids differently which will likely be important for future studies applying a multi-amino acid framework. It is possible that these reflect biases in conventional TP estimates (i.e., stomach content analysis) as proposed by McMahon (2015a) or there may be biases in controlled feeding studies. For example, growth rate of individuals in controlled feeding studies may not accurately reflect those in natural ecosystems which may lead to overestimates in trophic discrimination if they are higher in natural systems compared to controlled feeding experiments. This may be plausible in the Washington food web as consumption of juvenile fish is common at multiple trophic levels, and juveniles presumably have higher growth rates than adults.

**3. Methods for Multivariate Autoregressive State-Space (MARSS) Model**

A MARSS model was fit to herring stock spawning biomass and harbor seal stock population size. Harbor seal datasets and herring biomass were collected by stock (n = 7 and n = 20, respectively) but did not have observations for every for every year (Figure S8 and Figure S10). In order to get total population and total biomass estimates for these species for each year two MARSS models were fit to the data, one for each species, to estimate population and biomass for each year for each for each stock (Figure S9 and Figure S11). For both datasets process variance (Q) assumed equal variance and covariance across stocks, and observation error (R) was assumed to be equal across stocks. U and x0 were both set to unequal, thus assuming they vary across stocks. Model states (Figure S9 and S11) were summed across years for total biomass/population size estimates. Harbor seal data has not been collected since 2000 and we assumed the population has remained constant from 2000-2010.

**4. using multi-trophic amino acid analysis compared to only glutamic acid**

-Similar results, similar coefficients, improves model certainty.

-Tissue turnover primary driver for differences? Using lag and multi AA net averaging different turnovers? Aspartic Acid == LONG turnover probably why its so different. Important to consider turnover when selecting AAs to use

-PHE t0.5 is 780. Alanine (642), glutamic acid (940), valine (942) are the most similar in shrimp (Downs et al.). Compared to proline (369) and aspartic acid (1530). Look at lizard paper and Jens’s unpuplished paper (the one with sketchy data)

**-** We observed some variability likely due to turn over time. Should consider turnover time of amino acids. Ideally the tissue turnover time of the tissue being sampled should be known for a similar taxa but we really don’t have info.

-Alanine, glutamic acid, proline may be best for AA plus valine with phe. Jens recommended 4 in his meta analysis? Double check

-Omitting proline did not substantially change our results just like glutamic acid only didn’t, it only changed model certainty (all env had 2 models with support rather than 1 but all had the same covariates included)...biggest difference in results comes from adding ALA actually would get the same results for all models except prey availability year-0 with just GLU and ALA which are actually seem to compensate eachother for turnover time?

- May not need 4 AAs just carefullt selected ones in comparison to your source AA (making tissue turnover as similar to PHE as possible)

**Figure S1:** Analysis of seasonality of harbor seal trophic position for five different trophic amino acids (glutamic acid, alanine, aspartic acid, valine, and proline) calculated using the source amino acid phenylalanine and equation 2 (Table S2; Figure S3.2) with a weighted beta. Color corresponds to trophic amino acid, line shows the fit of a generalized additive model with a smoothed term by month (1 = January, 12 = December) and a k of 12. \* denotes a significant smoothed term.

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**Figure S2:** Distributions (density of probability, y-axis) of calculated trophic position (x-axis) for harbor seals in this study. Equations (1-4) refer to Table S1 and parameter values described in Table 1 of the main text. Colors correspond to trophic amino acids (*Tr*) and the grey box represents ecologically realistic trophic positions for harbor seals if they were to predate 1 trophic position above herring (trophic position of 2.5, minimum expected value) and one trophic position below killer whales (trophic position of 6, maximum). The value within the grey box corresponds to the percentage of observed trophic position values that fell within the ecologically realistic range.

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**Figure S3:** Distributions (density of probability, y-axis) of calculated trophic position (x-axis) for harbor seals in this study applying equations (1-4) in Table S1 with βW instead of βAq as described in Table 1 of the main text.



**Figure S4:** Time series of residuals by year for the three ocean condition models (year-0, year-1, year-2) with the most support for five different trophic amino acids (glutamic acid, alanine, aspartic acid, valine, and proline) calculated using the source amino acid phenylalanine. Color corresponds to trophic amino acid, line shows the fit of a generalized additive model with a smoothed term by year and a k of 6. \* denotes a significant smoothed term.



**Figure S5:** Time series of residuals by year for the three ocean condition models (year-0, year-1, year-2) with the most support for five different trophic amino acids (glutamic acid, alanine, aspartic acid, valine, and proline) calculated using the source amino acid phenylalanine. Color corresponds to trophic amino acid, line shows the fit of a generalized additive model with a smoothed term by year and a k of 6. \* denotes a significant smoothed term.



**Figure S6:** Residual plots for the year-0, year-1, and year-2 ocean condition models with the most support.



**Figure S7:** Residual plots for the year-0, year-1, and year-2 prey availability models with the most support.



**Figure S8:** Pacific herring spawning biomass by stock from Siple and Francis 2015 stored in “HerringSpawningBiomass.csv”. ****

**Figure S9:** MARSS model (described in Appendix S3) for Pacific herring spawning biomass by stock fit to “HerringSpawningBiomass.csv” using source file ‘MARSS\_Herring\_HarborSeal.R’.

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**Figure S10:** Harbor seal population estimates by stock from Jeffries et al. 2003 stored in “HarboSeal.csv”.

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**Figure S11:** MARSS model (described in Appendix S3) for harbor seal population counts fit by stock to “HarborSeal.csv” using source file ‘MARSS\_Herring\_HarborSeal.R’.

**Table S1**: Equations for single and multi-trophic discrimination factor parameterizations of trophic position and the associated assumptions for each parameterization using the values described in Table 1 in the main text. *Tr*  refers to an individual trophic amino acid. In addition to βAq (Figure. S1) the same equations were used with βW (equation 6, Figure S2). Applying βW has the additional assumption that both C3 and C4 plants contribute to the coastal food web in which harbor seals forage and that the contributions of each can be calculated from bulk δ13C data.

|  |  |
| --- | --- |
| Assumptions | Equation |
| 1. Assumes that all trophic transfers are best represented by the average TDF in this system |  |
| 2. Includes harbor seal TDF which assumes one trophic transfer is from high quality prey (and urea excretion) and assumes average TDF accurately represents all other trophic transfers |  |
| 3. Assumes harbors seal TDF is a better representation for all trophic transfers in this system (carnivores) compared to an average TDF. |  |
| 4. Assumes harbors seal TDF is a better representation for most trophic transfers in this system, includes a TDF similar to basal consumers (zooplankton / herbivores). |  |

**Table S2:** Covariates used to test ocean condition as a bottom-up driver of harbor seal trophic ecology. Total number of models tested = 53.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Time Series Description | Length | Source |
| Discharge | Total discharge from the Columbia River at Dalles, WA during summer months of high discharge (May-Oct) from monthly U.S. Geological Survey discharge data. | 1879-2018 | Data Source: [USGS 14105700](https://waterdata.usgs.gov/nwis/uv?site_no=14105700) |
| Sea Surface Temperature (SST) | Average of monthly NOAA Extended Reconstructed SST for summer (Jul-Sep) in coastal Washington (48°N, 125°W). | 1854-2019 | Data Source: [NOAA ERSST V5](https://www.esrl.noaa.gov/psd/data/gridded/data.noaa.ersst.v5.html)  SST data was obtained from NOAA\_ERSST\_V5 data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their Web site at https://www.esrl.noaa.gov/psd/ (Huang et al. 2017). |
| Upwelling | Mean coastal upwelling index (CUI) coastal Washington (45°N, 125°W) using Bakun upwelling calculation based on Ekman's theory of mass transport due to wind stress, for spring (Apr-Jun) and summer (Jun-Sep). | 1946-2019. | Data Source: [NOAA ERD SWFSC](https://oceanview.pfeg.noaa.gov/products/upwelling/dnld) |
| North Pacific Gyre Oscillation | 2nd dominant mode of sea surface height variability in the northeast Pacific. Correlates with fluctuations in salinity nutrients and chlorophyll-a. | 1950-2019 | Data Source: Di Lorenzo et al. 2008. [NPGO](http://www.o3d.org/npgo/) |
| Multivariate ENSO Index | The extended Multivariate ENSO Index (MEI) uses Principle Component analysis on six variables: sea-level pressure, u and v component of the surface wind vector, sea surface temperature and cloudiness fraction in the tropical Pacific. | 1950-2019 | Data Source: NOAA/ESRL (https://psl.noaa.gov/enso/mei.ext/table.ext.html) via California Current Integrated Ecosystem Assessment [MEI](https://www.integratedecosystemassessment.noaa.gov/regions/california-current/cc-indicator-climate-ocean-drivers) |
| Pacific Decadal Oscillation | Same as eastern Bering Sea | 1900-2018 | Data Sources: [PDO](http://research.jisao.washington.edu/pdo/PDO.latest.txt); Zhang et al. 1997; Mantua et al 1997 |

**Table S3:** Covariates used to test prey availability as a bottom-up driver of harbor seal trophic ecology. Total number of models tested = 59.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Time Series Description | Length | Source |
| Herring Biomass | Adult herring spawning biomass from egg deposition surveys for the estimated from Washington State Department of Fish and wildlife by Siple and Francis 2015. | 1973-2012 | Siple, M.C. and T.B. Francis. 2015. Population diversity in Pacific herring of the Puget Sound, USA. |
| Hake Biomass | Pacific Hake (whiting) relative spawning biomass in US and Canadian waters. | 1973-2012 | Berger et al. 2017. Table 8 total spawning biomass. |
| Chinook Salmon Spawners | Chinook salmon spawner summary data including all populations with a time series with data from at least 1973. Includes: Cedar River, Coweeman River, Elochoman River, Grays and Chinook Rivers, Green River, Kalama River, Lewis River, Lower Cowlitz River, Lower and Upper Sauk River, Lower and Upper Skagit River, McKenzie River, Mid-Hood Canal, Nisqually River, Puyallup River, Skokomish River, Skykomish River, Snoqualmie River, Suiattle River, Toutle River, Upper Gorge Tributaries, White River and White Salmon River. | 1973-2012 | Northwest Fisheries Science Center, 2020: SPS Abundance - Salmon spawner abundance data compilation and database management, [https://www.webapps.nwfsc.noaa.gov/apex/f?p=261:1:::NO::P1\_ARCHIVE\_NOTE\_CHECK:1&cs=1A0A1845C9A3C7202FD5C2934C6FD9410#](https://www.webapps.nwfsc.noaa.gov/apex/f?p=261:1:::NO::P1_ARCHIVE_NOTE_CHECK:1&cs=1A0A1845C9A3C7202FD5C2934C6FD9410) |
| Smolts | Hatchery release data from the Regional Mark Information System and Wild Salmon Production data summarized by Chasco et al. 2017. Data was summed across both datasets for total juvenile salmon production. | 1973-2012 | RMIS: https://www.rmis.org//rmis\_login.php?action=Login&system=cwt  Summarized: https://github.com/bchasco/COAST\_WIDE |
| Coho Salmon | Coho salmon spawner summary data including all populations with a time series with data from at least 1973. Includes: Coastal Estuaries, Eastern Bays, Hood Canal, Olympic Peninsula, Puget Sound, San Juan Islands, Strait of Juan de Fuca. | 1973-2012 | Northwest Fisheries Science Center, 2020: SPS Abundance - Salmon spawner abundance data compilation and database management |
| Chum Salmon | Chum salmon spawner summary data including all populations with a time series with data from at least 1973. Includes: Hood Canal, Strait of Juan de Fuca. | 1973-2012 | Northwest Fisheries Science Center, 2020: SPS Abundance - Salmon spawner abundance data compilation and database management |
| Harbor Seal Abundance | Harbor seal population estimates based on coastal estuary, eastern Bays, Hood Canal, Olympic Peninsula, Puget Sound, San Juan Islands, and the Strait of Juan de Fuca counts. | 1975-2012 | Jeffries, S., H. Huber, J. Calambokidis and J. Laake. 2003. Trends and status of harbor seals in Washington state: 1978-1999. The Journal of Wildlife Management 67: 207-218. |

|  |  |
| --- | --- |
| Amino Acid | Mean Precision |
| Phenylalanine | 0.34 |
| Glutamic Acid | 0.56 |
| Alanine | 0.46 |
| Proline | 0.48 |
| Valine | 0.38 |
| Aspartic Acid | 0.83 |
| Norleucine | 0.40 |

Table S4: Mean standard precision for amino acids.

Table S5: Pearson correlation coefficients for harbor seal trophic position calculated from five trophic amino acids.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trophic Amino Acid | Glutamic acid (Glu) | Alanine (Ala) | Aspartic Acid (Asp) | Proline (Pro) | Valine (Val) |
| Glutamic acid (Glu) | **-** |  |  |  |  |
| Alanine (Ala) | 0.79 | **-** |  |  |  |
| Aspartic Acid (Asp) | 0.25 | 0.29 | **-** |  |  |
| Proline (Pro) | 0.46 | 0.61 | 0.17 | **-** |  |
| Valine (Val) | 0.58 | 0.61 | 0.18 | 0.39 | **-** |

Table S6: Covariates included in the best models using standard linear models and only glutamic acid to calculate trophic position. Supported models is the number of models with delAIC < 1.97.

|  |  |  |  |
| --- | --- | --- | --- |
| Model | Covariates | Supported Models | Comparison to hierarchical models |
| year-0 Environment | Summer upwelling, Columbia River discharge \* | 14 | Summer upwelling was included in best model and 6 others |
| year-1 Environment | Sea surface temperature, spring upwelling \* | 3 | Sea surface temperature was included in all supported models, best model was the same as the hierarchical framework |
| year-2 Environment | Summer upwelling, MEI | 4 | Summer upwelling was included in all supported models, best model was the same as the hierarchical framework |
| year-0 Food web | Herring | 2 | Supported model was different than the model with the hierarchical model with most support |
| year-1 Food web | Chinook smolts | 3 | Best hierarchical model was included in the supported models |
| year-2 Food web | Chinook smolts † | 9 | Best hierarchical model was included in the supported models |

1. Alanine, glycine, proline, aspartic acid, leucine, isoleucine, valine, threonine, serine, glutamic acid, phenylalanine [↑](#footnote-ref-1)