

We appreciate the constructive comments on our manuscript titled "Delayed trophic response of a marine predator to ocean condition and prey availability during the past century" provided by the two anonymous reviewers and the editorial board. We have carefully considered and responded to all of the reviewer comments. In so doing, revised the manuscript to 1) include more explicit justification of assumptions for data interpretation, 2) provide more context for interpreting stable isotope analysis (tissue turnover, cause of death, feeding ecology), 3) provide greater ecological context for our results and 4) include more detailed data reporting.

Editorial Office Comments to the Author:

--Your appendix should be renamed to "Appendix_S1", with no other text or characters in the file name. References to the appendix as a single unit should use the format "Appendix S1".

Response: Revised

--At the beginning of the Appendix, add the journal name.

Response: Revised

--References to the Appendix in the Main Document must use the following format: "Appendix S1", "Appendix S1: Fig. S#", "Appendix S1: Table S#", "Appendix S1: Section S#", "Appendix S1: Eq. S#" and so on. Several references use "Appendix 1" currently.

Response: Revised

Reviewer #1 Comments to the Author:

This study uses compound-specific nitrogen stable isotope analysis applied on 153 harbor seal museum skulls to investigate abiotic and biotic factors associated with change in trophic position. The authors in particular investigate some plausible time delays between environmental covariates and trophic position.

I find it difficult to evaluate such a study because 1) one does not know anything about how the skulls were collected, and 2) a relatively large number of models were used on a rather limited and noisy dataset.

1. Sample of skulls: The only information available is that they were museum skulls, and that for most we might know the date, age or sex of the individuals. But how were the skulls collected? Do they represent animals shot by hunters (I guess that does not apply to recent years), animals found dead, etc?

Response: Specimen are primarily collected from animals found dead which includes strandings. Strandings can include trauma and disease or being shot (seals were the target of bounties before 1972, and seen as a nuisance species by many fishermen). Many specimens are collected by researchers and marine mammal stranding networks. A portion of stranded individuals were shot, which was estimated to constitute 30% of stranded individuals in 1979

(Stroud and Roffe 1979), even after it became illegal in 1972 with the passage of the Marine Mammal Protection Act.

A more recent analysis of pinniped strandings in San Juan County (Salish Sea) found most stranding cause of death for adult harbor seals was due to trauma (40%) or infectious disease (40%) with only 5% finding identifiable signs of nutritional distress in a necropsy (Ashley et al. 2020).

We agree that some more relevant information would be useful. We have included some information in lines 152-155. Unfortunately, detailed information is not available for every individual in each museum collection

2. Most seals were collected in the 1970s for Salish Sea and late 70s and 80s for “Coast”, any reason for that?

Response: The Northwest Marine Mammal Stranding Network began in the 1970’s which began as a collaboration with University of Puget Sound (UPS) Slater Museum’s mammal curator Murray Johnson and agencies such as NOAA and WDFW. Both the University of Washington Burke Museum and the UPS Slater Museum had a large number of specimens from this time period. Based on personal communication with the collection managers, specimens often come in associated with specific research projects or were collected by regional marine mammal stranding networks. In the 1970’s and 1980’s a number of specimens were collected by marine mammal biologists Steve Jeffries (employed by Washington Department of Fish and Game) and Terrell Newby (employed by University of Washington College of Fisheries and UPS alum). Some of these seals were associated with a research contract with UPS in the late 1970s to look at harbor seal biology which involved the collection of 100 seals from coastal Washington (Steve Jeffries personal communication).

Seemingly, interest in specimen collection peaked in the 70’s and 80’s likely as a result of increased marine mammal research and monitoring following low abundances and the introduction of stranding networks. Available specimens declined after this period, potentially due to limited space for collection.

3. Is there any systematic spatial variation (within a region) that should be accounted for when you investigate temporal effects (ie are some locations associated with specific values of the environmental predictors – that is are you sampling different areas in different years)?

Response: The only spatial bias in sampling is that more specimens came from the Salish Sea in the past two decades than coastal Washington (Figure 1). Within each region we targeted our sampling to be distributed across the region through time. We have added Appendix S1: Figure S1 to the appendix to provide greater detail regarding the spatio-temporal distribution of specimens within region.

Correlation between harbor seal trophic position and environmental predictors is indicative of bottom up forcing on the food web. Given their generalist foraging strategies and ability to forage 100 km from haul out sites, systematic spatial differences in the observed relationship would be expected based on differences in overall food web structure. As a result, we don't expect harbor seal trophic position to be associated with more localized environmental predictors than those tested in this study. We note that the tested environmental predictors have been associated with food web changes in previous studies that we would expect to influence harbor seals.

In an ideal sampling scenario, we would account for both spatial and temporal autocorrelation within the data, but due to the nature of museum specimen sampling our dataset is limited in its capacity to test finer scale spatial variation than the regions we delineated. In addition, testing finer scale spatial differences would require assuming restricted home ranges of harbor seals that we do not feel are accurate.

4. If some sample correspond to animals found dead because they were starving, could that influence estimates of trophic position? Or because you use bones, what is happening in the last few months should have no influence on the estimated trophic position?

Response: What happened to the animal in the last few months should have minimal influence on the estimated trophic position because we used bone.

If an individual was stranded due to starvation this would increase in ^{15}N in trophic amino acids in harbor seal tissues which would result in higher trophic position estimates. However, Bone collagen has a slow tissue turnover time and incorporates a stable isotope signature over the past 1-2 years of the animal's life and recent conditions (last few months) would likely have a minimal contribution to the bone collagen stable isotope signature. There are two reasons we do not think nutritional stress has a substantial influence on our data:

1. Starvation is not a common cause in adult harbor seal strandings. Since the 1990s the vast majority of stranded adults and sub adult harbor seals in Washington are attributed non-anthropogenic trauma (27%) or gross lesions from infectious disease that was not associated with emaciation (42%) (Ashley et al. 2020). 16% were associated with human incidence (this includes being shot, fisheries interactions, boat strikes) (Warlick et al. 2018). In the 1970s 43% of individuals in the region were shot, with another 9% showing signs of trauma and 21% indicating bacterial infection (Stroud 1979).

2. Based on the tissue turnover studies we cited, it takes at least a year for a dietary switch to be significantly correlated with the stable isotope value of bone tissue. The last few months of an individual life should have an unobservable influence in bone tissue stable isotopes.

We have added additional details in the supplement Appendix S1: Text S7 to further justify this assumption and state this assumption in the methods text lines 246-250.

5. I appreciate the value of using old specimens as they allow an investigation of effects over a wider range of time (and therefore more combinations of different environmental predictors), but at the same time there are sampling issues that need to be at least discussed.

You fit a relatively large model set (eg 35 models for ocean condition modelling, each set being evaluated with different time delays). These models have at most 4 predictor variables, but it is still a rather large model set for a small, noisy data set (as you acknowledge, at best 76% of the estimates of trophic position are “ecologically realistic”). I understand that it might be hard to use some form of cross-validation with an unbalanced data set (and simple cross validation like loo may not be very interesting here given this unbalance), but it would strengthen the inference. In particular some form of cross-validation in time (years) and/or space (regions+subregions) would be informative.

Response: We agree that the model set was large. Limiting the number of models while testing covariates that were known to impact the food web based on other studies was a challenge and we initially explored many approaches to deal with this (restricting model combinations, LASSO regression, BANOVA, Dynamic factor analysis). Due to the model uncertainty we chose to emphasize covariate estimates across models rather than a single model. In general, AIC should be an approximation to leave one out cross validation (see Stone 1977) so those results should be similar. Where cross validation would differ would be if we generated folds based temporal or spatial strata – results from this analysis would also be influenced by the choice of cross validation, however.

Values that were described as ecologically realistic are based on the absolute trophic position value falling within a defined range. While the absolute value may not be realistic, that does not mean the parameterization of the trophic position equation is not able to capture the variability between specimens – which would only change if and trophic enrichment factor or beta value specific to an individual sample was applied. Therefore, trophic position parameterization should have minimal impact on the modelling approach, especially considering the data was standardized about a mean of 0.

6. You mention at some point “extreme ocean conditions” (l. 298, 410), but it was not clear to me why you mention extremes – is it because you refer to specific years that are eg in the 5% of the distribution of environmental covariates (or if the distribution has some fat tails, some highly unexpected years), or because you suspect nonlinear effects (your model l 191 is linear, but of course it does not exclude nonlinear effects – you know additive models better than I do).

Response: The northeast Pacific has experienced extreme environmental change over the past decade that has been associated with food change (the ‘warm Blob’ starting in 2013 and described by Bond et al. 2015). We mention extreme events because our results show food web responses to environmental conditions are delayed on the order of years and so have some studies examining ecological responses to heat waves. This is important in the context of climate extremes as it indicates food web responses to extreme conditions may manifest

multiple years after the event. The predictors we used did not include the recent increase in marine heatwaves over the past decade but we expect environmental conditions to have even more drastic food web consequences in future conditions that may include non-stationary dynamics or non-analogue futures. Based on our results we expect responses to these changes to take multiple years to propagate through the food web.

We recognize this was unclear from the text. We have added additional context and discussion in lines 338-343 to address this for future readers.

7. You mention in the introduction that harbor seals have been recovering from very low levels and your population reconstruction (Figure S14) seems to confirm this. But you do not appear to have any long-term trends that could be linked to this large change in population size. Any explanation?

Response: The increase in harbor seal population in this region is due to reduction in mortality from the implementation of the Marine Mammal Protection Act which was passed in 1972. This legislation severely restricted anthropogenically induced mortality of harbor seals. Prior to 1972 (and for at least a decade after based on documented stranding reports, Stroud and Roffe 1979) it was not uncommon for people to kill harbor seals and bounties even existed. Harbor seal population recovery was not related to them taking advantage of a new resource (which would be indicated by a long-term trend in trophic position) but instead caused by legislative action that reduced mortality. We mentioned the MMPA in the introduction in lines 116 and have further emphasized the reduction in mortality as it relates to population recovery in line 117 to make this clearer to future readers that this is the known cause for population recovery.

I have a few queries that may reflect my limited knowledge of the field:

8. - When you give the equation used to estimate trophic position, you refer to McMahon 2015 but this paper is about corals and different bacteria/algae. I agree that the bacteria/algae in McMahon et al. 2015 have different trophic positions, but I could not find any justification why the equation used here is justified. I guess I might have overlooked it.

Response: We appreciate the attention to detail! Here we are describing the multi-TEF approach which was proposed to reduce variability in CSIA-derived trophic positions in McMahon 2016 not 2015. We have corrected the citation here, and elsewhere in the manuscript.

9. - You use month as a continuous covariate in Section S3, but that means that December is not close to January. There is clearly little evidence for any seasonal variation, but you could consider some cyclic predictor rather than a simple continuous predictor.

Response: We agree. Our initial test of seasonality applied a multivariate autoregressive state-space model with a seasonal predictor. Temporal gaps in the data resulted in the model not converging and the gam model was used as an alternative approach. To further verify this, we tested a linear model with a cyclic predictor of trophic position. Like our generalized additive

model analysis, we found no evidence of seasonality in our data (not a significant predictor). We have elected to keep only the gam analysis in our supplement, but are willing to add the cyclic predictor results to the supplement at the request of the reviewers or editor.

Tested cyclic predictors:

$$\cos\left(\frac{2\pi * Month}{12}\right)$$

$$\sin\left(\frac{2\pi * Month}{12}\right)$$

Details:

L 160: I guess a ; after harbor seals and not . to be consistent with the rest of the sentence?

Response: Revised. We agree ; seems correct

I. 193: random effect

Response: Revised.

I. 237, 238, 240, 245, 248: I would give two digits always (ie -0.20 and not -0.2 etc)

Response: It is typical for decimal places to only be reported with one decimal place based on the machine precision on which they were run, so the sample reporting does not exceed that of the machine precision. For most stable isotope data, this means they should be reported to one decimal place (unless they were run on an exceptionally precise machine). See “Reporting instrument precision and accuracy” in Bond and Hobson 2012 and our standard precision in Appendix S1: Table S13.

Bond, A. L., & Hobson, K. A. (2012). Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. *Waterbirds*, 35(2), 324-331.
<https://doi.org/10.1675/063.035.0213>

Section S2, second line: omnivory

Response: Revised.

Reviewer #2 Comments to the Author:

Delayed trophic response of a marine predator to ocean condition and prey availability during the past century

This study used $\delta^{15}\text{N}$ AA-CSIA of archived harbor seal bone samples collected from coastal Washington and the Salish Sea to estimate TP and relate TP estimates to environmental and biological data. Samples ranged from 1928-2014 (n=153), with most samples coming from the 1970s to 2000s. This period was identified as a period of 10-fold population increase. 1, 2 and 3 year time lags were applied to upwelling, SST, freshwater discharge and abundance indexes for Pacific hake, Pacific herring, and Chinook salmon. Overall, delayed responses of TP were detected for upwelling, SST, freshwater discharge, and prey availability. This is an ambitious study that attempts to assess the influence of a complicated mix of environmental and

biological factors on harbor seal TP using a novel compound-specific stable isotope analysis approach. Overall, I think it is an interesting dataset that warrants publication, but feel that additional information should be incorporated to strengthen findings. I have included some of these general comments below as well as specific line-by-line comments and edits.

General Comments:

1. Incorporation of Complementary Studies: Given the challenges of interpreting AA-CSIA-derived TP estimates in relation to a variety of biological and environmental variables and also trying to account for time lags associated with isotopic turnover and delayed biological responses, study results would greatly benefit from direct comparison to more conventional diet and TP data. Any available evidence from published studies in this region (e.g., directed scat/diet studies) that can be used to support observed TP trends would greatly strengthen confidence in the causality of observed correlations. I would suspect that such data must be available, at least for the data from the past twenty years. As another example, this study observed a lower TP in the year following summer upwelling. Does this match with documented fish communities and available prey associated with upwelling conditions?

Response: Revised

We discussed the previously documented prevalence of Pacific herring, Pacific hake, and Chinook salmon in harbor seal diet in lines 363-379 and lines 385-387 along with documented preferences of adult versus juvenile age classes of those species that justify the mechanisms we propose in Figure 3. No studies we have identified have examined the delayed responses of harbor seal diet to environmental conditions or prey availability. Scat and diet sample studies are highly limited through time and typically only include 1-2 years of data and a single season whereas our dataset are time normalized over the course of a year. This is what makes this study novel compared to previous work that has focused on scat samples

We also note that we reference studies that have documented changes in fish and plankton communities along with other predators in response to the tested environmental variables (Columbia River discharge, sea surface temperature, upwelling) in Washington in lines 345-361 and 377-380). We recognize this description may not have been explicit enough and we have revised this section to make it clearer to make the connections and supporting evidence clearer to readers.

2. Turnover Rate Interpretation: Including a time lag makes sense given the estimated slow turnover rate of bone collagen, but if harbor seals are continually feeding across seasons, it would seem to make more sense to try to relate measured TP to an average of the year leading up to the time of collection, rather than a full year separation from collection date. Relatedly, the slow turnover of bone collagen would not seem well suited for trying to detect any sort of seasonal environmental or biological phenomena (e.g., seasonal environmental conditions or prey availability). The Discussion would benefit from further consideration of the presumed

turnover rate of bone collagen in the context of timing and duration of environmental and biological changes.

Response: Revised.

Date of collection was not available for all specimens (only year) and there was no evidence that trophic position varied throughout the year for the subset of specimens that had month data. The subset of specimens that did have month of collection data are in Appendix S1: Figure S2. For all prey data, abundance is only reported annually and calculating a seasonal metric for prey is not possible although it is possible for environmental conditions. We estimate the time of a diet shift to be measurable in bone to be approximately a year (see lines 245-251 and Appendix S1: Text S7) but we note that turnover time is a continuous process that is time averaged over 1-2 years based on previous studies (Riofrío-Lazo and Aurióles-Gamboa 2013). Our analysis of trophic position by month using a gam for the subset of specimens that had a collection date did not have any evidence that trophic position derived from bone varied throughout the year and we feel this justifies using a 1-year time lag to account for a physiological delay. Applying a cyclic predictor (see Reviewer 1's comment #9) verified this assumption as well. We also ran the model selection process on our dataset without including a 1-year delay and there was high model uncertainty.

We agree that the value over a year would not detect short-term seasonal diet shifts unless they were substantially different than what the harbor seal was eating during the rest of the year but we disagree that our data would not detect seasonal environmental conditions from a whole food web perspective. For example, spring upwelling is a seasonal condition that is associated with juvenile recruitment of many fish species (i.e. Pacific herring) and the abundance of herring for the entire year (and even years into the future) is partially determined by seasonal conditions. In a year when Pacific herring have strong recruitment those fish do not disappear after the upwelling season. By testing spring upwelling, we are not showing that harbor seal trophic position (and presumed diet) changes in spring due to upwelling, instead we are showing that spring upwelling exerts strong bottom up forcing on the food web that is measurable in the trophic position of a top predator (in this case harbor seals). Environmental conditions such as summer SST or spring upwelling do not only influence the food web during that one specific season, but rather alter abundance that persists. The duration of biological change in response to environmental change does not occur on the same temporal scale as the duration of the environmental change, meaning, a change in fish abundance in response to the environment will persist for longer than the season if it is propagating through the food web.

We agree with the reviewer that this warrants a greater discussion and we feel the text we added to the manuscript and appendix to address reviewer 1's comment 1, 4, and 9 has offered a greater explanation of this and is more explicit about our assumption.

3. Movement Effects: Harbor seal ecology should be briefly further described in the Introduction. In particular, home range for this area should be defined. Do individuals undertake any seasonal migrations or are they foraging in the near vicinity of their collection

locations year-round? This is important as any migratory component would further complicate attempts at inferring TP relationships to local biological and environmental drivers.

Response: Revised.

We agree that this needs to be further developed in the introduction. Washington harbor seals are divided into distinct stocks, and exchange between coastal Washington and inland (Salish Sea) stocks is unlikely based on movement patterns. Home ranges are estimated to be up to 100 km. It is possible individuals have foraged away from where they are collected, but it is unlikely that they foraged outside of the delineated regions of this study (coastal WA versus Salish Sea). See lines 119 - 122 for revisions.

4. Data Reporting: It would be helpful to include a summary table either in the main article or as a supplement that reports TP estimates by year and also includes sample size (similar to Fig. 1). TP estimates should include the range as well as SD with propagated error (see, for example, Choy et al. 2015).

Response: Revised.

We agree, we have added the standard deviations used to propagate error in Appendix S1: table S2 in addition to the analytical error reported in Appendix S1: Table S13. We have included a table that shows year, mean trophic position, and standard deviation which incorporates variability between specimens and propagates analytical error in addition to error for beta values and TEFS (Appendix S1:Table S16). We note we also have a plot that reports TP estimates by year (Appendix S1:Figure S7) for each amino acid and both regions which shows the range of trophic position estimates for each amino acid.

There are multiple trophic amino acids in this study, and as a result the summary table becomes unwieldy quickly so we only included trophic position calculated from glutamic acid. Given we also have Appendix S1:Figure S6 which shows the ranges for each amino acid, Figure 1 that reports the sample size by year, and the data are available on dryad we think including glutamic acid should suffice but would consider adding the additional amino acids at the request of the reviewers or the editor.

Line By Line Comments:

Line 57 – Change “system” to “systems”

Response: Revised

Line 87 – It would be helpful to include a reference here that covers the concept of isotopic turnover rates.

Response: Revised

Line 104 – Include scientific name.

Response: Revised

Line 182 – Change “were included” to “was included”

Line 187 – Why only use a single source AA rather than a suite as applied to the trophic AAs?

Response: We agree including additional source amino acids would have been beneficial. We did not measure other source amino acids which is why they were not included in our analysis (measured AAs are described in Appendix S1:Text S1).

There are only four “true” source amino acids, phenylalanine, lysine, tyrosine, and methionine. Glycine and serine were initially thought to be source amino acids in previous studies but there has been strong evidence that they do show trophic enrichment. Due to our analytical methods (esterification and derivatization of amino acids, and the polarity of our GC column) we were not able to identify lysine. Methionine and tyrosine occur in bone collagen in very low concentrations, and we would have needed 2X the mass of bone collagen to measure them which would require greater destruction to the museum specimens. In addition this would have required running all samples twice to produce clean chromatogram (avoid co-elution of GC peaks and utilize a standard at a similar concentration as the individual amino acids in the samples) for both the low concentration amino acids and the amino acids that occur at higher concentrations.

Line 193 – Change “effects” to “effect”

Response: Revised

Lines 215-216 – Since the nitrogen is undergoing continual turnover, it would seem more appropriate to consider the TP to be representative of the year leading up to the time of collection, rather than a full year prior to collection.

Response: We revised Appendix S1: Text S7 to provide more details. Please see response to comment 2

Line 300 – Add period.

Response: Revised

Line 312 – Change “alters” to “alter”

Response: Revised

Line 328 – Add “the” before “herring spawning season”

Response: Revised

Line 346 – Add “in” before “the ocean”

Response: Revised

Line 347 – Change “represents” to “represent”

Response: Revised

Line 353 – Change “they species” to “species they”

Response: Revised

Line 361 – Start new sentence with “Thus”

Response: Revised

Line 378 – Change to either “equations” to “the trophic position equation”

Response: Revised

Figure 1 – The color gradation for different sample sizes is difficult to see. Maybe consider different shapes?

Response: The color gradation only shows overlapping points/specimens (it is not a different color for distinct number of n but rather the points overlap and become darker as they are

transparent). We agree the legend was confusing and we have elected to remove and add additional details to the caption to make it clearer.