Spring 5-22-2021

Modeling biliary polycyclic aromatic hydrocarbon metabolites in fish using high performance liquid chromatography with fluorescence detection, principal component analysis, and partial least-squares analysis

Joshua C. Padilla
Seattle Pacific University

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by

Joshua C. Padilla

Faculty Advisor, Dr. Karisa Pierce

Honors Program Director, Dr. Christine Chaney

A project submitted in partial fulfillment of the requirements for the Bachelor of Arts degree in Honors Liberal Arts

Seattle Pacific University

2021

Presented at the Seattle Pacific University Honors Research Symposium

May 22, 2021

Preface

The present work is a chemometric exploration into the modeling and monitoring of environmental pollutants in fish from Seattle waterways and the north Pacific Ocean. This was motivated by a sense of responsibility for the stewardship of nature as a whole: that we, as humans, are specifically called to be carers and keepers among the rest of creation, and our contemporary scientific understanding of the world allows us to more faithfully and substantially fulfill that calling. While this research is a rather niche pursuit of chemistry within the broader environmental sciences, a firmer understanding of the world and its ecosystems are requisite to protect and remediate them. It is a search for better ways to track, model, and monitor environmental pollutants, in an effort to ensure the health of aquatic ecosystems. My response, in attentiveness to the world around us, is to pursue science so that we may better keep in honoring the lives of other creatures and the flourishing of life on earth. This to me, as someone who is deeply involved in the sciences, is part of what it means to live as a faithful Chritsian in our time.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) constitute a diverse class of highly toxic, ubiquitous environmental pollutants, and are thus of high interest in environmental monitoring and regulation. In this study, biliary samples of English soles *Parophrys vetulus* and smallmouth bass *Micropterus dolomieu* from Seattle waterways, and chum salmon *Onchorynchus keta* from the north Pacific Ocean were analyzed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) to gauge PAH exposure. Samples were profiled in three broad molecular weight categories, to capture naphthalene-like (NAPH), phenanthrene-like (PHEN), and benzo[a]pyrene-like (BAP) metabolites. While quantification was not achieved for the chum salmon, the semi-quantitative measurements of biliary PAH metabolites in English soles and smallmouth bass revealed differences in exposure between the two species. The fish also exhibited generally lower levels of BAP than NAPH and PHEN. Principal component analysis (PCA) of the bile data was able to capture differences in chromatogram profiles between all three species for each PAH metabolite group. Finally, the PAH metabolite concentrations of the smallmouth bass were modeled and predicted using partial least-squares (PLS) regression models applied to their HPLC-FLD chromatogram data. The leave-one-out cross validation models were able to make fairly accurate predictions of BAP (R^2 = 0.9483) and PHEN (R^2 = 0.9394) concentrations but performed slightly worse with the NAPH data (R^2 = 0.8944). These results indicate the potential for automated chemometric screening of bile data to determine PAH contamination in fish.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large and diverse class of organic compounds that are also ubiquitous environmental pollutants[1-2]. PAHs are produced to some extent during natural occurrences such as volcanic eruptions and oil seeps, but mostly originate in industrial processes and

other anthropogenic activities involving the incomplete combustion of fuels and organic materials, atop oil spillage events[3-4]. These compounds are typically hydrophobic and adsorb strongly to organic particles. Freshwater and marine sediments therefore act as PAH sinks, and many studies have examined the fate and effects of PAHs in aquatic environments, especially those near urban areas[5]. In those environments, species can be exposed to PAHs in water or sediment by contact or direct ingestion. PAHs are highly toxic, and elevated environmental PAH levels have been strongly linked with increased occurrences of carcinogenic, mutagenic, teratogenic, and immunotoxic effects in fish[5-6]. With a host of such deleterious properties, PAHs are of high interest in environmental regulation and the monitoring of aquatic ecosystems. This present study considers English soles *Parophrys vetulus* and smallmouth bass *Micropterus dolomieu* of Seattle waterways, two regularly monitored species, to measure PAH exposure to fish in urban areas. Additionally, chum salmon *Onchorynchus keta* of the north Pacific Ocean were screened to capture possible PAH exposure at sea.

PAH levels in the aquatic environment itself can be measured in the sediment and water, however measuring the exposure of fish to PAHs is more complicated. Fish and other aquatic vertebrates rapidly metabolize PAHs[7], so accumulation in tissues is low, and tissue PAH levels tend to correlate poorly with exposure[8]. Instead, bile is the major excretion route for biotransformed PAH metabolites, and so biliary PAH metabolite levels are used to gauge exposure in fish samples. This biliary data can provide vital information about the severity of exposure and the geographic scope of pollution, and continued monitoring aids in maintenance or tracking remediation. Since PAHs are highly fluorescent, bile samples in this report were screened using high performance liquid chromatography with fluorescence detection (HPLC-FLD). Yet there are countless PAH metabolites, and very few of these compounds have standards. To account for this, quantification is made in three broad categories at different fluorescence excitation/emission pairs to selectively measure PAH metabolites in different molecular weight ranges: a) naphthalene-like metabolites (NAPH) to represent the low molecular weight range, b) phenanthrene-like metabolites (PHEN) to represent the mid-range molecular weights, and c) benzo[a]pyrene-like metabolites (BAP) to represent the higher molecular weight range[9]. In the chromatograms, fluorescent

peaks can include the parent PAH compound, their metabolites, alkylated derivatives of both, and polycyclic aromatic compounds containing nitrogen, sulfur, or oxygen[10]. PAH metabolite concentrations are then determined semi-quantitatively by calibrating the sum of peak areas against a spiked standard of a parent PAH compound.

After quantification, the bile chromatograms were subjected to principal component analysis (PCA). PCA works by mathematically identifying the greatest sources of variation in a given data set and reducing the dimensionality of the data while retaining those variations in features called principal components (PCs). The first PC will capture the greatest source of variation in the data, and every PC thereafter will explain less of the variation. Applied to the bile data, PCA will identify the retention times where peak intensities vary most. PCA and other clustering analyses have previously been used to profile biliary samples of fish from different habitats, locations, and sources of PAH exposure[11], and also to identify key PAH metabolites in the bile[12].

Finally, partial least-squares (PLS) analysis was applied to the smallmouth bass chromatograms with the purpose of creating a model that can predict biliary PAH metabolite concentrations. PLS works, in this case, by mathematically loading retention times at which the signal intensity correlates with variations in the PAH metabolite concentration. Few works have considered PLS in predicting PAH metabolite concentrations through bile. Some studies have used PLS to predict PAH content in crustaceans through tissue and passive samplers[13-14], and one study has used PLS to measure PAH exposure by the bile proteome profiles rather than the fluorescence data[15]. In any case, a model that can accurately predict PAH concentrations based on chromatograms may have applications in the continuous and long-term environmental monitoring of specific species and areas of interest.

Experimental Methods

All fish specimens were retrieved in Seattle, WA. English soles (n = 3) were collected in June 2019 from Myrtle Edwards Park, Elliot Bay. Smallmouth bass (n = 14) were collected in September 2019 from the

Lake Washington, SR 520 floating bridge west high-rise. Viscera from fresh, wild-caught chum salmon (*n* = 30) were obtained from Independent Packers Corp., in October 2020. Bile samples were extracted from fish gall bladders using a syringe. The sample matrices were kept on ice until delivery to the King County Environmental Laboratory (Seattle, WA), where they were frozen at −20 °C until analysis.

All bile samples were then diluted by a factor of 10 and analyzed on an Agilent (Santa Clara, CA) 1290 Infinity II LC system equipped with an Agilent 1260 Infinity fluorescence detector. Separations were achieved in a C18 reversed-phase column (Synergi™ 4 µm Hydro-RP 150 × 4.6mm, Phenomenex, Torrance, CA). The mobile phase consisted of a gradient from 100% water to 100% methanol over 15 minutes, then a 7-minute hold at 100% methanol, and finally a return to 100% water over 3 minutes, for a total run-time of 25 minutes. Each subsequent run was followed by a re-equilibration time of 10 minutes. Every bile sample was subjected to three separate runs with different excitation/emission pairs for benzo[a]pyrene-like (BAP, 380/430 nm), phenanthrene-like (PHEN, 256/380 nm), and naphthalene-like (NAPH, 290/335 nm) metabolites. Additionally, the protein content of each sample was determined using a Pierce™ Coomassie (Bradford) Protein Assay kit (Thermo Scientific, Waltham, MA).

For the English sole and smallmouth bass samples, the fluorescent peak areas from each excitation/emission pair were summed and calibrated against the response of the parent PAH compound to obtain semi-quantitative measurements of each PAH metabolite equivalent. These concentrations were normalized by the protein content of the corresponding sample.

For further data analysis, the chromatographic data was imported into MATLAB R2020b (MathWorks, Inc., Natick, MA) equipped with PLS_Toolbox 8.9 (Eigenvector Research, Inc., Manson, WA). For initial preprocessing, all chromatograms were normalized according to the corresponding protein concentration in each sample, then baseline-corrected and mean-centered. Data from all three fish species were submitted to PCA in a single matrix. For this submission, the high-density English sole and smallmouth bass data were smoothed by a boxcar averager and reduced by keeping only every 8th signal to fit on an array with the less dense chum salmon data. The data were submitted to PCA in 2D arrays, wherein each row corresponded to the chromatogram of one bile sample. Lastly, PLS was applied to the

non-smoothed and non-reduced smallmouth bass data (SIMPLS algorithm), using leave-one-out cross validation. Two latent variables (LVs) were kept for the BAP and PHEN models, while 4 LVs were used in the NAPH model. All calculations were repeated for each PAH metabolite equivalent excitation/emission pair.

Results and Discussion

Before any treatment of results, it should be noted that the concentration of PAH metabolites in chum salmon were not obtained, and as such will not appear in this report. The chum salmon samples were collected in October 2020, analyzed in February 2021, then refrozen. After two months, select samples were spiked with standard PAH parent compounds and reanalyzed, although with a new column. Replacing the column was necessary since the lifespan of the old column was shortened by the bile samples clogging the instrument. It was then discovered that the same samples responded and separated differently on the two columns (see Supplementary Information, Figure S1). Thus the standard prepared on the new column could not be used to determine PAH concentrations based on sample peak responses from the old column. Furthermore, it was deemed unfeasible to reanalyze all the chum salmon samples on the new column due to time constraints and frequent clogging. The reason for the difference in response is currently unknown. While indeed some degree of variation is expected between an older, worn column and a newer one, both columns were of the same model and make. It is possible that the repeated thawing and freezing of the chum salmon samples also altered the sample matrices to some extent. However, according to one stability experiment, PAH metabolites should remain stable in the bile matrix for at least 13 months[16] at −20 °C. In the same study, no significant changes in PAH metabolite concentrations were observed even after 2 months at +40 °C. The chum salmon samples clogged more readily in the new column, so perhaps some moisture loss occurred in the more low-volume, high surface-area samples. Yet, this too would not explain the extent of changes between the old and new scans. It may be worth pursuing another stability experiment that involves repeated freezing and thawing of bile samples over various time

intervals. In any case, the key takeaway from this situation is to minimize and remain conscious of possible sources of variation, like such time-, storage-, and instrument-related parameters.

Table 1. Concentrations of Biliary PAH Metabolite Equivalents in English Soles

Sample	[Protein] (mg/mL bile)	[BAP equiv.] (µg/mg protein)	[PHEN equiv.] (µg/mg protein)	[NAPH equiv.] (µg/mg protein)
ES 1	1.40	0.10	1.14	0.96
ES 2	0.31	1.03	10.60	9.49
ES 3	0.34	1.02	5.33	7.26

Table 2. Concentrations of Biliary PAH Metabolite Equivalents in Smallmouth Bass

Sample	[Protein] (mg/mL bile)	[BAP equiv.] (µg/mg protein)	[PHEN equiv.] (µg/mg protein)	[NAPH equiv.] (µg/mg protein)
SMB 1	1.43	0.21	1.74	2.05
SMB 2	2.33	0.11	0.44	1.20
SMB 3	0.65	0.23	1.57	2.88
SMB 4	1.43	0.07	0.59	0.92
SMB 5	0.55	0.54	3.75	5.30
SMB 6	1.43	0.93	4.95	5.69
SMB 7	0.52	1.01	4.56	4.58
SMB 8	0.61	0.23	1.28	2.15
SMB 9	0.95	0.33	1.62	2.16
SMB 10	1.00	0.71	3.01	3.64
SMB 11	15.08	0.01	0.05	0.12
SMB 12	0.78	0.49	1.96	2.95
SMB 13	0.75	0.27	1.63	2.43
SMB 14	0.95	0.52	2.98	2.60

Semi-quantitative measurements of PAH concentrations in the English soles and smallmouth bass are featured in Tables 1 and 2, and reported in µg of PAH metabolites per mg of protein in the bile. Biliary levels of fluorescent aromatic compounds are largely affected by the feeding status of individual fish, and normalizing the data according to protein content can account for these differences in feeding status[17-18]. Based on the concentration tables alone, the English sole bile samples generally appear to have greater PAH concentrations than the smallmouth bass. This is to be expected. PAHs are typically insoluble in water and readily adsorb to freshwater and marine sediments. English soles are benthic fish and thus have a greater chance of exposure to PAHs through sediment, while smallmouth bass live and feed higher in the water column. These samples were collected in a metropolitan area, so at least some PAH exposure for both species is warranted. It also appears that the fish tend to have lower levels of BAP-like metabolites than the NAPH- and PHEN-like metabolites. The likelihood of exposure to larger PAHs like BAP is lower than the other two groups, since the aqueous solubility of PAHs decreases with molecular weight[19]. BAPs and other large PAHs can still attach to the sediment, so this may explain the fractionally higher levels of BAP in the English soles compared to the smallmouth bass. Indeed, the PAH concentration data may capture habitational differences between the English soles and smallmouth bass, although more English sole samples would be helpful in solidifying these trends.

The normalized HPLC-FLD chromatograms for all three fish species at each excitation/emission pair and depicted in Figures 1A (BAP), 1B (PHEN), and 1C (NAPH). Chromatograms for the individual species are in Figures S2-S4. Four of the chum salmon samples repeatedly clogged the original column, so chromatogram data for only 26 of the 30 collected samples appear. At their respective excitation/emission pairs, standard benzo[a]pyrene is expected to elute at ~19.4 min, standard phenanthrene is expected to elute at ~16.8 min, and standard naphthalene is expected to elute at ~15.5 min. The chromatograms in Figure 1 do not have discrete peaks for these parent compounds, as much of it elutes earlier in the form of metabolites and other derivative structures.

While the PAHs in the chum salmon samples could not be quantified, a rough idea of their relative PAH content can be gathered through qualitatively comparing their normalized chromatograms

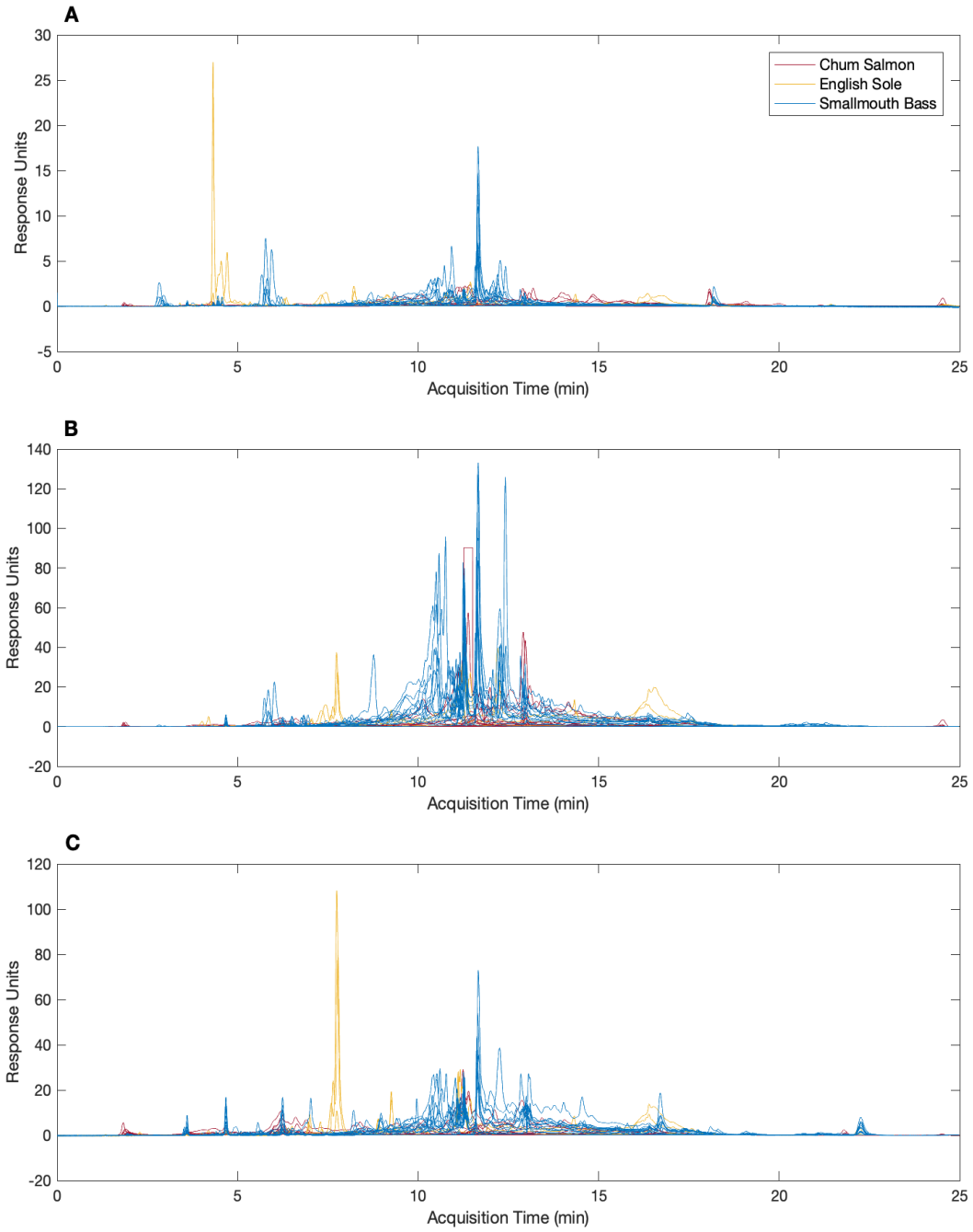

Figure 1. The overlaid, normalized chromatograms of all three species at (A) BAP ex/em 380/430 nm, (B) PHEN ex/em 256/380 nm, and (C) NAPH ex/em 290/335 nm.

with those of the other two species. Of the 26 chum salmon samples that appear in Figure 1, very few appear to have peak areas comparable to that of the English soles and smallmouth bass. This feature is most pronounced in the BAP scans (Figure 1A), where even the most populated chum salmon chromatograms have rather small peak areas. On the other hand, chum salmon have more fluorescing peaks in the PHEN (Figure 1B) and NAPH (Figure 1C) scans. This may indicate generally higher biliary levels of smaller PAHs, owing to the decreased solubility of large PAHs, much like what was observed in the other two species. Perceived peak area for the chum salmon is still comparatively small to the other fish in the NAPH scans, but more of the PHEN chromatograms appear to be populated. Nevertheless, the chum salmon appear to have lower PAH levels than the other two species all around. This is to be expected—whereas the English sole and smallmouth bass specimens were collected directly in a metropolitan area, the chum salmon were caught in the north Pacific Ocean, where PAH exposure is less likely. It is worth considering that chum salmon are migratory fish and can be exposed to PAHs if they migrate through urban estuaries**,** which might explain the few samples that seem to be more contaminated. Outmigrant juvenile chum salmon have previously exhibited elevated biliary PAH levels[20-21], although specimens in such studies were collected from urban waterways rather than the open ocean. The more PAH-exposed chum salmon in this report could have recently traveled from contaminated freshwater sites. However, the maturity and freshwater origin of the chum salmon in this present study are unknown, so this is mere speculation of the contamination source. In sum, the chum salmon samples are expected to, and appear to be less contaminated than the other two species.

All the preceding qualitative observations are rather troublesome to make, due to the distinct chromatogram profiles of each fish species. At the same time, the easily distinguishable profiles are helpful in that they may reflect differences in level and source of PAH exposure[11], in addition to the habitational differences as previously discussed. In the chromatograms, each of the three species have characteristic peaks and other features. Theoretically, these profiles can be more definitively and quantitatively captured by PCA.

The PCA score plots and associated loadings in Figure 2 include data from all three fish species at the BAP excitation/emission pair scans. Axes on the score plot (Figure 2A) are principal components (PCs) 1 and 2, which explain the two greatest sources of variation in the data set by percentage. The loadings (Figure 2B) are analogous to correlation coefficients for the given PC. The independent variable

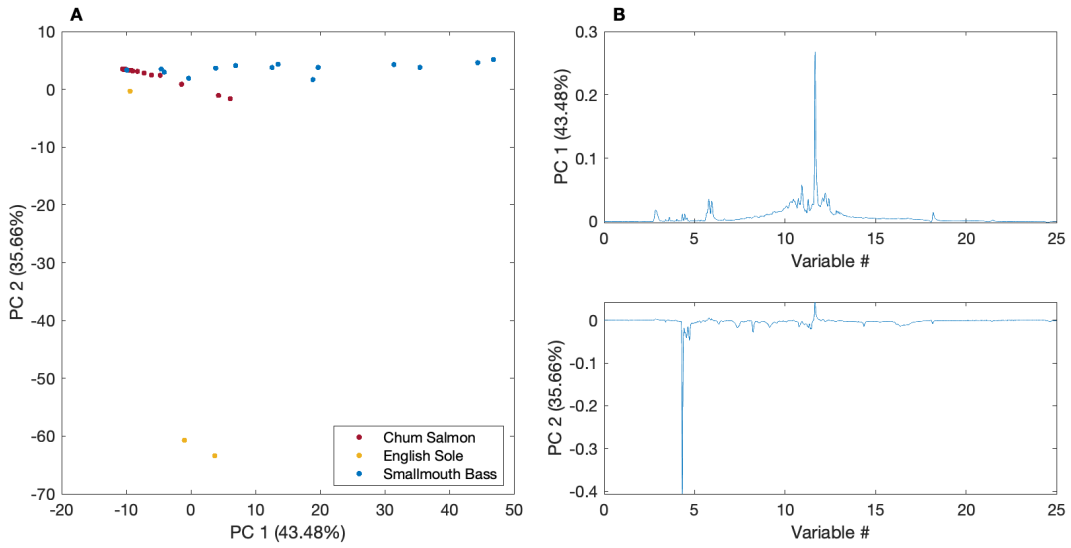

Figure 2. (A) BAP PCA score plot and (B) loadings plots for PC 1 and PC 2.

number in the loadings plots correspond to chromatogram retention times, and retention times with the largest loadings are the most responsible for the variation captured by the PC. For the BAP scans, PCA does not appear to form traditional clusters for each species. Rather, the smallmouth bass vary almost exclusively across PC 1, while the English soles vary almost exclusively across PC 2. Per the loadings, the two largest sources of variation are the peaks at ~11.6 (43.48%) and ~4.3 min (35.66%). This is easily explained by referring to the overlaid BAP chromatograms, where the smallmouth bass seem to vary at the later peak, while the English soles can be distinguished by the earlier peak. PCA of the smallmouth bass samples alone also confirms the ~11.6 min peak variation as their most defining feature (Figure S5). While retaining information about these two species is helpful, it also creates a rather artificial clustering of the chum salmon data. Compared to the other two species, the chum salmon simply do not vary to the

same extent at any peak, likely due to lower BAP content. There are a few points that extend from the main chum salmon "cluster" across PC 1, that correspond to the more populated chromatograms. However, PCA of the chum salmon data alone (Figure S6) reveals that the species hardly varies by the ~11.6 min peak, and far more variation is captured elsewhere.

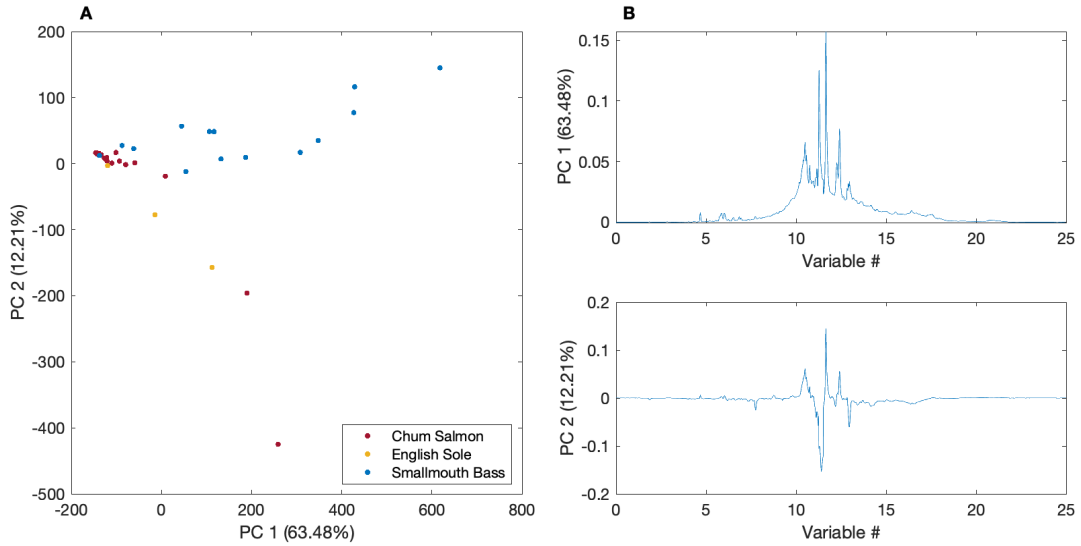

Figure 3. (A) PHEN PCA score plot and (B) loadings plots for PC 1 and 2.

The PHEN scan PCA results are slightly different. These are depicted in Figure 3. PC 1 can still be attributed to the ~11.6 min peak in the smallmouth bass chromatograms, but they explain a higher 63.48% of the variation across the whole data set. Meanwhile, PC 2 accounts for far less variation, but both the English soles and chum salmon vary across this PC. The highest loadings on PC 2 are for peaks at ~11.3 and ~12.2 min, which also happen to be the highest loadings on PC 1 for the chum salmon PHEN data alone (Figure S6). Data for the English soles were not submitted separately to PCA, due to low sample numbers, but the ~11.3 and ~12.2 min peaks are also the most prominent for this species as well. The fact that the English soles and chum salmon share these two prominent peaks, despite their habitational differences, perhaps reveals some important intermediate in the PAH metabolism process. Additionally, the shared peaks might appear to cause issues for distinguishing the English soles from the

chum salmon PHEN samples. However, the English soles have another characteristic peak at ~7.7 min, as well as a weak, broad band between ~16 and 18 min. Both of these features do not appear in the chum salmon data. This may not even be a problem in the first place since English soles are expected to exhibit greater PAH metabolite levels than the chum salmon in general. Thus, the two species might split into their own clusters in the PCA plot if more samples of English soles are obtained.

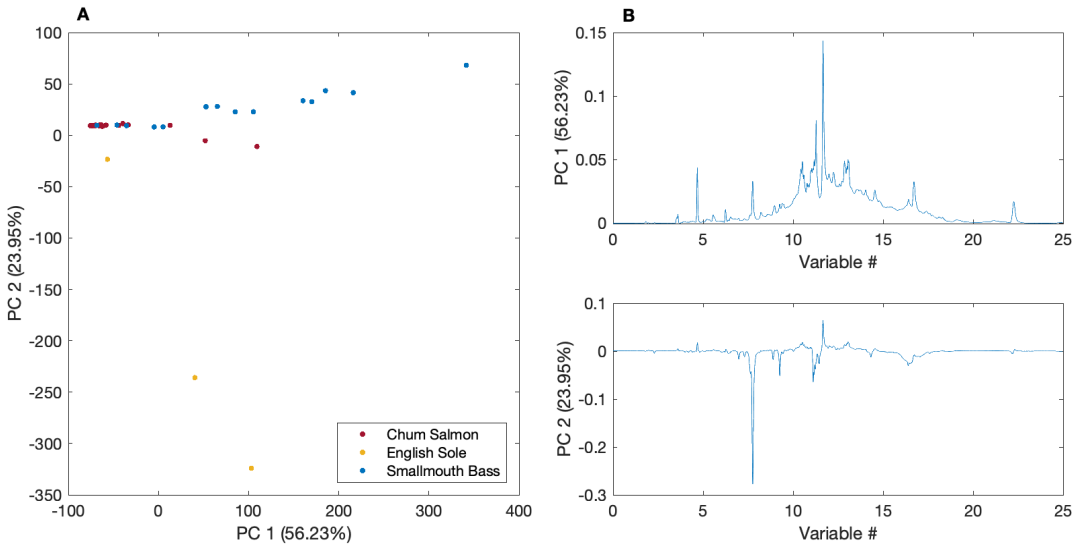

Figure 3. (A) NAPH PCA score plot and (B) loadings plots for PC 1 and 2

The NAPH scan PCA results, shown in Figure 4, are much like those for the BAP scans. Once again, the largest source of variation is the ~11.6 min peak from the smallmouth bass, and this species varies almost exclusively across PC 1. A few of the chum salmon samples are also spread along PC 1, but most of the variation among the chum salmon alone is due to a different peak (Figure S6). A peak at ~7.7 min in the English sole chromatograms contributes most to PC 2, although the English soles do vary slightly across PC 1 as well. In all, many of the most highly loaded retention times on the first PCs may indicate key metabolites within and between fish species.

For all three score plots, the ~11.6 min peak in the smallmouth bass chromatograms is the primary contributor to variation in the entire data set. The exact identity of this peak is unknown, but its

prevalence in all three PAH group scans mark it as a point of interest. There appears to be a near linear relationship between the calculated PAH metabolite concentration of the smallmouth bass sample and the position of that sample along the PC 1 axis, with very few exceptions. These exceptions are unsurprising, since the ~11.6 min peak is not the only peak on the chromatogram, but it may nevertheless be a key metabolite or major indicator of biliary PAH content for smallmouth bass. In an attempt to capture this relationship, the smallmouth bass chromatogram data was analyzed by PLS regression. For this analysis, the number of latent variables (LVs) were chosen to minimize the root-mean-squared-error of cross-validation (RMSECV). This yielded 4 LVs for the NAPH data, but only 1 LV for both the BAP and PHEN data sets. A minimum of 2 LVs were kept for BAP and PHEN to ensure that enough information from the chromatograms were retained. All models were built by leave-one-out cross-validation, wherein a) one-by-one, each chromatogram was pulled from the data, b) the model was rebuilt with the remaining chromatograms, and c) the extracted chromatogram was submitted to the model to predict PAH metabolite concentration. This process was repeated for each chromatogram in the data set.

The results of the smallmouth bass data PLS analysis, for all PAH metabolite groups, appears in Figure 5. The leave-one-out cross-validation plots (Figures 5A, 5C, and 5E) contain the predicted PAH metabolite content against the actual measured values. The regression of this plot is depicted with a red line. Meanwhile, the loadings plots (Figures 5B, 5D, and 5F) indicate the weights of each variable on LV 1, where the most highly loaded variables are the retention times most useful for predicting the PAH metabolite concentration. The peak at ~11.6 min consistently has the highest loadings on LV 1 for all three PAH groups, perhaps indicating that the associated metabolite is a prominent marker for PAH content in general. This relationship is the strongest for the BAP data (Figure 5A), where the regression has an $R^2 = 0.9483$, the highest of the three plots. In the corresponding loadings plot (Figure 5B), the ~11.6 min retention time is essentially the only major contributor to LV 1. The regression fits just slightly less for the PHEN scans (Figure 5C), which has an $R^2 = 0.9394$. In addition to the ~11.6 min peak, the PHEN loadings plot (Figure 5D) also shows retention times ~10.5, ~11.3, and ~12.4 min to have significant loads on LV 1. Meanwhile, the NAPH group cross-validation plot (Figure 5E) features the

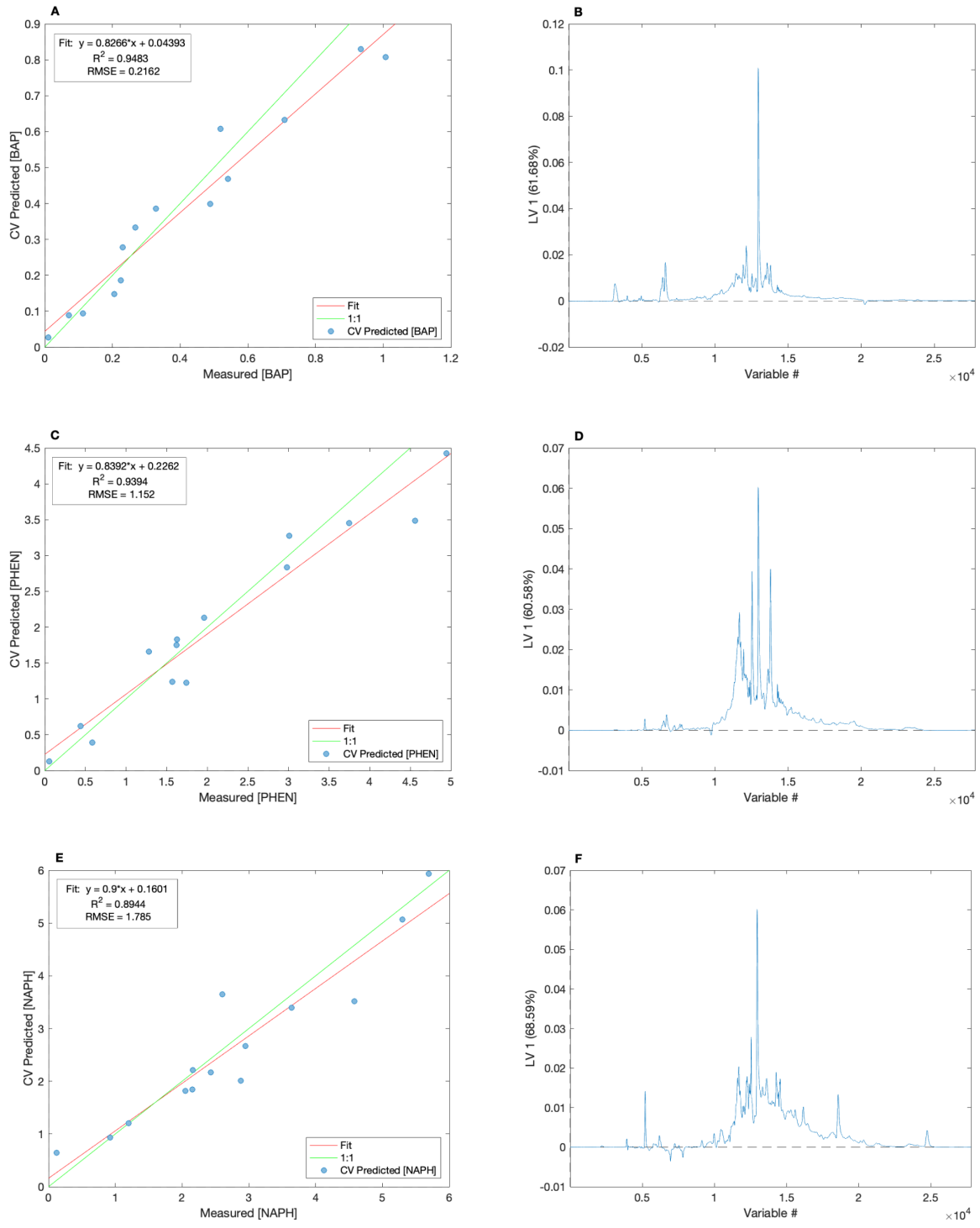

Figure 5. Smallmouth bass (A) leave-one-out cross validation and (B) loadings plots for BAP. (C) Cross validation and (D) loadings pots for PHEN. (E) Cross validation and (F) loadings plots for NAPH.

most variation and smallest R^2 value, at 0.8944, even though the NAPH model was built with more (4) LVs. Per the loadings plot (Figure 5F), no other retention times are nearly as highly loaded as the ~11.6 min peak, but there are more variables with non-insignificant loads on LV 1 here compared to the other two PAH groups. Indeed, variations in the ~11.6 min peak appear to be especially decisive markers for BAP concentration, but it appears that other variables are at hand for the PHEN and most definitely the NAPH groups. It may be possible to build stronger models with more samples of smallmouth bass, and the accuracy of the models could be further validated with a test set. Ideally, models for the English soles and chum salmon would be prepared as well. Unfortunately, there are not enough samples of English sole to build a reliable model, and the lack of PAH concentration data for the chum salmon renders the PLS analysis for predicting concentrations impossible. Ultimately, it seems that the intensity of the ~11.6 min peak has a sufficiently strong relationship with PAH metabolite concentration, and this metabolite may be an important mark of PAH exposure. This is reflected by the fairly accurate predictions of PAH metabolite concentrations in the smallmouth bass by PLS for all three PAH groups.

Several suggestions for future studies will be made here. For one, more samples for all species should be considered. Both PCA clustering and PLS regressions might be improved if multidimensional separations were performed on the bile samples. The HPLC-FLD chromatograms contain many unresolved overlapping peaks, and multidimensional separation might provide data that can further distinguish the samples. Accordingly, this may lead to more definitive PCA clustering, and a more robust PLS regression model that can predict biliary PAH concentrations with higher accuracy. It may also be helpful to pool data that varies by species, habitat, location, and contamination source, which are factors that have been previously shown to alter the chromatogram profiles. This may further expand on the idea that PCA can capture these differences, but targeted comparisons within a larger data set can also determine the usefulness and generalizability of the PLS models outside of specific, local fish populations or niche applications.

Conclusions

In this study, bile samples from chum salmon, English soles, and smallmouth bass were analyzed by HPLC-FLD at three different excitation/emission pairs to selectively scan for small (NAPH), mid-sized (PHEN), and large (NAPH) PAH metabolites. The chromatogram data was used to quantify these metabolites and their equivalents for the English soles and smallmouth bass. It was discovered that the English sole bile samples tended to be more contaminated than the smallmouth bass, and both species tended to have higher levels of PHEN and NAPH metabolites than BAP. The chromatograms from all three species were then subjected to PCA, which captured differences in chromatogram profiles and clearly distinguished between the English soles and smallmouth bass. Meanwhile, chum salmon could be distinguished by their major lack of variation in comparison with the other two fish. In any case, the concentration and clustering trends are both attributed in part to habitational differences between species. Finally, the smallmouth bass data was analyzed by PLS regression. This revealed that the variations in a single peak at retention time ~11.6 min covaried strongly with the metabolite concentration for all three PAH groups. The BAP cross-validation model had the strongest regression and most significant load on LV 1 with the ~11.6 min peak, while the PHEN and NAPH models were slightly weaker and affected by other factors. Nevertheless, all models had an $R^2 \geq 0.8944$, indicating their ability to make fairly accurate predictions of metabolite concentrations for all three PAH metabolite groups in smallmouth bass, assuming future specimens will have similar chromatogram profiles. Chemometric models offer an objective and quantitative form of analyzing bile and screening bile sample fingerprints to predict PAH exposure, which can potentially reduce the amount of manual sample preparation and manual analysis that scientists currently must perform.

Acknowledgements

The author acknowledges Dr. Karisa Pierce for guidance and assistance, as well as Dr. Elizabeth Frame and the King County Environmental Laboratory for instrument time and further insight.

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Modelling biliary polycyclic aromatic hydrocarbon metabolites in fish using high performance liquid chromatography with fluorescence detection, principal component analysis, and partial least-squares analysis

Supplementary Information

Joshua C. Padilla

Department of Chemistry and Biochemistry, Seattle Pacific University, Seattle, WA, 98119

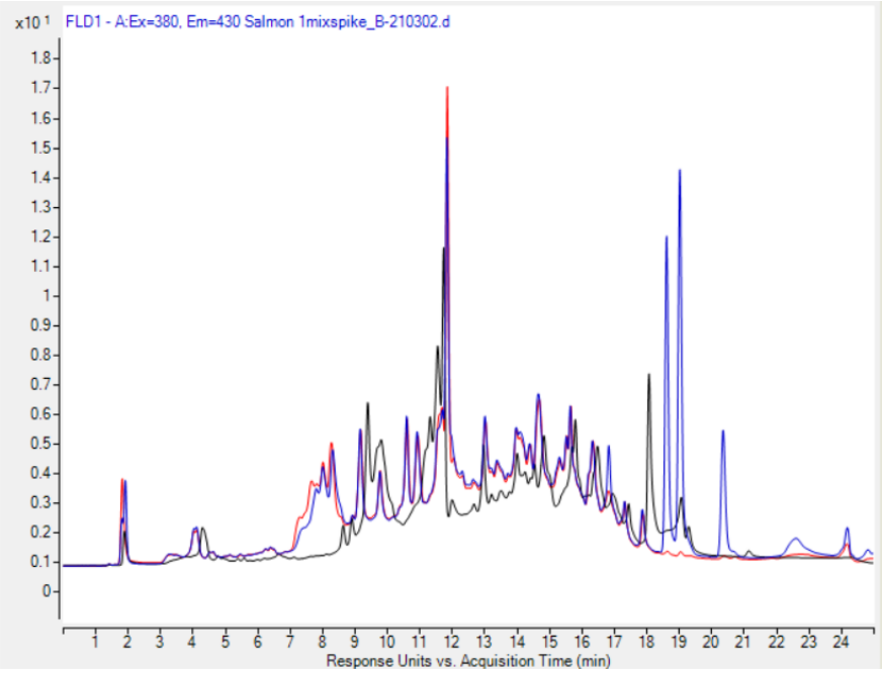

Figure S1. Overlaid chromatograms depicting a chum salmon sample, run on the new column with (blue) and without (red) an added standard PAH mix, and the scan from the original column (black), at the excitation/emission pair 380/430 nm.

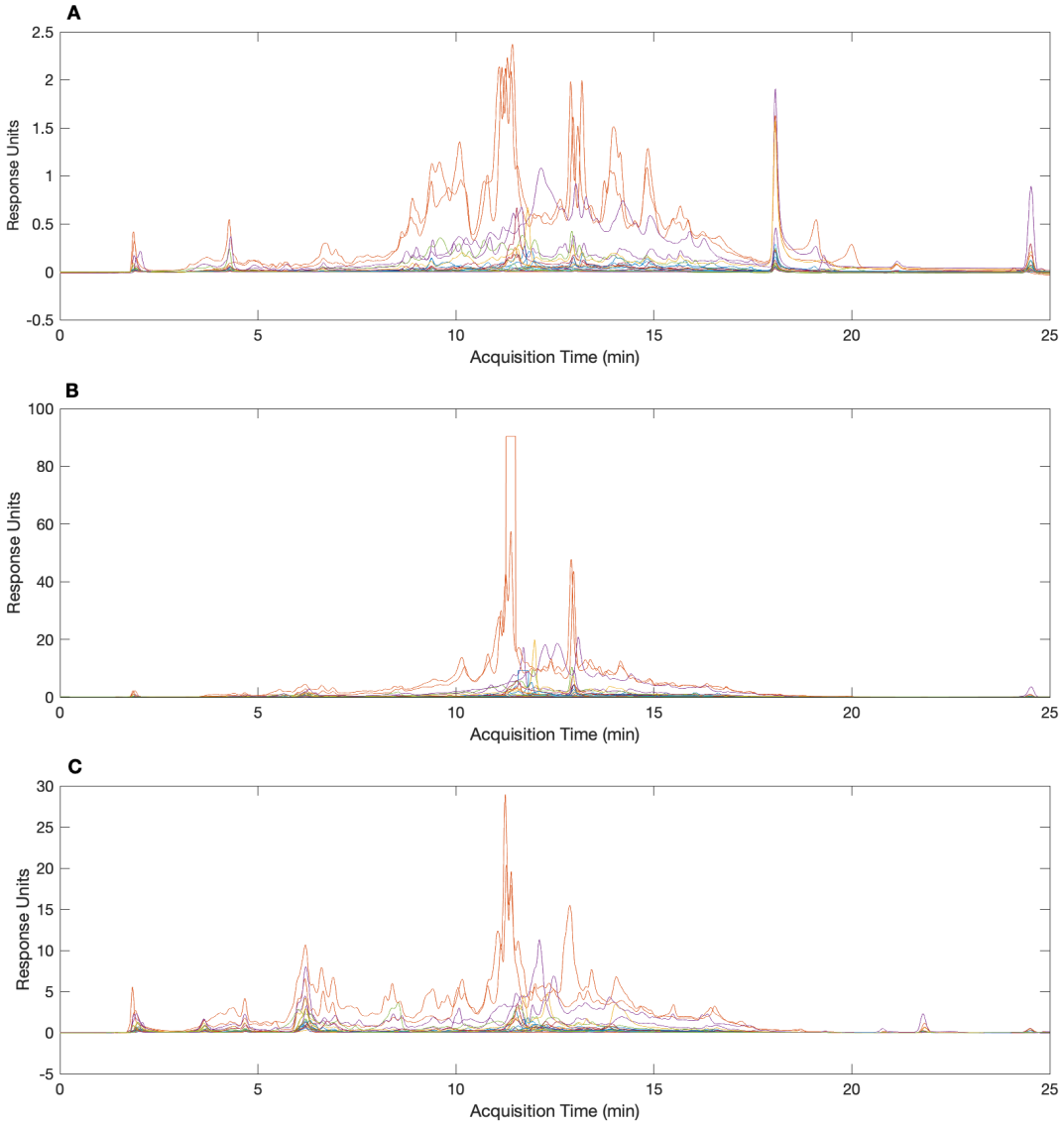

Figure S2. Overlaid, normalized chum salmon chromatograms for the (A) BAP, (B) PHEN, and (C) NAPH excitation/emission pairs.

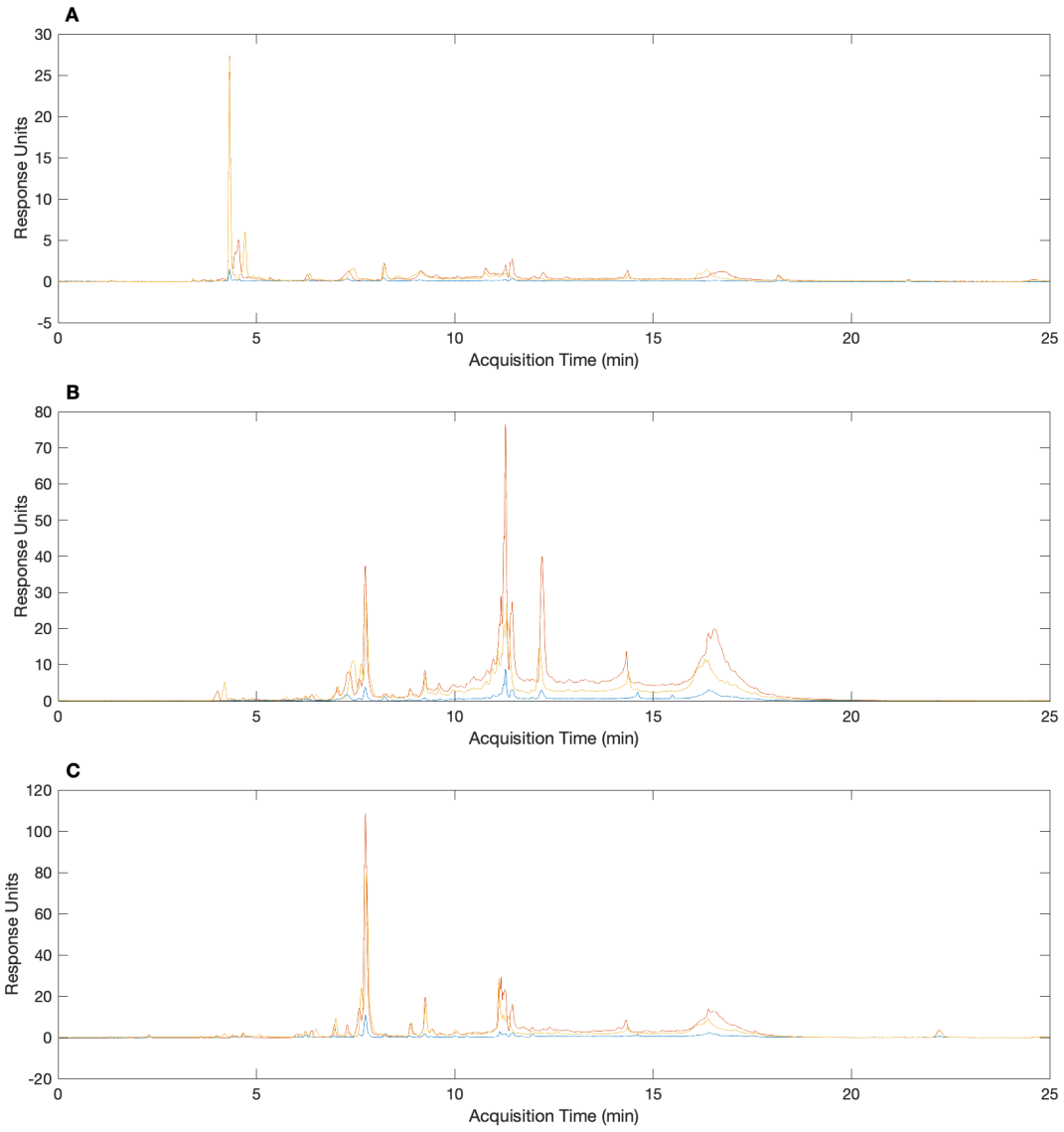

Figure S3. Overlaid, normalized English sole chromatograms for the (A) BAP, (B) PHEN, and (C) NAPH excitation/emission pairs.

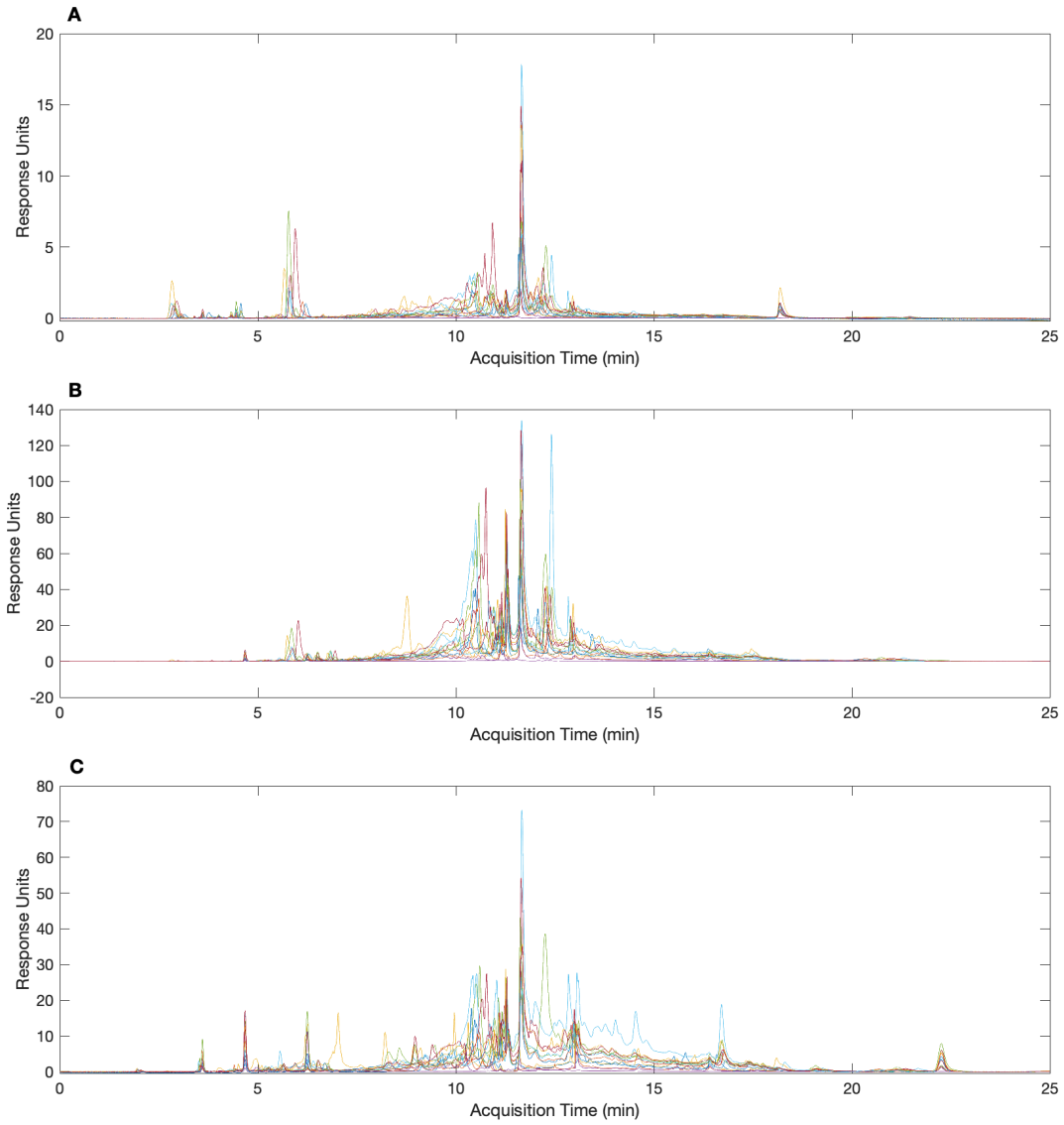

Figure S4. Overlaid, normalized smallmouth bass chromatograms for the (A) BAP, (B) PHEN, and (C) NAPH excitation/emission pairs.

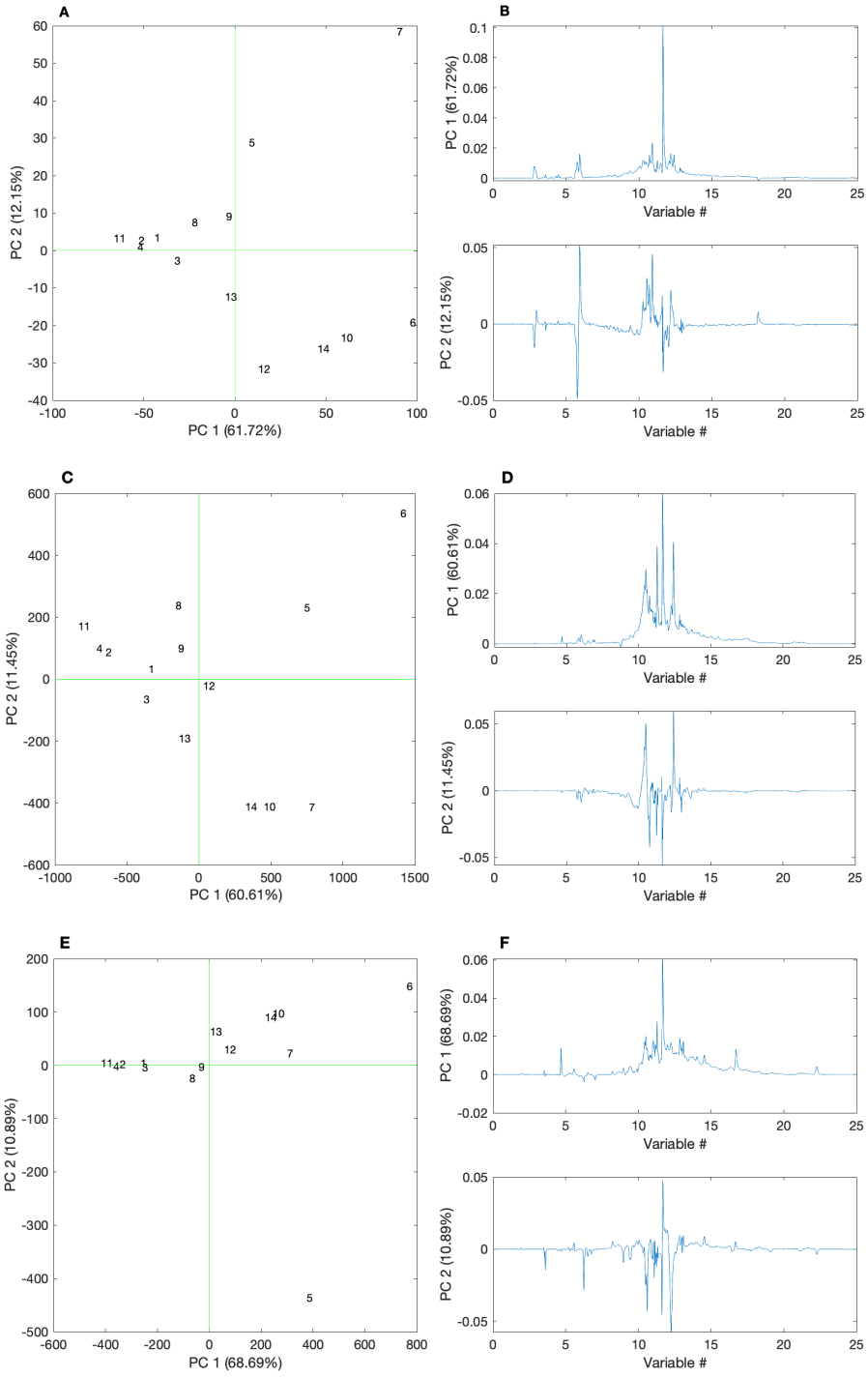

Figure S5. Smallmouth bass (A) score plots and (B) loadings plots for BAP. (C) Score plots and (D) loadings plots for PHEN. (E) Score plots and (F) loadings plots for NAPH.

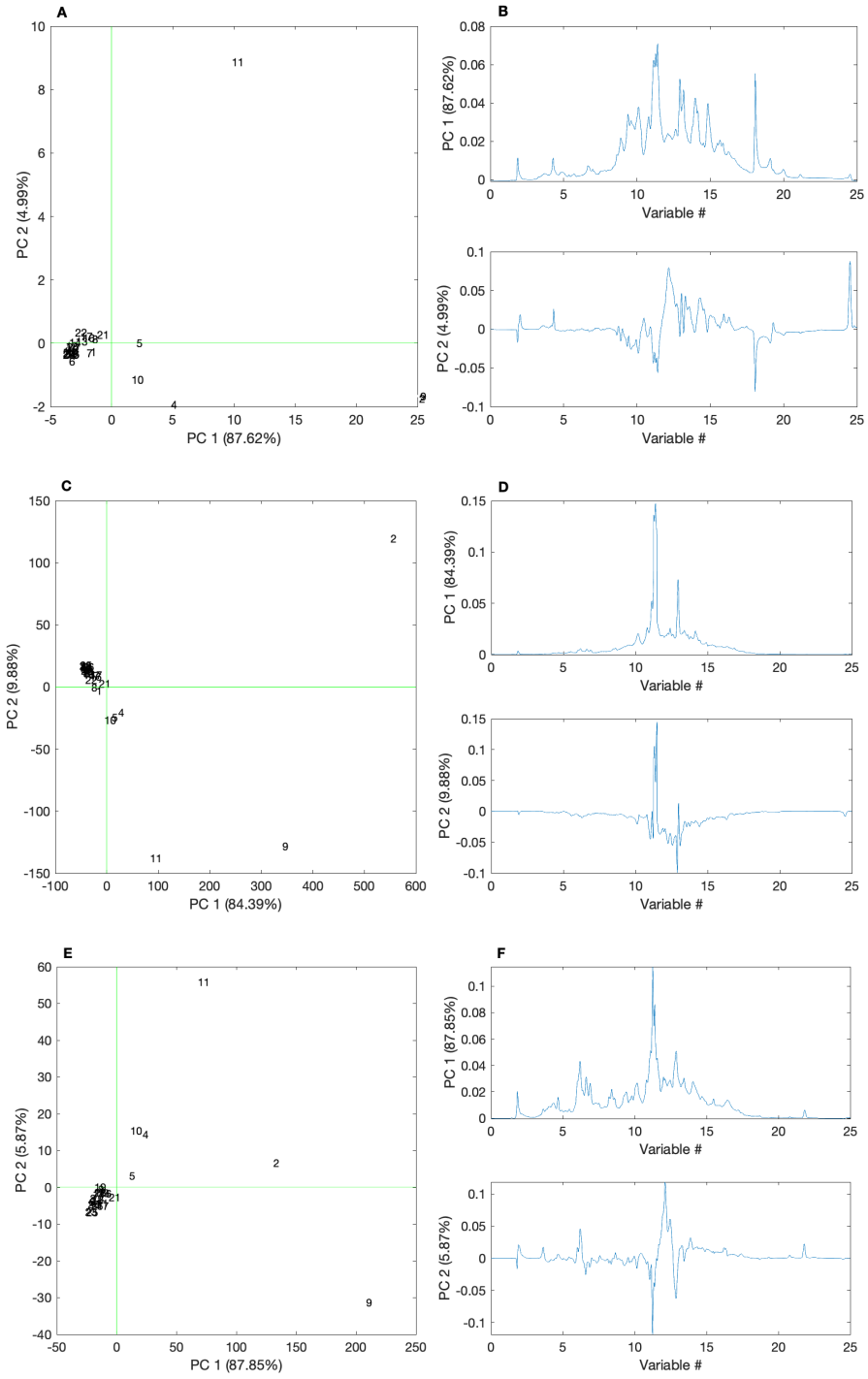

Figure S6. Chum salmon (A) score plots and (B) loadings plots for BAP. (C) Score plots and (D) loadings plots for PHEN. (E) Score plots and (F) loadings plots for NAPH.

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Honors Symposium Presentation, "Intersections" Transcription

Joshua C. Padilla

Department of Chemistry and Biochemistry, Seattle Pacific University, Seattle, WA 98119

On the Intersections Between Belief and the Present Work

With the short time I have left, I'd now like to address the theme of our panel and frame my work within what I believe to be our calling as stewards of creation. My research here is a very specific analytical chemical exploration within the much broader issue of pollution. Even that is one of many issues contributing towards the unprecedented environmental degradation of our time, for which the blood is mostly on our hands. In becoming more attentive to those issues in the scientific sense, I also wondered what the Christian faith had to say to them, if anything. I consider myself both a Christian and an aspiring scientific scholar, so I believe that there exists some cross-dialogue between the two; that they are, in some way, mutually informative. What I find is that the Christian faith orients us in a particular way towards the world, and some of our scientific understanding of that world today fits well within that vision. That is, caring for the earth is not only a pressing scientific issue, but it is also something inextricably bound to the gospels from beginning to end. In scripture we are called to be stewards among creation and taught how to respond in hope to the brokenness within it, and our contemporary scientific understanding of the world allows us to faithfully and substantially fulfill that calling.

Christians claim to honor God as the maker of earth, but there have been an odd amount of people in the church showing little inclination to live like it. Perhaps Christian indifference over the ecological

crisis, and even hostile attitudes towards environmental activism can be attributed to the notions of dominion found in Genesis 1, or the idea that everything will be renewed in the end anyway. It does not help that in our cultural context, we also tend to speak of environmental issues as being ones of political party. Yet for the Christian, these views are astoundingly anthropocentric. Scripture reminds us that God is the one *in whom* and *through whom* all things are created, and the one *for whom* all things exist -- not us. God affirms the goodness of creation sevenfold in Genesis 1, so we know that nature has value before God beyond mere human utility. The Psalmists celebrate creation, and Job reminds us of that goodness as well. Thus when God grants us dominion over the earth, it is not our license to exploit and abuse, rather it is a call to service as in Genesis 2: that our purpose among creation is to jealously protect it just as God does us. All of creation glorifies God by being what they were created to be, and we as part of creation must also take our appropriate place within it. Here we find our calling as the carers, the keepers—the stewards of creation. To reject that calling by disrespecting and dishonoring the earth is to be out of alignment with the creator who called it good.

This does not disappear when we move to the New Testament. God's purposes, the gospel preached by Jesus in Luke are all-encompassing and extend to the whole of creation. The death and resurrection of Christ, then, mean the renewal of all things and the reconciliation of all things. For indeed, our relationship with the earth is broken. Isaiah 24: "The earth lies polluted under its inhabitants; for they have transgressed laws, violated the statutes, broken the everlasting covenant." Moving to Romans 8, we learn that creation groans and suffers under humans who have become destroyers of the earth. Yet at the same time, creation groans in hope for the revelation of the children of God; for us to become the people we were created to be. Thus if we claim to be children of God, then we ought to live like it and embody the life of the new creation in the now—and the life of the new creation means our reconciliation with the earth.

What then, does an appropriate Christian response both to what scripture and science tell us about the nature of our world? As believers, we can start by being attentive to the groaning of creation, and recognize its beauty and goodness despite our brokenness within it. My research falls here, as I search for

ways to better monitor environmental pollutants, in an effort to ensure the health of aquatic ecosystems. Christians should respond in kind to what science tells us about the nature of our world, since that faithful stewardship of the earth requires a firm knowledge of how its ecosystems work. Science provides tools that, through wisdom, allow us to better keep in honoring the lives of other creatures and the flourishing of life on earth. Then, we can act. Of course, the stewardship of creation does not only mean caring for the natural environment, as we have seen in my wonderful colleagues' presentations, but this is where my discipline and my faith intersect. Pursuing both in hope and love, I am responding to what I have been called to do in attentiveness towards the world around us; to who we are; to the people we love. This to me, as a person deeply involved in the sciences, is what it means to live as a faithful Christian in our time.

Thank you for listening.

Final Acknowledgements

To my loving family, my professors, and the University Scholars class of 2021, thank you for sharing this journey with me throughout my time at Seattle Pacific. I extend a special thanks to Henry Duchow, Kelsey Furiya, Victor Hanson, Jennifer Havens, Angie Vazquez-Busching, and Tristan Wine—my remarkable friends, whose aura was always so warm and comfortable. These are the people with whom I never felt alone; the people who made me feel at home. They are absolutely wonderful, and I love them dearly.