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Understanding the Connection Between Water, Fish, and PFAS Concentrations: Implications of Fish Diet and Species-Specific **Variability**

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UNDERSTANDING THE CONNECTION BETWEEN WATER, FISH, AND PFAS CONCENTRATIONS: IMPLICATIONS OF FISH DIET AND SPECIES-SPECIFIC

VARIABILITY

by

Ece Yeldan

A Thesis Submitted in Partial Fulfillment of the Requirements for a Degree with Honors (Wildlife Ecology)

The Honors College

University of Maine

May 2024

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ABSTRACT

Perfluoroalkyl and polyfluoroalkyl substances, commonly known as PFAS or "forever chemicals," have emerged as a significant concern for both human health and the environment. These persistent compounds permeate various aspects of commerce, leading to widespread exposure and the cycling of these compounds through environmental feedback routes. Among the sources of exposure to humans, fish consumption stands out prominently. Recreational and sustenance fishing, cherished by Maine residents and indigenous communities alike, underscores the need to comprehend PFAS dynamics in fish and water. To explore the variability of PFAS accumulation in fish, water, and sediment, I conducted a comprehensive literature review. The study's findings not only shed light on existing gaps in knowledge but also pave the way for more focused investigations in future PFAS research.

DEDICATION

I dedicate this thesis in loving memory to my sister, Selen. Not a moment passes where I do not think of you, rest well.

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I am deeply thankful to my advisors, Christina Murphy and Erik Blomberg for providing unconditional support and being there every step of the way. Steve Coghlan, associate professor, and mentor, I want to thank you for encouraging me to think critically and ask the necessary questions. Dr. Caroline Noblet, thank you for being so accommodating and always providing a listening ear. Bob Klose, professor and friend, you have remained my greatest supporter throughout the years, thank you for helping me recognize my true potential. The staff at the Office of International Programs, I want to thank you for welcoming me in with such warmth from the first days I set foot on campus. My dear friends: Thea Tengstrom, Tristan Mcmerty, and Kalina Kinyon, it is an honor to have built such a strong bond with you. My parents, none of this would be possible without you, everything I do is to be deserving of your unconditional love and pride.

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INTRODUCTION

Per- and poly-fluoroalkyl substances (PFAS) are a large class of persistent anthropogenic compounds that have been widely used in many sectors of commerce since the 1950s. (Buck et al., 2011; Pickard et al.; 2020, Goodrow et al.; 2020). Application of PFAS include but are not limited to common products such as textile stain and soil repellents, processing aids, coatings, fire extinguishers, grease-proof food wrappings, etc. Due to their extensive use, PFAS and their corresponding emissions from production have become an emergent concern in wildlife, the environment, and human health. (Buck et al., 2011) Though PFAS contains a diverse class of substances, they are all highly stable and often referred to as 'forever chemicals' due to their extreme resilience to environmental and metabolic degradation. (Shi et al., 2009) Current research suggests that certain levels of PFAS exposure amongst humans lead to decreased fertility, hormonal imbalance, weakened immune system, and increased risk of certain cancers. (Pelch et al., 2019). Though the general populace has relatively low levels of exposure to PFAS, certain communities may be subject to elevated levels of PFAS by ingesting contaminated water and fish (Post et al., 2012; Fujii et al., 2015).

With recreational fishing playing a vital role in cultural and economic contexts, it is important to understand the role of fish as a source of contamination. Many regions in the USA have released, and are developing, further PFAS advisories regarding water and fish consumption guidelines with the intent of reducing exposure risks and informing consumers of fish to make educated decisions. However, the state of knowledge surrounding the bioaccumulation of PFAS in biota is largely unknown and controversial, as there is great variability amongst the bioaccumulation factors. While this variability

could be credited to differences in methodologies, unexplored differences in accumulation patterns across species and their interactions with their environment could be a potential factor of the observed irregularity. (Babut et al., 2017) Existing studies reveal that PFAS are proteinophilic, and thus tend to accumulate in the fish's protein-rich tissues (muscle, liver, blood). This accumulation of PFAS within the fish will likely have seen and unseen consequences, namely disruptions in the structure of aquatic food webs. These disruptions may harm ecosystem processes, fish communities, and basic biological interactions between species. (Ng and Hungerbühler, 2013; Martin et al., 2003; Shi et al., 2012).

A major source of PFAS contamination has been the spread of wastewater effluent that contains these compounds, in which the water cannot be effectively treated (George et al., 2023). PFAS have an affinity for sorbing to sediment particles and organic matter, which then settle at the bottom of water bodies. This creates a long-lasting reservoir of PFAS that can cycle within the environment for decades (Pickard et al., 2022). To better understand how PFAS biomagnifies in fish and its consequences within the food web, it is necessary to characterize the relationship between PFAS in water and sediment. In comparing the findings of PFAS concentrations detected in water and sediment samples, we can gain a more comprehensive understanding of the bioaccumulation at play within the fish in contaminated waters (George et al., 2023).

As markets continue to adapt to the ever-growing economy, the utilization of PFAS across many sectors and consequently, adverse ecological impacts, is expected to increase. (George et al., 2023). The state of Maine has made significant efforts to break away from the use of PFAS; this action is needed at a global scale to prevent further

environmental degradation (Maine Department of Environmental Protection, 2024). Therefore, it is vital to understand the role of PFAS in the ongoing crisis of detrimental environmental impacts. My study aims to better understand the capacity of how PFAS operates in aquatic ecosystems by drawing conclusions from multiple studies and highlighting the gaps in knowledge. Building this foundational understanding is crucial for ongoing monitoring and evaluating future PFAS management strategies, particularly for rare or precious species where nonlethal sampling is vital for conservation efforts and protecting biodiversity. (George et al., 2023)

OBJECTIVES

I chose the following objectives for this study to better understand the variability present with PFAS research. These objectives address key points across all reviewed publications and will yield answers on what gaps of knowledge exist along with detectable patterns of PFAS research.

1. Distinguishing how different tissues play a role in the accumulation of major compounds of PFAS within freshwater fish.

2. Understanding how the concentration of PFAS within sediment relates to concentration in surface waters.

3. Evaluate the differences in PFAS accumulation across different freshwater species.

METHODS

Literature review

I conducted an informal review of PFAS accumulation in water, sediment, and fish literature to assess any patterns that might be indicative of how PFAS becomes more or less concentrated in fish. The review of the literature was restricted to publications dated 2004 and later, as PFAS were not documented in environmental samples until the early 2000s despite being manufactured since the 1950s (ITRC, 2020). Google Scholar (http://scholar.google.com) was used to search for terms pertaining to; PFAS, perfluoroalkyl substances, freshwater fish, water, sediment, variability, diet, biomagnification, and bioaccumulation. I further explored the citations in the chosen literature summary for further relevant publications within the selected literature. The goal for this practice was not to perform an extensive review, but rather to produce an adequate sample size that reflects major findings and draws discernable patterns from a multitude of different sources. Publications that were deemed not relevant were excluded, on the basis that they did not 1) provide supplementary data that could be utilized 2) the objectives of the study centered around human exposure rather than fish variability 3) the provided supplementary data could not be transformed into this study 4) the study was unpublished or non-peer-reviewed literature. A total of 23 publications were reviewed and the chosen literature was compiled and organized in Microsoft Excel. I recorded the findings from the publications into the following categories: source of publication, year of publication, title, continent, study area, units of measure of water/sediment/tissue samples, minimum and maximum PFAS levels reported for surface water, minimum and maximum PFAS levels reported for sediment, minimum and maximum PFAS levels

reported for fish tissues, objectives of the study, sampling bias reported, trophic levels reported, tissue type of the sampled fish, diet consisting of invertebrates, diet consisting of fish, reported species, key points of the study, and discussion points of the study. The chart was utilized to informally categorize the different findings and arguments presented on the issue at hand. These criteria were deemed important when organizing the literature composite as it helped filter relevant studies to each other, and group publications in a way to address the stated objectives.

Assessing PFAS Accumulation

To center around the objectives of the study, I modified the synthesis matrix to yield different visualizations of genera-specific variability, water-sediment relationships, and trophic-level relationships. For the sake of consistency across all publications, I calculated a 'pseudo-mean' as an average of the given minimum and maximum concentrations. These means were used for the construction of the figures and tables. I chose to focus on the major compounds perfluoro octane sulfonate (PFOS), perfluorooctanoic acid (PFOA,) and total PFAS, rather than a wide array of these compounds. In the literature composite, these compounds were present across all publications, mostly because PFOS and PFOA are the most widely studied compounds of PFAS (EPA, 2017). Despite the literature composite being constructed from 23 publications, not all of these sources pertained to the needed minimum and maximum concentrations on liver and muscle samples within the same fish sample. Species-specific concentrations were grouped at the genus level to determine patterns on a broader scale. I plotted a box and whiskers chart using the pseudo-mean concentrations of all tissues for the major compounds of PFAS to assess the variability across genera. 48 species were

collected across the publications, however, not all of them were utilized for this experiment as there were not enough data points to create a box within the figure. To understand the specific diet of the species Table S13 from Munoz et al., 2022 was used and combined with the additional data from the literature composite. The numerical value in the feeding category within the modified table was designated as Top trophic level piscivore: 4, Lower level piscivore: 3, Lower level insectivore/piscivore: 2, and Lower level insectivore: 1. These numerical values were set following the guidelines set by Babut et al., 2017. When trying to distinguish the relationship between water and sediment, I chose to 1) look at the compounds PFOA and PFOS separately to determine whether the nature of the compound played a significant role in how PFAS deposited in the two variables (Figures 3 & 4) and 2) determine whether there was a relationship at play in how the two compounds deposited in the sediment and surface water respectively.

Statistical Analysis

I used Pearson Correlation Coefficients to assess how the concentrations of PFOA and PFOS in water were related to the concentrations found in the sediment within the same water body. I conducted this test twice when considering the relationship between water and sediment for the compound PFOS, as I detected an outlier. This test was utilized again to determine liver-muscle and blood-muscle relationships. Similar to what I encountered with the water and sediment relationship, there was an outlier found in liver and muscle samples, hence I ran the analysis again without the outlier sample. When assigning the strength of correlation, I used the National Library of Medicine's guidelines(2012) which define r=0.0 to r|0.3| as a negligible relationship, $0.3\n-0.5 \pm$ weak, and 0.5-0.7 \pm moderate strength, 0.7-0.9 \pm strong correlation, and 0.9> \pm as very high

strong correlation. Any value falling under this scale was determined as having no correlation. Additionally, I used linear regression to evaluate the relationship of PFAS concentration in the surface water and sediment and interpreted the R2 values as a measure of the proportion of the variance of the criterion that can be explained by the predictors in my regression model. Considering the R2 values, I can assume that the higher the value R2, the greater the explanatory power of the regression equation, and therefore the better the prediction of the liver tissue concentrations dependance on muscle tissue concentrations. This assumption can be made if the regression model is properly applied and estimated (Cohen, 1988).

RESULTS

Liver and Muscle Tissue Correlation

I reviewed 23 publications that matched my search criteria and retained data from 7 publications that reported min and max concentrations for minimum and maximum concentrations on liver and muscle samples within the same fish sample. 13 species across 7 different publications were used to assess whether there is a discernible relationship between the accumulation of PFAS in the muscle tissue and the liver tissues. The species identified within the publications for the use of these figures were: *Tilapia spp., Hypophthalmichthys nobilis, Cyprinus carpio, Labeo rohita, Channa striata, Labeobarbus aeneus, Squalius cephalus, Pylodictis olivaris, Siluriformes spp., Tanichthys albonubes,* and *Dicentrarchus labrax.*

I observed a strong positive correlation between the muscle tissue's PFAS concentrations and that of the liver tissues $(r=0.87)$ and the linear regression produced a strong fit to the data ($r2=0.78$). (Figure 1.) However, these values might have been influenced by the outlier sample of smallmouth yellowfish (*Labeobarbus aeneus*). This sample, reported by Groffen et al., 2018, had 230.06 ng/g of PFOS in the liver tissue and 22.91 ng/g in the muscle tissue, which fell 51.6 times beyond the range of the rest of the data. Once the outlier was removed, I found only a weak negative correlation of -0.15 and R₂ 0.022 (Figure 2). The difference between the two analyses suggests that since the liver/muscle relationship accounts for 78% of the variation (Figure 1.), hardly any of the variation in the data is explained by the liver/muscle relationship when smallmouth yellowfish samples are taken out.

Figure 1a: Linear regression of how the concentration of major compounds of PFAS; PFOS and PFOA, are found within the liver and muscle tissues of fish. Concentrations were determined using the pseudo-means (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Figure 1b: Linear regression of how the concentration of major compounds of PFAS, PFOS and PFOA, are found within the liver and muscle tissues of fish when excluding outliers from smallmouth yellowfish samples. Concentrations were determined using pseudo-mean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Blood and Muscle Tissue Correlation

From the literature composite, only 5 species were reported with paired blood and muscle tissue samples. These samples were provided by Hoa et al., 2022 and Hung et al., 2018. The species in question were *Tilapia spp., Hypophthalmichthys nobilis, Cyprinus carpio, Labeo rohita,* and *Siluriformes spp.* I found that the concentration of major compounds of PFAS had a strong positive correlation with how they accumulate in the blood and consecutively, the muscle (r=0.92). The R2 value of 0.85 accounts for 85% of the variance in my data from this linear regression (Figure 2.) Though my results indicate that as the variable of blood increases, so does muscle, this correlation does not imply causation.

Figure 2. Linear regression of how the concentration of major compounds of PFAS; PFOS and PFOA, are found within the blood and muscle tissues of fish. Concentrations were determined using pseudo-mean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Water and Sediment Correlation

The accumulation of PFOA in water was not substantially correlated with the accumulation in the sediment ($r=0.02$). Similarly, I observed a very weak to no correlation between PFOA and PFOS concentration deposited in surface water (Figure 6). When looking at the behavior of PFOS, I found a strong positive correlation of 0.75 for concentrations found in surface water and sediment (Figure 4). This strong relationship persisted at 0.72, even after I ran the statistical analysis again without the reported pseudo-mean 35.00 ng/g as it was determined an outlier.

I observed that the nature of how PFOA and PFOS accumulate in the sediment yields a more moderate correlation value of 0.45, as PFOA and PFOS resulted in no

correlation ($r=0.09$) in surface water. When interpreting the relationship of PFAS accumulation in the water (Figure 6) within the bounds of this study, PFOS concentration appeared to increase proportionally in both the surface water and sediment while PFOA samples do not. This may be attributed to the chemical natures of the different compounds as opposed to the surface water and sediment relationship per se.

Figure 3. Scatter Plot of how the concentration of major compounds of PFAS; PFOA, is found within the Water and Sediment. Concentrations were determined using pseudomean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Figure 4. A comparison of the concentration of major compounds of PFAS: PFOS, is found within the water and sediment. Concentrations were determined using pseudomean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Figure 5. Scatter Plot of how the concentration of different compounds of PFAS; PFOS, and PFOA accumulate in the sediment. Concentrations were determined using pseudomean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

Figure 6. Scatter Plot of how the concentration of different compounds of PFAS; PFOS, and PFOA accumulate in the surface water. Concentrations were determined using pseudo-mean (average of minimum and maximum values) to keep consistency, as not all publications reported mean values.

PFAS Accumulation Across Genera

Excluding the Esox and Carassius genera, I observed a consistent positive skew across the remaining genera (Figure 7). Essentially, this indicates that the data for the Tilapia, Cyprinus, Channa, Squalius, Leopomis, Ictalurus, and Perca genera predominantly consists of high-valued scores. Within this study, species in these genera were found to have higher concentrations of PFAS.

I observed the genus *Esox* distribution to be fairly neutral, compared to the rest of the samples. Thus, this genus is more abundant with lower concentrations of PFAS. This observation, however, might have been influenced by one large data point in the smaller sample size of Esox (n=4). Along with *Esox, Lepomis, Perca, Channa,* and *Ictalurus* also had smaller sample sizes $(n=4)$. Within these samples, these genera shared a commonality of having at least one data point that was a very high concentration. Because the sample sizes consist of one or two very large data points, the results may not be representatives of the true population. *Carassius* was the only genus that I observed to have a normal distribution. This can be attributed to the fact that this genus had the most data points, and species within this group were most commonly studied across the publications. I detected 3 outliers in the genera *Carassius, Cyprinus, and Squalius*, these being very high values of 118.68 ng/g, 294 ng/g, and 552.8 ng/g respectively.

Figure 7. Box and Whisker plot of the distributions of PFAS concentrations across 9 Genera. Concentrations were determined using the pseudo-means, as opposed to given means, to keep consistency across all publications. Genera that did not have sufficient data points (<4 data points) were excluded from the scatter plot. Concentrations of PFAS include muscle, blood, and liver tissues.

Trophic Level and Biomagnification

I repurposed Table S13 from Munoz et al. to better illustrate any species-specific variability through diet. Regardless of the type of prey, I observed that average PFOS concentrations were higher than PFOA. That being said, 4 entries had no available data on PFOS concentrations for smallmouth bass, *Micropterus dolomieu,* which may have skewed my interpretation. (Table 1.) Fish that consume invertebrates had higher PFOS concentrations than individuals consuming fish prey. *Micropterus dolomieu* had the highest value of 2.3 ng/g when consuming fish prey (round goby, *Neogobius melanostomus*) had a drastic increase to 16.2 when consuming invertebrate prey (crayfish, *Palinuridae spp.*) Again, this pattern can be observed with rock bass,

Ambloplites rupestris, with a high of 14.6 ng/g of PFOS concentration with invertebrate prey and 2.8 ng/g with fish prey, and for northern pike *(Esox lucius)*, 6.1 ng/g for invertebrate prey and 0.9 ng/g for fish prey. (Table 1.)

PFOA however, yielded a different pattern of how this compound accumulates in fish prey as opposed to invertebrate prey. For rock bass, *Ambloplites rupestris,* I observed that the concentration of PFOA was higher if the sample consumed fish prey; 0.8 ng/g from juvenile yellow perch (*Perca flavescens*) and 4 ng/g from minnow (*Cyprinidae spp.*), compared to invertebrate prey; 0.06 ng/g from insect (*Insecta spp.)* and 0.1 from crayfish (*Palinuridae spp.*) This again was observed with yellow perch (*Perca flavescens*) accumulating 1.7 ng/g of PFOA when consuming round goby (*Neogobius melanostomus*) and northern pike (*Esox lucius) was* found with 0.5 ng/g when consuming round goby (*Neogobius melanostomus*). Within this study, I observed the pattern that higher PFOS concentrations are correlated with an invertebrate diet, and higher concentrations of PFOA are correlated with a diet composed of fish.

Table 1. Modified Table of Concentrations of PFOA and PFOS of prey and predator couples of a freshwater food web from Munoz et al., 2022. Biomagnification was deemed significant based on the equation BMF = contamination level in predator ([x]) to that of its prey ([y]). The feeding category was assigned based on the trophic level of the species, with 4 being top carnivores and 1 being lowest-level insectivores

DISCUSSION AND INTERPRETATION

The results from this literature review were unexpected, as I had predicted there to be more discernible patterns on how major compounds of PFAS become more or less concentrated in fish, water, and sediment. However, as researchers encounter difficulties when studying PFAS chemicals due to their occurrence in complex mixtures and various everyday products, it is not surprising that this study could not produce clear connections between the publications (NIEHS, 2024). It is also necessary to acknowledge that the reviewed publications all had different objectives, sampling methods, and biases that could have been additional factors that influenced my interpretation of the results. For example, the authors of the chosen publications may have deliberately chosen sites known to be highly contaminated (near factories, military bases, etc.) which could explain the outliers found across the data, and why there might have been a general positive skew in Figure 7.

With 380,000 fishing licenses pursued annually in the state of Maine, it is important to focus future PFAS research on genus-specific and species-specific studies that can better characterize the pattern in which PFAS are more or less abundant in fish (Maine Department of Environmental Protection, 2024). Within this study, I observed the genus *Squalius* to have the highest concentrations of PFAS within their tissues. Even when acknowledging sampling bias, it is important to recognize this genus as a potential source of high concentrations of PFAS accumulating within the environment these species occupy. Further research into the plausibility of the findings from this study could better inform policymakers and communities that partake in freshwater fishing, about the state of PFAS accumulation throughout the environment.

With the two figures and correlation tests yielding different results, it is difficult to definitively conclude how PFOS and PFOA accumulate in the liver and muscle within the fish. An additional aspect to consider as a limit to PFAS research is that the dataset for certain tissues (Figure 1b.) might be insufficiently large to yield meaningful insights. While conducting my results, I noted that there was significantly more data available on the whole body filet, or muscle of the fish sample as opposed to blood or liver. Across the 24 publications, 2 studies provided relevant information on blood-muscle concentrations and 7 studies on liver-muscle concentrations. Much of PFAS research primarily focuses on whole-body filet or muscle tissue analysis to understand the underlying impacts on human health and how this chemical moves across the environment. With the high volume of research needed for PFAS, it is no surprise that this issue is proving to be financially burdensome with the tests conducted requiring complex lab equipment and trained staff members to identify (EPA, 2023). Though liver and blood samples provide more accurate readings of toxicology (National Library of Medicine, 2010), research done on muscle tissue may be more common to address human health concerns and avoid high costs. Despite the lack of concrete results from this study, I would recommend targeting liver sampling and muscle sampling on fish to better understand the underlying relationship, as the liver has been suggested to be a reservoir for PFAS in additional publications.(He et al., 2015; Ali et al., 2021; Chen et al., 2021; Luebker et al., 2002). My findings indicate the highly variable nature of PFAS within the liver, thus, it is vital to further assess the claim of PFAS binding to the liver.

PFAS circulates through the environment through various sources of exposure, thus it is important to further understand how the water and sediment relate to each other

and fish contamination (Pickard et al., 2022). We must discover any relationship between these variables to gain an understanding of how PFAS bioaccumulates through ecological interactions. This study observed differences in two of the common compounds of PFAS: PFOA and PFOS and their concentrations in the water vs. the sediment. Figures 3 and 4 shed light on how the different chemicals behave differently within the same water body. These findings further emphasize the difficulty in distinguishing PFAS patterns as much variability is present within the chemical group (Shi et al., 2009). These differences in chemical behavior were again observed in Table 1., as PFOS and PFOA were found to accumulate differently in the prey and predator couples depending on the type of prey ingested. More discernible patterns on PFOS were observed as opposed to PFOA, which may have resulted in PFOS being more commonly studied across the publications used within this study.

CONCLUSION

The findings from this study underscore the urgent necessity for PFAS research. However, due to significant variability observed across the publications, it remains challenging to draw definitive conclusions regarding discernable patterns even within the available data.

Based on the observations from these results, I would suggest more target research into the compound PFOA as the nature of this chemical within the water and sediment seems largely unknown. The variability within the results of this study emphasizes the need for a more focused approach to PFAS accumulation in fish tissues, water, and sediment.

Despite a lack of concrete evidence within this study, it should not lead to inaction. The state of Maine has made efforts to decrease the widespread use of PFAS, by prohibiting the use of PFAS in any product unless it is designated as unavoidable effective January 1st, 2030. (Maine Department of Environmental Protection, 2024). Though this is a significant positive effort, until then more directed efforts are essential to ensure further exposure is limited. Approximately 45.1 % of all US citizens have never heard of PFAS, and 31.6% have stated that despite hearing about PFAS, do not understand the nature of the chemical or its implications (Berthold et al., 20203). With the current state of knowledge on PFAS being less than desirable, it is vital to have transparency in how information regarding PFAS is made available to the general public.

APPENDICES

Appendix A- Minimum, maximum and calculated pseudo-means of PFOS in the blood

and muscle tissues.

Appendix B - Minimum, maximum and calculated pseudo-means of PFOS and

PFOA in surface water and sediment.

Appendix C - Minimum, maximum and calculated pseudo-means of PFOS in the

liver and muscle tissues.

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