

# Evaluating Perfluorinated Compound Contamination in Mussel Samples from the Puget Sound Region

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## Introduction

- Perfluorinated alkyl substances, also known as PFAS, are a large class of synthetic compounds that have been used for a long time in industrial and consumer products.
- Applications include products such as Teflon, carpets, and waterproof rain jackets, and food handlings
- These compounds have been of increasing concern recently due to their persistence in the environment, their bioaccumulation, their detection in human blood samples worldwide, and the health risks they pose to humans and other animals.
- Recently, PFAS have been detected in drinking waters around the United States and Washington state.
- This study aims to quantify PFAS in mussel samples obtained from various bays around western Washington.
- Bivalves are known filter feeders and thus can serve as indicator species for contaminants.
- Previous research has been scarce in illuminating the degree of PFAS concentrations in marine bivalves, especially in urban waterways like the Puget Sound in Washington state.
- In this study, we validated an extraction method known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and evaluated the contamination of PFAS in mussel samples from urban and non-urban bays in the Puget Sound.
- The range of analytes measured included 12 perfluorinated alkyl substances, ranging from 4 to 14 carbon chain lengths. (In Figures 1 and 2,  $n$  is between 3 and 12).

Compound Names	Acronyms	Molecular Weight (Transition Measured)
Perfluorotetradecanoic Acid	PFTeDA	713>669
Perfluorotridecanoic Acid	PFTrDA	663>619
Perfluorododecanoic Acid	PFDaA	613>569
Perfluoroundecanoic Acid	PFUnDA	563>519
Perfluorodecanoic Acid	PFDA	513>469
Perfluorooctane Sulfonic Acid	PFOS	499>99
Perfluorononanoic Acid	PFNA	463>419
Perfluorooctanoic Acid	PFOA	413>369
Perfluorohexane Sulfonic Acid	PFHXS	399>99
Perfluoroheptanoic Acid	PFHpA	363>319
Perfluorohexanoic Acid	PFHxA	313>269
Perfluorobutane Sulfonic Acid	PFBS	299>99

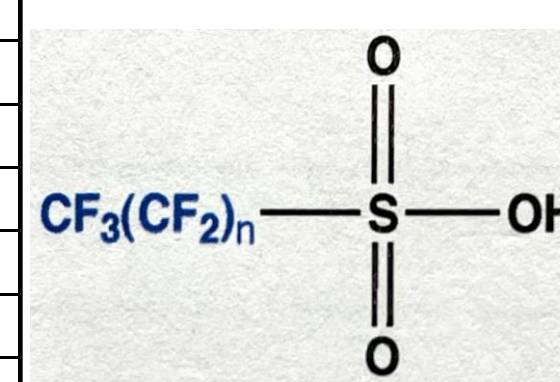
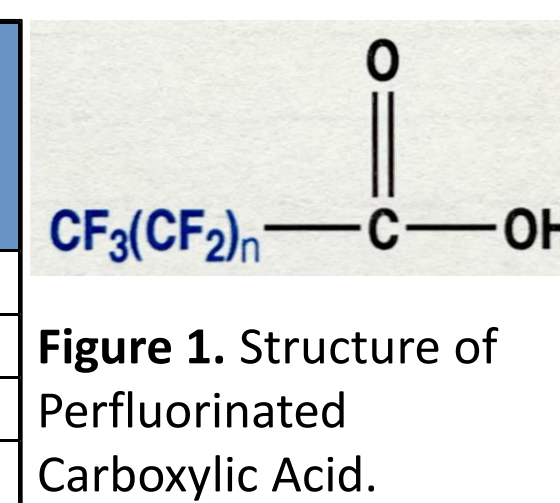


Table 1. List of Perfluorinated Alkyl Compounds analyzed, with acronyms and molecular weights (including transition weight).

## Objective

- Validate and optimize extraction and clean-up methods for mussel tissue samples collected from urban bays in order to measure perfluorinated acid contamination on these organisms. Spike and recovery experiments will be performed with the goal for >70% recovery for the majority of the analytes of interest.
- Quantify 12 PFAS analytes of interest from mussel samples collected from bays around the Puget Sound region. Samples will be extracted using the validated method from previous objective and analyzed using a liquid chromatography tandem mass spectrometry.

## Methods

### Collection

- In the winter of 2012/2013 Washington Department of Fish and Wildlife conducted a synoptic, Puget Sound-wide assessment of toxic contaminants on nearshore biota.
- Used native mussel species (*Mytilus trossulus*) as an indicator for the degree of contamination.
- Placed cages of transplanted mussels at 108 different locations in November of 2012.
- Mussel cages were collected in January of 2013 and homogenized for analysis.
- An aliquot from each location, averaging about 5 grams, was donated to Dr. Dinglasan-Panlilio for this project.
- Mussel samples were stored at -80°C up until this point.

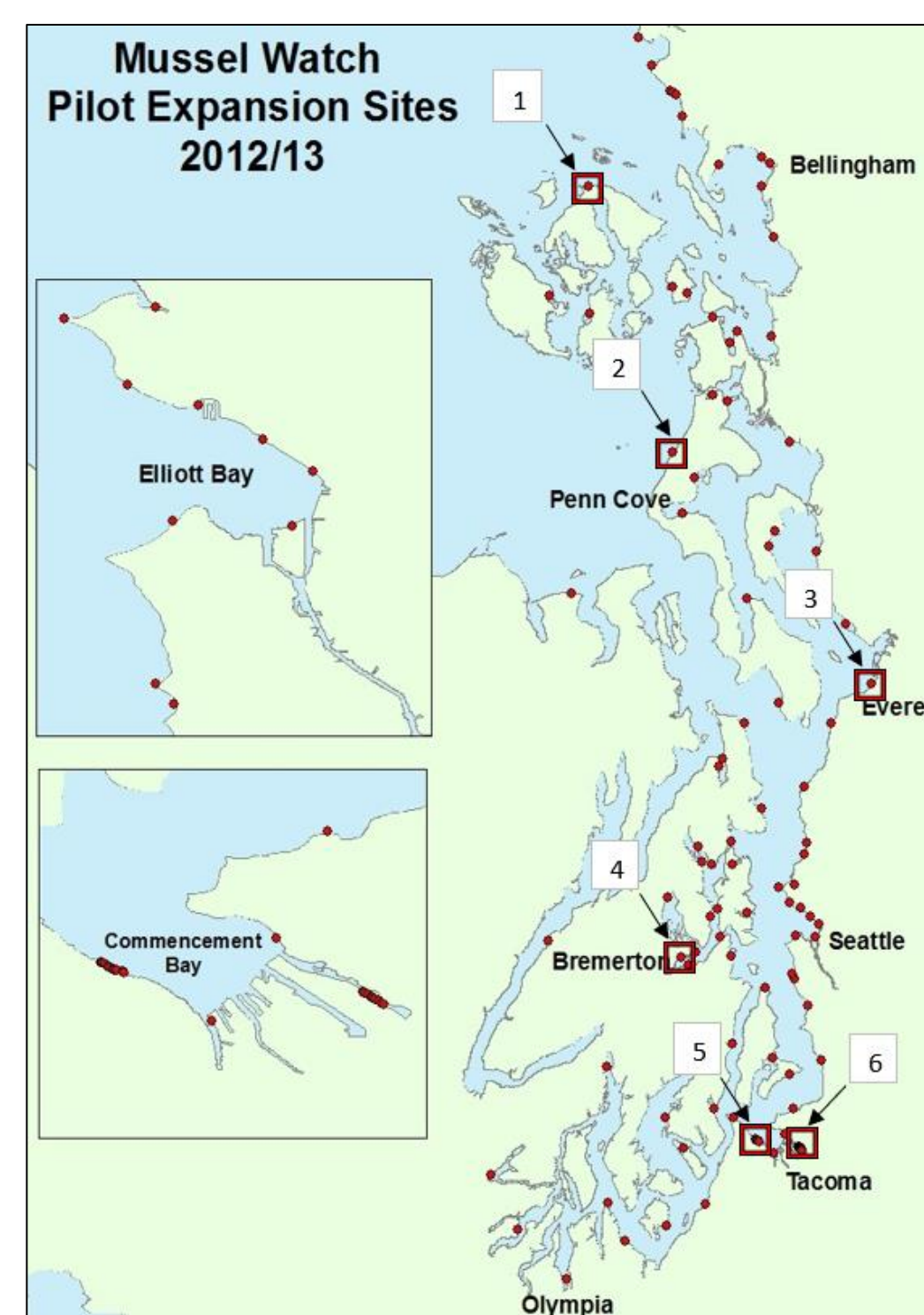


Figure 3. Map of mussel collection sites across the Puget Sound area.

1	OINS	North Shore, Orcas Island
2	MIAR	Smith and Minor Islands Aquatic Reserve, Joseph Whidbey State Park
3	EH	Everett Harbor
4	BSCB	Bremerton Shipyard, Charleston Beach
5	RW8	Tacoma Ruston Waterfront 8
6	HWY8	Hylebos Waterway



Figure 4. Image of caged mussels.  
Table 2. Key of mussel collection sites analyzed in this study by number, acronym, and location.

## Methods (continued)

### Extraction (QuEChERS) and Clean-up

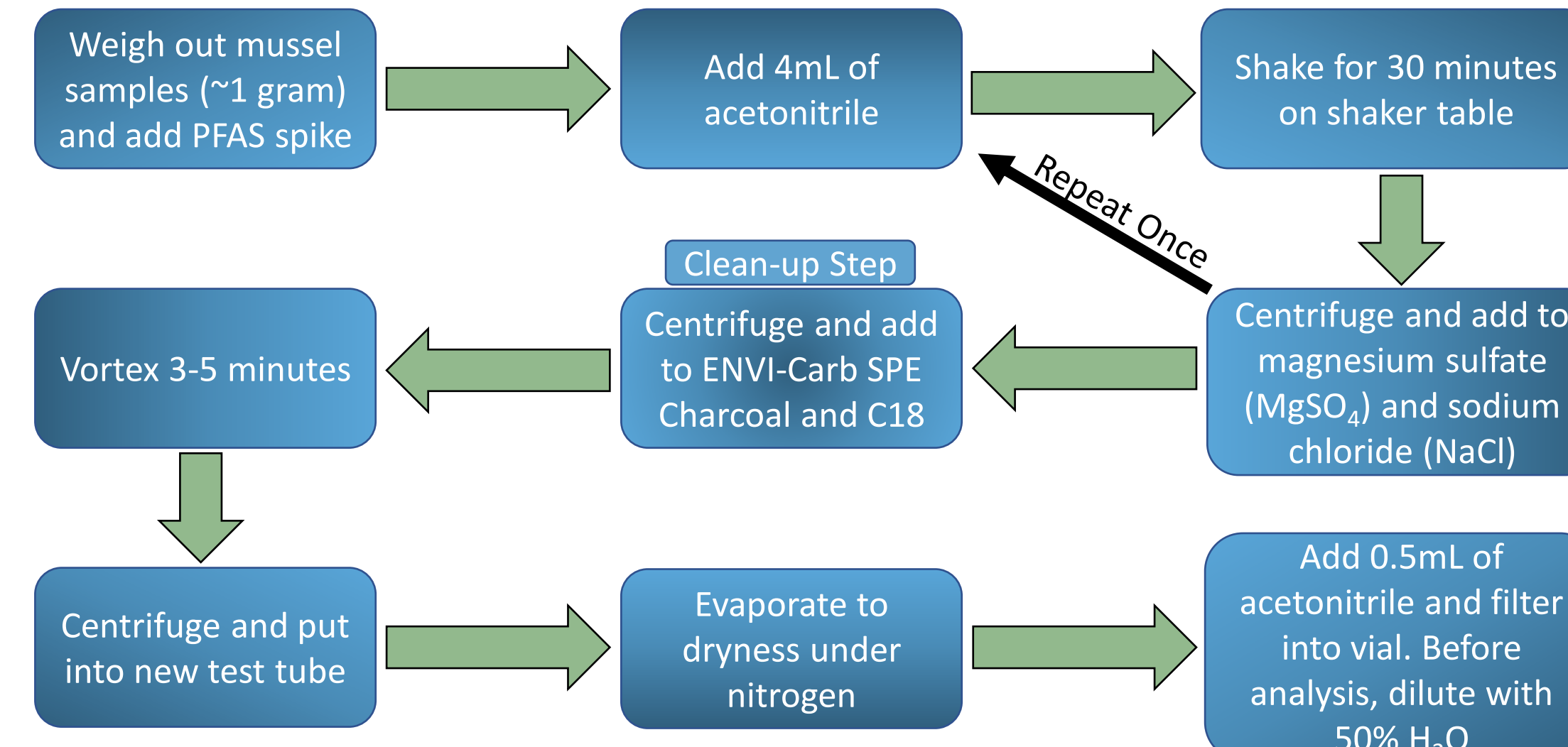


Figure 5. Schematic of QuEChERS mussel extraction and clean-up method.

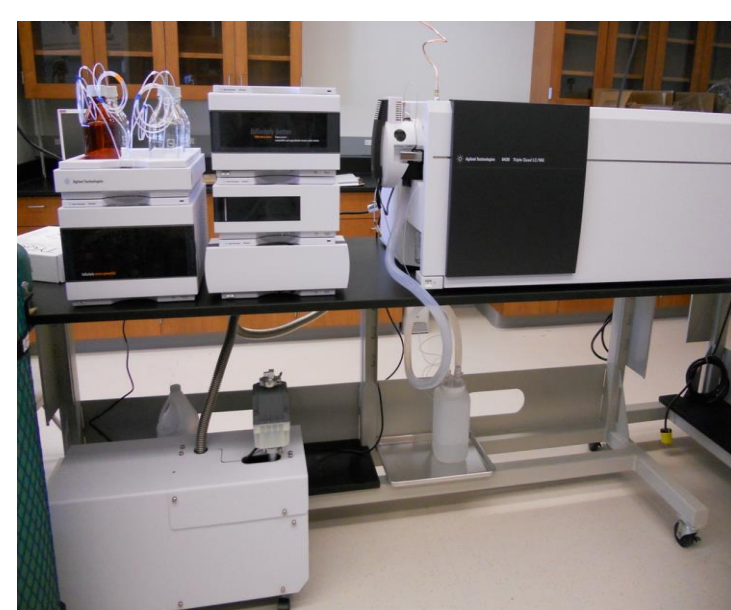


Figure 6. Image of Agilent 6430 Triple Quadrupole LCMS/MS.

### Analysis with LCMS/MS

- Analyzed samples with Agilent 6430 Triple Quadrupole LCMS/MS
- Negative Electrospray – Multiple Reaction Monitoring Mode (MRM)
- Column Info: Agilent ZORBAX Eclipse Plus C18 Column Rapid Resolution 4.6x50mm 3.5 micron (P.N. 959943-902)
- Data analyzed with Agilent MassHunter Qualitative Analysis

## Results

### Spike and Recovery Experiment

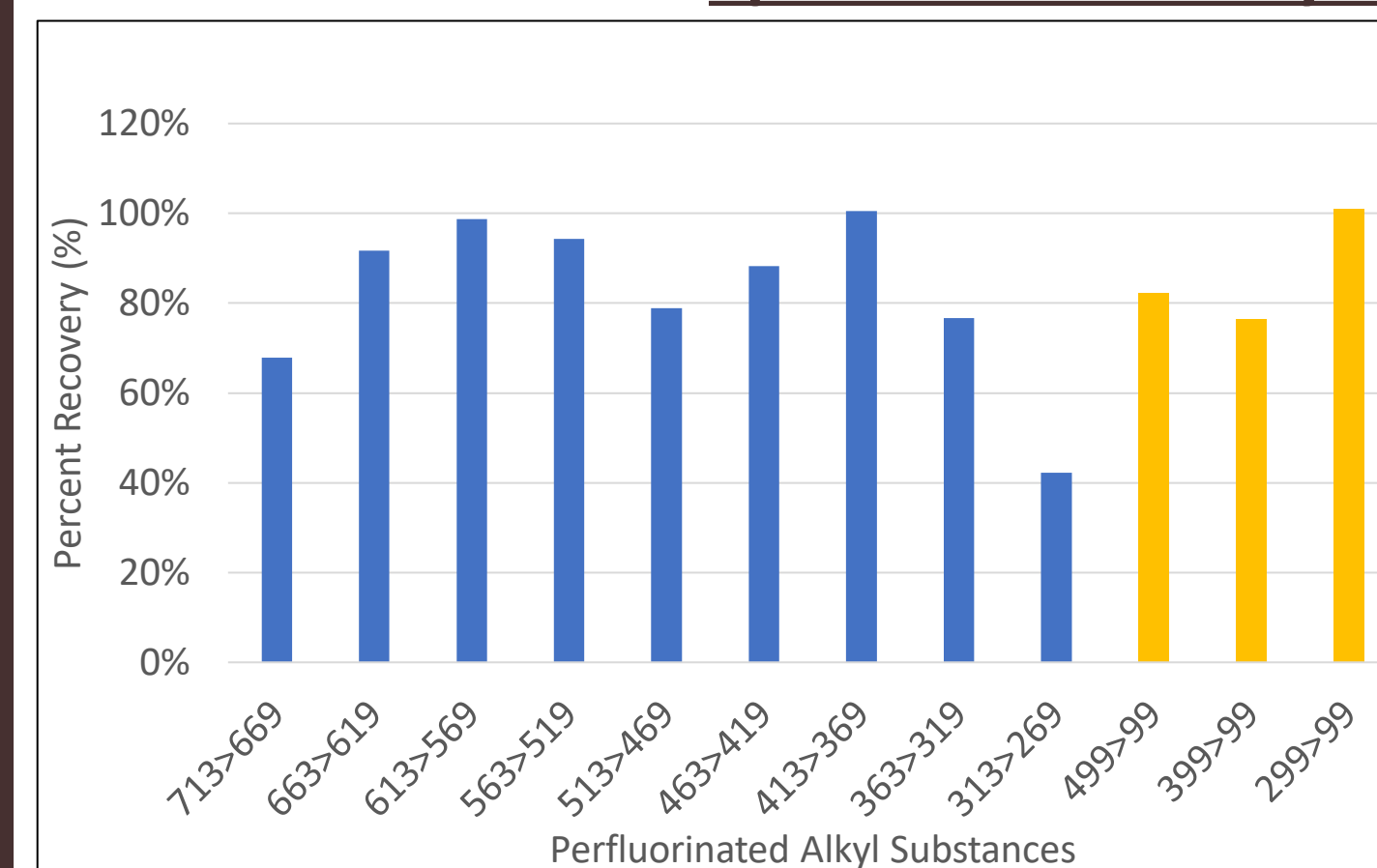
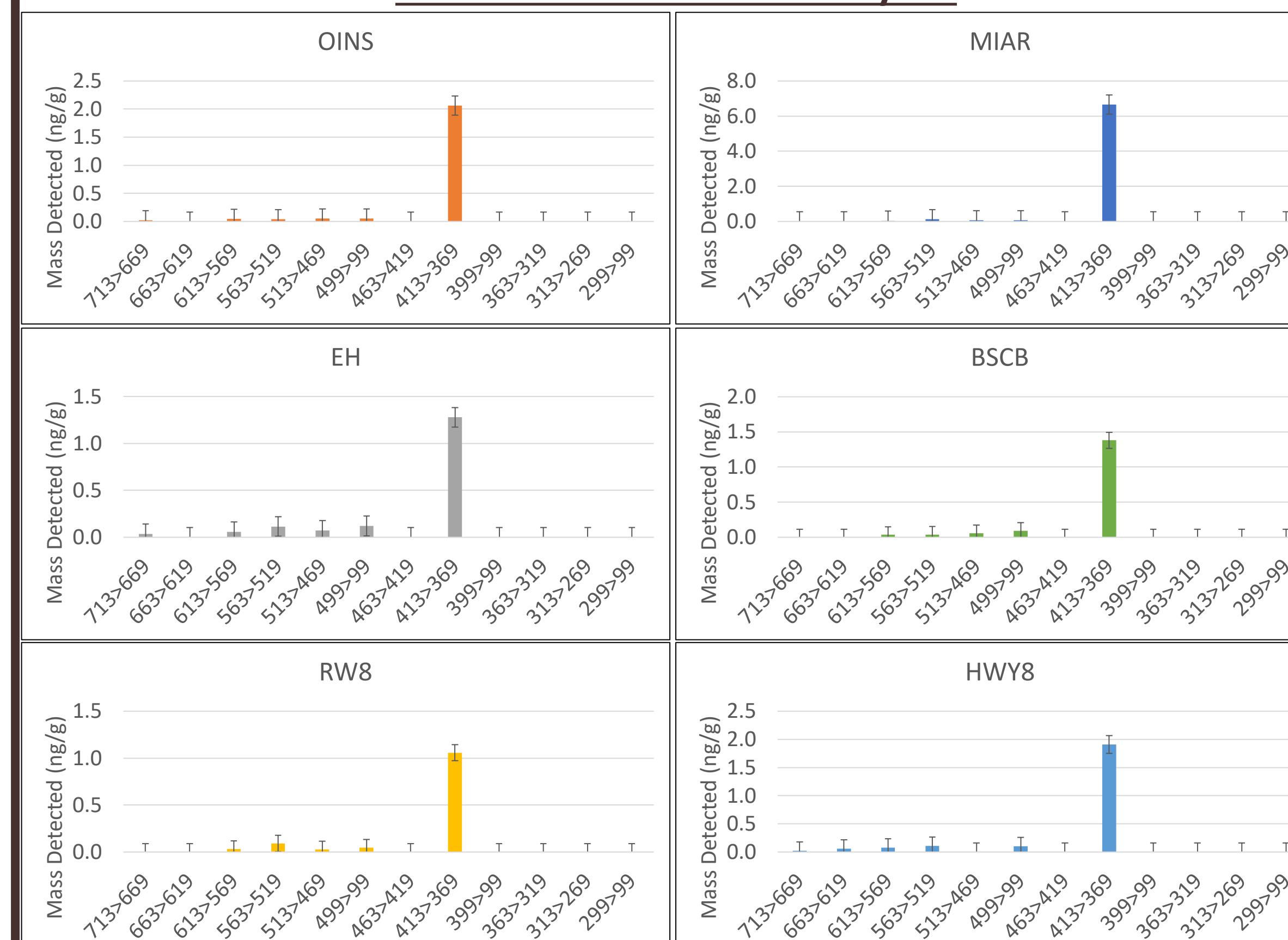


Figure 7. Average of percent recoveries for PFAS analytes of interest. (Perfluorinated sulfonic acids in yellow and in blue are perfluorinated carboxylic acids).

### Select Mussel Tissues Analyzed



Figures 8-13. Mass of detected PFAS from six selected sites around the Puget Sound region.

## Results (continued)

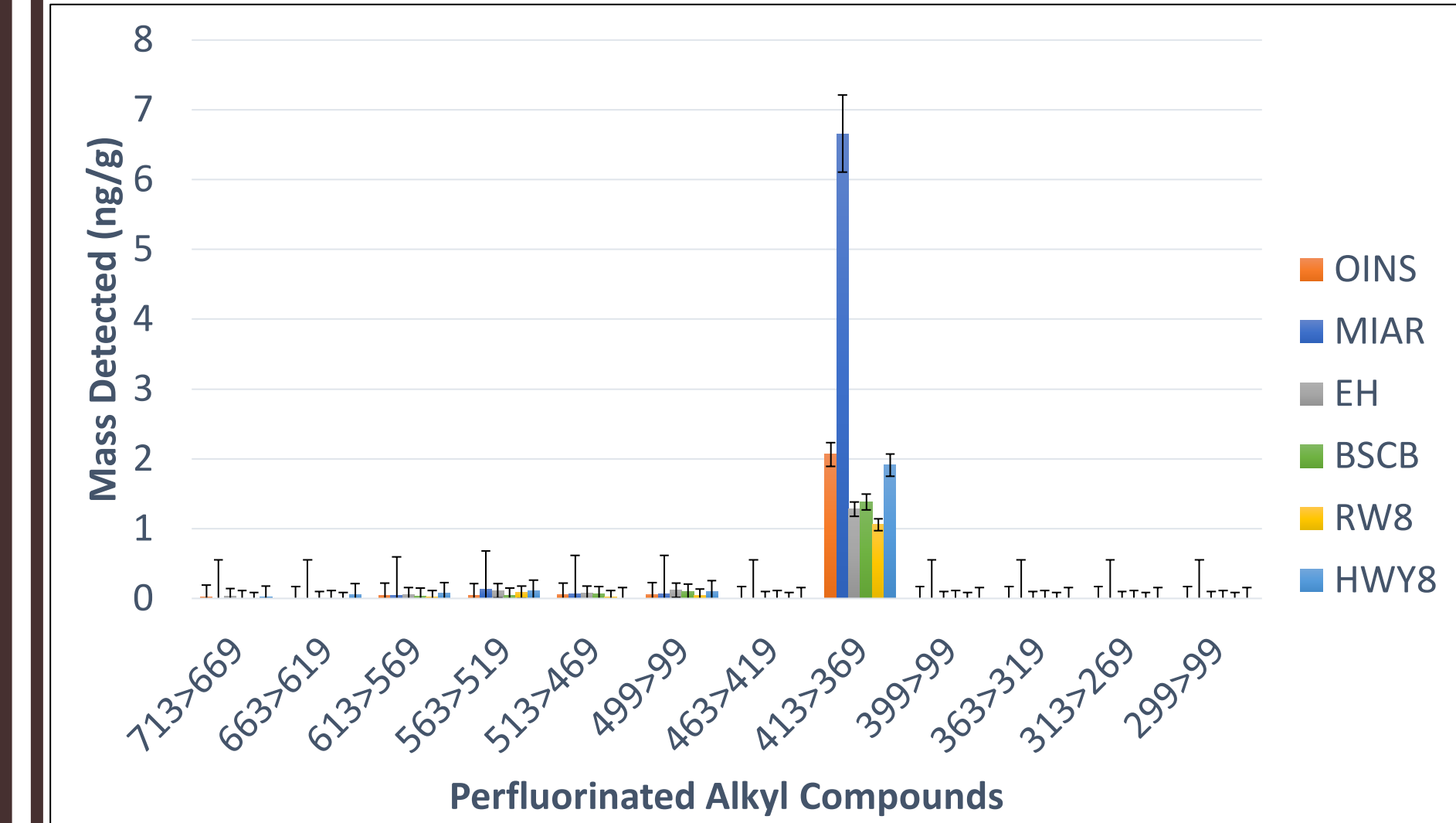


Figure 14. All mussel collection sites showing the mass of PFAS detected for each perfluorinated alkyl substance.

Figure 15. Zoomed in graph of the perfluorinated alkyl substances with trace amounts of mass detected for all mussel collection sites.

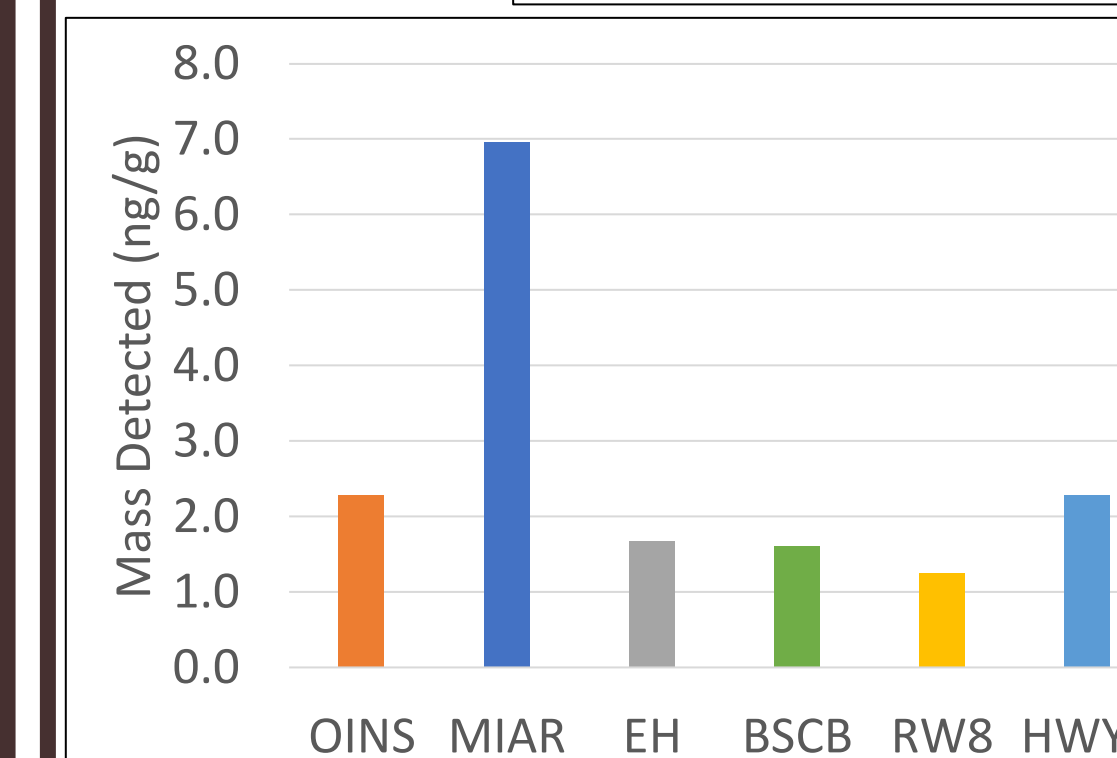
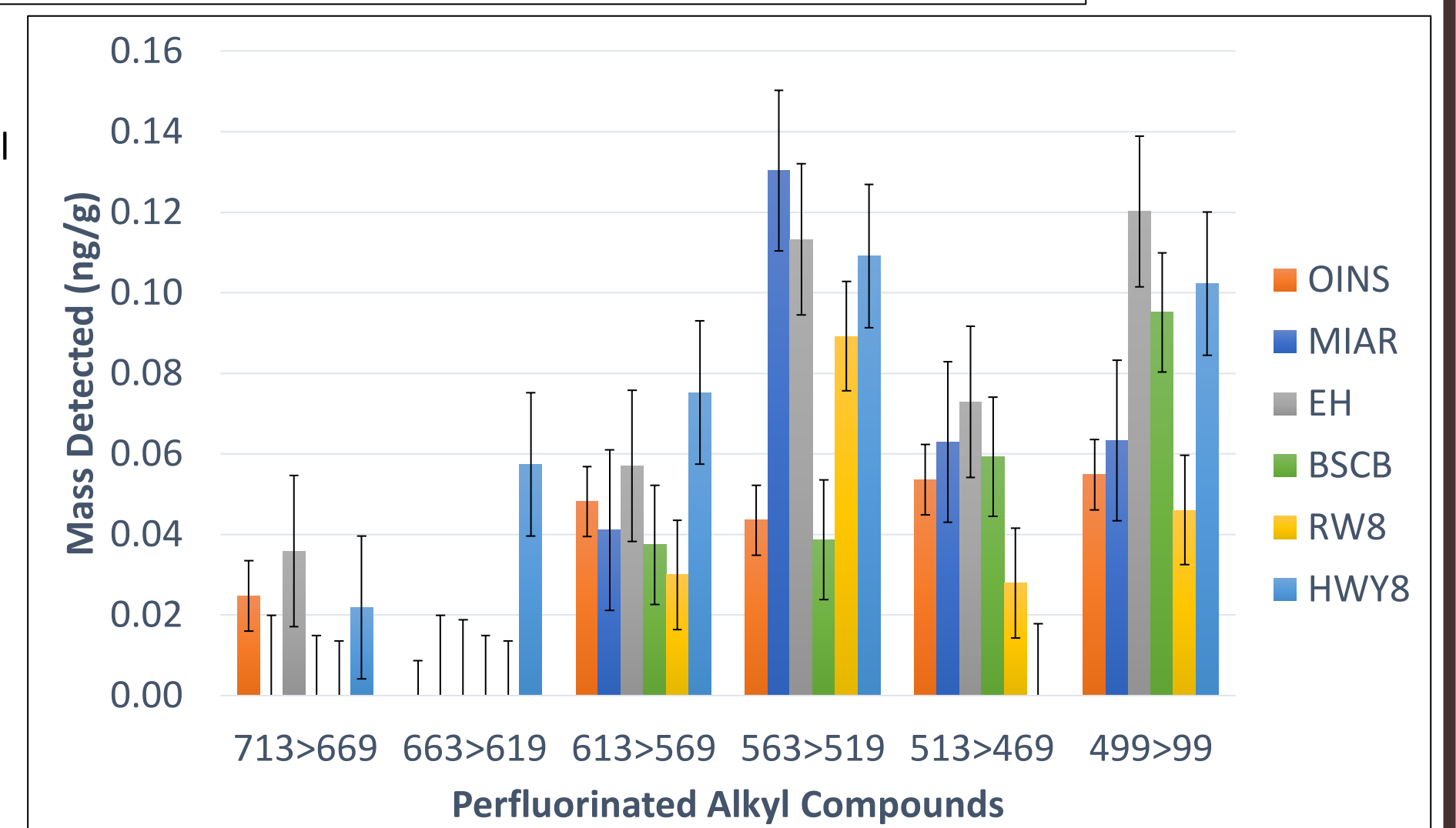


Figure 16. Total mass of detected PFAS for each mussel collection site.

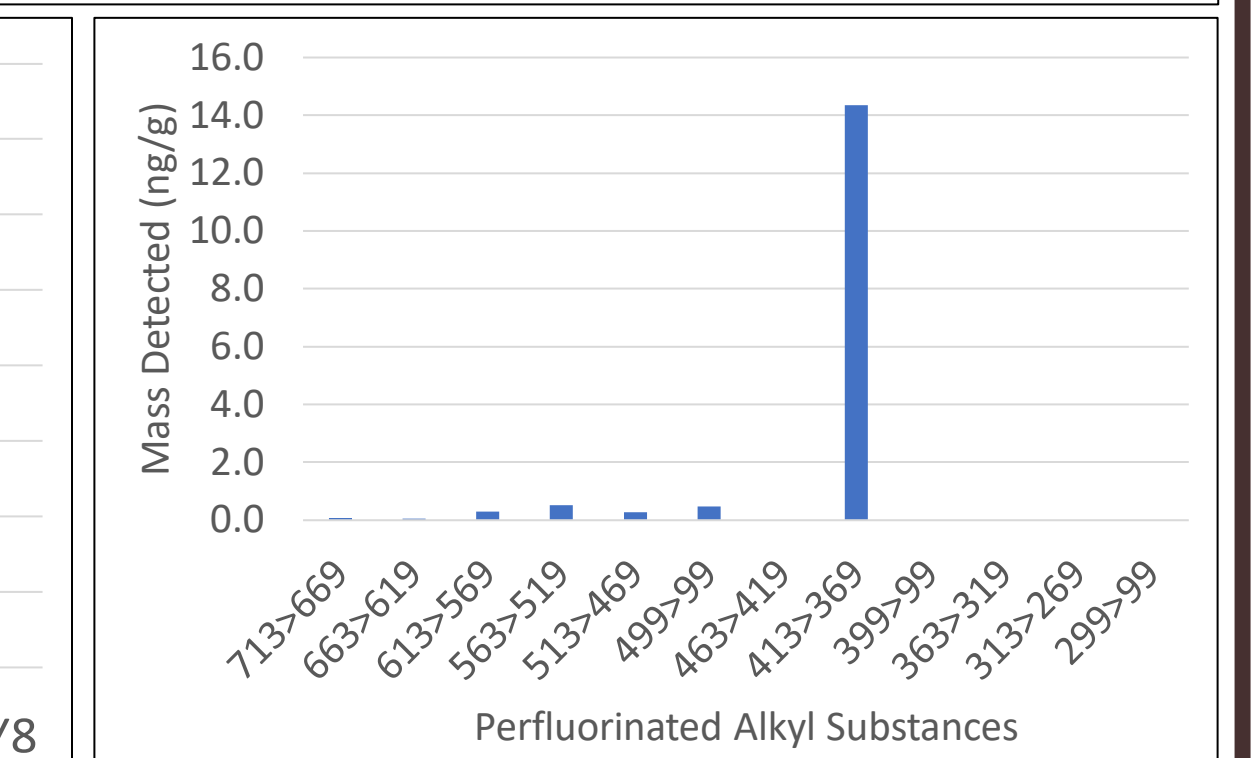


Figure 17. Total mass of detected PFAS for each perfluorinated alkyl substance.

## Discussion and Conclusion

- Spike and recovery experiment yielded recoveries ranging from 42% to 101% for samples spiked with 10ng of PFAS. Most of the analytes achieved well above the 70% recovery benchmark.
- In the select mussel tissues analyzed, there were 7 out of 12 perfluorinated alkyl substances detected, which included PFOA, PFOS, PFDA, PFUnDA, PFDaA, PFTeDA, and PFTeDA.
- Five PFAS were below the minimum quantification limit (>MQL), such as PFBS, PFHxA, PFHpA, PFHXS, and PFNA.
- Four PFAS were detected in all samples, which included PFOA, PFOS, PFUnDA, and PFDaA.
- The most prevalent of the substances was PFOA, which was detected as high as 6.66 ng.
- Of the substances detected, all but PFOA were detected in trace amounts, i.e., lower or equal to 0.13 ng.
- The mussel collection site with the highest total amount of PFAS detected was MIAR at 6.95 ng, followed by OINS at 2.29 ng, HWY8 at 2.28 ng, EH at 1.68 ng, BSCB at 1.61 ng, and RW8 at 1.25 ng.
- The blank samples in this study showed no evidence of contamination.
- Future work will continue to analyze more collection sites to further quantify the level of PFAS concentrations in mussels around the Puget Sound.
- This data will contribute to limited studies done on PFAS contamination from shellfish collected around the Puget Sound region and can provide further information to assess possible routes of PFAS exposure to humans.

## Literature Cited

Lin Wang, Xizhi Shi, Qiaoling Zhao, Aili Sun, Dexiang Li, Jian Zhao. Determination of lipophilic marine toxins in fresh and processed shellfish using modified QuEChERS and ultra-high-performance liquid chromatography-tandem mass spectrometry. Food Chemistry, Volume 272, 2019, 427-433, ISSN 0308-8146.

Katarzyna Sznajder-Katarzyńska, Magdalena Surma, Wiesław Wiczowski, Mariusz Piskula. Determination of perfluoroalkyl substances (PFAS) in fats and oils by QuEChERS/micro-HPLC-MS/MS. Food Research International, Volume 137, 2020, 109583, ISSN 0963-9969.

Isabel Cunha, Philippe Hoff, Kristin Van de Vijver, Lucia Guilhermino, Eddy Esmans, Wim De Coen. Baseline study of perfluorooctane sulfonate occurrence in mussels, *Mytilus galloprovincialis*, from north-central portuguese estuaries. Marine Pollution Bulletin, 2005, 1121-1145.

Jian-Li HE, Tao PENG, Jie XIE, Han-Hui DAI, Dong-Dong CHEN, Zhen-Feng YUE, Chun-Lin FAN, Cun LI. Determination of 20 Perfluorinated Compounds in Animal Liver by HPLC-MS/MS. Chinese Journal of Analytical Chemistry, Volume 43, Issue 1, 2015, Pages 40-48, ISSN 1872-2040.

Surma M, Hliwa P, Sznajder-Katarzyńska K, Wiczowski W, Topolska J, Zieliński H. Perfluoroalkyl Substance Contamination Levels of Pike (*Esox lucius* L.) and Roach (*Rutilus rutilus* L.) from Selected Masurian Lakes in Eastern Europe. Environ Toxicol Chem. 2021 Dec;40(12):3317-3327.

Jennifer A. Lankbury, Laurie A. Niewolny, Andrea J. Carey and James E. West. Toxic Contaminants in Puget Sound's Nearshore Biota: A Large-Scale Synoptic Survey Using Transplanted Mussels (*Mytilus trossulus*). Puget Sound Ecosystem Monitoring Program, 2014.