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**Ecological Risk Assessment of Tire Wear Particles in the San Francisco Bay Using a  
Bayesian Network Relative Risk Model**

By

Emma E. Sharpe

Accepted in Partial Completion  
of the Requirements for the Degree  
Master of Science

ADVISORY COMMITTEE

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## **Master's Thesis**

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Emma E. Sharpe

09/27/2022

**Ecological Risk Assessment of Tire Wear Particles in the San Francisco Bay Using a  
Bayesian Network Relative Risk Model**

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Emma E. Sharpe

September 26, 2022

## **Abstract**

Here we present an ecological risk assessment for a specific type of microplastic in the San Francisco Bay. There has been an increased interest in understanding and managing the impacts that microplastics may have on ecological systems because recent studies have shown that plastic particles are widespread in the environment and that exposure to these particles has toxicological effects. Until now, an ecological risk assessment for microplastics that meets the current standards for risk assessment, has not been completed. This study lays the groundwork for future ecological risk assessments of microplastics and identifies key uncertainties that need to be addressed. Using a Bayesian network relative risk model (BN-RRM), we determined the risk tire wear particles present to juvenile Chinook salmon and Northern anchovy. In past studies, BN-RRM has been a successful framework for regional scale ecological risk assessments of multi-stressor systems, allowing for the creation of a model with predictive capability and adaptive potential as new data become available. The BN-RMM is parameterized for each risk region with tire wear particle environmental concentration data collected by the San Francisco Estuary Institute, plastic particle toxicity data generated by Oregon State University, and site-specific water quality, chemical, and land use data from regional databases. Relative risk was then calculated for each risk region and spatial gradients of risk were determined. Results indicate a relatively low risk for juvenile Chinook salmon and Northern anchovy at current tire wear particle concentrations in the San Francisco Bay. This risk assessment confirms that, with the data that is currently available, a quantitative, spatially specific risk assessment is possible. Additionally, Bayesian networks are an excellent tool for modeling the complex and uncertain nature of microplastics. This study is funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.

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## Acronyms and Abbreviations

BN	Bayesian network
BN-RMM	Bayesian Network Relative Risk Model
CDFW	California Department of Fish and Wildlife
CEDEN	California Environmental Data Exchange Network
CPT	Conditional Probability Tables
CPUE	Catch-per-unit-effort
FTIR	Fourier Transform Infrared
GIS	Geographic Information System
HC5	Hazardous Concentration for 5% of the Species
hpf	Hours Post Fertilization
HUC	Hydrological Unit Code
IEP	Interagency Ecological Program
NASEM	National Academy of Sciences, Engineering, and Medicine NASEM
NOAA	National Oceanic and Atmospheric Administration
RRM	Relative Risk Model
SFEI	San Francisco Estuary Institute
SURF	Surface Water Database
TP	Tire Particle
TWP	Tire Wear Particle
TWP-BN	Tire Wear Particle Bayesian Network
USEPA	United States Environmental Protection Agency
WWTP	Wastewater Treatment Plant

## **1. Introduction**

### *1.1 Ecological risk assessment and microplastics*

Microplastics are an emerging contaminant that is widespread in the environment. Growing public concern around microplastics has resulted in an increased interest in understanding and managing the impacts that microplastics may have on ecological systems. An ecological risk assessment is the only way to determine the actual risk of microplastics to the environment and support the creation of management goals to mitigate risk. The National Academy of Sciences, Engineering, and Medicine (NASEM) committee on gene drive research in non-human defines ecological risk assessment as the, “probability of an effect on a specific endpoint or set of endpoints due to a specific stressor or set of stressors” (NASEM 2016). The data that is currently available on microplastics has yet to be applied to management strategies of microplastics in the environment. As jurisdictions begin to regulate microplastics, geographically specific risk assessment such as this one, provide critical information on risk to endpoints of interest. The goal of this project was to conduct an ecological risk assessment for microplastics in the San Francisco Bay. This study also lays the groundwork for future ecological risk assessments of microplastics and will identify key uncertainties that need to be addressed.

### *1.2 Introduction to Microplastics*

#### *1.2.1 Definition of Microplastics*

Currently, there is not an agreed-upon definition for microplastics, however it is important that a single definition is used for the purposes of this risk assessment. I will define microplastics in accordance with the San Francisco Estuary Institute’s (SFEI) definition developed for their recent microplastic monitoring study in San Francisco Bay. In this definition, they distinguish between microparticles and microplastics. Microparticles are smaller than 5 millimeters in at least one dimension. Particles that are smaller than 0.1 micrometers are considered

nanoparticle. In order to confirm whether the microparticle is a microplastics, Raman or Fourier Transform Infrared (FTIR) spectroscopy must be used to determine if the composition is a synthetic polymer of anthropological origin (Sutton et al. 2019). Examples of synthetic polymers that are included in this definition are polyethylene, polypropylene, polyamide, polystyrene, polyethylene terephthalate, and polyvinyl chloride, just to name a few. Synthetic rubber from sources such as tire wear particles is also included in this definition. Shapes are also specified and include fragments, sphere/pellets, films, foams, and fibers. Naturally derived particles, even if they have been modified with synthetic additives of anthropogenic origin (e.g., synthetic dyes), will not be considered plastic particles (Sutton et al. 2019). I will refer to microparticles, microplastics, and tire wear particles (TWP) throughout this document as they related to the above definitions. I will use microplastics as a more general term that includes TWP and will only delineate between the two when it becomes necessary.

California is one of the first jurisdictions to officially approve a definition for microplastics. I have chosen to use the SFEI definition for microplastics rather than California's definition because the monitoring data use for this risk assessment was collected and analyzed by SFEI. The definition being used for this thesis differs from the State of California's Definition of Microplastics in Drinking Water (CSWRCB 2020) in two ways:

- The California State Water Resources Control Board definition specifies that the plastic particles must be smaller than five millimeters in all three dimensions whereas the SFEI definition specifies that they must be smaller in at least one dimension
- The California State Water Resources Control Board definition specifies that particles that are derived naturally but have been chemically modified are considered microplastics. In contrast, SFEI specifies that naturally derived particles that have been chemically modified with synthetic additives, are not considered microplastics.

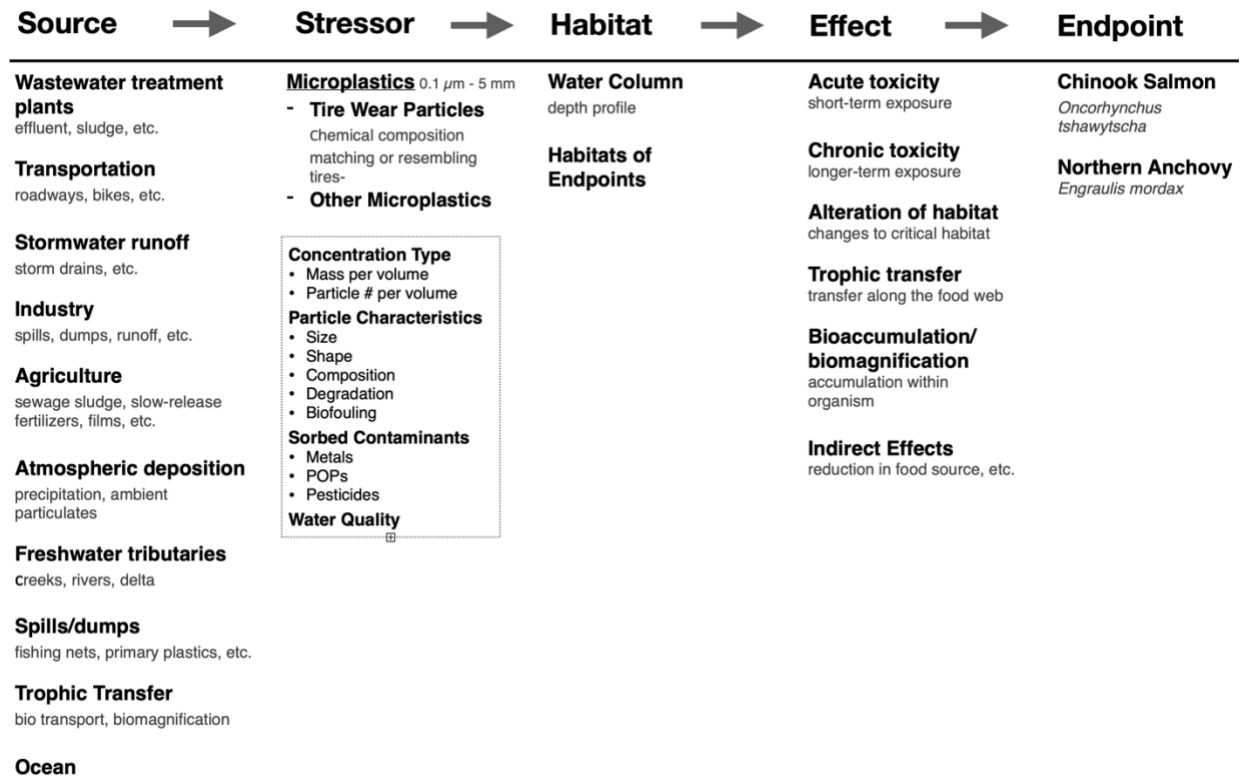
### *1.2.2 Characteristics*

Plastic is a synthetic polymer that is most commonly made with large chains of hydrocarbons formed from petroleum products. However, manufacturers will often add other chemicals into the plastic to allow it to have different properties (e.g., adding colorants to achieve different colors or fire retardants to reduce flammability)(Andrady and Neal 2009). Microplastics come in wide variety of shapes, sizes, colors, and composition all of which can impact exposure and toxicity (Jeong et al. 2016, Fisner et al. 2017, Gray and Weinstein 2017). These properties may also affect degradation, sorption of contaminants, and the likelihood of ingestion. Effects of water quality parameters such as temperature (Kolomijeca et al. 2020), pH (Bakir et al. 2014), and salinity (Hartmann et al. 2017) have also been shown to affect concentrations and types of chemicals that leach out of microplastics. Other environmental effects such as degradation, could change microplastic toxicity and bioavailability (Hartmann et al. 2017). This includes photo, mechanical (Kolomijeca et al. 2020), and biotic degradation. Any model that is used for microplastic risk assessments must include the characteristics of microplastics and the effects from the environment mentioned above. These characteristics are currently included in the conceptual model (Figure 1) used as framework for this risk assessment case study.

### *1.2.3 Abundance*

Increased awareness of microplastics has prompted more monitoring studies to begin looking for them all over the world. Results of these studies suggest that microplastic are present near both urban and non-urban areas (Yonkos et al. 2014). Microplastics have been identified in a wide range of environments including freshwater (Alimi et al. 2018, Li et al. 2018), marine (Auta et al. 2017), and terrestrial ecosystems (He et al. 2018). TWP in particular have been identified as a higher percentage of the microplastics found in a several field studies (Sutton et al. 2019, Goßmann et al. 2021). Estimates indicate that TWP are being released into the environment from roadways in large concentrations (Wagner et al. 2018). The abundance would suggest that

the opportunities for exposure between organisms and TWP may be quite high. Increased exposure of aquatic organisms to TWP also increases the likelihood of human exposure through seafood (Rochman et al. 2015).



**Figure 1.** Broad conceptual model micro in the San Francisco Bay.

### 1.2.4 Effects

Toxicity caused by TWP may occur through different routes of exposure including ingestion and respiration. Additives leach out TWP into the water, sediment, or organism. Exposure to either the particle or the leachate causes toxicity. For some organism, such as Coho salmon, the leachate is the primary cause of toxicity and has a significant impact on survival (Tian et al. 2022). Other organisms do not appear to be as sensitive to the leachate (McIntyre et al. 2021). For these organisms, both the particles and leachate cause toxicity (Cunningham et al. 2022)

but it is relatively minimal when compared with Coho salmon. Chemicals and microbes can sorb to microplastics. In this way, microplastics can act as vectors for both biotic and abiotic contaminants (Bakir et al. 2014, Hartmann et al. 2017, Yeo et al. 2020). Although there is debate about the degree to which these contaminants affect toxicity, it is an important factor that must continue to be explored and included in risk assessments where possible. Most toxicity studies on TWP, expose organisms to plastic leachates and relatively few have exposed organisms directly to particles. Additionally, techniques for measuring the effects of microplastic exposure have also varied immensely, making it difficult to draw conclusions about the toxicity of TWP to organisms.

### *1.3 Previous Risk Assessments for Microplastics*

Although several studies have been published that purport to be microplastic risk assessments, they fall short of the requirements for an ecological risk assessment defined in National Academies of Sciences, Engineering, and Medicine. A risk assessment must be probabilistic, use regional values and management goals to determine endpoints, and include an evaluation of the uncertainty involved in model (NASEM 2016).

Wik and Dave (2009) conducted a risk assessment for TWP using limited data on toxicity, environmental concentrations, and exposure. Everaert et al. (2018) used modeled concentrations of microplastics in the marine environment to determine the present and future global impact of buoyant microplastics given various plastic waste production scenarios. Everaert et al. (2020) used measured concentrations to assess percentage of risk to the global oceans' surface from buoyant microplastics. Tamis et al. (2021) focused on TWP, and other contaminants associated with road runoff using estimated and measured concentration in European waters. Wik and Dave (2009), Everart et al. (2018), Everart et al. (2020), and Tamis et al. (2021) all used a non-probabilistic approach to conduct their assessments. Variability and



uncertainty are minimally considered and reported when they are included at all. Adam et al. (2018, 2021) performed a probabilistic risk assessment for microplastics in marine waters however probability distributions were only included in a small portion of the overall analysis.

All five studies concluded presently a low impact from microplastics with the exception of areas that have acted as sinks for microplastics. However, they all used the quotient method to estimate risk which relies on predicted no-effect concentrations and/or species sensitivity distributions. Quotients are not probabilistic, they use of a single number either as a denominator or numerator underrepresents variability and may not adequately describe an exposure-response relationship (Dale et al. 2008). Even when probabilistic data is used for quotients, variability and probability distributions are immediately reduced to a single number or a range of numbers. Additionally, the use of the Species Sensitivity distribution assumes that the hazardous concentration for five percent of the species (HC5) is a meaningful attribute. Although widely used to approximate impact, the HC5 lacks specificity. Species Sensitivity distribution were originally developed for determining water quality benchmarks parameters and are limited in their usefulness for risk assessments (Fox et al. 2021).

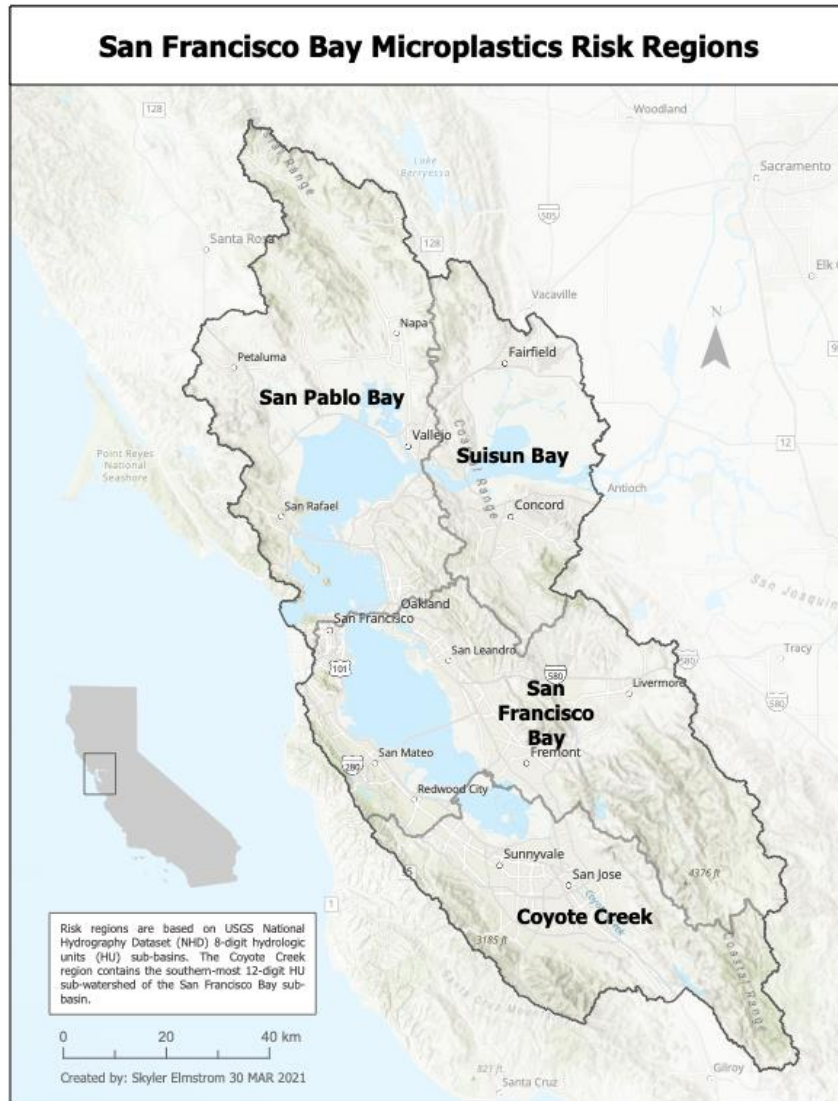
These studies also conducted their assessments on global or continental-scale which makes it nearly impossible to consider microplastic concentrations gradients that may exhibit contagion in the distribution (Everart et al. 2020). The uncertainty in the exposure estimation is likely to be underestimated. In addition, because there is no single regulatory body for these large spatial areas, these studies neglected to focus on specific management goals and endpoints when considering risk of impact. Xu et al. (2018) also conducted an assessment for measured concentrations of microplastics in the Changjiang Estuary in China. Their study is geographically constrained which allowed them to conduct a finer grain assessment however, they never actually calculated risk. Instead, they performed a hazard assessment to compare

the relative concentrations of microplastics in the Changjiang Estuary to that of the adjacent sea. A difference in exposure does not necessarily mean an increase in risk depending on the exposure to the key endpoints specific to that habitat. A hazard assessment is a useful initial step, but it does not constitute a comprehensive risk assessment. Although attempts have been made, an ecological risk assessment has not yet been conducted for microplastics up to the standards outlined by NASEM.

#### *1.4 Case Study: San Francisco Bay*

The study site selected for this ecological risk assessment is the San Francisco Bay. This site was selected because of the availability of microplastic data from recent monitoring studies (Sutton et al 2016, Sutton et al. 2019) conducted through the SFEI. I have generously been granted access to this data and have worked closely with the researchers at SFEI. Data on contaminants, water quality, habitat, and species parameters are also prevalent for a large temporal and spatial range for the San Francisco Bay area through databases such as the California Environmental Data Exchange Network (CEDEN) and California Surface Water (SURF). Data availability is critical in formulating a comprehensive and accurate model, therefore, a site that is rich in relevant data is ideal.

San Francisco Bay, located just north of central California (Figure 2), is one of the largest estuaries on the west coast of the United States (CSCC 2010). Approximately forty percent of the fresh water moving through California goes through the San Francisco Bay, draining from Sierra Nevada Mountain range into the Sacramento-San Joaquin River Delta before gathering in the bay (SFBCDC 2020). The San Francisco Bay is also connected to the Pacific Ocean, allowing for transfers of nutrients, organisms, and contaminants between the two bodies of water. The Bay is made up of 4 major basins referred to as the South Bay, Central Bay, San Pablo Bay, and Suisun Bay.



**Figure 2.** A map of the study site divided into risk regions. Boundaries for risk regions were developed based on hydrological unit code 8 watershed boundaries.

#### 1.4.1 Wildlife and Habitats

The San Francisco Bay provides critical habitat and migration routes for a wide range of organisms. Two-thirds of California’s salmon pass through the bay as they migrate to and from their spawning grounds in the creeks and tributaries of the Sacramento-San Joaquin River Delta (USEPA About the Watershed). This includes chinook salmon, steelhead trout, and sturgeon. A variety of fish and invertebrates, such as the native Olympia Oysters and California mussels,

use the San Francisco Bay as a year-round habitat. Dungeness crab, California halibut, and other important fishery organisms use the bay as nurseries, allowing young organisms to grow and feed in the relative safety of the bay (CSCC 2010). These fish and invertebrates also provide food for larger marine mammals and fish such as seals, sharks, porpoises, and gray whales. San Francisco Bay is a critical wintering stop on the Pacific Flyway, where migrating birds can rest and eat before they continue their journey (SFBCDC 2020). Many unique habitats are present in the San Francisco Bay, including tidal marshes and salt ponds, provide critical locations for wildlife to grow, feed, and nest (CSCC 2010).

#### *1.4.2 Human activities and inputs*

San Francisco Bay is a major urban, industrial, marine, and recreational hub. Nine counties and more than 40 cities containing approximately 7.75 million people in total, surround the San Francisco Bay (SFBCDC 2020). These diverse communities rely on the San Francisco Bay for a wide variety of ecosystem services including food, transportation, jobs, tourism, recreation, and cultural interests. Land use around the bay includes high density and suburban housing, agriculture, public and protected lands, and industrial and commercial infrastructure (SFBCDC 2020). The combination of community, industrial, agricultural, and environmental interests that merge around the San Francisco Bay results in a diverse set of stakeholders and interest groups with converging and diverging goals.

#### *1.4.3 Sources of Microplastics*

There are many potential sources of microplastics in the San Francisco Bay. Water bodies in close proximity to high density urban and industrial centers are likely to have higher concentrations of microplastics than more rural areas (Yonkos et al. 2014). Recent biomonitoring studies for microplastics have confirmed their presence in San Francisco Bay water, sediment, prey fish (Sutton et al. 2019) and bivalves (Miller et al. 2017). Many of the

concentrations were found to be higher than other urban locations in North America (Sutton et al. 2016). Studies looking for potential sources have identified microplastics in wastewater effluent discharging directly or indirectly into the San Francisco Bay (Mason et al. 2016, Sutton et al. 2016). Microplastics in wastewater treatment plant discharge has been recorded in other studies (Carr et al. 2016). Sutton et al. (2019) identified urban stormwater runoff as another large source of microplastics in the San Francisco Bay. They found that the majority of microplastics entering the San Francisco Bay were black rubber that were likely TWP. Although not yet confirmed, other likely sources of microplastics into the San Francisco Bay include precipitation/atmospheric fallout (Dris et al. 2016), delta/other freshwater inlets (Vermaire et al 2017, Alimi et al. 2018), and spills/dumps (Galafassi et al. 2019). The Pacific Ocean might also be a source of microplastics in San Francisco Bay due to ambient concentrations of microplastics in the open ocean (Pan et al. 2019).

### *1.5 Ecological Risk Assessment*

Ecological risk assessment was developed to create tools that use data-driven models to help guide decision-makers when making management and regulatory decisions. They used scientific data and modeling to calculate the impact on predetermined endpoints. Endpoints are derived from cultural and social values that are linked to the ecosystem of interest and connected to regionally specific ecosystem management goals (Landis and Weigers 2005). Ecological risk assessments as defined in the 1993 and 1998 USEPA framework (USEPA 1993) and guidelines (USEPA 1998), have been used to analyze the relationship between a stressor and an endpoint in a contaminated ecological structure. However, the approach outlined in the USEPA framework has some limitations when the assessment of risk is taking place at a larger scale with multiple stressors. Ecological structures are often much more spatially complex, containing multiple stressors, receptors, and habitats in one region. Therefore, there was a need to develop a method of ecological risk assessment for multiple stressors in complex ecological

structures. Wiegiers et al. (1998, Landis and Wiegiers 1997) developed the Relative Risk Model (RRM) in which a study area is broken down into smaller subregions and risk is determined for the whole study using rankings (Landis 2021). Landis and Ayre (2012) further refined this method into a framework called the Bayesian network relative risk model (BN-RRM) for assessing risk at a regional scale using Bayesian networks rather than ranks. I have chosen to use the BN-RMM because the large scale of the San Francisco Bay requires a method that is able to consider multiple habitats, stressors, and endpoints.

### *1.5.1 Bayesian Networks*

The Bayesian network relative risk model framework uses Bayesian network (BN) models to calculate probabilistic risk. They are a powerful tool for understanding relationships between variables in complex systems using probability distributions. BNs are acyclic graphs made up of nodes linked by probability relationships represented as arrows between the nodes (McCann et al. 2006). The relationships are informed by conditional probability tables (CPT) that represent dependent relationships between variables (Ayre and Landis 2012). BNs are particularly useful for modeling the complex causal relationships between variables in ecological structures (McCann et al 2006). Although BNs have been used widely for many years in fields, such as medicine and artificial intelligence (Barton et al 2012), their use in the environmental science is relatively new (Aguilera et al. 2011). Even more recently Bayesian networks have been applied to ecological risk assessments in a wide range of subjects (Kaikkonen et al. 2021). Bayesian networks are a uniquely good choice for ecological risk assessments because different types of information can be included in them without increasing the uncertainty of the model (Kaikkonen et al. 2021). Values can be entered into the Bayesian Networks that are different than those found in the actual data to hypothesize potential affect. States in the output nodes can be selected to represent various management or mitigation scenarios and the required input can be determined. They also allow for the inclusion of new information and data as they become

available, increasing their usefulness as a long-term management and decision-making tools. All variables and relationships in a Bayesian network model are clearly defined and illustrated in the model, allowing for transparent and unambiguous communication of results (Chen and Pollino 2012).

### *1.5.2 Bayesian Network Relative Risk Model*

The BN-RMM is a probabilistic, spatially specific framework for assessment ecological risk. It was designed to consider risk at a large spatial scale where multiple habitats, endpoints, and stressors are involved in complex ecological systems. Although one stressor may be the focus of an assessment, in this case, TWP, additional stressors can be included if they have an effect on the endpoint or the stressor of interest. The BN-RRM has the capability to consider variation in risk over large temporal and spatial ranges. After a study area is chosen, a conceptual model is developed that outlines the causal relationships between stressors and impacts on management endpoints. A Bayesian network is then constructed based on the initial conceptual model and available data for the study area. The selected study area is then divided into smaller region down called risk regions that are based on natural (e.g., watersheds, habitats) and socially constructed (e.g., management areas, landownership) boundaries. The Bayesian network is then parameterized with data from each risk region so that spatial variations in risk can be assessed. The BN-RRM has successfully been used as an ecological risk assessment and management tool in a wide variety of applications since its development in 2012 (Landis 2021).

### *1.5.3 Convergence in Ecological Risk Assessment*

Ecological risk assessment relies on the knowledge and data of many other fields of study. A robust risk assessment will combine data and expertise from toxicology, chemistry, environmental science, statistics, ecology, and data science just to name a few, with the goal of

supporting decision-makers and regulatory bodies. The BN-RRM is an ideal framework for supporting and highlighting convergence in the risk assessment process as each node in the model can represent data and knowledge from many different fields and the arrows represent the converging relationships (Landis 2021).

### *1.6 Study Objectives*

The main objectives of this study are:

- Conduct an ecological risk assessment for microplastics using the San Francisco Bay as a case study.
- Create a framework for microplastic risk assessment that can be used for future case studies in other regions.
- Identify the uncertainties and data gaps involved in determining the risk of microplastics in the San Francisco Bay.
- Show that it is possible to perform a spatially specific, quantitative ecological risk assessment for microplastics given the data that is currently available

### *1.7 Summary of Findings*

A broad summary of the findings from this study are:

- A spatially specific, quantitative ecological risk assessment for microplastics is possible given the data that are currently available.
- TWP present a relatively low risk to out-migrating Chinook salmon and juvenile Northern anchovy in the San Francisco Bay.
- Season is more important to output in the fish affected nodes than TWP concentration.
- In high concentration scenarios, acute exposure still had a relatively minimal effect on the fish output node.



- Future microplastic and nanoplastic toxicity studies should prioritize data collection sufficient to for dose-response modeling.

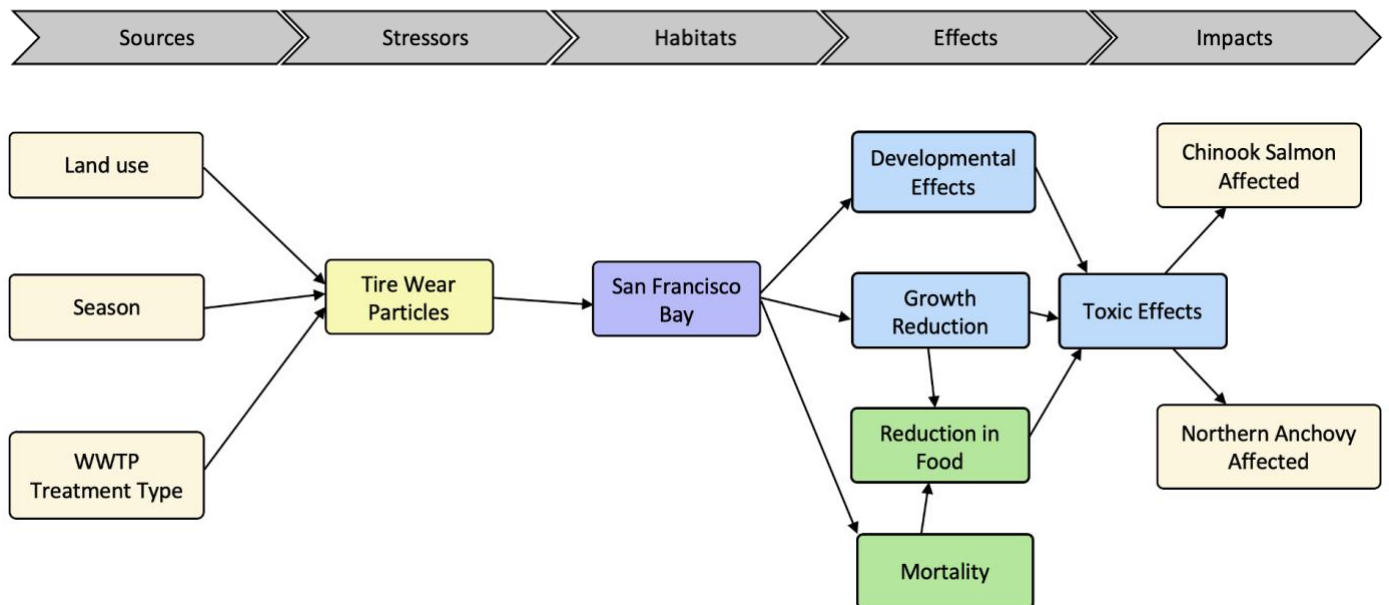
## **2. Methods**

### *2.1 Study Area*

The study area includes the South Bay, Lower Bay, Central Bay, San Pablo Bay and Suisun Bay basins. The outer boundary of the study area follows the California State Water Resources Control Board Region 2 boundaries (Figure 2). The only exception to this is along the eastern portion of the study area where the study area boundary uses the Hydrological Unit Code (HUC) level 8 sub-basin boundaries. I chose this outer boundary because HUC boundaries represents a large watershed boundary, and the California State Water Resources Control Board Region 2 boundaries represent a regional regulatory boundary. Both types of boundaries are important when considering a region-scale analysis of risk. The study area was then broken down into four risk regions based on HUC level 8 sub-basin boundaries: Suisun Bay, San Pablo Bay, San Francisco Bay, and Coyote Creek (Figure 2). These boundaries were chosen because watershed characteristics such as size, land use type, and so on. have the potential to effect microplastic abundance and type (Su et al. 2020).

### *2.2 Conceptual Model*

The first step in model construction is to use the framework of the BN-RRM to create a conceptual model that places the stressor into context within the system (Figure 3). More specifically, the framework for the conceptual model illustrates the causal relationships between the sources of the stressor, characteristics of the stressor, habitat of the endpoint, effects from the stressor to the endpoints, and impacts to endpoints.



**Figure 3.** Box and arrow conceptual model used to build the Bayesian network model. The framework from the broader conceptual model (Figure 1) is retained in this figure. Beige colored boxes represent input and output variables. The input variables are sources, and the output variables are impacts on the endpoints. The yellow box represents stressor concentrations. The purple box represents habitat of the endpoints. The blue boxes represent fish toxicity responses, and the green boxes represent invertebrate toxicity responses.

### 2.2.1 Sources and Stressors

I determined sources of MP based on studies specific to the study site. These sources include wastewater treatment plants (WWTP) (Sutton et al. 2016, Sutton et al. 2019, Zhu et al. 2021) and stormwater runoff (Sutton et al. 2019, Werbowski et al. 2021, Zhu et al. 2021). Additional sources were added based on studies that were not specific to the study site but other likely sources of MPs into similar regions. These sources include atmospheric deposition (Dris et al. 2016, Wright et al. 2020), agriculture (Grbić et al. 2020), industry (Zhou et al. 2020), freshwater tributaries (Leads and Weinstein 2019), transportation (Unice et al. 2019), spills/dumps (Lusher and Pettersen 2021), and trophic transfer (Carbery et al. 2018, Nelms et al. 2018). Additionally, I included the ocean as a potential source given that the San Francisco Bay is directly connected to the ocean through a large channel.

Microplastics are a complex group of contaminants, requiring an approach that considers the many variables that may affect the overall risk they present to endpoints. Therefore, I have included particle characteristics, sorbed contaminants, and water quality parameters in the conceptual model. Particle characteristics such as size (Jeong et al. 2016, Gray and Weinstein 2017, An et al. 2021), shape (Gray and Weinstein 2017), composition (Renzi et al. 2019), degradation (Zou et al. 2020), and biofilm (Johansen et al. 2019) all have the potential to affect the toxicity, transport, and uptake of the microplastic particles. Contaminants such as pesticides (Wang et al. 2020), pharmaceuticals (Puckowski et al. 2021), and metals (Guo et al. 2020) can sorb and desorb to microplastics and may affect the overall toxicity. Additionally, it is important to consider the environment and how this could affect exposure and toxicity. Some of these environmental factors include water quality parameters such as temperature (Bakir et al. 2014, Kolomijeca et al. 2020), pH (Bakir et al. 2014), and salinity (Hartwell et al. 2000). Finally, contaminants and water quality parameters may also indirectly impact the effects of microplastic exposure by changing an organism's prevalence in that environment or exposure response ability.

### *2.2.2 Effects*

The effects were chosen for this conceptual model based on observed and hypothesized effects in microplastic literature. Direct acute and chronic toxicity are the most common types of studied effect. Alteration of habitat has been examined in a handful of laboratory studies (Green et al. 2017, Corinaldesi et al. 2021) but has yet to be tested in the field. Trophic transfer has been demonstrated in laboratory experiments (Athey et al. 2020, Hasegawa and Nakaoka 2021, Stienbarger et al. 2021) and has been hypothesized in the field (Nelms et al. 2018). Bioaccumulation (Goswami et al. 2020) has been recorded in tissues in organisms and

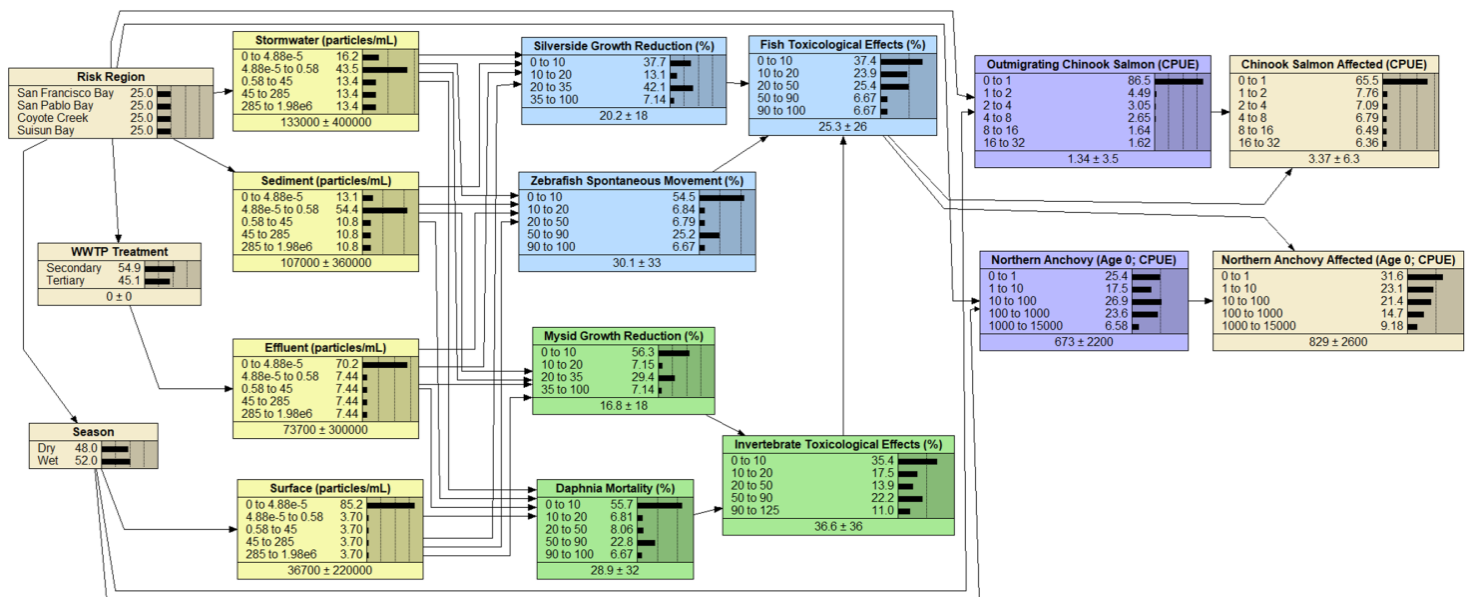
biomagnification has been hypothesized for organisms based on result of trophic transfer studies (Farrell and Nelson 2013).

### *2.2.3 Endpoints*

Endpoints can be broken down into entities, the valued ecological organism or service, and attributes, the characteristic of the entity that risk is being assessed for (USEPA 1998). Entities and attributes are chosen based on ecosystem resources that are considered important to stakeholder and decision makers within the study area. Two fish species were selected as the entities, Chinook salmon and Northern anchovy. The attributes are any toxic effects to these fish caused by TWP exposure. These fish species spend time in the San Francisco Bay during critical life stages and have substantial importance to the region. Additionally, both species are migratory and spend much of their life outside of the San Francisco Bay region. Therefore, impacts to these fish populations that occur within the San Francisco Bay have the potential to effect populations outside the study area as well. Chinook salmon and Northern anchovy are historical, ecological, commercial, cultural, and economic significance in the region.

Chinook salmon migrate through the San Francisco Bay to and from their spawning grounds in the San Juaquin and Sacramento rivers and tributaries. Historically there have been four runs of Chinook salmon: fall, late fall, winter, and spring. However, currently most of the migrating fish are part of the fall-run (Jahn 2011). Specifically, I will be evaluating risk to juvenile out-migrating Chinook salmon because recent analysis suggests that outmigration survival through the San Francisco estuary might have a larger influence on overall population dynamics and success than variables effecting adults in their marine environment (Michel 2019). Chinook Salmon were chosen as endpoint because of their considerable historical and present importance to the culture, economy, (Yoshiyama 1999) and ecosystems in the San Francisco Bay.

There are three subpopulations of Northern anchovy along the west coast of North America. The central subpopulation ranges from Point Reyes, California to Punta Baja, Baja California (Kuriyama et al. 2021) which includes the study area. As the most abundant fish species in the San Francisco Bay, Northern anchovy were chosen primarily because of their importance as forage fish, supporting a diverse ecosystem as a primary food source for many organisms. Northern anchovy and other small planktivore fish, make up approximately 48% of Chinook salmon diet by weight (Default et al. 2009). Additionally, while demand for Northern anchovy is relatively low compared to historical numbers, they are still caught for bait, human consumption, and fish meal (Kuriyama et al. 2021).



**Figure 4.** Bayesian network model for tire wear particles in the San Francisco Bay. Beige colored nodes indicate input and output nodes. Yellow nodes are environmental concentrations parameterized with SFEI microplastic monitoring data. Green nodes are invertebrate toxicity nodes and blue nodes are fish toxicity nodes parameterized with OSU toxicity data. Purple nodes are fish abundance nodes parameterized with CDFW data converted to CPUE.

### 2.3 Development of the Bayesian Network Relative Risk Model

In this step, the conceptual model developed for the stressor is used to guide the development of a Bayesian network model (Figure S22) using Netica 6.07 Software (Norsys Software Corp

2020). Ideally, all variables included in the conceptual model would also be included in the Bayesian network model, however this is generally not possible. Data availability is the limiting factor and is often not available for many of the variables included in the conceptual model. Below I will discuss which variables were included and how the Bayesian network model was parameterized with available data. I have chosen to create two Bayesian network models: one for TWP and one for all other plastics. This is due to likely differences in sources, toxicity, and transport. In this report, I focus on the tire wear particle Bayesian network (TWP-BN)(Figure 4).

### *2.3.1 Parameterizing the TWP-BN*

#### *2.3.1.1 Environmental Concentrations*

Data collected by SFEI were used to parameterize the nodes representing environmental concentration of TWP in the environment. Data were collected in five different environmental compartments across the study area: stormwater runoff, wastewater effluent, surface water, sediment, and prey fish. Samples were counted and a subset of particles were analyzed using FTIR or Raman spectroscopy to chemical composition. The resulting report (Sutton et al. 2019) from the SFEI study contains further information about methods and results. The SFEI was unable to confirm that any particles were from tires, however, they were able to determine that some particles were composed of rubber or potential rubber. Data from these two categories were added together and used to parametrize the TWP Bayesian network for each environmental compartment. Samples from prey fish were not included in this model because only a single rubber or potential rubber particle was found in any of the prey fish samples. While this could indicate an actual lack of ingestion of these particles, there is evidence to suggest that fish in similar environments do ingest TWP at low concentrations (Parker et al. 2020). Additionally, sampling data for prey fish were only available for two of the four risk regions and therefore a spatial analysis of risk for the whole study area would not be possible. Given the possibility that particle counts of TWP in prey fish were estimated too low in this study and a

general lack of data on fish ingestion outside of the laboratory setting, I chose to take the approach that assumes that fish ingest all potential particles coming into the ecosystem.

SFEI also collected data on season for the surface water samples and wastewater treatment type for the wastewater treatment effluent samples. I have included this as part of the model to show how these variables might also influence risk. The data was input into the model using casefile learning, which is a feature in Netica that allows CPT to be populated using a dataset (Norsys Software Corp 2020). Uncertainty can also be incorporated using this method.

Sediment data was collected in grams per liter dry weight, but I have converted it to particles per milliliter using the conversion factor 0.5 kilograms per liter. This was the sediment conversion factor suggested by the SFEI (D. Lin, personal communication, February 16, 2022).

#### 2.3.1.2 Effects

The toxicity data used to construct the toxicity nodes were produced by the Harper and Brander laboratories at Oregon State University in collaboration for this risk assessment. The species used for toxicity testing were *Daphnia magna*, zebrafish (*Danio rerio*), inland silverside (*Menidia beryllina*), and mysid shrimp (*Americamysis bahia*). The data represented in this model are from acute exposures to tire particles in the microparticle scale. Particles in this section will be referred to as tire particles (TP) rather than TWP because they were produced via cryomilling rather than natural tire wear. Only responses that showed a clear concentration-response relationship with TP concentration were included in the model and discussed in this paper.

Cunningham et al. (2022) exposed zebrafish embryos to twelve TP concentrations ranging from  $1.0 \times 10^4$  to  $1.29 \times 10^6$  particles/ml or approximately 0.63 to 81.18 mg/L. Exposures occurred up to 120 hours post fertilization (hpf) and behavioral and developmental endpoints were assessed

at 24 hpf and 120 hpf. The only response that showed a clear concentration-response relationship was for spontaneous movement at 24 hpf. They also exposed neonatal *D. magna* to six TP concentrations ranging from  $1.3 \times 10^5$  to  $8.59 \times 10^5$  micro particles/ml or approximately 8.18 to 54.05 mg/L. Exposures took place for 48 hours and mortality and immobilization were recorded at 24 and 48 hours. The only response that showed a clear concentration-response relationship was for mortality at 48 hours.

Siddiqui et al. (2022) exposed silversides and mysid to three different concentration of TP for seven days and ninety-six hours respectively. The three different concentrations were 60, 6000, and 60000 particles/mL or 0.0038, 0.378 and 3.778 mg/L. Behavioral endpoints and aspect ratio were measured at the end of the exposure time. For both silversides and mysids, the only response that showed a clear concentration-response relationship was aspect ratio of the organisms.

Using the data from Cunningham et al. (2022) and Siddiqui et al. (2022), I created concentration response models using the *drc* package in R Version 4.0.5 (RStudio 2021) for each species and the associated response. The selection of the model type was determined based on which model makes the most sense given the type of data being modeled. The lowest Akaike information criterion values derived using the *mselect* function in R studio, was used to help further determine which model would be the best choice. I then fit a model to the data, derived the equation for each model, and inputted them into their associated toxicity node in the Bayesian network. Netica is then able to use the equation to develop a CPT for the node that allows the Bayesian network to calculate specific outputs give various inputs.

I converted final aspect ratio data for mysids and silverside to growth reduction percentages. Growth reduction is an easier metric to interpret and can be used to predict impact to fecundity



and survival success. To calculate growth reduction, I first determined the average final aspect ratio for the control groups for each organism. I then created an equation that uses the model output at zero particles per liter to divide final aspect ratios in the test concentration groups. This value can then be subtracted by one to determine a value for growth reduction in decimal form. I then multiplied by one hundred to convert to a percentage. This is represented in Eq. 1:

$$\text{Growth Reduction} = 100 \left( 1 - \frac{\text{Final AR}}{\text{Average AR of controls}} \right) \quad (1)$$

To input this into Netica, Eq. 1 was modified to use the concentration-response relationship equation for final aspect ratio for each organism. Eq. 2 shows the concentration-response model for silverside final aspect ratio and TP. Eq 1. was combined with Eq. 2 to create Eq. 3. Eq 3 was input into Netica to calculate growth reduction for Silversides where the average of controls was 0.480667.

$$F(x) = 0.321372 + (0.480667 - 0.321372) * \left( \exp \left( -\frac{X}{119.773263} \right) \right) \quad (2)$$

$$\text{Growth Reduction} = 100 \left( 1 - \frac{0.321372 + (0.480667 - 0.321372) * \left( \exp \left( -\frac{X}{119.773263} \right) \right)}{0.480667} \right) \quad (3)$$

In Netica, the additive value of TWP concentrations from the environmental concentration nodes are input for X and growth reduction can be calculated.

Next, I summarize the toxic effects in the intermediate nodes. Intermediate nodes are useful in Bayesian networks because they help to reduce the number of input nodes going into

downstream nodes allowing smaller and more comprehensible CPTs (Chen and Pollino 2012). To summarize the toxicity information, I used weighted values to modify the effect response as in Liu et al. (2013) who weighted different responses in zebrafish to illustrate relative importance to biological impact. The weighing included spontaneous movement which was weighted at 0.2 out of 1 and mortality which was weighted 1 at 24 hpf and 0.95 at 120 hpf. Based off this paper, I weighed zebrafish spontaneous movement at 0.2 and daphnia mortality at 0.95. Similarly, I elicited expert judgment from Dr. Stacey Harper (S. Harper, personal communication, March 23, 2022) to weigh the other responses values used in the model out of 1. Growth reduction was weighed at 0.2 for both mysid and silversides. Reduction in food as a measurement of invertebrate effect was weighed at 0.1.

#### *2.3.1.3 Endpoints*

Data from the California Department of Fish and Wildlife (CDFW and IEP 2021) was used to parameterize the species abundance nodes for our chosen endpoints. Fish catch data was available from otter trawls and midwater trawls. I corresponded with Kathryn Hieb, a senior marine biologist with the CDFW, and she advised that only midwater trawl data should be used because it is more accurate than otter trawl data for pelagic and strongly schooling fish species (S. Harper, personal communication, December 2, 2021). Data was queried for Chinook salmon and Northern anchovy from January 1<sup>st</sup>, 2015 to July 27<sup>th</sup>, 2021 from the midwater trawl dataset. For Northern anchovy, total catch data is available as well as data broken down by age of fish. The included age categories are age 0, fish above the minimum length but less than a year old, and age one plus, all fish caught that were not in the age 0 category. For Chinook Salmon, data was only available for total catch of out-migrating smolt. The CDFW recommends that catch data be converted to catch-per-unit-effort (CPUE) when comparing fish abundance temporally or spatially (CDFW 2021). To calculate CPUE, I used the equation suggested by the CDFW for midwater trawl data:

$$\text{CPUE} = \left( \frac{\# \text{ caught}}{\text{tow volume}} \right) * 10,000$$

Season was added in as a variable to model fish abundance by using the month the fish sample was collected. November through April were selected as part of the wet season and May through October were selected as part of the dry season. Months were determined to be wet or dry based on average precipitation across the last 10 years (NOAA 2022). The fish abundance nodes were parameterized using the casefile learning function in Netica.

#### *2.3.1.4 Node Discretization*

Before parameterizing the nodes, discrete states must be created for the continuous data. This is usually done based on regulatory values and effect concentrations. Currently, there are no regulatory values, management goals, or benchmarks for microplastics of any type for our study area or any other regions. Therefore, I used effect concentrations for the most and least sensitive responses. I also used the high estimate for floating microplastics in the ocean in 2100 from Everart et al. (2018) and the highest concentration found in the study site. For the fish abundance and fish affected nodes states, a log-scale was chosen for the discretization because it would allow variability in the lower states to be more visible.

#### *2.4 Risk Calculation*

Risk is calculated by comparing different exposure scenarios of the stressor to the management goals for each endpoint organisms. Most management goals are related to population scale effects. The current scope of this model does not capture changes at the population scale for the endpoint organisms, therefore it is not possible to determine how different exposure scenarios may affect populations scale goals. Additionally, management goals for Chinook salmon and Northern anchovy in the San Francisco Bay are complex and often unspecific.

Therefore, I have chosen to calculate risk based on large changes in the distributions in the fish affected nodes.

### *2.5 Sensitivity Analysis*

I performed a sensitivity analysis using the feature in Netica called *Sensitivity to Findings*. This feature allows the user to select a specific node and then calculate which upstream nodes have the most effect on the selected nodes output. The metric used to evaluate sensitivity is mutual information, a measurement of mutual dependence between two variables. Mutual information can then be compared between nodes across the model to determine relative mutual information as a percent for each node. Netica can also calculate sensitive for various model configurations by selecting states in different nodes. I calculated and compared sensitivity for each risk region for the Chinook Salmon Affected node, the Northern Anchovy Affected node, and the Fish Toxicological Effects node.

### *2.6 Counterfactual analysis*

In a counterfactual analysis, values are entered into the model that are different than those found in the actual data to hypothesize potential affect. In this case, I selected states in the TWP environmental concentration nodes to see how this would affect probability distributions in the Chinook Salmon Affect node and the Northern Anchovy Affect node. I decided to have three scenarios: lowest, current, and highest. In the lowest scenario, states in all environmental concentration nodes are select at the lowest concentrations, 0 to  $4.88e^{-5}$ . In the current scenario, none of the states in the environmental concentration nodes are selected. This allows for the comparison of the current environmental concentrations with the higher and lower scenarios. In the highest scenario, states in all environmental concentration nodes are select at the highest concentration in the nodes, 0 to  $1.98e^6$ .

## *2.7 Uncertainty Analysis*

I used the definitions of uncertainty outlined in Regan et al. (2002, 2003) to document uncertainty in this case study. There are two types of uncertainty that are important to recognize in an ecological risk assessment: epistemic and linguistic. Epistemic uncertainty can be further broken down into four categories: measurement error, model uncertainty, systematic error, and natural variation (Regan et al. 2002).

To quantify epistemic uncertainty, I used quantitative measurements where possible and qualitative communication where quantitative measurements are not an option. Quantitative uncertainty is described for every step of the model and quantified as part of the distribution in the final output nodes. Uncertainty is also recorded qualitatively in the results section of this thesis. Linguistic uncertainty arises during communication of methods and results of the model. This can include specificity, vagueness, context dependence, unclear terminology, and ambiguity (Regan et al. 2002). I address linguistic uncertainty by communicating model methods and results in a manner that is transparent and appropriate to the audience. I also communicate all uncertainties and limitations associated with the model.

## **3. Results**

### *3.1 Understanding the Model Output*

The Bayesian Network model can produce four useful types of output. The first is observing the variation in risk by selecting different inputs to determine how this affects the probability distributions in the downstream nodes. The second is the sensitivity analysis, which shows which nodes are most important to the output. The third is the counterfactual analysis where values that are not present in the data are selected as input in the model and distribution

upstream or downstream are analyzed. The fourth is the uncertainty analysis which quantifies uncertainty and indicates where it is present in the model.

### *3.2 Variation in Risk*

The endpoint nodes in this model are Chinook Salmon Affected and Northern Anchovy Affected (Figure 1). These nodes represent the relative abundance of each fish species modified by the effect distribution in the fish toxicity node. Different input nodes in the model can be selected to see how the output in the impact nodes change. Slight variations may not be indicative of real impact because of high uncertainty in the model however, large changes in the node distributions could indicate real affects from TWP concentrations or other variables such as season and risk region. I selected each state in the risk region node and season node to see how it would affect the distributions in the fish affected nodes.

#### *3.2.1 Spatial Variations of Risk*

There is no substantial variation in risk across risk regions for Chinook salmon. By selecting each risk region, distributions in the fish affected node for Chinook shift minimally (Figure S8). The distribution for Coyote Creek is shifted slightly towards a higher number of fish affected.

The risk varied more clearly by risk region for Northern anchovy (Figure S8). Distributions are similar in San Francisco Bay and Coyote Creek. Both risk regions have the highest percent fish affect in the 1 to 10 state. In both San Pablo Bay and Suisun Bay, the distributions are skewed towards the lowest states with Suisun Bay having 49.7 percent and San Pablo Bay having 28.4 percent of fish affected in the 0 to 1 range. When different seasons are selected within each risk region, clear seasonal changes in distributions appear in some risk regions.

#### *3.2.2 Seasonal Variations of Risk*

When the season variable is added in, the degree to which the distributions in the fish affected node differs depending on risk region and organism. For Chinook salmon, there is no clear difference between the wet season and the dry season in number of fish affected (Figure S9). For Northern anchovy, distributions in all four risk regions are skewed lower in the wet season than they are in the dry season (Figure S10). This seasonal effect is most apparent in Suisun Bay and San Pablo Bay.

### *3.3 Sensitivity Analysis*

I performed the sensitivity analysis using mutual information as a measurement of entropy reduction to determine the relative importance each variable in the model has on the two fish affected nodes: Chinook Salmon Affected and Northern Anchovy Affected. I then selected each risk region to see how the relative importance of variable changes across the study area. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the node that the sensitivity analysis was performed for.

#### *3.3.1 Chinook Salmon Sensitivity Analysis*

In all four risk regions, season was either the most important or second most important variable in the analysis (Figure S13). The season variable was the highest for San Pablo Bay. Season was less important in Coyote Creek and Suisun Bay.

Of the toxicity responses, zebrafish spontaneous movement had the highest mutual information in Coyote Creek and Suisun Bay and the second highest in San Francisco Bay and San Pablo Bay (Figure S13). In all risk regions, Silverside growth reduction and Daphnia mortality had the following highest mutual information.

Of the TWP environmental sample matrices nodes, stormwater has the highest percent mutual information followed by sediment, effluent, and surface water respectively (Figure S14). In San Francisco, this pattern is slightly less clear because the mutual information is very low in all four sample matrices.

Wastewater treatment plant treatment type has the lowest percent mutual information in all risk regions (Figure S13).

### *3.3.2 Northern Anchovy Sensitivity Analysis*

In all four risk regions, season has the highest mutual information in the analysis (Figure S15). When compared to Chinook salmon, mutual information is higher for season for the Northern Anchovy Affected node and this is maintained across every risk region.

The percent mutual information for toxicity response nodes (Figure S15) in the Northern anchovy sensitivity analysis have the same pattern found for Chinook salmon sensitivity analysis. Zebrafish spontaneous movement has the highest mutual information followed by Daphnia mortality, silverside growth reduction, and mysid growth reduction.

The sample matrix nodes (Figure S16) also follow a similar pattern to the Chinook salmon analysis where stormwater has the highest mutual information and sediment, effluent, and surface water follow respectively. The only exception is in San Francisco Bay where sediment has the highest mutual information.

### *3.3.3 Sensitivity Analysis for Fish Toxicological Effects Node*

I performed a sensitivity analysis for the Fish Toxicological Effects node to determine how important input variables, such as season and risk region are to the output in this node. All



patterns from this sensitivity analysis are the same as in the previous sensitivity analyses for Northern anchovy and Chinook salmon apart from the season node (Figure S17). The season node has zero percent mutual information in all four risk regions.

#### *3.3.4 Overview of Sensitivity Analysis*

For both organism there is a clear pattern between risk regions where the mutual information from each node apart from season, is proportionally similar, but the magnitude differs. For Chinook salmon in San Francisco Bay, the mutual information for all nodes is very small whereas in Coyote Creek it is much larger (Figure S13 & S14). This is similar for Northern anchovy, however the variation in magnitude is less pronounced between risk regions (Figure S15 & S16) .

#### *3.4 Counterfactual Analysis*

For Chinook salmon, changes in concentration scenarios do not appear to have any substantial effect on probability distributions in the Chinook Salmon Affect node (Figure S11). This is true for all four risk regions. Only very small increases, in most cases less than a percentile, are observed in the highest concentration scenario when compared with the current or lowest scenarios. Patterns seen in other the risk variation results (Figure S8) appear in the results of this analysis as well. Coyote Creek continues to have distributions with a slightly lower frequency of values in the 0 to 1 state.

For Northern anchovy, changes in concentration scenarios have a much more pronounced effect on probability distributions (Figure S12). These changes also are variable by risk region. The lowest concentration scenario is very similar to the current scenario. Patterns in the distributions within risk region are maintained between the lowest and current scenario with a slightly higher frequency of values falling into higher states in the current scenario. In the

highest concentration scenario, probability distributions are all skewed higher than in the current and lowest concentration scenarios.

### *3.5 Uncertainty Analysis*

In the next sections I detail the types of uncertainty involved in this risk assessment and the varying degrees to which uncertainty impacts the results of the model. As described in the methods section of this paper, I am using the definitions for uncertainty from Regan et al. (2002, 2003).

#### *3.5.1 Epistemic Uncertainty*

I used probability distributions for each variable in the model to quantify measurement and systematic error. By including the full distributions rather than a single number, variability that is the result of different types of uncertainty can be delineated. The uncertainty within each probability distribution is carried through the model to the final nodes, Chinook Salmon Affected and Northern Anchovy Affected. The distributions in the final nodes quantify the measurement and systematic uncertainty for the entire model. Probability distribution also demonstrate the uncertainty from natural variation in TWP and fish abundance. This is further illustrated using risk region and season to assess temporal and spatial variations. Model uncertainty is an additional type of epistemic uncertainty.

##### *3.5.1.1 Model Uncertainty*

A lack of microplastic data from every sample matrix in each risk regions is a primary cause of model uncertainty. Where sample numbers are relatively small, uncertainty is higher. In Coyote Creek, there are relatively few data for fish abundance because there are only two CDFW stations in that risk region. The result is an undue influence of states not represented in the data. This is illustrated in the Chinook Salmon abundance node in Coyote Creek (Figure S8).

Because all the values are zero and there are low sample numbers for this risk region, the distribution in the other states in the node are relatively high, therefore over representing the number of fish in that risk region. In this case, additional fish data for Coyote Creek, if available, could be added to the model to reduce uncertainty.

Similarly, for the microplastic dataset samples sizes were very small across all risk regions and sample matrixes. Uncertainty is represented as larger values in states above actual concentrations of TWP found in the study region. In the model this appears as even percentages in the higher states (Figure S9). This value varies by risk region and sample matrix because there are different numbers of samples and therefore different levels of uncertainty. For example, stormwater samples from Coyote Creek have some of the highest uncertainty because there were only two sample collected from that risk region.

Finally, in some cases there are no data present. For example, there were no stormwater samples collected in Suisun Bay (Figure S9). This is illustrated in the model by creating an equal distribution across all states in the stormwater concentration node to indicate that the model does not have knowledge to create a distribution. In both cases, more sampling within that risk region (e.g., Suisun Bay) for the specific sample matrix (e.g., stormwater) will decrease model uncertainty.

A major source of model uncertainty is insufficient data on the actual ingestion of TWP by the fish in this study meaning I was unable to derive a dose-response relationship. This is true both in the laboratory toxicity studies as well as the field samples. In the laboratory data used for this study, organisms are exposed to varying concentrations of tire particles in the surrounding water. Intake of particles was visually confirmed in all organisms however, a link was not drawn between the water concentrations and actual dose for every organism (Cunningham et al. 2022,

Siddiqui et al. 2022). Therefore, it is unclear what the actual dose is and how water concentrations may affect toxicity simply by effecting dose. In the field microplastic data, fish samples were analyzed for particle concentrations but only one rubber particle was found in all prey fish samples. These data were excluded, and no other data was available to indicate field exposure to our study organisms.

Particles from the San Francisco Estuary Institute microplastic monitoring study were analyzed for composition but none of the particles could be confirmed to be from tires. Therefore, for this study I have had to assume that all particles categorized as rubber or potentially rubber are TWP. It is possible that particles in these categories were not all from tires adding uncertainty to the environmental concentrations.

An additional source of model uncertainty arises from using model laboratory organisms to extrapolate effects to different endpoint organism. The field data use for fish abundance were also from a different the age class than those used for laboratory tire particle toxicity tests. Although this is not an uncommon practice in risk assessment, there is always inherit uncertainty when this is done. All organisms tested in the lab were either in eggs or recently hatched neonates and larvae (Cunningham et al. 2022, Siddiqui et al. 2022). In the study area, Chinook salmon are not present in this age class and are generally out-migrating smolt when they pass through the San Francisco Bay. Northern anchovies are present in the study area in multiple age classes including the eggs and larval stages. The Northern anchovy data used to parameterize this model is for fish estimated by the CDFW to be greater than or equal to the minimum length for the Age 0 age class based on length. I have had to extrapolate laboratory toxicity results for age classes not represented in the CDFW fish abundance data used to parameterize this model. As more toxicity studies are conducted for tire particle exposure on

other fish species in a variety of age classes, the model can be updated with this data and uncertainty can be reduced.

In the laboratory toxicity testing, cryomilled TP were used instead of TWP from roadways. Particles generated from actual road wear can be a different shape than particles generated from a cryomilling (Wagner et al. 2018). On average, particles from laboratory generation were also smaller than those collected in the field (Figure S4) because of limitations of collection devices. Differences in microplastic shape (Gray and Weinstein 2017) and size (Cunningham et al. 2022) can affect toxicity and uptake therefore, this is a source of uncertainty in the model. Additionally, new tires were used rather than used tires for toxicity testing. Halle et al. (2021) found that particles from new tires were more toxic than used tire particles. Since most tires in the environment are from used tire, this adds model uncertainty.

### *3.5.2 Linguistic Uncertainty*

There are several sources of possible linguistic uncertainty present in the model. The use of CPUE as the measurement for fish abundance and affect may make it difficult for an audience to understand how it relates to actual numbers of fish. Additionally, epistemic uncertainty in the model can be difficult to communicate. When epistemic uncertainty in a model is incorrectly understood it can result in confusion in interpreting and understanding the model results. Finally, communicating risk to Chinook salmon and Northern anchovy carries a degree of linguistic uncertainty because management goals for these organisms in the San Francisco Bay are somewhat ambiguous. This means that level of risk may be misunderstood or interpreted differently depending on the stakeholder group.

## **4. Discussion**

### *4.1 Variation in Risk*

#### *4.1.1 Spatial Variation of Risk*

The distribution for Coyote Creek is shifted slightly towards higher number of fish affected because of high levels of uncertainty calculated by the model. This is a result of an extremely low sample number for Chinook salmon abundance in that risk region (Figure S4). There are only two CDFW sampling stations in that risk region. When the sample number is low, uncertainty can be high because the system is known to be dynamic and two samples is unlikely to represent these dynamics.

The patterns in Northern anchovy risk variation by risk region can be explained by the relative abundance distributions of Northern anchovy in each risk region. Suisun Bay has the lowest number of Northern anchovies (Figure S5). San Pablo Bay and San Francisco Bay have the highest. Where there are more fish present, there are more fish available to be affected by the presence of TWP. It is important to also note that the Fish Affected node states are discretized in log scale which may make it difficult to see smaller variations in states with higher values (Figure S4).

#### *4.1.2 Seasonal Variations of Risk*

Selecting different seasons in the model does not change the risk in each risk region for Chinook salmon. This would indicate that season is not an important factor for out-migrating salmon in the San Francisco Bay however, Jahn (2011) found that most outmigration took place in the dry season. I defined season based on the last ten years of precipitation data for the study area however precipitation in California is unpredictable in certain months (NOAA 2022). It is possible that the seasonality of out-migrating salmon does not fit distinctly into the wet and dry seasons that I identified. At this time, numbers of chinook salmon are relatively low

compared to other species, therefore some variation could be hidden, making it difficult to see seasonal patterns.

The seasonal changes in abundance for Northern anchovy are most apparent in Suisun Bay and San Pablo Bay (Figure S5). During the wet season there is an increase in freshwater flow from the Sacramento-San Joaquin River Delta resulting in a decrease in salinity in these two risk regions (Cloern et al. 2017). In the San Francisco Bay, Northern Anchovy have responded to a decrease in prey caused by the introduction of *Potamocorbula amurensis*, an invasive clam species from Asia, by shifting their range into higher salinities where primary producers are more abundant (Cloern et al. 2017). Therefore, it is likely that the seasonal changes in Northern anchovy abundance are related to salinity changes in the San Francisco Bay.

## 4.2 Sensitivity analysis

### 4.2.1 Chinook Salmon Sensitivity Analysis

For Chinook salmon, the importance of season in the sensitivity analysis diverges from the findings in the risk variation section where season has no impact on the Chinook salmon endpoint node. This result is possible because a sensitivity analysis determines relative importance compared to other variables in the model. Therefore, while season has little effect on the Chinook salmon endpoint node it has more of an effect than most other variables.

For the toxicological response nodes, zebrafish spontaneous movement had the highest mutual information. The driving factor in the high mutual information of the Zebrafish Spontaneous Movement node is that the concentration response curve model (Figure S19) for this response. It includes higher percentage effected than the growth response models (Figure S18 & S21) which only go up to approximately thirty percent. It is higher than Daphnia mortality (Figure S20) because it is directly connected to the Fish Toxicological Effects node whereas the invertebrate

node is an intermediate node that reduces the influence has on the fish affected nodes (Figure S4).

Patterns in the sample matrix nodes were similar across all four risk regions (Figure S14). The highest concentrations of TWP were found in sediment and stormwater runoff (Figure S2) therefore it makes sense that these two sample matrices have the highest mutual information. However, small sample sizes for sediment and stormwater samples in each risk region (Table S1) could mean that high levels of uncertainty are also driving this result.

TWP numbers are very low in wastewater effluent data (Figure S2). This limits the importance of effluent treatment type to the fish affected nodes in the sensitivity analysis. Particle types that are more prevalent in wastewater treatment effluent may see greater variation based on wastewater effluent treatment type.

#### *4.2.2 Northern Anchovy Sensitivity Analysis*

The varying importance of season to the Northern Anchovy Affected node in the sensitivity analysis can be explained by variations in freshwater flow affecting salinity. This agrees with my previous analysis in the risk variation section that seasonal changes in primary producers caused by salinity changes is an important factor in Northern anchovy abundance in the San Francisco Bay.

For the sample matrix nodes, San Francisco Bay deviates from the other risk regions because percent mutual information is highest for sediment (Figure S15). In all other risk regions, stormwater has the highest percent mutual information. In San Francisco Bay the stormwater node does not have the highest uncertainty of all the sample matrices. The sediment node has the highest uncertainty for that risk region. In contrast, for all other risk regions, stormwater has



the highest uncertainty. This is driving the mutual information up for sediment in San Francisco Bay because the higher uncertainty leads to higher percent likelihood that the concentrations are in the higher states.

#### *4.2.3 Sensitivity analysis for Fish Toxicological Effects Node*

The mutual information for season is zero in the sensitivity analysis for the Fish Toxicological node (Figure S17). This suggests that season plays an important role in fish abundance but not in TWP concentration. The only TWP concentration data with a seasonal component is surface water sample data. Therefore, most of the changes in distribution caused by season were due to changing abundance of fish and not changing concentrations in TWP concentrations.

Seasonal sampling for each sample matrix could change the influence of season in the model. If seasonal sampling data were available for stormwater, it is likely that season would play a more important role in the Fish Toxicological Effects node.

#### *4.2.4 Overview of Sensitivity Analysis*

While there are differences in the sensitivity analysis results for Chinook salmon and Northern anchovy, many patterns are retained between species. The model does not include factors to account for potential differences in Chinook salmon and Northern anchovy sensitivity or likeness to the model organism used, therefore this result is expected. However, future iterations of this model could include variables to represent differences in exposure or sensitivity for each species. Currently, this is not possible given limited data on these topics.

For all sensitivity analyses performed, the toxicity response nodes had higher mutual information than the sample matrix nodes. The sample matrix nodes are further downstream than the toxicity response nodes and therefore have less influence on the final output in the fish affected nodes (Figure S4).

Magnitude changes in mutual information for nodes between risk regions can be attributed to the varying number of fish in each risk region. Fewer fish results in a reduction in magnitude of influence from all the model variables but it does not change which variables have the highest mutual information in each risk region. There are the fewest salmon in Coyote Creek (Figure S4) however low sample numbers from that risk region, cause uncertainty to be high. This skews the probability in the Chinook salmon abundance nodes into the higher states resulting in the appearance of higher abundance in the model. Suisun Bay has the highest number of out-migrating salmon smolt followed by San Pablo Bay and San Francisco Bay. As fish swim farther into the marine part of the estuary their abundance decreases. For Northern Anchovy the pattern is less apparent because their relative abundance is higher in every risk region (Figure S5). This results in lower uncertainty and more normally distributed distributions in the northern anchovy abundance nodes in the model (Figure S6).

#### *4.4 Counterfactual Analysis*

There are very few changes from the lowest concentration scenario to the current concentration scenario for both fish species (Figure S9 & S10). This is because the current scenario has very low concentrations of TWP and is very similar to the lowest concentration scenario. The highest concentrations scenario produces more of a change because it is many times higher than either the lowest or current concentration scenario. Variations in all scenarios are more apparent for Northern anchovy than Chinook salmon because the number of Chinook salmon is much lower than Northern anchovy.

#### *4.5 Uncertainty in the Model*

There are many sources of uncertainty in this risk assessment. Uncertainty is inherent to any ecological model because it is an imperfect representation of a stochastic system. Additionally,

microplastics present their own unique challenges to model. Much of this uncertainty can be attributed to how new the field of microplastics is, how difficult and expensive it is to conduct microplastic environmental monitoring studies, and a lack of quality microplastic toxicity data. The Next Steps sections will outline some ways in which microplastic monitoring and toxicity studies can reduce uncertainty in future iterations of this model or other models built for microplastic risk assessment.

#### *4.6 Summary of conclusions*

At the concentrations found in the San Francisco Bay, TWP present a relatively low risk to out-migrating Chinook salmon and juvenile Northern anchovy. Environmental variables such as season play a larger role in the output of the fish affected nodes than TWP concentration did. At concentrations many times higher than currently found in the environment, acute exposure still had a relatively minimal effect on the fish output node. Higher numbers of Northern anchovy were affected when compared to Chinook salmon. Although uncertainty is high throughout the model a quantitative, spatially specific risk assessment is possible given the current microplastic data that is available. Future microplastic toxicity studies need to focus on developing data for dose-response modeling. Future monitoring studies need to consider how their data will be used in a risk assessment and what entities they are interested in predicting risk for.

#### *4.7 Contributions*

This study gives perspective and context to a contaminant of growing concern to the public. This style of risk assessment allows the integration of multiple stressors and also lists the knowledge gaps that lead to uncertainty. It demonstrates that a quantitative risk assessment is possible and should be prioritized for microplastics. The conceptual model and Bayesian Network create a framework that can be built upon and adapted for different study areas, endpoints, and decision-making goals. The field of microplastics lacks direction resulting in studies that do not contribute

to critical gaps in research. This study and related publications and presentation will help to focus future studies on microplastics to the areas where data is needed the most.

#### *4.8 Limitations*

The scope of this study was limited to the species chosen as endpoints and to acute toxicity responses. It did not consider contaminants or water quality parameters that might affect toxicity or an organism ability to mitigate the effects of exposure to TWP. TWP are only one type of microplastics. They do not represent other microplastics and results should not be extrapolated to other types of plastic. Similarly, this study is specific to the San Francisco Bay and therefore extrapolations of results from this study to other sites or regions are not possible.

Additionally, the scope of the study was limited TWP and does not consider risk involved in other parts of the plastic lifecycle. When considering the overall environmental risk that microplastics presents, it is important to remember the entire lifecycle of plastic. The production and end-of-life phases of the plastic cycle can be highly intensive and produce large amounts of greenhouse gases that contribute to climate change (Cabernard et al. 2021, Ford et al. 2022). Chemical contaminants produced and released during the extraction of oil and production/end-of-life add additional stressors to the environment. The environmental risk of the entire plastic life cycle is not covered in this risk assessment and may not be a necessary part of every risk assessment for microplastics. However, when considering the comprehensive risk of a stressor as complex as microplastics, it is important to remember the bigger picture.

#### *4.9 Next Steps*

This risk assessment was focused on building a spatially specific, quantitative model that could continue to be developed as more information and data become available. In the next steps, I would like to add co-contaminants into the model so that they can be directly compared to TWP

toxicity. I would also like to determine how leachate data might be added into a risk assessment model with particle exposure data. Chronic toxicity data would be a useful addition because many organisms are in the San Francisco Bay for longer periods of time. Finally, I would like to use the monitoring and toxicity data available to me to create models like this one for other microplastic types. Another risk assessment is underway for the Sacramento-San Joaquin River Delta which will use the model in this thesis as its framework.

#### *4.9.1 Environmental Concentration and exposure needs*

The microplastic research community needs to consider how their work will be used for risk assessments. Currently, most data from microplastic studies would not be useful to a risk assessment framework. Many monitoring studies have neglected to sample study areas to meet the requirements of a risk assessment. Sampling sites are often not selected to cover the whole study area or relevant environmental compartments. Sampling should also sample across seasons because it is likely an important variable in most regions and may become more important with climate change. Finally, sampling methods must be developed to consider the specific management goals for the region.

Toxicity studies generally do not produce enough data to create a dose-response model. Dose-response models must be used in the place of point estimates such as EC50s. Point estimates reduce useful data to a single point and introduce large amounts of uncertainty. Dose-response models allow for a probabilistic analysis with predictive capabilities for varying concentrations. Additionally, a lack of set standards for microplastic toxicity testing has resulted in little continuity between laboratory studies. Protocols that ensure toxicity testing is conducted accurately are not always followed. For example, microplastics should be dialyzed prior to toxicity testing to remove chemicals that are sorbed weakly to the surface and would likely not be present on an environmental sample.

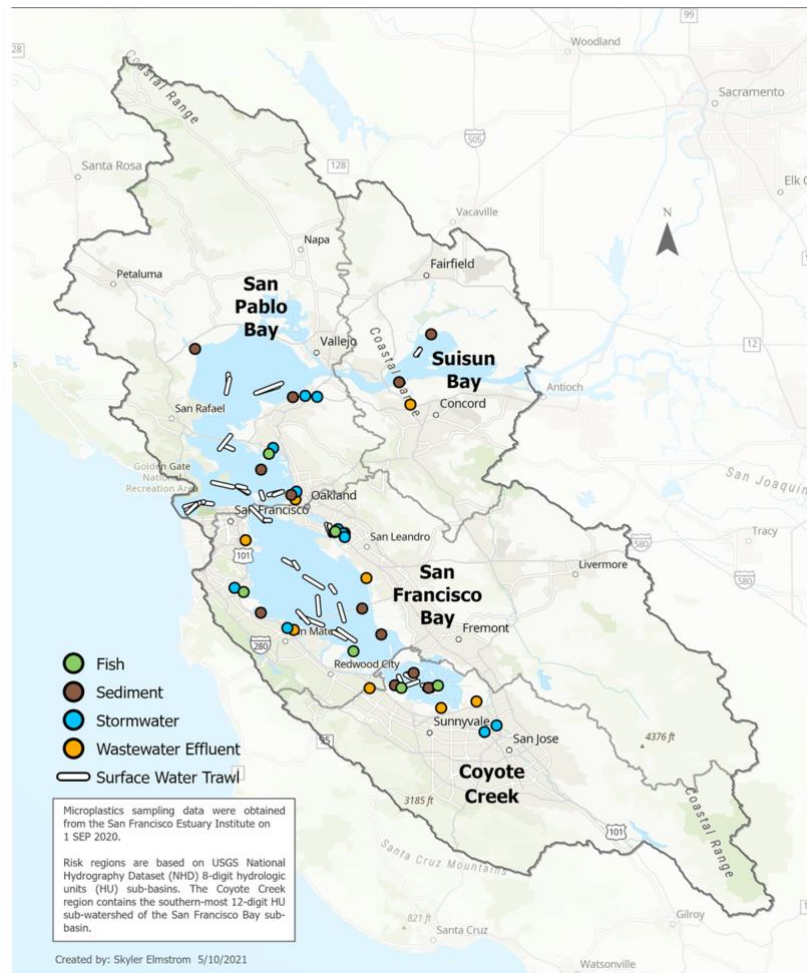
Risk assessments conducted for microplastics must also use better practices to accurately characterize risk. Microplastics should be considered a contaminant group rather than a single contaminant. There is strong evidence to suggest that differing composition, size, and shape can significantly change the transport of microplastic in the environment and the toxicity upon exposure. Microplastic risk assessments that fail to consider the unique ways in which microplastic characteristics affect risk will fail to produce useful risk assessments. Many microplastic risk assessment have attempted to assess risk for large spatial scales rather than specific regions. This method entirely neglects to consider the importance of management goals in defining risk and the many localized variables that affect risk for microplastics. Risk assessment must be spatially specific to accurately characterize risk.

#### *4.10 Microplastics and Risk Assessment*

Microplastics are a major concern to the public and have prompted governments to begin regulatory processes. However, decision making should not occur without first understanding the actual risk a stressor presents. Risk assessments are critical for determining the risk TWP and other microplastics present to the environment. They contextualize the stressors in the environment and provide insight for decision makers. Without information on risk, individual actions and regulatory decisions related to microplastics may not be useful or may even make the problem escalate. Therefore, it is imperative that future microplastic studies focus on producing results that will support comprehensive risk assessment.

## Figures and Tables

### SM1. Plastic Sampling and Fish Abundance Data

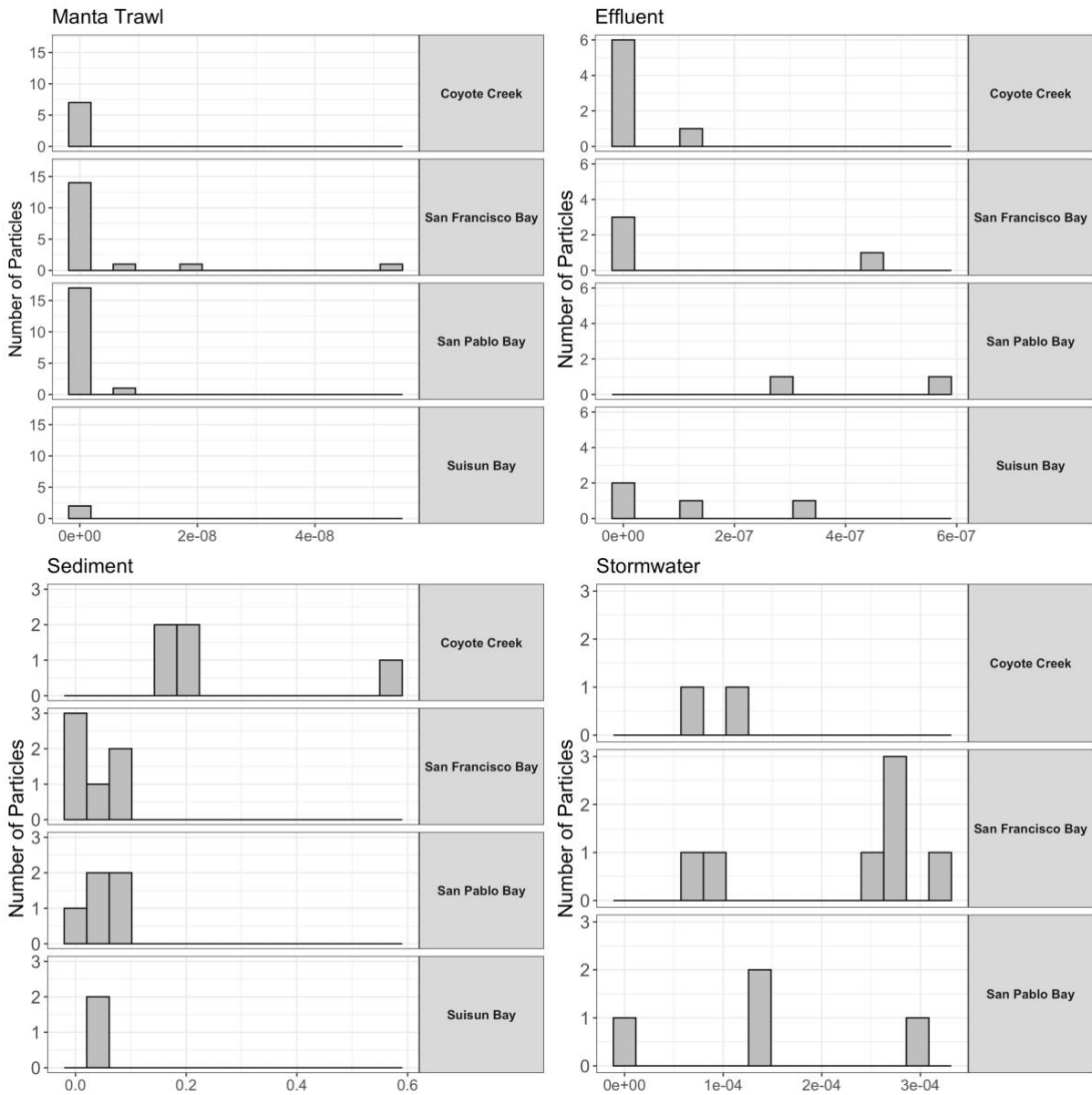


**Figure S1.** Map of SFEI microparticle sample sites in each risk region. Locations of fish, sediment, stormwater, wastewater effluent, and surface water trawls are shown. Effluent sample locations are shown for the collection point and not for effluent outfall location.

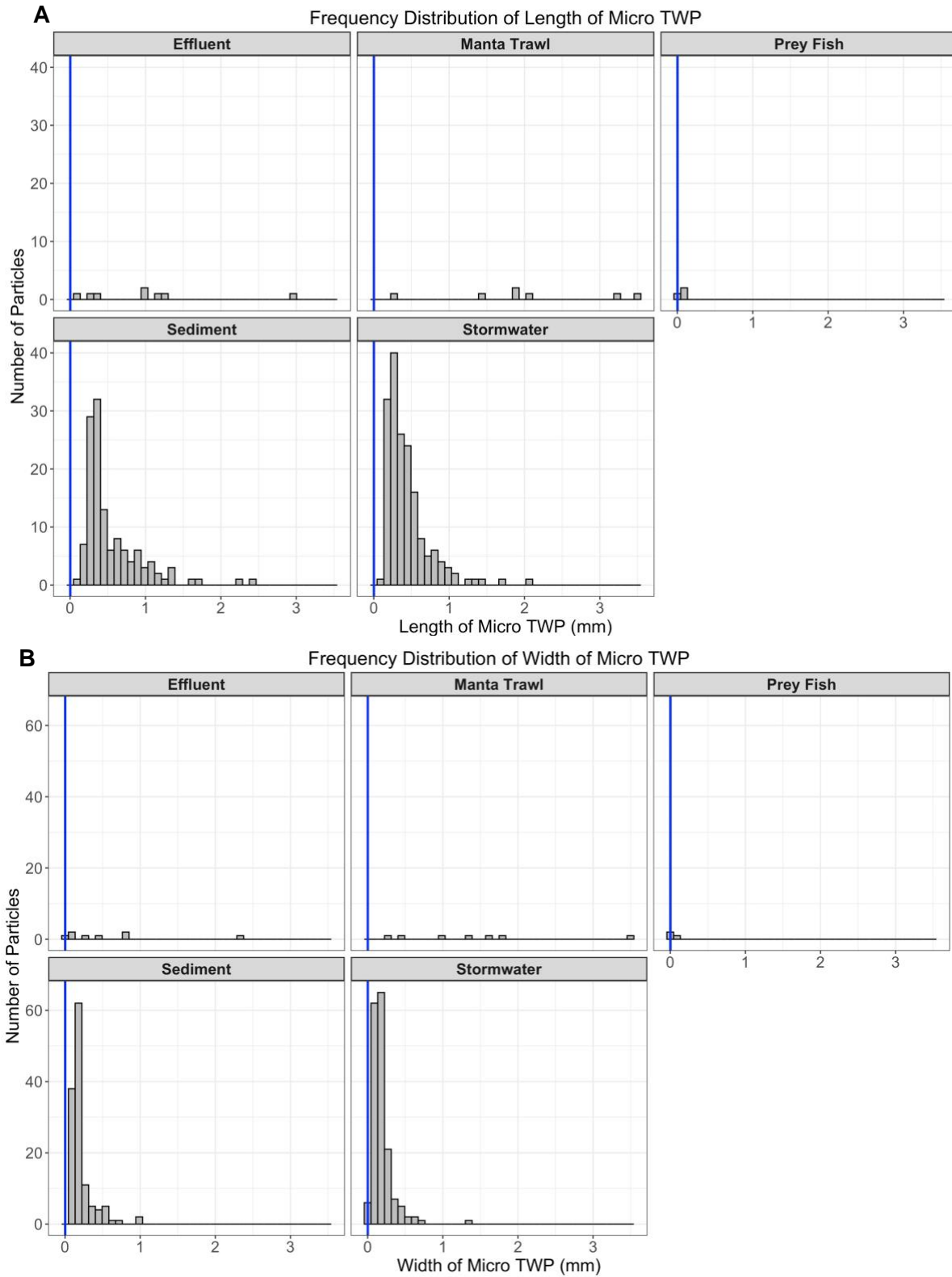
**Table S1.** Number of samples taken in each risk region and sample matrix for the SFEI microparticle study. Effluent counts are for outfall locations and not sample collection points.

Sample matrix	Suisun Bay	San Pablo Bay	San Francisco Bay	Coyote Creek	Total
Effluent	4	2	4	7	17
Surface	2	18	17	7	44
Stormwater	0	4	7	2	13
Sediment	2	5	6	5	18

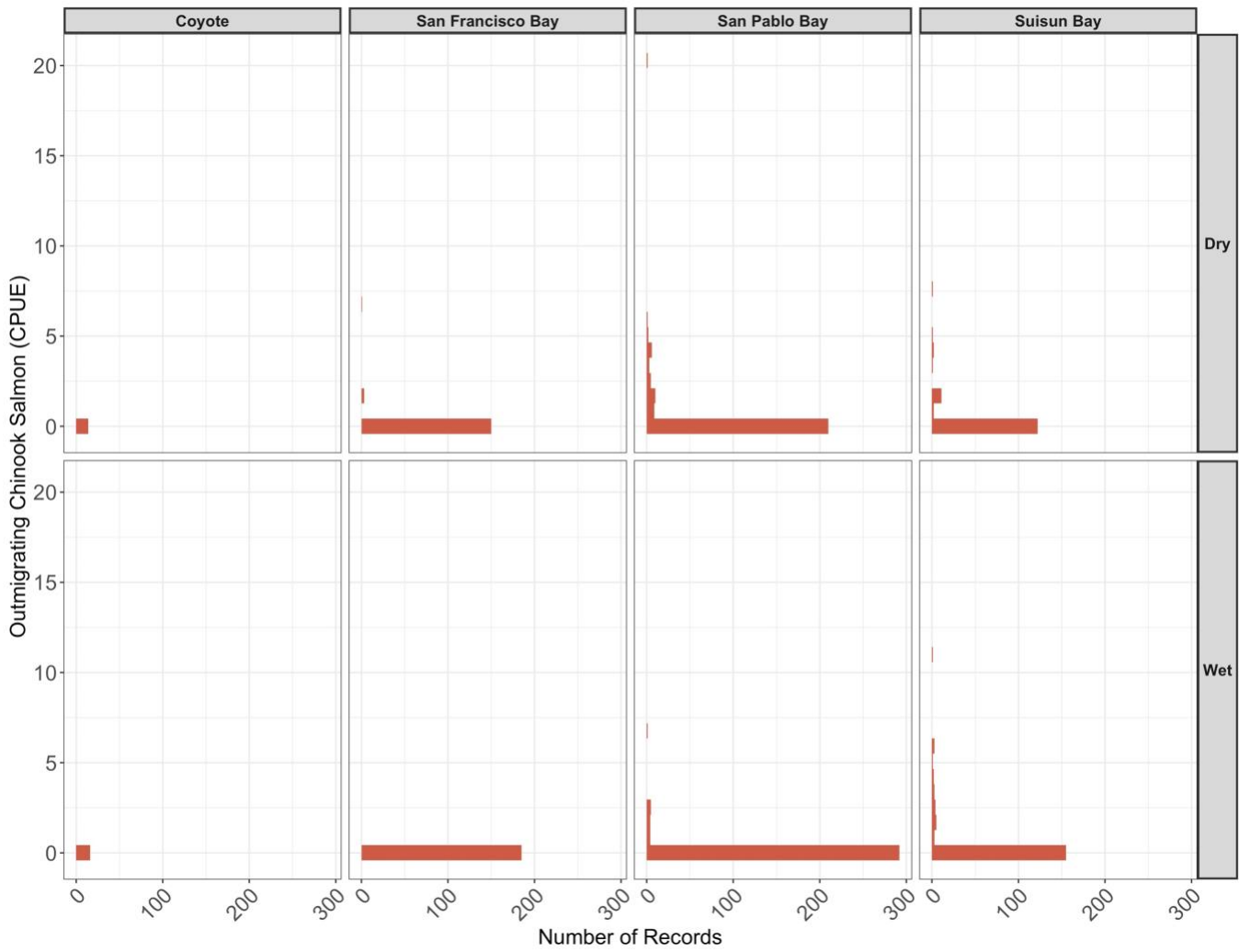




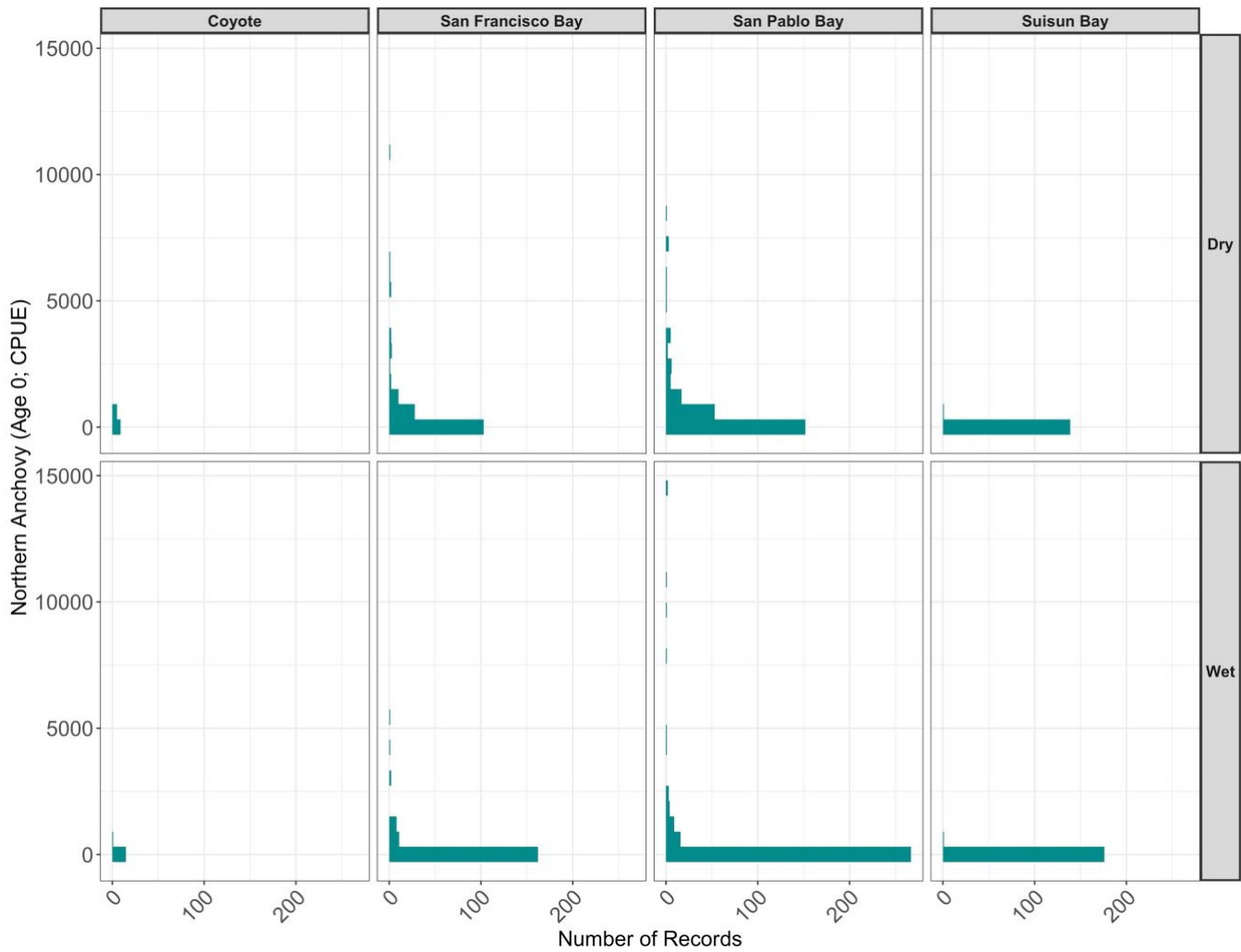
**Figure S2.** Frequency distributions for rubber and potentially rubber (together) particles concentrations by risk region and sample matrix. There were no stormwater data collected for Suisun Bay. Note that the x-axis is not scaled the same for each sample matrix graph so that variations in distributions are apparent.



**Figure S3.** Frequency distributions for the (A) length and (B) width of rubber and potentially rubber particles collected from the study site broken down by sample matrix. The blue line shows the average diameter of tire particles used in toxicity study for Cunningham et al. (2022).

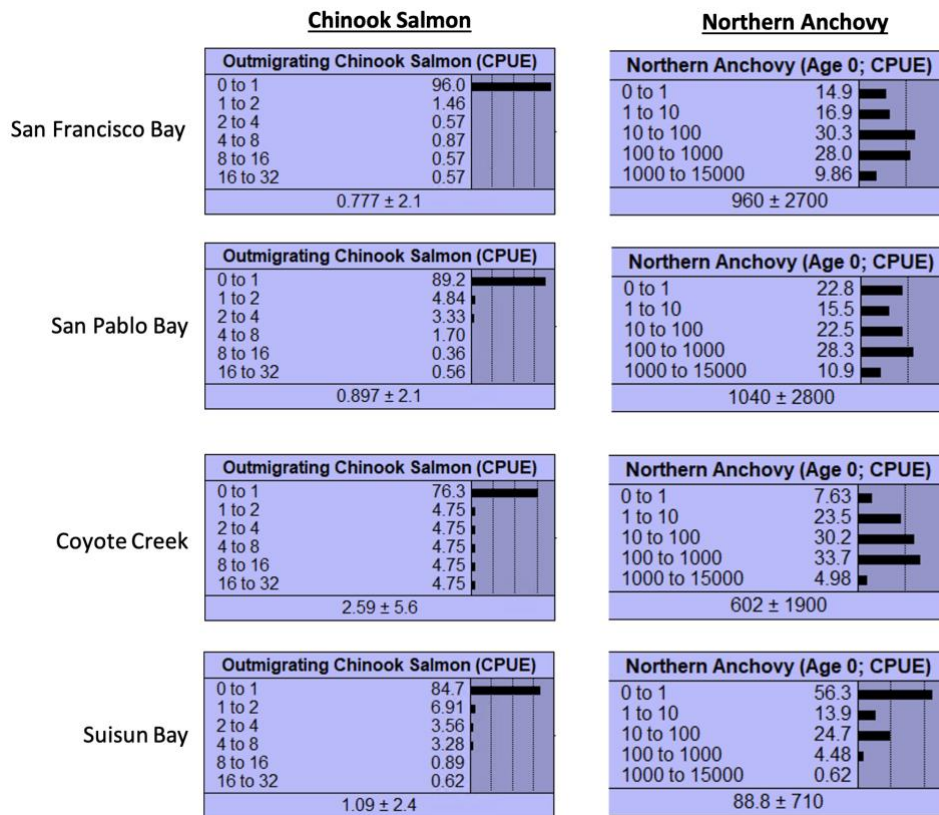


**Figure S4.** Histograms of out-migrating Chinook salmon abundance (CPUE) by number of records in the CDFW dataset for out-migrating Chinook salmon abundance. Graphs are separated by season and risk region to see spatial and temporal variations. Chinook salmon abundance is highest in Suisun Bay and San Pablo Bay. Clear patterns in seasonal variations are not present.

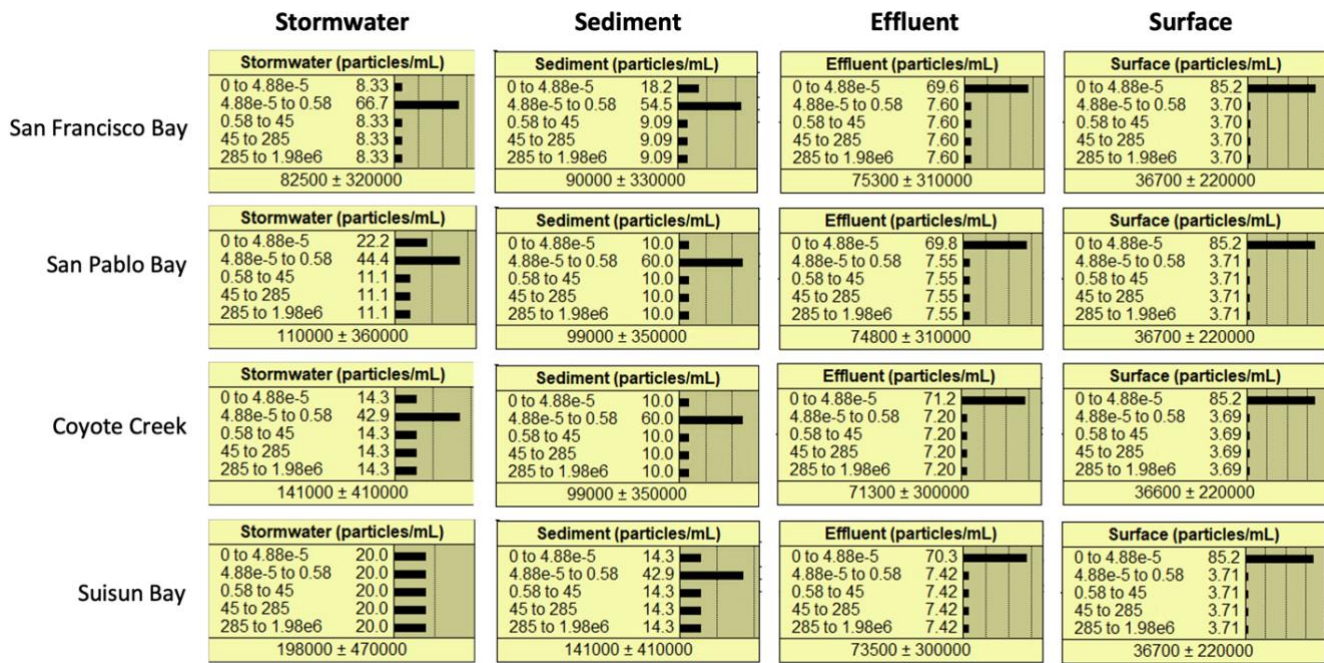


**Figure S5.** Histogram of Age 0 Northern anchovy abundance (CPUE) by number of records in the CDFW dataset for Northern anchovy abundance. Graphs are separated by season and risk region to see spatial and temporal variations. Northern anchovy abundance is highest in San Francisco Bay and San Pablo Bay with clear seasonal variations in both these regions.

SM2. Bayesian Network Node Distributions

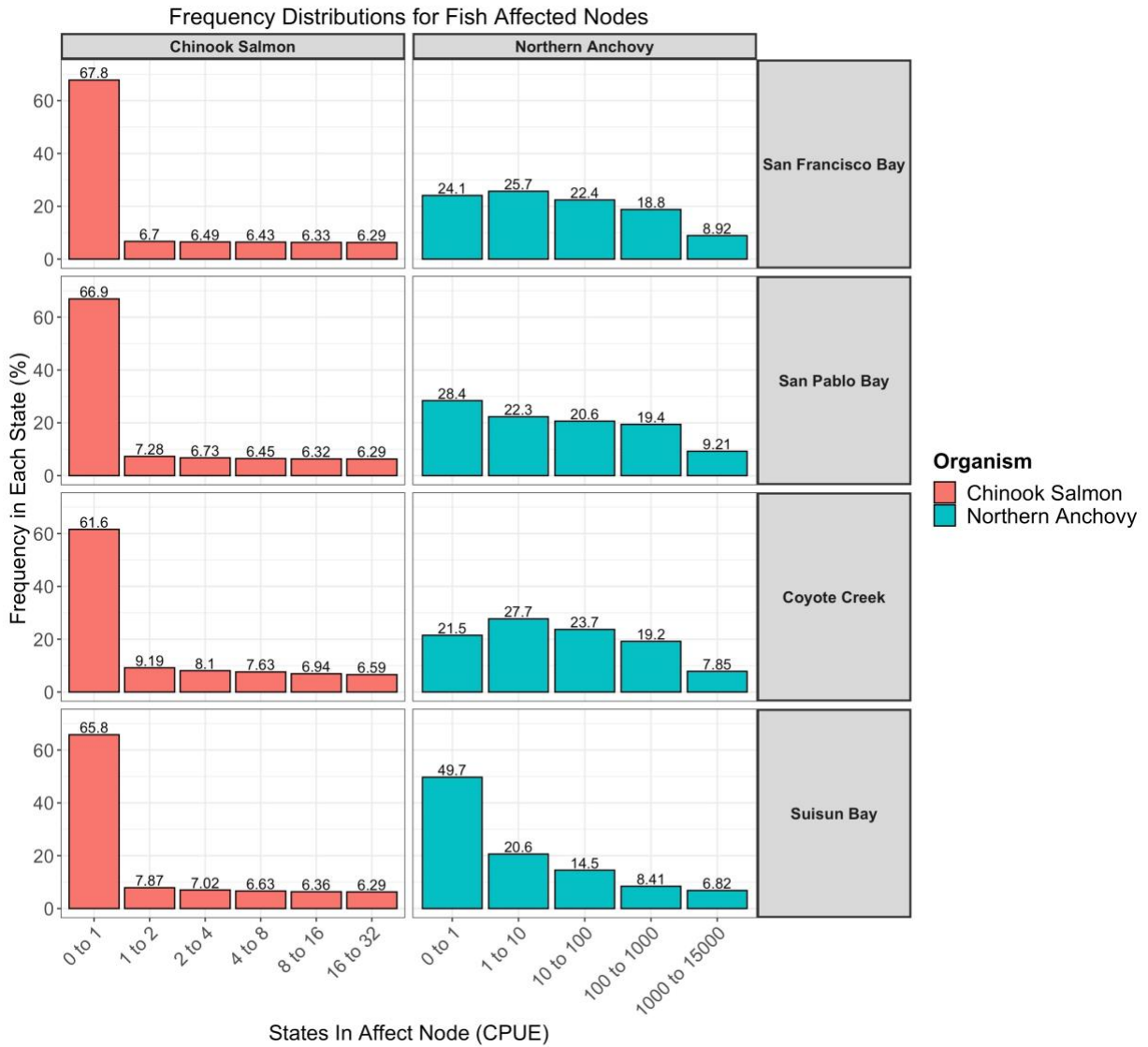


**Figure S6.** Fish abundance nodes from the Bayesian Network model. I selected each risk region in the model and the resulting outputs in the fish abundance node are presented in this figure. Chinook salmon distributions change minimally for each risk region with Coyote Creek having the highest uncertainty. Variations in the distributions are more apparent for Northern anchovy with Suisun Bay having the lowest abundance of fish. Note that the states are discretized into log scale.

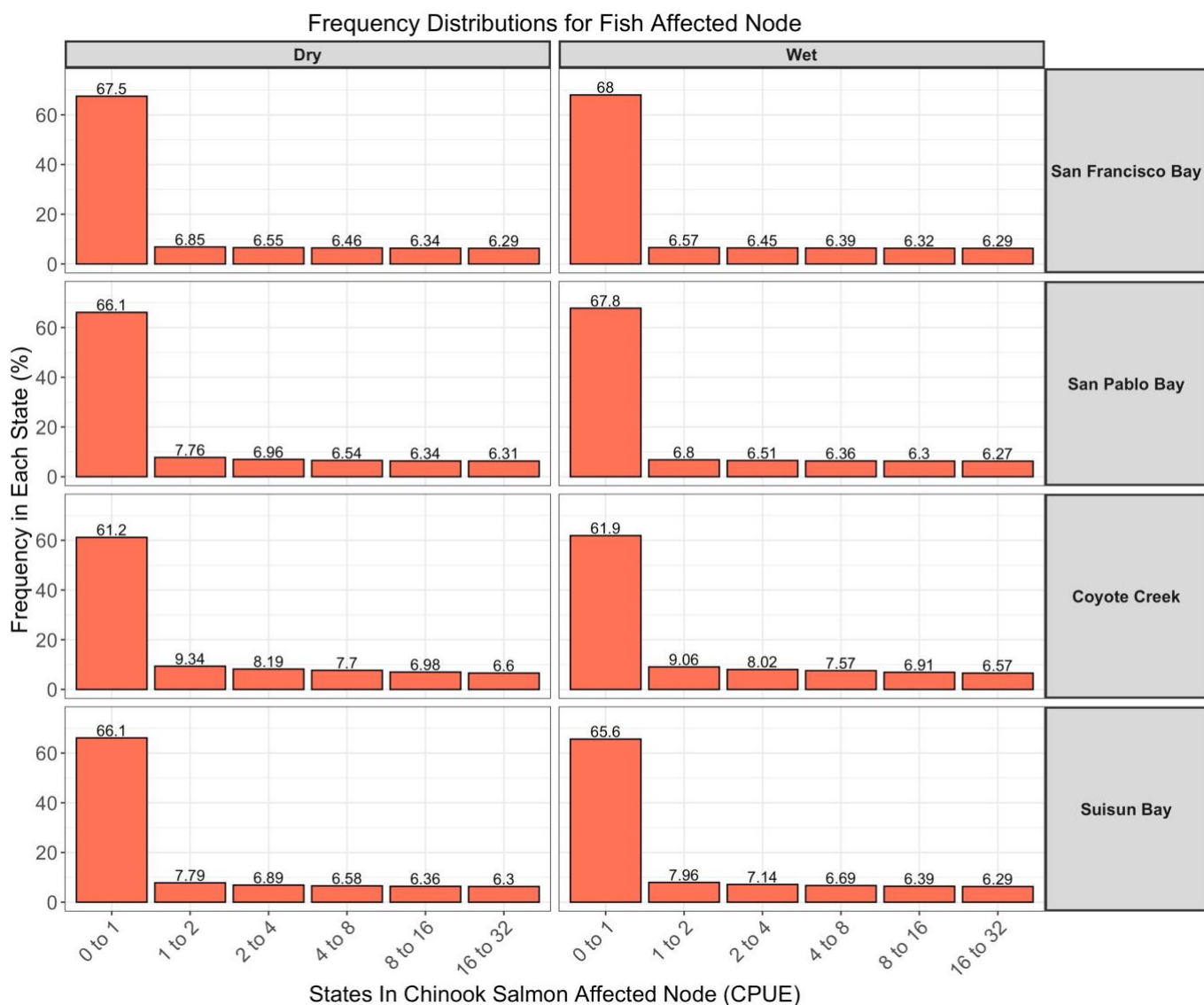


**Figure S7.** TWP concentration nodes from the Bayesian Network model. I selected each risk region and the resulting outputs in the TWP concentration nodes are presented in this figure. The most particles were found in stormwater and sediment. No data was collected for stormwater in Suisun Bay so the model inputs an even distribution to indicate a lack of data.

SM 3. Fish Affect Node Distribution Figures

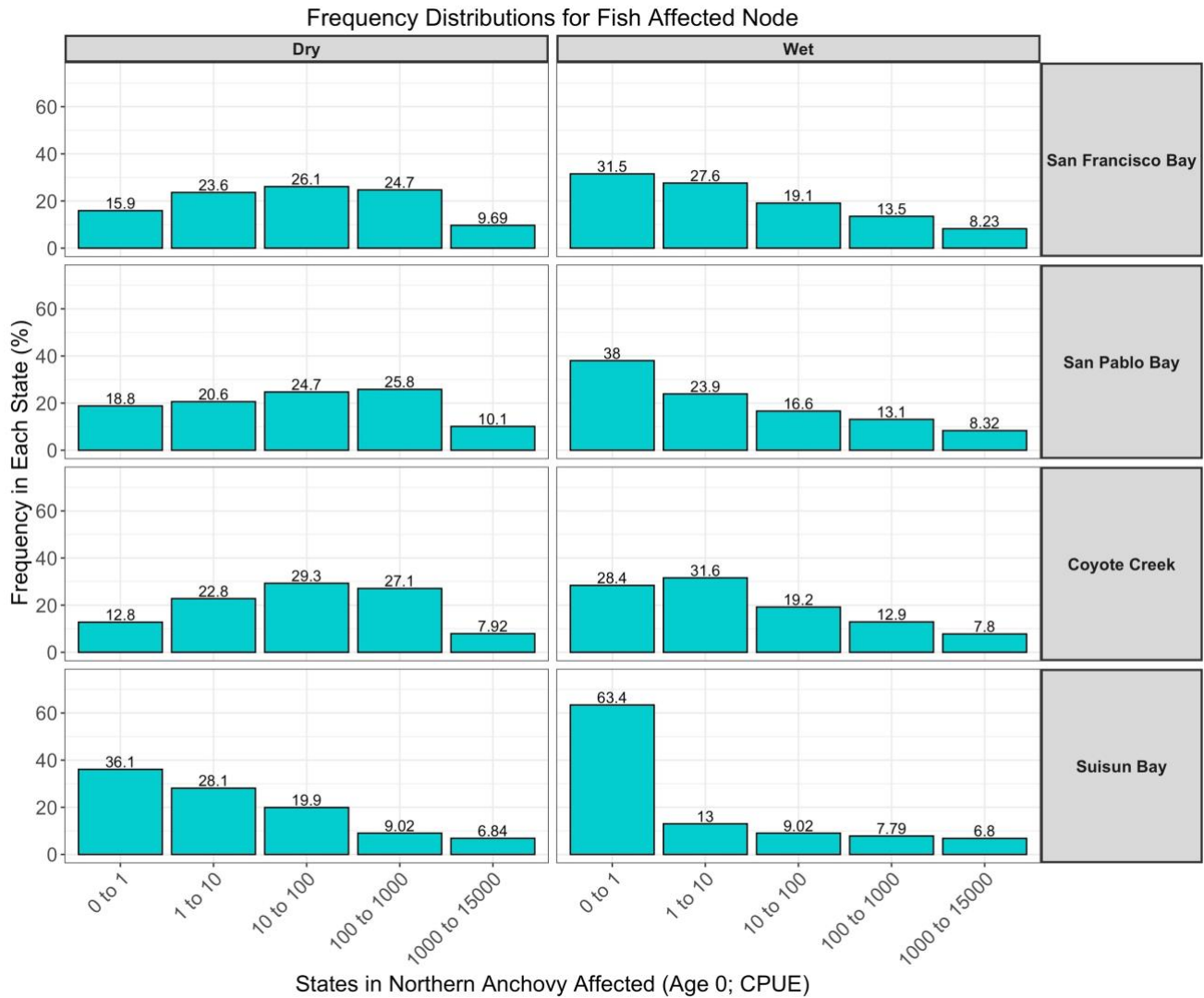


**Figure S8.** Frequency distributions for Chinook Salmon Affected and Northern Anchovy Affected nodes from the Bayesian Network model. I selected each risk region in the model and graphed the distribution in the fish affected nodes. The states in those nodes are on the x-axis and the y-axis is the percent of values in each of those states. The number of Chinook salmon affected changes minimally between each risk region, but variation is more apparent for Northern anchovy. Suisun Bay has the lowest number of Northern anchovies effected because it has the highest number of values in the 0 to 1 node. The other risk regions have distributions skewed towards the higher states.

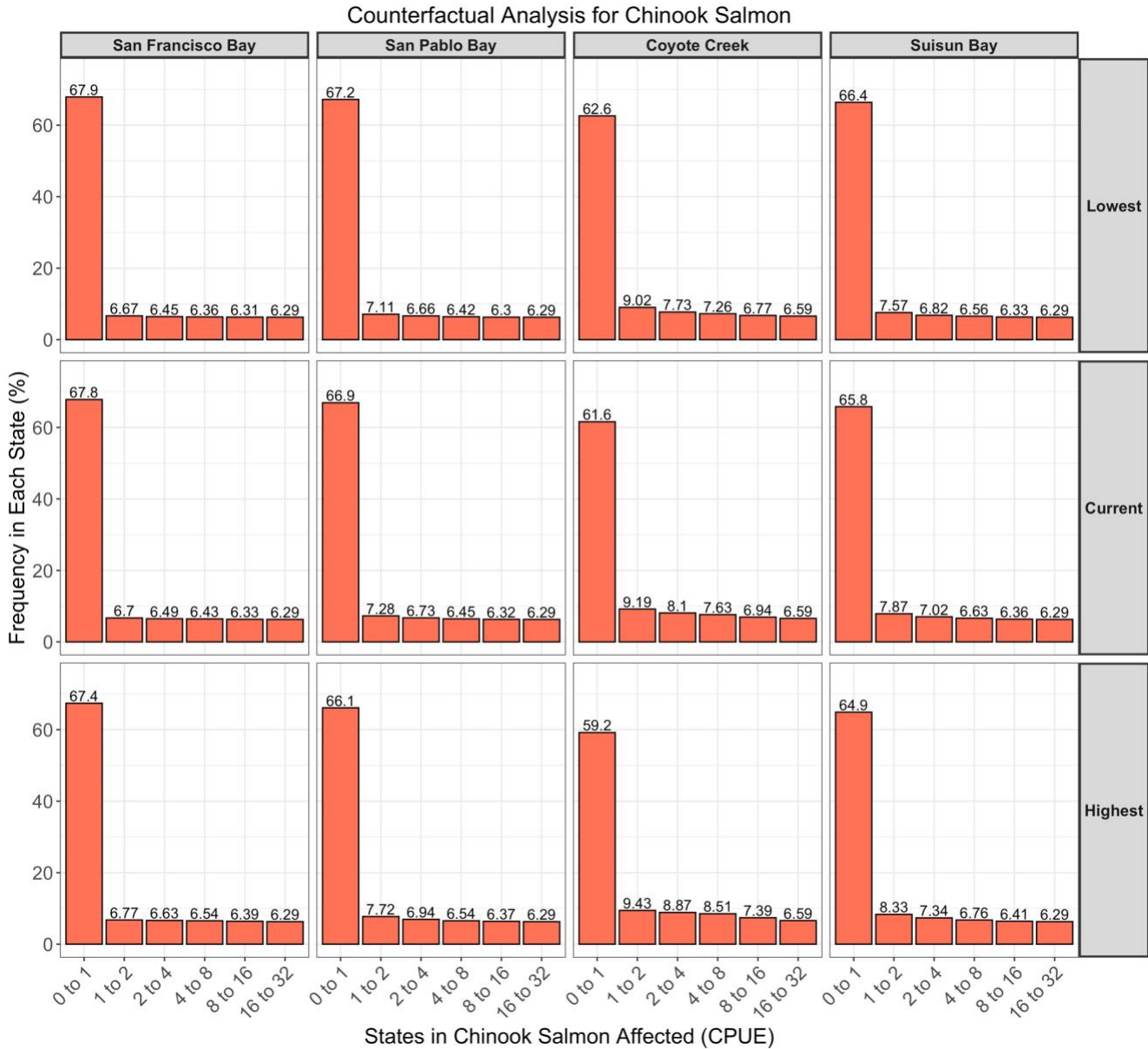


**Figure S9.** Frequency distributions for the Chinook Salmon Affected node from the Bayesian Network model. To see seasonal variation, I selected each risk region and each season. I then graphed the output for the Chinook Salmon Affected node. Variation is minimal across all seasons and risk regions.

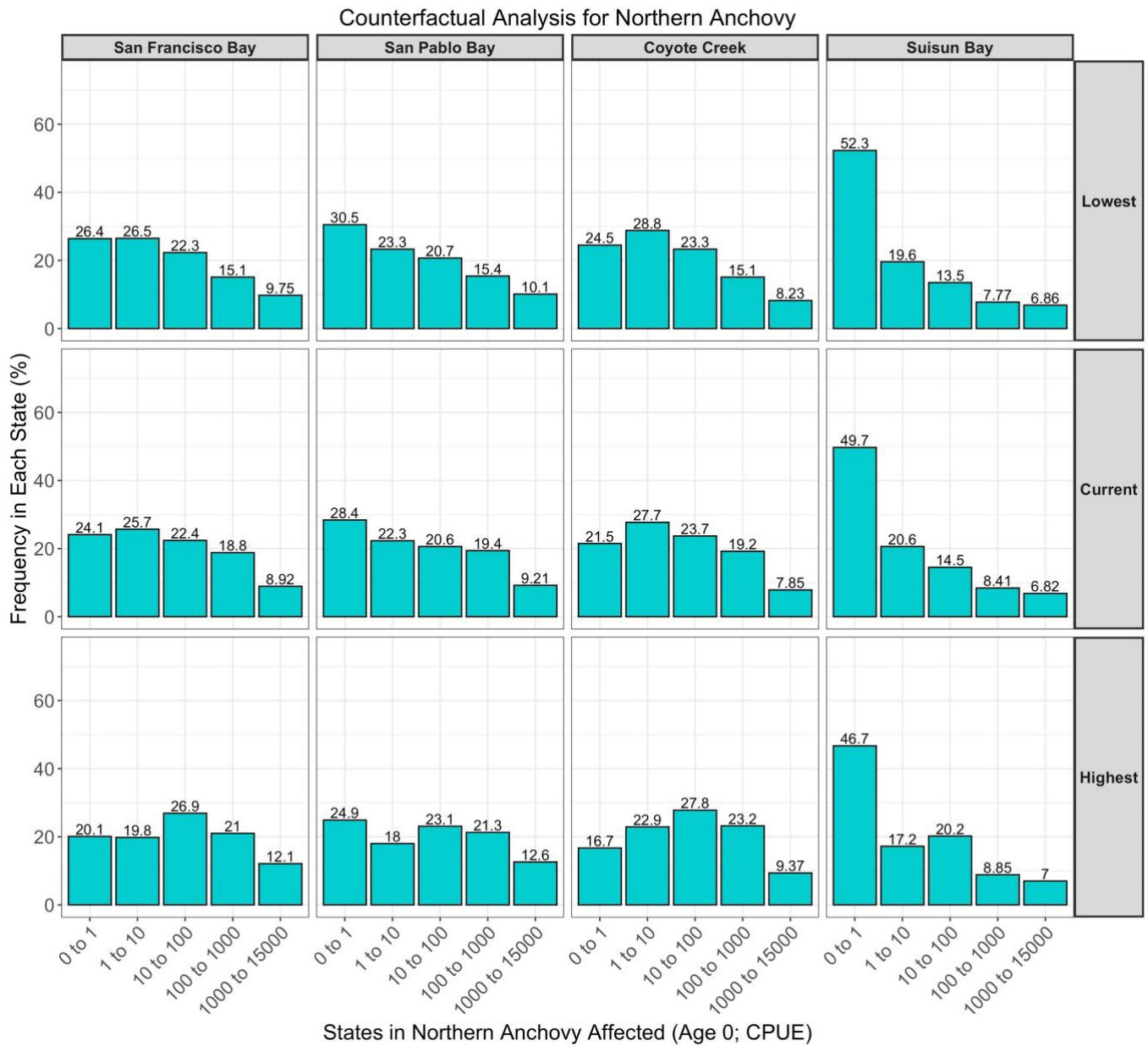




**Figure S10.** Frequency distributions for the Northern Anchovy Affected node from the Bayesian Network model. To see seasonal variation, I selected each risk region and each season. I then graphed the output for the Chinook Salmon Affected node. In all risk regions, values are skewed towards lower states in the wet season when compared to the dry season.

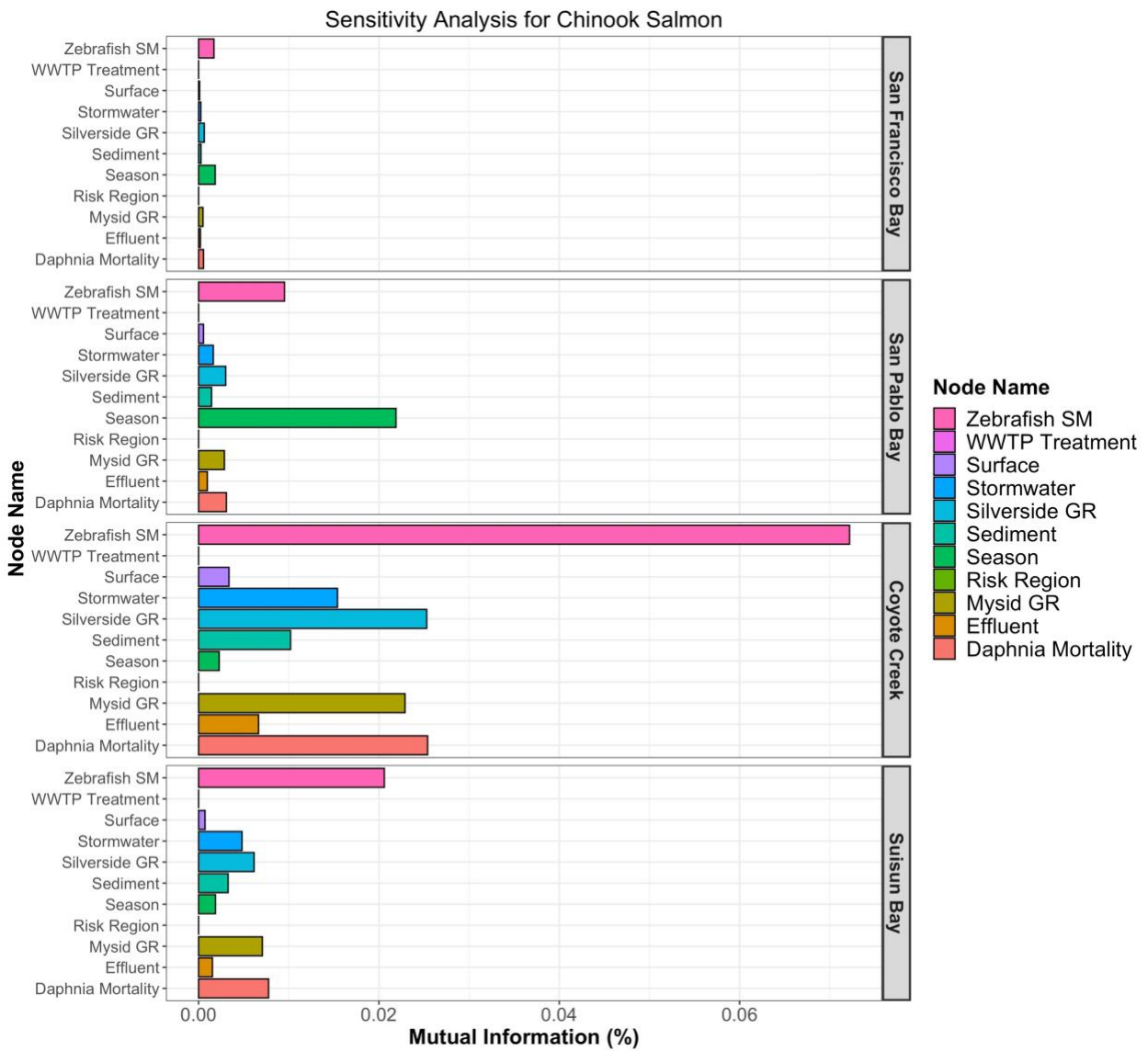


**Figure S11.** Frequency distributions for the Chinook Salmon Affected node from the Bayesian Network model. To create different exposure scenarios, I selected different states in the TWP concentration nodes and graphed output. The lowest scenario is  $0$  to  $4.88 \times 10^{-5}$  particles/mL, the current scenario is all states left as they are in Figure S7, and the highest state is  $285$  to  $1.98 \times 10^6$  particles/mL. I also selected each risk region to see how this would affect the different scenarios. Distributions shifted minimally for all scenarios and risk regions with slightly more fish affected in the highest concentration scenario.

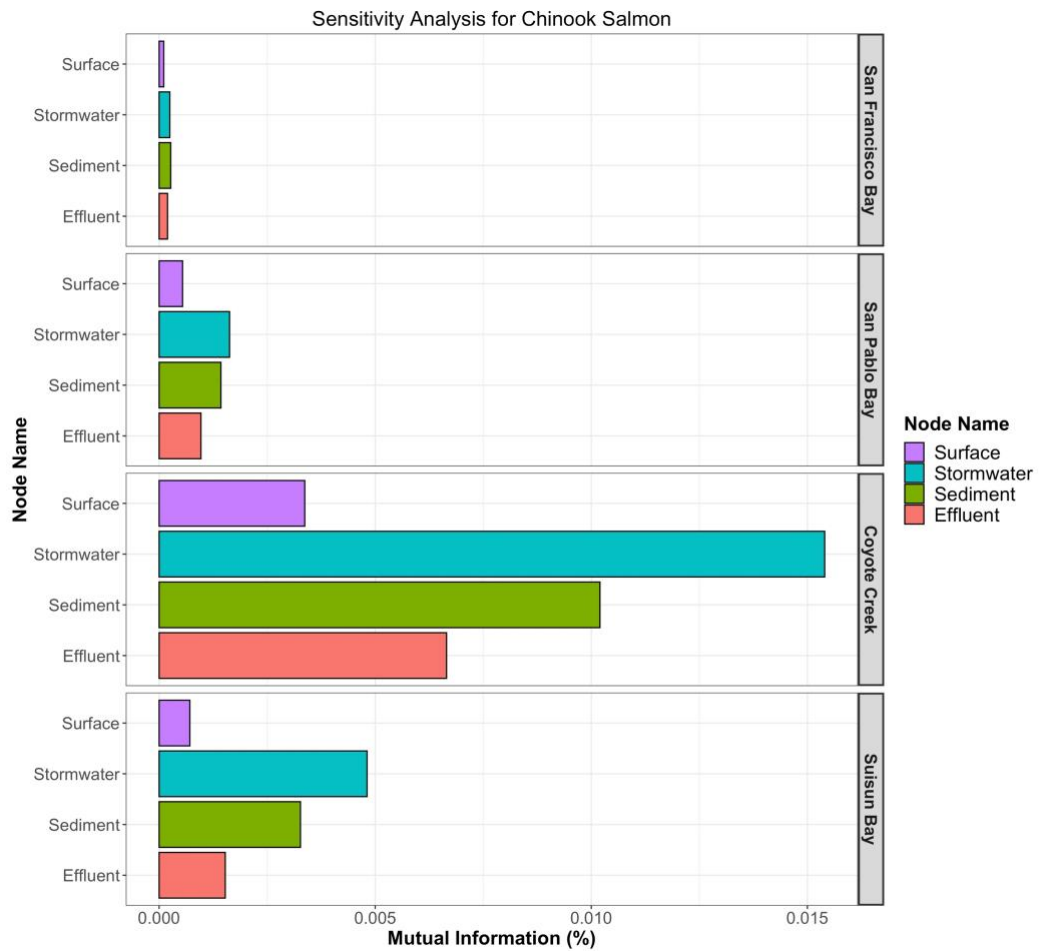


**Figure S12.** Frequency distributions for the Northern Anchovy Affected node from the Bayesian Network model. To create different exposure scenarios, I selected different states in the TWP concentration nodes and graphed output. The lowest scenario is 0 to  $4.88 \times 10^{-5}$  particles/mL, the current scenario is all states left as they are in Figure S7, and the highest state is 285 to  $1.98 \times 10^6$  particles/mL. I also selected each risk region to see how this would affect the different scenarios. Distributions shifted minimally between the lowest and current scenario and shift higher for the highest concentration scenario.

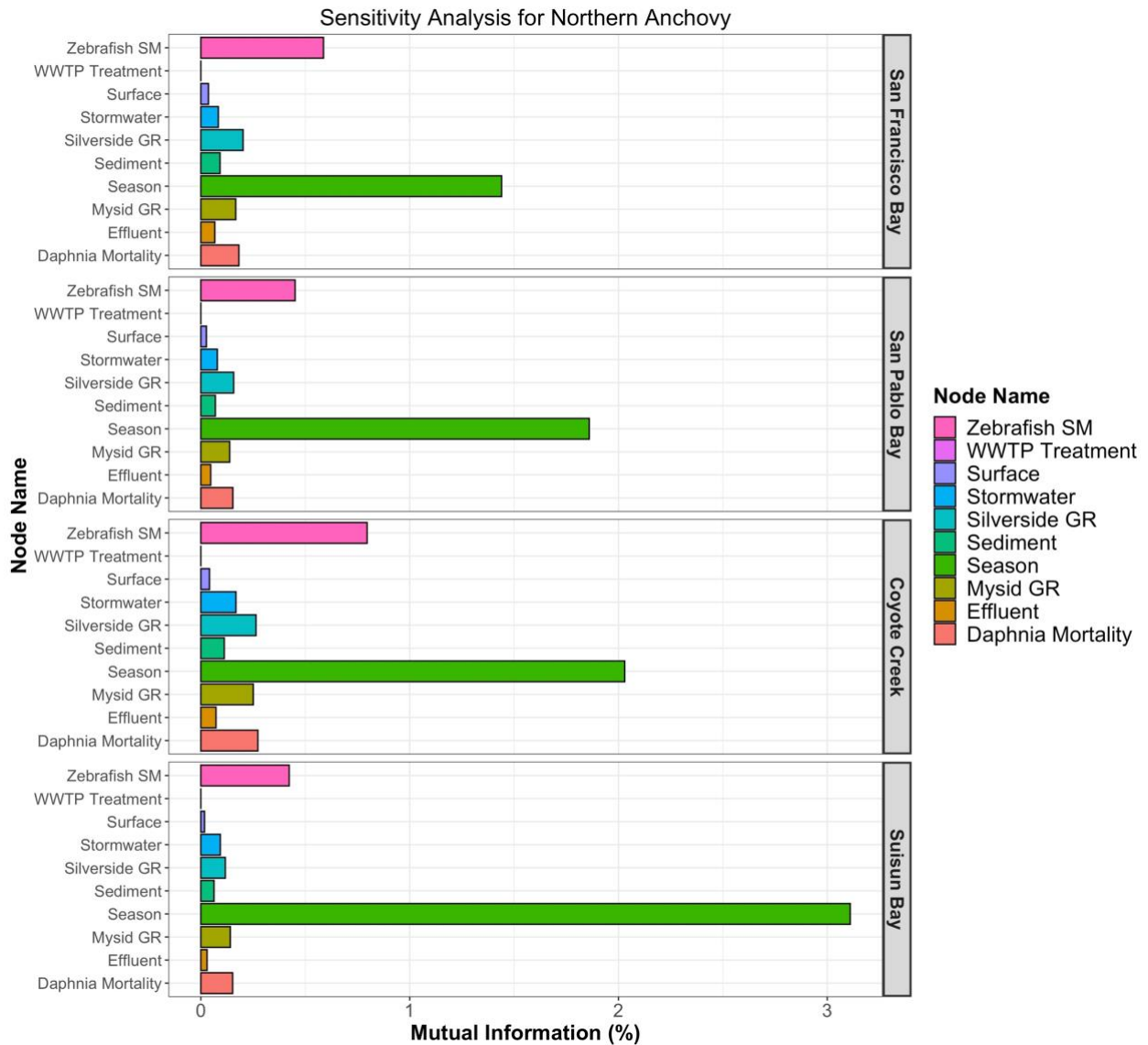
SM4. Sensitivity Analysis Figures



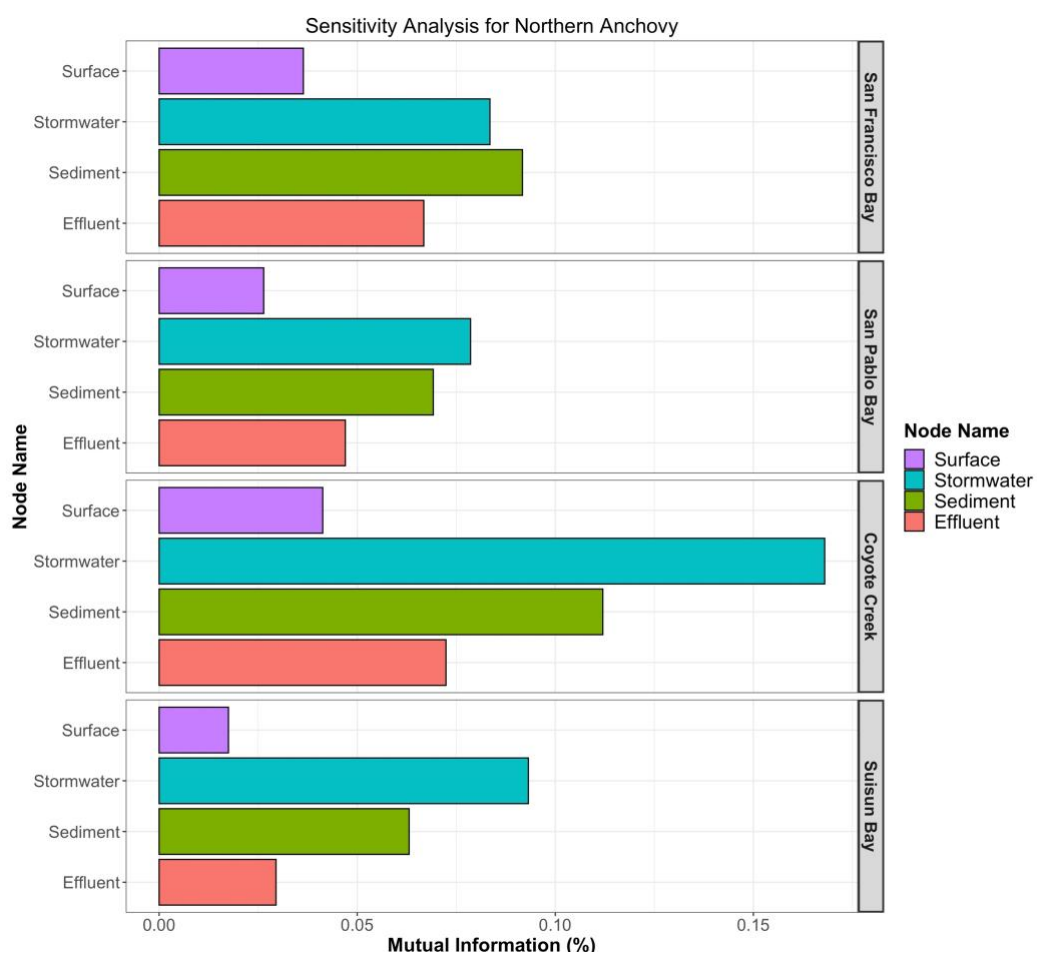
**Figure S13.** Sensitivity analysis for the Chinook Salmon Affected node by risk region. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the Chinook Salmon Affected node. GR = Growth reduction, SM = Spontaneous movement.



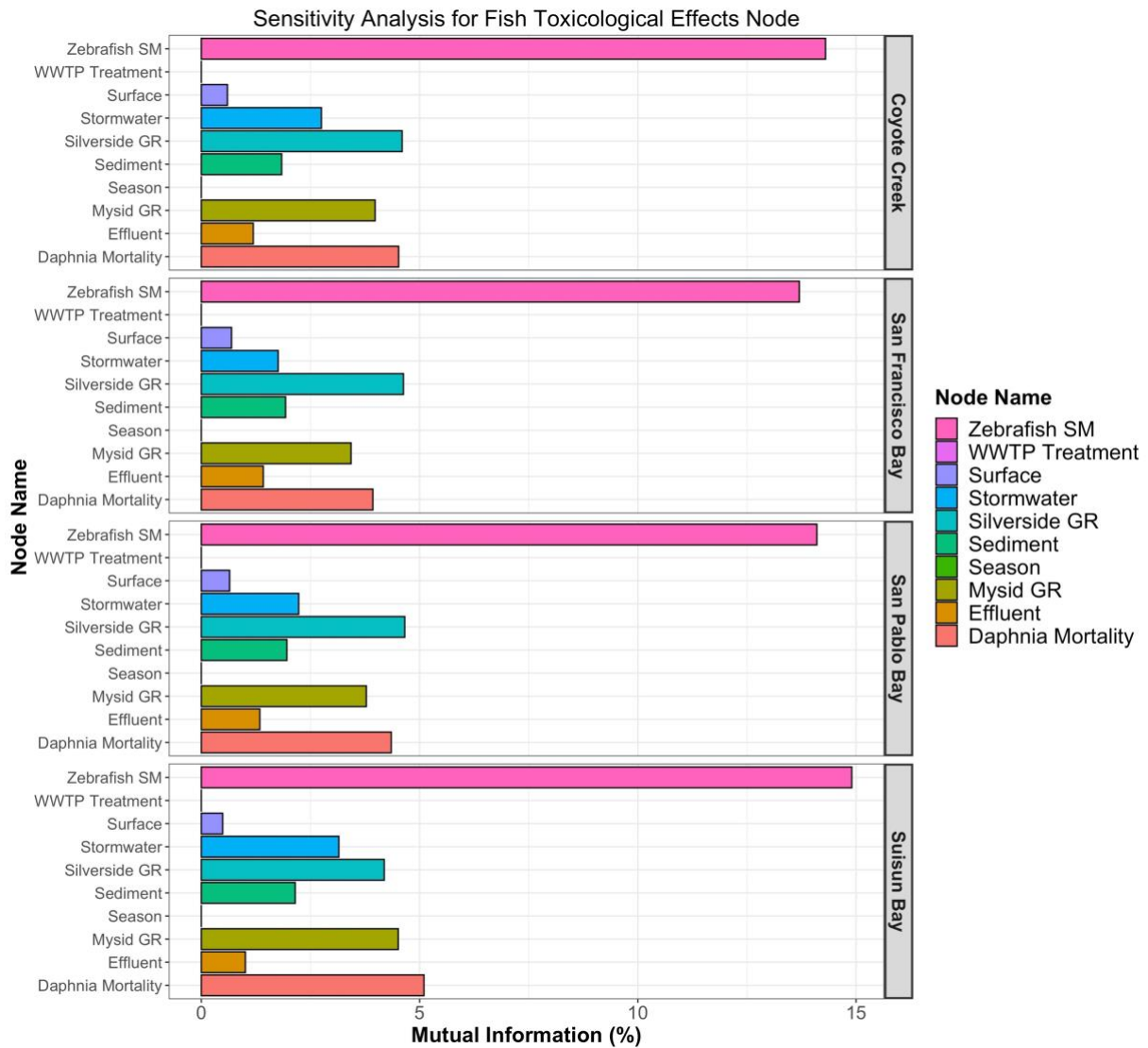
**Figure S14.** Sensitivity analysis for the Chinook Salmon Affected node by risk region for the sample matrix nodes. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the Chinook Salmon Affected node. GR = Growth reduction, SM = Spontaneous movement.



**Figure S15.** Sensitivity analysis for the Northern Anchovy Affected node by risk. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the Northern Anchovy Affected node. GR = Growth reduction, SM = Spontaneous movement.



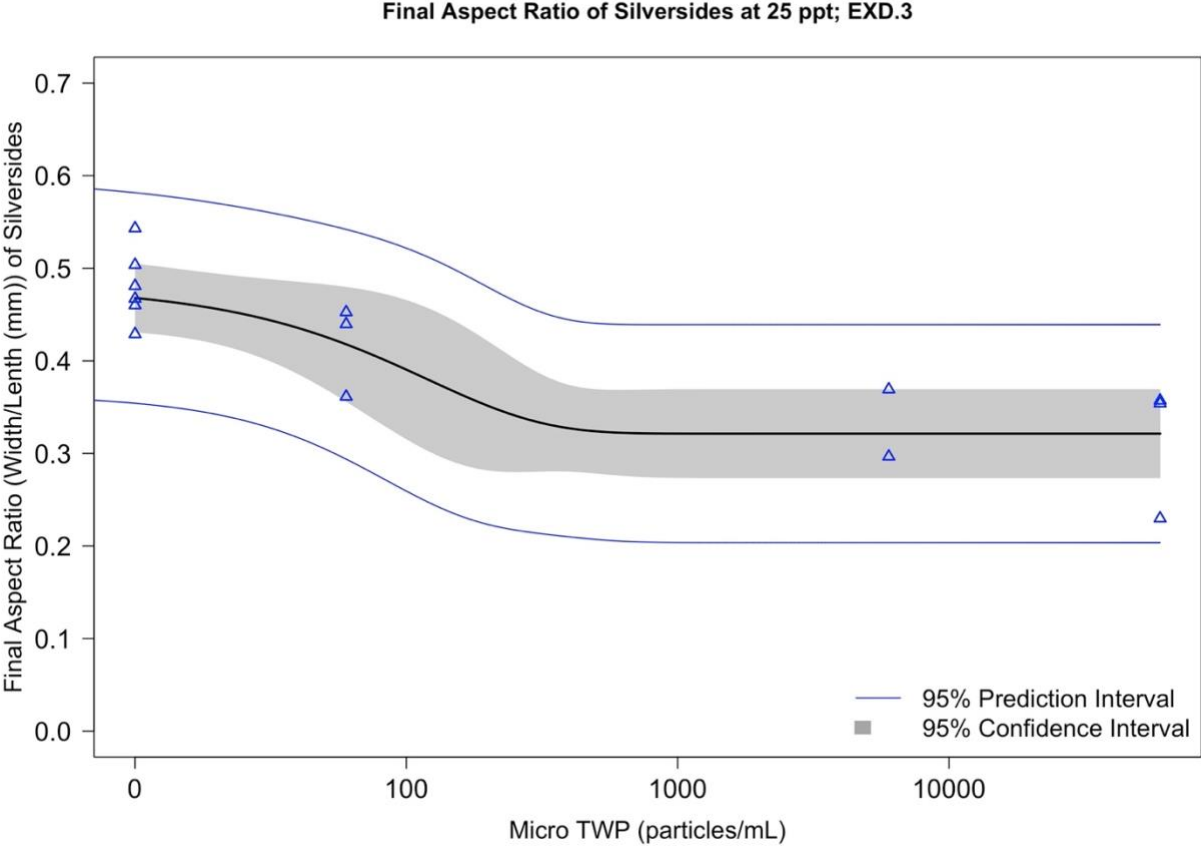
**Figure S16.** Sensitivity analysis for the Northern Anchovy Affected node by risk for the sample matrix nodes. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the Northern Anchovy Affected node. GR = Growth reduction, SM = Spontaneous movement.



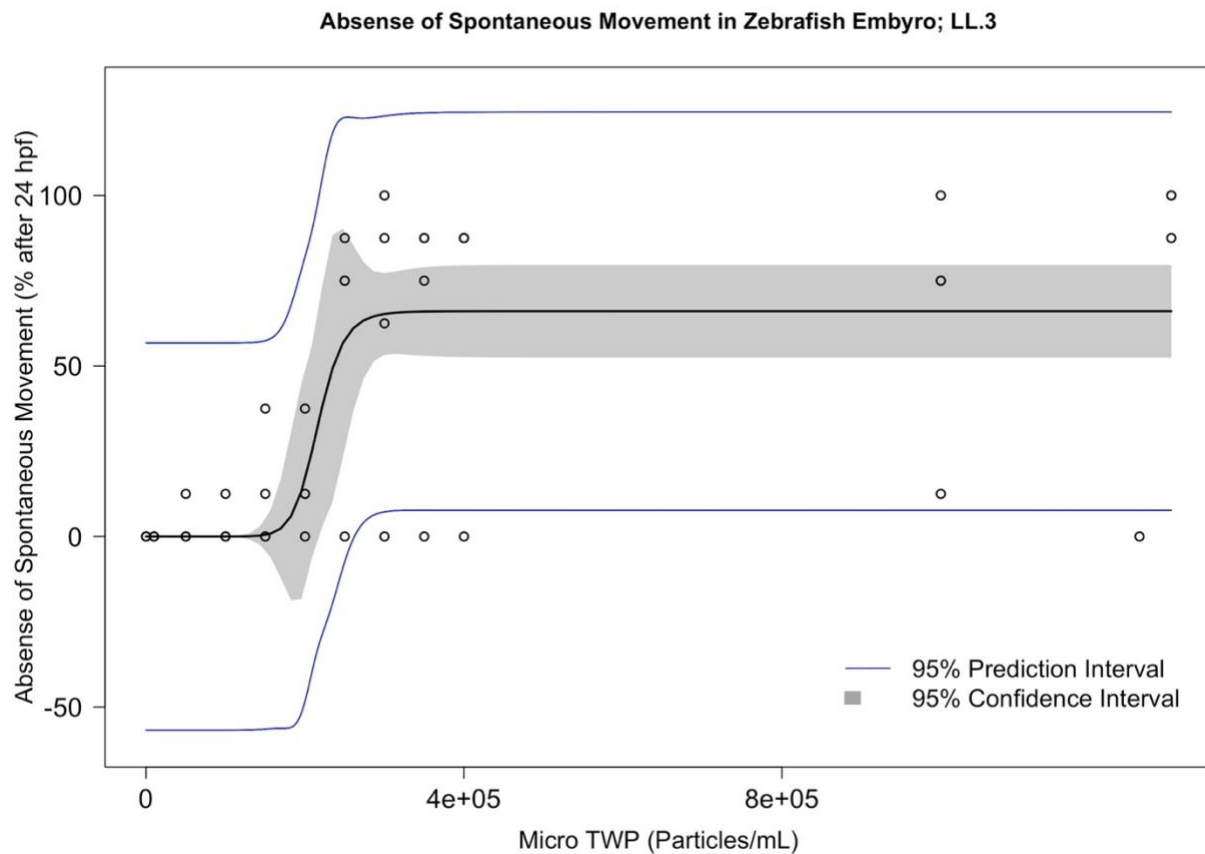
**Figure S17.** Sensitivity analysis for the Fish Toxicological Effects node by risk region. Percent mutual information is the measurement used to evaluate dependence between two nodes. Higher mutual information indicates a higher level of dependency between that node and the Fish Toxicological Effects node. GR = Growth reduction, SM = Spontaneous movement.



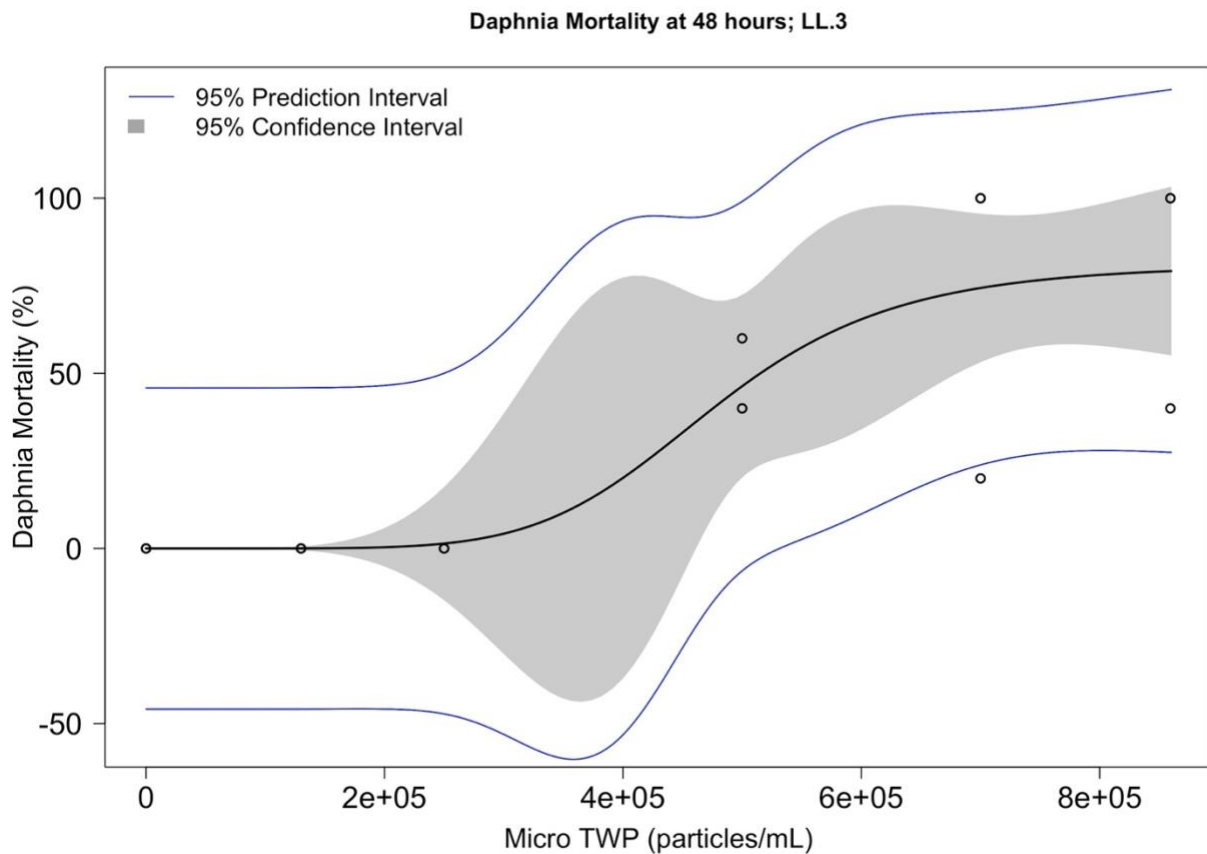
SM5. Dose response curves for fish and invertebrate exposed to micro TWP



**Figure S18.** Concentration response curve for final aspect ratio (width/length) of silversides (*Menidia beryllina*) exposed to TWP. The DRC package in Netica was used to create the concentration-response curve using an exponential decay model with three parameters. The parameters are  $c = 0.321372$ ,  $d = 0.480667$ ,  $e = 119.773263$ .

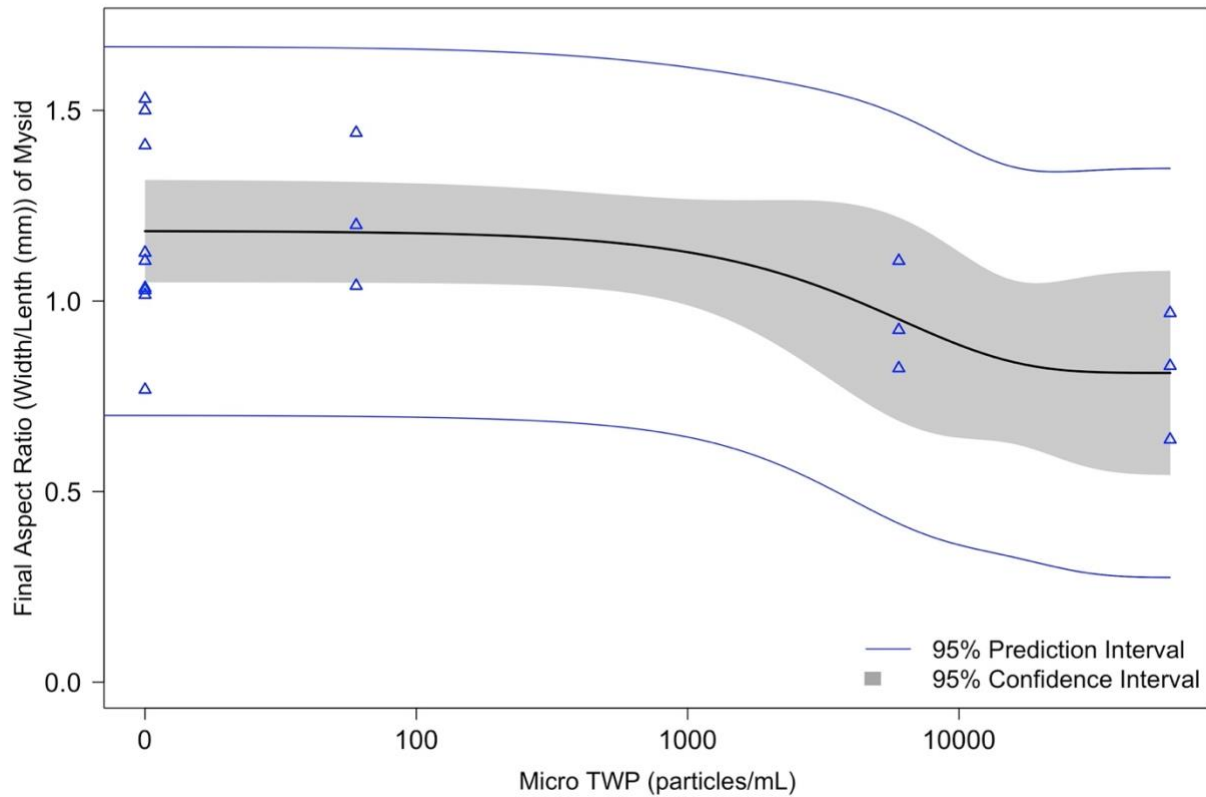


**Figure S19.** Concentration response curve for absence of spontaneous movement for Zebrafish (*Danio rerio*) exposed to TWP. The DRC package in Netica was used to create the concentration-response curve using a log logistic model with three parameters. The parameters are  $b = -13.4781$ ,  $d = 66.0637$ ,  $e = 216584.2768$ .



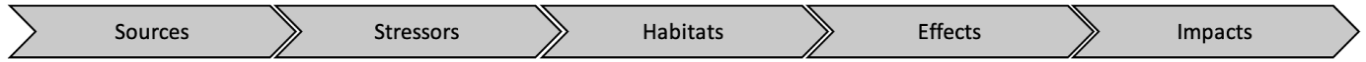
**Figure S20.** Concentration response curve for mortality of *Daphnia magna* exposed to TWP. The DRC package in Netica was used to create the concentration-response curve using a log logistic model with three parameters. The parameters are  $b = -6.2454$ ,  $d = 81.2282$ ,  $e = 477828.5167$ .

Final Aspect Ratio of Mysid at 25 ppt; EXD.3

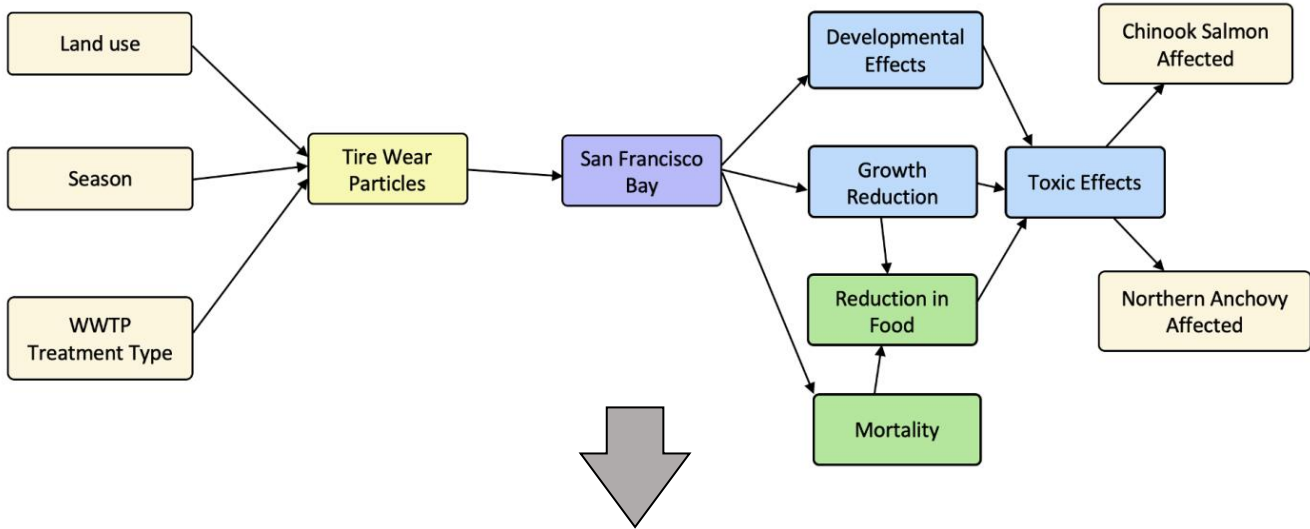


**Figure S21.** Concentration response curve for final aspect ratio of mysid (*Americamysis bahia*) exposed to TWP. The DRC package in Netica was used to create the concentration-response curve using an exponential decay model with three parameters. The parameters are  $c = 8.1116e^{-1}$ ,  $d = 1.1837$ ,  $e = 6.1722e^{-3}$ .

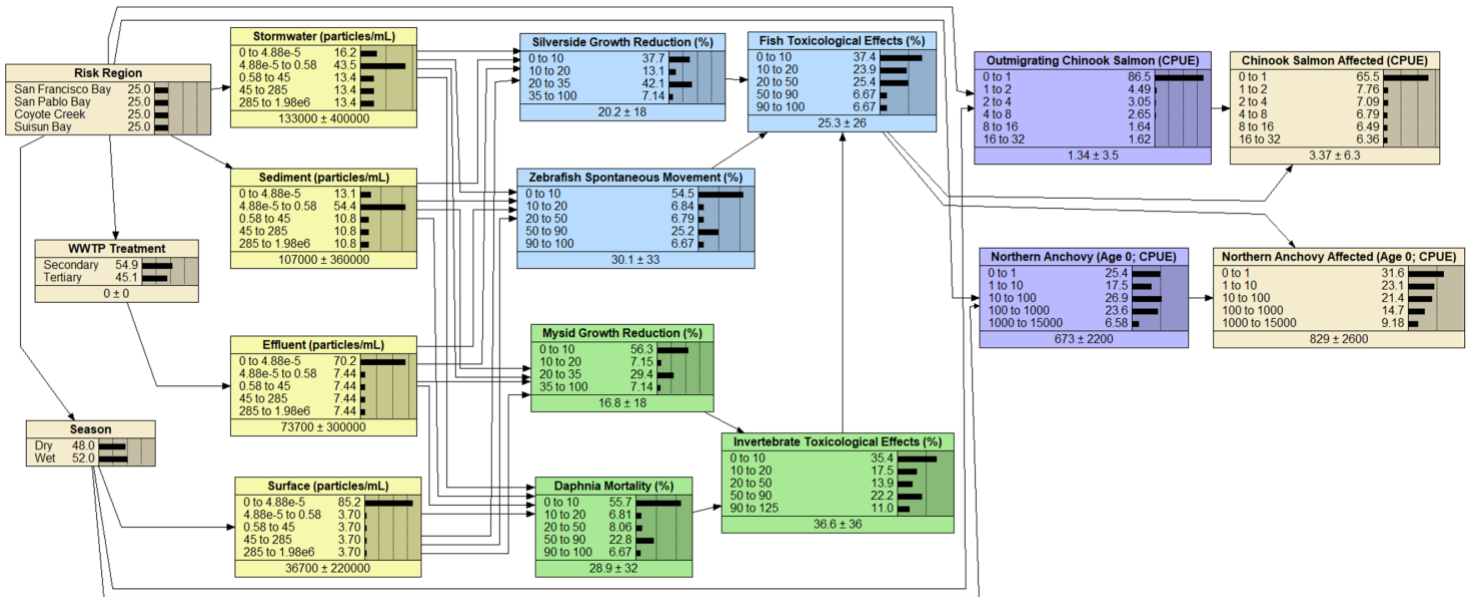
A. Relative Risk Model Framework



B. Tire wear particle conceptual model



C. Tire wear particle Bayesian Network Model



**Figure S22.** The connection between the conceptual model and the Bayesian network model.

(A) The framework that connects the major pieces of the system, (B) the conceptual model before data is added in, and (C) the Bayesian network model that uses data to populate the

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