The effects of three petroleum products on Pacific oysters (*Crassostrea gigas*)

by

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Abstract

Canada is one of the largest producers and exporters of petroleum products in the world. With the rapidly increasing petroleum transportation planned through marine routes in North America, understanding the toxicological repercussions of petroleum exposure and risk to wildlife is essential. Pacific oysters (*Crassostrea gigas*) were selected as a model bivalve species to determine the ecological implications of potential petroleum spills using marine diesel (MD), crude oil (CO), and diluted bitumen (DB). Toxicity endpoints included the scope for growth (respiration rates, food assimilation, and clearance rates), condition index, health assessment index, gonadal and digestive gland histopathology, as well as larval development. CO water-accommodated fractions (WAFs) contained the highest initial total polycyclic aromatic compound (TPAC) concentrations (350 µg/L), followed by MD (150 µg/L) and DB (128 µg/L). Oyster tissue TPAC concentrations reflected these trends; CO (29 µg/g), MD (4 µg/g), and DB (3 µg/g). No WAF or time-related effects were observed on the measured endpoints in Pacific oysters following a sub-chronic exposure to WAFs of CO, MD, or DB. These results suggest that despite being used as a biomonitor species in the past, Pacific oysters do not retain TPACs for long durations after being removed from a petroleum spill. Consequently, further studies with different exposure conditions and bivalve species are required to assess the impact of these contaminants in a marine environment.

Keywords: Petroleum; Pacific oysters; scope for growth; histopathology; larval development; morphometric indices

Dedication

This thesis is dedicated to my family.

To Amari, I am so blessed that you came into my life during this masters. I cannot wait to see you grow up, my shining bright light.

To my parents, grandparents, Harkaran, and Sarah, who have stood by my side every step of the way. All the support I have received from you is unfathomable. Wherever I have reached today is because of you. I cannot express how much it means to me to always have you in my corner, looking out for me.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

AE	Absorption efficiency
API	American Petroleum Institute
BC	British Columbia
BTEX	Benzene, toluene, ethylbenzene, and xylene
CCCM	Canadian Centre for Culture of Microorganisms
CI	Condition index
CLWB	Cold lake winter blend
CO	Crude oil
COOGER	Centre for Offshore Oil, Gas and Energy Research
CR	Clearance rate
DB	Diluted bitumen
DI	Deionized
GDP	Gross domestic product
GE	Guillard's f/2 enrichment
HAI	Health assessment index
HEWAF	High energy water accommodated fraction
HMW	High molecular weight
LMW	Low molecular weight
MAH	Monocyclic aromatic hydrocarbons
MD	Marine diesel
MO_2	Oxygen consumption
PAC	Polycyclic aromatic compounds
PADDS	Petroleum Administration for Defense Districts
PAH	Polycyclic aromatic hydrocarbons
PO_2	Oxygen partial pressure
POM	Particulate organic matter
SFG	Scope for growth
SFU	Simon Fraser University
TPAC	Total polycyclic aromatic compounds

VIU	Vancouver Island University
VOC	Volatile organic compounds
WAF	Water accommodated fractions
WCSB	Western Canada Sedimentary Basin

Chapter 1. General Introduction

1.1. Canadian oil industry

1.1.1. Value and size

Canada has one of the largest energy supplies in the world with high quantities of natural resources, a large spatial area, and a small population size (NRC, 2022). Consequently, it is the fourth-largest producer and third-largest exporter of oil in the world, fulfilling 5.9% of the global requirements (CER, 2022; NRC, 2022). Oil and gas contributed $133 B to the Canadian gross domestic product (GDP) in 2021 and were responsible for the direct and indirect employment of 178,000 and 415,000 individuals, respectively (CAPP, 2023; NRC, 2022). The daily total production of all crude oil resources within Canada is around 4.4 M barrels, while the total exportation of these oil products is 4.03 M barrels (Crude Oil, 2023). These products accounted for 16% of Canada's total exports in 2020, which added $86 B to the Canadian economy (NRC, 2022). Moreover, oil and gas extraction capital expenditures within Canada was $35 B (Crude Oil, 2023). At the end of 2020, 1,700 B barrels of crude oil were detected in Canada with the largest reservoirs found in the oil sands at the Western Canada Sedimentary Basin (WCSB), a primary source of crude oil and oil sands production (CER, 2022; NRC, 2022; Yang et al., 2011). Rich in oil and gas, the WCSB is 1.4 M km^2 in area, and covers almost the entirety of Alberta, along with parts of Manitoba, Saskatchewan, British Columbia (BC), Yukon, and Northwestern Territories (CER, 2022). The oil produced there is further characterized based on the chemical composition and physical properties as conventional light and heavy, field condensate, as well as mined and *in situ* bitumen (CER, 2022).

The most common processes for extracting these oil products are through mining or *in situ* methodologies (Federal Government, 2013; NRC, 2022). The mining process comprises scooping oil sands and transporting them to extraction plants for separating oil tailings from sand with the aid of steam. Since crude bitumen can be too viscous, *in situ* techniques are utilized that involve drilling horizontally or vertically into the oil wells and injecting steam to facilitate oil flow. This process is widely used for producing 50% of the overall petroleum products, as well as 80% of oil sand resources (CAPP Extraction, 2022; NRC, 2022). Once these crude oil products have been extracted, they are transported to

upgraders for refinement, which alters their physiochemical properties (NRC, 2022). Within Canada, the total upgrading capacity of such oils is 1.33 M barrels/d (NRC, 2022). Refinement of over 30% of Canadian oil products occurs within Canada, however, the remainder is exported to refineries in the United States (CAPP Extraction, 2022). These crude petroleum products are converted into a wide range of products through processes such as distillation, product blending, catalytic cracking, hydrotreating, reforming, and coking (Yang et al., 2011). Subsequently, the refined products are utilized as transportation fuels (gasoline, marine diesel, and heavy fuel oil), cooking fuel, liquified petroleum gases (propane and butane), petrochemical feedstock, along with other products such as kerosene, lubricating oils, greases, waxes, and asphalt (CAPP Extraction, 2022; CER, 2022; NRC, 2022).

1.1.2. Output and transportation

Among petroleum products, conventional crude oil and refined petroleum, including marine diesel, can easily flow through wellbores and pipelines, due to their liquid state at atmospheric temperature and pressure (CAPP Extraction, 2022). This is not the case with heavier crude oils and bitumen, which require additional processes such as heating or incorporating diluents, such as condensates, that alter their viscosity and density to allow transportation through pipelines (CAPP Extraction, 2022; Zhong et al., 2022). The total conventional crude oil exportation from Canada consists of 957,000 barrels/d, while 2.4 M barrels of diluted bitumen are exported daily (Government of Canada, 2022). The quantities for distillate fuels such as marine diesel are relatively low, with 196,000 barrels being exported daily from Canada (Refined Petroleum Products, 2023). Canada has also become one of the largest foreign suppliers of crude petroleum to the US, accounting for 61% of the total US crude oil and 23% of their refined oil product imports (NRC, 2020).

Within Canada, 19,000 km of oil pipelines are operated and regulated that cross provincial or international borders (CER, 2022). While rail and marine transportation comprised 4.6% and 7.8% of crude oil exports from Canada in 2020 respectively, oil pipelines were responsible for transporting the remaining 87.6% (CER, 2022). Currently, there are three pipeline projects (Keystone XL, Kinder Morgan Trans Mountain Expansion, and Enbridge Line 3 Replacement) being proposed, which will substantially increase the

number of petroleum products being exported from Canada (CER, 2022; Federal Government, 2013).

Marine tankers are responsible for carrying large quantities of oil from the western and eastern coasts of Canada every year (CER, 2022; Transport Canada, 2016). Marine crude oil exports averaged 279 M t/d in 2020 (CER, 2022). These transports from Burnaby, BC, allow the shipment of 2.2 M t of crude oil to Asia and the US west coast, while the 23 Atlantic Canada ports are responsible for transporting over 82 M t of produced offshore petroleum products to Europe and the US Petroleum Administration for Defense Districts (PADDS; CER, 2022; Transport Canada, 2016). Similarly, from the 39 ports in Quebec about 25 M t of crude and other petroleum products are moved (Transport Canada, 2016). About 180 large commercial vessels travel within 300 km of Canadian shores daily, which can considerably increase the risk of petroleum spills occurring in the marine environment (Transport Canada, 2016). With the expansion of petroleum routes planned for marine transportation, there is a high potential for accidental spillage of the extensively produced and exported crude and refined petroleum products to occur in the marine environment, including conventional crude oils, diluted bitumen, and marine diesel (CER, 2022; Crude Oil, 2023; Environment Canada, 2021; Refined Petroleum Products, 2023; Transport Canada, 2016; Zhong et al., 2022).

1.1.3. Marine diesel

Marine diesel (MD) is a heavy gas distillate fuel oil that is typically utilized by medium to high-speed marine diesel and propulsion engines (Marine Diesel Fuel Oil, n.d.). Daily, around 746,000 barrels of marine diesel are produced in Canada, with 500,000 barrels being consumed, and 196,000 barrels being exported (Refined Petroleum Products, 2023). While the constituents for MD could vary based on the origin location and physiochemical properties, one product for instance, can comprise 88% w/w saturated, 11% w/w aromatic, 2% w/w resin, and 0% w/w asphaltene hydrocarbons, in addition to 4.9% waxes in a fresh state (Environment Canada, 2021). Contrary to diluted bitumen and conventional crude oils, the concentrations of C_{16-34} are the highest, followed by C_{10-16}, C_{6-10}, and ($> C_{34}$) hydrocarbon fractions (Environment Canada, 2021). Some of the common hydrocarbon classes present in MD include normal, branched, and cyclo-alkanes, especially naphthalene and biphenyls, as well as isoprenoids, aromatics, and polar compounds containing sulfur, oxygen, or nitrogen (Mackay et al., 1985; Onwurah et

al., 2007). The high concentrations of two to three-ring polycyclic aromatic hydrocarbons (PAHs) in MD can partition readily into the water column (Neff et al., 2000). Additionally, monocyclic aromatic hydrocarbons (MAHs) such as benzene, toluene, and xylenes, along with low concentrations of trimethylbenzenes are present in MD (Neff et al., 2000). Other non-hydrocarbon components of marine diesel include porphyrins and their derivatives, biomarker compounds pentacyclic terpenes and steranes, along with metals like nickel, iron, zinc, vanadium, cobalt, copper, and titanium that form an association with the porphyrins (Alderman et al., 2017; Callot & Ocampo, 2000; Chicarelli et al., 1990; Onwurah et al., 2007; Yang et al., 2011).

1.1.4. Conventional crude oil

Conventional crude oil (CO) is one of the primary energy sources in the world, with a net field production of 3,300,000 barrels/d across Canada in 2022 (CER, 2022; Zhong et al., 2022). CO can be categorized as light, medium, and heavy-grade crude oils based on their chemical composition and physical properties (Crude Oil, 2023; Federal Government, 2013; Neff et al., 2000). Within Canada, around 826,000 barrels of light and medium-grade crude oil are being produced daily with 701,000 barrels/d being used for oil refineries (Crude Oil, 2023). Some of the compounds present in high concentrations within CO include two to five-ring polycyclic aromatic compounds (PACs), such as naphthalene, anthracene, phenanthrene, dibenzothiophene, fluorene, chrysene, pyrene, perylene, resin, and asphaltene, along with their alkylated homologs (Lee et al., 2015; Madison et al., 2017; Philibert et al., 2016; Stoyanovich et al., 2019; Yang et al., 2011). One such medium-grade CO is Alaska North Slope (ANS) crude, which has a composition of 58% w/w saturated, 32% w/w aromatic, 6% w/w resin, and 4% w/w asphaltene hydrocarbons, as well as 4% w/w waxes in an unweathered state (Environment Canada, 2021). Moreover, the highest concentration of hydrocarbon fractions detected in ANS crude were C_{16-34}, followed by C_{10-16}, (> C_{34}), and C_{6-10} (Environment Canada, 2021).

1.1.5. Diluted bitumen

Canada has one of the largest bitumen reserves in the world, holding almost 70% of this global resource (Zhong et al., 2022). As a result of depleting CO reserves, the petroleum oil sand production has substantially increased since 2010, where the daily production of oil sand products across Canada, including *in situ*, mined, and non-upgraded

crude bitumen were 1.79 M, 1.76 M, and 2.04 M barrels in 2022, respectively (CER, 2022; Crude Oil, 2023; Yang et al., 2011). One of the heaviest forms of petroleum, 98,000 barrels/d of diluted bitumen (DB) are consumed in Canada for refineries, of which 47% are upgraded into refined products in Alberta (Crude Oil, 2023; NRC, 2022). Bitumen refinement is performed with diluents to alter its chemical and physical composition by converting it into a semiliquid form with reduced density and viscosity (Federal Government, 2013; Stoyanovich et al., 2019; Yang et al., 2011; Zhong et al., 2022). The diluents used for its refinement are natural gas condensates composed of BTEX (benzene, toluene, ethylbenzene, xylene), and naphthalene, along with $C_{3\text{-}10}$ alkane hydrocarbons, metals, and naphthenic acids (Alderman et al., 2017; Lee et al., 2015; Speight, 2010; Stoyanovich et al., 2019; Zhong et al., 2022). Moreover, the addition of water during the bitumen dilution process makes it easier to transport in pipelines (Ashrafizadeh & Kamran, 2010). One of the highest-volume diluted bitumen products transported within Canada is the Cold Lake Winter Blend (CLWB) DB that originates from Alberta (Environment Canada, 2021; Federal government, 2013). In an unweathered state, CLWB has 46% w/w saturated, 30% w/w aromatic, 11% w/w resin, 13% w/w asphaltene hydrocarbons, and 3% w/w waxes. The majority of alkylated and priority PACs detected in CLWB include naphthalene, phenanthrene, dibenzothiophene, fluorene, benzonaphthothiophene, and chrysene, while other priority PACs can consist of perylene, pyrene, biphenyl, acenaphthene, acenaphthylene, fluoranthene, and anthracene (Environment Canada, 2021). Furthermore, amongst the hydrocarbon fractions detected in CLWB, $C_{16\text{-}34}$ had the highest concentrations, followed by $C_{10\text{-}16}$, ($> C_{34}$), and lastly, $C_{6\text{-}10}$ (Environment Canada, 2021). Some other non-hydrocarbon compounds present in DB include sulfur, and nitrogen, along with vanadium and nickel that organically bind to asphaltene, a high molecular weight hydrocarbon (Chevron, 2021; Hounjet et al., 2018).

1.1.6. Environmental fate

In the aftermath of an oil spill in a marine environment, the fate of oil is dependent on a complex interplay of various physical and chemical processes that significantly impact the ecosystem (Federal Government, 2013; Neff et al., 2000; Zhong et al., 2022). The discharge of petroleum products into aquatic ecosystems results in its weathering, where processes such as spreading, drifting, partitioning, evaporation, dissolution, photochemical oxidation, biodegradation, sedimentation, and emulsification can occur

(French-Mccay, 2004; ITOPF, 2021; Lee et al., 2015; Neff et al., 2000; Shiu et al., 1990; Stoyanovich et al., 2019; Zhong et al., 2022). Additionally, the fate of oil products is influenced by environmental factors such as weather condition, wind speed, tidal current, salinity, temperature, solar insolation, geomorphology, available nutrients for microbial degradation, presence of biota, along with the extent of remediation performed at the spill site (Mackay & Mcauliffe, 1989; Yim et al., 2002). The major constituents of oil, including aromatic and alkane hydrocarbons of different molecular weights, as well as volatile organic compounds (VOCs) can undergo distinct transformations as they interact with the surrounding water, air, and sediment (Baussant et al., 2001; Cripps & Shears, 1997; French-Mccay et al., 2019; Stoyanovich et al., 2019; Yim et al., 2002).

Evaporation is one of the initial processes that act upon the oil slick present on the water surface, particularly with lighter hydrocarbons such as volatile alkanes and aromatics (French-Mccay, 2004; ITOPF, 2021; Lee et al., 2015; Stoyanovich et al., 2019). These compounds have low boiling points and readily vaporize into the atmosphere, forming airborne plumes (French-Mccay et al., 2019; Stoyanovich et al., 2019). During the initial phases of oil weathering lighter petroleum products can lose up to 75% of their initial volume, while medium-grade and heavier residual oils can lose up to 40% and 5% of their respective initial volumes (Federal Government, 2013). This is highly dependent on the water temperature and wind conditions (Hounjet et al., 2018; ITOPF, 2021; Mackay & McAuliffe, 1989). It has also been determined that after a week on the sea surface, between 23-100% of crude and refined oil mass can be lost due to evaporation (Neff et al., 2000). Weathering of conventional crude oil, diluted bitumen, and marine diesel can cause a progressive loss of low molecular weight (LMW) and volatile MAHs and PAHs, including the C_{10-13} alkanes, benzene, naphthalene, and their C_{1-2} alkyl homologs, while the less volatile phenols and high molecular weight (HMW) hydrocarbons remain more persistent (Federal Government, 2013; Neff et al., 2000). The rapid reduction of volatile BTEX compounds is highest in MD, followed by CO and then DB (Cripps & Shears, 1997; Environment Canada, 2021; Hounjet et al., 2018; Neff et al., 2000; Zhong et al., 2022).

Spreading occurs concurrently, driven by the oil density, viscosity, as well as volume spilled (French-Mccay, 2004; ITOPF, 2021; Mackay & Mcauliffe, 1989). Lighter oil fractions tend to spread rapidly, creating thin surface slicks that can cover large surface areas, while heavier fractions tend to remain in the water column or sink (ITOPF, 2021; Lee et al., 2015; Zhong et al., 2022). The higher proportions of heavier molecular weight

resins and asphaltenes present in DB, compared to CO and MD can increase the oil density and viscosity with the weathering processes (Hounjet et al., 2018; Stoyanovich et al., 2019; Yang et al., 2011). This further results in DB having a lower and slower spreading potential relative to the other products after an oil spill (Onwurah et al., 2007; Stoyanovich et al., 2019; Zhong et al., 2022).

Emulsification is another long-term process that occurs following evaporation, which can lead to the formation of highly viscous oil-water emulsions (French-Mccay, 2004; ITOPF, 2021; Lee et al., 2015; Stoyanovich et al., 2019). Mixing and turbulence results in the dispersion of oil into tiny droplets suspended in the water (Mackay & Mcauliffe, 1989; Stoyanovich et al., 2019; Zhong et al., 2022). Such emulsified oil particles would be more persistent in the environment after a spill (ITOPF, 2021). Moreover, emulsification is dependent on petroleum release conditions, including depth, rate, and volume of oil spilled; environmental conditions, such as wind action, aqueous phase pH, temperature, and tidal currents; physiochemical properties of oil (Ashrafizadeh & Kamran, 2010; ITOPF, 2021). The proportion of oil remaining afloat on the water surface in the form of a stable emulsion is found to be higher for DB than CO and MD since it has a lower rate of dispersion in water due to the presence of resins and asphaltene (Hounjet et al., 2018; Zhong et al., 2022). Moreover, since CO and MD have a higher proportion of volatile components than DB, bitumen likely persists longer in an aqueous environment (Environment Canada, 2021; Hounjet et al., 2018; Stoyanovich et al., 2019).

Meanwhile, dissolution comes into play after a spill, as water-soluble components such as VOCs and some lighter hydrocarbons dissolve rapidly into the water phase (French-Mccay et al., 2019; Hodson et al., 2019; Mackay & Mcauliffe, 1989). The rate and extent of complex dissolved hydrocarbon mixtures being released in the water column are dependent on the oil composition, viscosity, surface tension, droplet size, water temperature, wave turbulence, and degree of oil dispersion (French-Mccay, 2004; ITOPF, 2021). Although the dissolved oil fraction is usually smaller than the total oil mass, it can intimately contact aquatic organisms and have a toxic impact (Shiu et al., 1990). The most soluble petroleum component is BTEX which rapidly dissolves, whereas PACs partially dissolve in the water column, while some surface alongside oil droplets (French-Mccay et al., 2019). Consequently, light petroleum products, such as MD have a higher dissolution rate as compared to heavier and more viscous CO and DB due to the plateauing PAC concentrations in the water column (Stoyanovich et al., 2019).

Sinking is also often associated with the heavier components of oil, particularly high molecular weight hydrocarbons, including resins and asphaltenes (Hounjet et al., 2018; Stoyanovich et al., 2019; Zhong et al., 2022). Some major mechanisms that can result in the sinking of petroleum products are evaporation, water temperature, photooxidation, and water molecule uptake (Federal Government, 2013). These components can aggregate with suspended particles, sediments, and organic matter, causing them to settle on the seafloor (Federal Government, 2013; ITOPF, 2021; Zhong et al., 2022). This could impact the carbon biogeochemical cycling by increasing the available nutrients and organic matter for sustaining benthic organisms in a marine ecosystem (Baussant et al., 2001; Mackay & Mcauliffe, 1989; Onwurah et al., 2007; Yim et al., 2002). It has been determined that petroleum products with an American Petroleum Institute (API) gravity lower than 6.0 and density exceeding 1.035 g/mL are more likely to sink, which would suggest that fresh CO, DB, and MD would float on the seawater surface after a spill (Environment Canada, 2021; Federal Government, 2013; Hounjet et al., 2018; Zhong et al., 2022). However, the evaporation of lighter PACs and the hydration of oils can increase their viscosity and density, causing the eventual sinking of heavier oils over a longer timeframe (Stoyanovich et al., 2019; Zhong et al., 2022).

Additionally, microbial degradation contributes to the loss of PACs from petroleum spills in the water column (Atlas, 1975; Neff et al., 2000). This biodegradability by indigenous microbiota is dependent on water temperature and depth; oil surface area, physiochemical characteristics, and dissolution potential; oxygen and nutrients (primarily nitrogen and phosphorous) availability (Atlas, 1975; French-Mccay et al., 2019; ITOPF, 2021). Lighter and water-soluble components of oil, such as BTEX are rapidly biodegraded compared to heavier oils that have greater resistance to microbial degradation due to the presence of complex and heavier hydrocarbons, such as resin, hopane, and asphaltene (Atlas & Hazen, 2011; Federal Government, 2013; Zhong et al., 2022). This results in lighter oils like MD having higher biodegradation rates compared to heavier CO and DB that tend to persist longer in the marine environment (Cripps & Shears, 1997; ITOPF, 2021; Onwurah et al., 2007).

Another weathering process for petroleum spills is photochemical oxidation that rapidly degrades terpenes and steranes, along with the alkylated homologs of some PACs under intense sunlight (Neff et al., 2000; Stoyanovich et al., 2019; Zhong et al., 2022). Ultraviolet radiation can cause the activation of chemical residues remaining after an oil

spill and increase the bioaccumulation of PACs in aquatic organisms (Calfee, et al., 1999; Hodson et al., 2019; Onwurah et al., 2007). Photo-oxidation results in the formation of oxidized compounds, including aliphatic and aromatic ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxides, sulfoxides, sulfones, phenols, anhydrides, quinones, along with aliphatic and aromatic alcohols (Lee, 2003). Photolysis can also cause the loss of petroleum hydrocarbons based on their chemical structure and oil properties, resulting in MD having the highest photolytic rate of decomposition compared to CO and DB (Zhong et al., 2022).

Ultimately, knowledge regarding the weathering processes acting on spilled oil is essential for designing effective spill response strategies and mitigating their ecological and economic consequences. By studying the environmental fate of oil components, better predictions on the extent of contamination, potential exposure pathways for aquatic organisms, and long-term impacts on ecosystem health are made. This understanding also informs regulatory decisions, risk assessments, and development of remediation technologies that minimize the adverse effects of oil spills on marine environments.

1.2. Biological impact

1.2.1. Bivalves

Extensive research has been performed on the impact of petroleum products on numerous aquatic species, but it is essential to understand their effects on bivalves. Bivalves are a group of aquatic mollusks encompassing clams, mussels, oysters, and scallops that play pivotal roles in aquatic ecosystems as filter feeders and are crucial components of food chains, while being economically vital (Lüchmann et al., 2011; Orban et al., 2004; Soniat et al., 2011). Their ecological and economic significance and potential vulnerability make investigating the impacts of oil spills on bivalves imperative. In 2019, the aquaculture of these organisms generated approx. $116 M within Canada, based on the total production of 43,000 t of bivalves (Statistics Canada, 2022). Bivalves provide several ecological services that include the building of reefs used as a shelter, habitat, and food resources for various fish and shellfish species, along with stabilizing shorelines and enriching biogeochemical cycling (Newell, 2004; Powers et al., 2017; Proffitt et al., 2011; Stefansson et al., 2016; Viginier et al., 2015). The deposition of feces in these reefs also stimulates bacterial growth, which aids in the denitrification of nitrogen in the water

(Grabowski et al., 2017; Piehler & Smyth, 2011). Additionally, bivalves can improve water quality through the filtration of suspended particulate that enhances light penetration, as well as promote the deposition of nutrients in the environment for supplementing the growth of algae and aquatic plants (Finch et al., 2016; Grabowski et al., 2017; Loh et al., 2018; Newell, 2004; Stefansson et al., 2016). Bivalves also increase the recruitment and growth of other ecologically and economically valuable species by providing them sustenance as prey or releasing suspended particles in the form of excretion or pseudofeces (Fukuyama et al., 2000; Grabowski et al., 2017; Loh et al., 2018; Proffitt et al., 2011; Sun et al., 2020).

Bivalves have an expansive distribution around the globe, a sedentary lifestyle, robust size, and suspension feeding ability that increases their susceptibility to environmental pollutants (Loh et al., 2018; Mondol et al., 2015; Orban et al., 2004; Peterio et al., 2007; Soniat et al., 2011; Sun et al., 2020). These broadcast spawners have an increased likelihood of being exposed to spilled petroleum components during their early developmental stages since gametes are typically free-floating near the water surface (Finch et al., 2016). Bivalves have a reduced capacity for the oxidative biotransformation of petroleum compounds such as PACs, along with a slow metabolism that allows the retention of higher PAC concentrations in their tissues over time (Baussant et al., 2001; Loh et al., 2018; Soniat et al., 2011). Consequently, bivalves can be used as biomonitor organisms for assessing the biological and ecological impacts of petroleum contamination in an aquatic environment (Lüchmann et al., 2011; Orban et al., 2004; Soniat et al., 2011).

1.2.2. Petroleum spill impacts on bivalve ecology

Every year approximately 4.63 M t of petroleum products are discharged into the marine environment, as a result of accidental spills from tankers, drilling rigs, and oil wells (Li et al., 2022; Sun et al., 2020). Some notable large-scale spills that induced mass mortality and had long-term toxicological implications on bivalves were the Exxon Valdez (EV) spill in 1989 (Downs et al., 2002; Fukuyama et al., 2000; Trowbridge et al., 2001) and Deepwater Horizon (DWH) oil blowout in 2010 (Atlas & Hazen, 2011; Beyer et al., 2016; Langdon et al., 2016; Powers et al., 2017; Soniat et al., 2011). During the EV spill, release of crude oil resulted in a combination of chemically induced toxicity, anoxic conditions, and smothering that induced a decline in the total abundance, biomass, richness, diversity, and densities of bivalves in Prince William Sound, Alaska (Driskell et

al., 1993; Jewett et al., 1999; Peterson et al., 2003). In particular, there was a 40% mortality of blue mussels, *Mytilus trossulus* (Andre et al., 1993; Soon & Ransangan, 2019). It was further estimated that 40-60% of bivalves had been sub-lethally affected by oil exposure (Peterson et al., 2003). Although the quantities of EV spilled oil had reduced to less than 2% of initial amounts on the Prince William Sound beaches a decade after the spill, bivalves were still displaying sub-lethal effects (Downs et al., 2002). It was also proposed that the complete recovery of bivalve populations post the EV spill would take place over three to six generations, or around 30 years (Fukuyama et al., 2014).

Similarly, the DWH spill caused an impairment in bivalve species richness, diversity, and evenness in the Gulf of Mexico with improvements observed in these biological parameters with increasing distance from the oil wellhead (Baguley et al., 2015; Reuscher et al., 2017; Washburn et al., 2017). The DWH spill resulted in mass mortality of 77% bivalves in Barataria Bay, Gulf of Mexico (Soon & Ransangan, 2019), while causing a reduced density in 68% spat, 32% seed, and 24% adult bivalves at Barataria Bay post-spill (Grabowski et al., 2017; Powers et al., 2017). Moreover, there was a 27% decline in the abundance of adult bivalves after a year of the spill at four locations in the peninsular Florida Gulf of Mexico estuaries due to reduced oyster habitat (Grabowski et al., 2017; Proffitt et al., 2011). The bivalve population in these locations continued to have a slow recovery 6 years after the blowout with a complete recuperation of their densities estimated to occur a decade after the DWH spill (Fleeger et al., 2018).

1.2.3. Oil toxicity in bivalves

After an oil spill, bivalves encounter petroleum products through various exposure routes and pathways (Lüchmann et al., 2011; Mondol et al., 2015; Orban et al., 2004; Soniat et al., 2011). These routes encompass passive exposure *via* the mantle cavity during food ingestion (Redmond et al., 2016; Schmutz, 2018) and passive diffusion across their gill epithelium during respiration due to the extensive surface area and lipid-rich membranes (Baussant et al., 2001; Li et al., 2022; Luna-Acosta et al., 2011; Redmond et al., 2016; Schmutz, 2018). Suspended oil particles trapped in bivalve gills can also transfer to their gut, where they become incorporated into endocytic vacuoles within digestive glands, ultimately assimilating into tissue lipids (Baussant et al., 2001; Luna-Acosta et al., 2011). Moreover, bivalves can absorb freely dissolved petroleum constituents from the

water column through dermal surfaces and cross the biological membranes (Li et al., 2022; Loh et al., 2018; Shiu et al., 1990).

The biological impact of petroleum products on bivalves is contingent upon oil constituents, their physiochemical properties, weathering, spill conditions, exposure duration, and ultimately, bioavailability. The feeding habits, lifestyle, and developmental stage of the exposed species are also influential factors (Baussant et al., 2001; Lee & Page, 1997; Mackay & Mcauliffe, 1989; Pathak & Mandalia, 2012; Yim et al., 2002). These contaminants subsequently induce both lethal and sublethal toxicity in bivalves through diverse mechanisms, including nonpolar narcosis and activation of the aryl hydrocarbon receptor (AHR), which can impact gene expression, DNA integrity, enzymatic activity, histopathology, physiology, morphology, reproduction, and behavior (Baussant et al., 2001; Cripps & Shears, 1997; Dupuis & Ucan-Marin, 2015; Jesus et al., 2022; Onwurah et al., 2007; Prescott et al., 1996; Soon & Ransangan, 2019; Widdows et al., 1990; Widdows & Staff, 2006). The direct physical effects of oil exposure can also lead to bivalve asphyxiation by clogging gills or binding their velum (Vignier et al., 2015).

Lethal effects

Previous research has linked bivalve mortality resulting from petroleum exposure to the presence of lighter molecular weight PACs, such as naphthalene and acenaphthene, along with BTEX compounds (Di Toro et al., 2007; Neff et al., 2000; Schrandt et al., 2018; Stefansson et al., 2016; Vignier et al., 2016). The weathering process of these oil products leads to the removal of a substantial portion of these acutely toxic components, causing the persistence of HMW hydrocarbons in the environment, which exhibit a lower toxic potential (Stefansson et al., 2016). LC_{50} values in bivalves from field and laboratory petroleum exposures range from 10-247 µg/L in *Crassostrea virginica* D-veliger larvae (24 h exposure to crude oil; Laramore et al., 2014), 7.9-2540 µg/L in *Mulinia lateralis* to four crude oil products (Pelletier et al., 1997), and 715-2814 µg/L in three developmental stages of *Crassostrea virginica* exposed to a high energy water accommodated fraction (HEWAF) of crude oil (Viginier et al., 2016). Moreover, advanced bivalve development can increase their tolerance to oil products, since smaller individuals have a higher surface area to volume ratio that allows a greater uptake of dissolved petroleum components *via* passive diffusion (Laramore et al., 2014).

Molecular and biochemical effects

Exposure to petroleum compounds can have deleterious molecular and biochemical consequences on bivalves, which can impact their detoxification response toward PACs (Dos Reis et al., 2020; Gan et al., 2021; Lima et al., 2019; Zacchi et al., 2019). Given the limited genotoxic studies performed with petroleum mixtures, the reported impacts on bivalve gene expression are based on individual PAH species. Exposure to fluorene and pyrene can increase the CYP2AU1, CYP2AU2, GST, and SULT transcripts in bivalve gills (Dos Reis et al., 2020; Zacchi et al., 2019). Phenanthrene exposure can also elevate the CYP2AU1 transcripts in the gills, mantle, and digestive tract (Dos Reis et al., 2020) while reducing the GSTΩ transcript (Lima et al., 2019). Moreover, petroleum contamination can induce the expression and production of many response genes responsible for PAC metabolism through interactions with the aryl hydrocarbon receptor nuclear translocator (ARNT; Denison & Nagy, 2003; Jesus et al., 2022). This induction further alters the CYP1 family metabolic pathways, which are mediated by the aryl hydrocarbon receptor (AHR) transcription factors (Conney, 1982; Jesus et al., 2022). The activation of these transcripts can impact biotransformation, which can comprise of increased ethoxy resorufin-O-demethylase (EROD), aryl hydrocarbon hydroxylase (AHH), and microsomal GSH transferase (MGST) activity in gill tissue (Dos Reis et al., 2020; Li et al., 2022; Zacchi et al., 2019). Variations in gene expression due to petroleum contamination can also induce genotoxicity in bivalves, which is enhanced after biotransformation due to an increased accumulation of toxic PAC metabolites (Baussant et al., 2001; Capuzzo et al., 1988). Altered gene expression can even cause DNA strand damage (Baussant et al., 2001; Dupuis & Ucan-Marin, 2015; Sarkar et al., 2017) and impair DNA replication (Gan et al., 2021; McCarrick et al., 2019; Sarkar et al., 2017). Exposure to lighter hydrocarbons can also decrease the heat shock protein 90 (*HSP90*) levels, causing transcriptional or translational inhibition (Lüchmann et al., 2011).

Elevated antioxidant enzyme activity due to petroleum exposure can also allow the elimination of oxygen radicals in bivalve tissues (Li et al., 2022). This antioxidase response can result in the upregulation of various respiratory and electron transfer chain enzymes such as superoxide dismutases (SOD), glutathione S-transferases (GST), and catalases (CAT) while causing a reduction in the peroxidase (POD) and glutathione reductase (GR) activity (Downs et al., 2002; Jiang et al., 2017; Lima et al., 2019; Luna-Acosta et al., 2017; Sarkar et al., 2017). Another oxidative stress reaction that occurs due to metabolic

impairment in bivalves is lipid peroxidation (Li et al., 2022; Lüchmann et al., 2011; Luna-Acosta et al., 2011). PAC exposure can also induce an immune response with a reduction in granulocyte counts, phagocytic capacity, and reactant oxygen species (ROS) production (Bado-Nilles et al., 2008; Donaghy et al., 2010; Volety et al., 2016). These exposures can further cause alterations in the biochemical composition of bivalve carbohydrates and proteins while utilizing excessive glycogen reserves to combat stress related to chemical exposure (Mondol et al., 2015; Patel & Eapen, 1989; Peterio et al., 2007; Smolders et al., 2004; Stekoll et al., 1980).

Cellular effects

At the cellular level, petroleum exposure has been associated with disturbances in bivalve immunosuppression (Baussant et al., 2011; Schmutz et al., 2021; Sun et al., 2020; Widdows et al., 1982). Impairment of the bivalve immune response upon oil exposure can be attributed to hemocyte apoptosis, syncytia formation, and inflammation, accompanied by an increased number of agranular hemocytes and a reduction in granular hemocytes (Croxton et al., 2012; Gan et al., 2021; Vignier et al., 2018). Furthermore, the elevation of ROS production in hemocyte immune effector cells due to oil exposure can compromise the cytoskeleton and membrane integrity, leading to disrupted phagocytic capability for xenobiotic molecules (Sun et al., 2020; Tang et al., 2020). This ROS induction subsequently downregulates immune system-related molecular signaling pathways (e.g., NF-κB) and triggers the apoptosis process in cells, impacting their survival, differentiation, and viability (Dayem et al., 2017; Downs et al., 2002; Jiang et al., 2017; Sun et al., 2020). Additionally, the absorption of hydrocarbon compounds can influence the permeability and fluidity of lipid bilayer membranes, as well as induce the enlargement and destabilization of lysosomal membranes (Hwang et al., 2008; Li et al., 2022; Luna-Acosta et al., 2017; Moore et al., 1987; Schmutz et al., 2021). Given that lysosomal activity plays a crucial role in the degradation of ingested contaminants *via* phagocytosis, these effects can collectively have a detrimental impact on bivalve immunoreactivity (Li et al., 2022; Schmutz et al., 2021). Exposure to PACs can also trigger the development of renal cell, gill filament, circulatory system, gastrointestinal, and gonadal tumors in bivalves (Gan et al., 2021; Gardner et al., 1991).

Histopathological effects

Petroleum contaminants have been linked to bivalve tissue damage (Aarab et al., 2011; Schmutz et al., 2021). Following an oil spill, inflammation, lesions, necrosis, and atrophy in the digestive gland and gonadal epithelium tissue have been observed in *Ostrea edulis* and *Crassostrea gigas* (Berthou et al., 1987; Neff et al., 1987; Schmutz et al., 2021; Trowbridge et al., 2001). *Mytilus edulis* has shown hemocyte cell infiltration into bivalve follicles, severe neoplasia, and atresia in mussel oocytes with petroleum exposure (Aarab et al., 2011; Bignell et al., 2011; Schmutz et al., 2021; Smolarz et al., 2017). Hemocytic infiltration is also observed in the digestive gland and gill tissues (Joshy et al., 2022). Littleneck and butter clams exhibit histopathological alterations like tubular vacuolation and granulocytosis of digestive glands, disorganization of lamellar cells, vacuolization, and hyperplasia of epithelial cells, gill tissue edema, along with increased susceptibility to parasitosis (Anderson, 1988; Dos Reis et al., 2020; Joshy et al., 2022; Khan et al., 2015; Trowbridge et al., 2001). Tubular atrophy of the digestive gland occurs after the enlargement of tubular lumen and thinning of the tubular lining, due to petroleum stress-induced morphological alterations of the epithelium lining and increased lipid accumulation (Cajaraville et al., 1992; Joshy et al., 2022; Ogunola, 2017; Wu et al., 2019).

Physiological effects

The physiological implications of petroleum contamination in bivalves include an impact on their metabolism and growth potential (Baussant et al., 2001; Mondol et al., 2015; Widdows & Johnson, 1988). Exposure to petroleum mixtures can result in the decline of bivalve scope for growth (SFG), a quantitative assessment that incorporates various energy acquisition and expenditure processes (Kang et al., 2015; Mondol et al., 2015; Widdows & Donkin, 1991). This includes reduced clearance rates causing starvation (Jeong & Cho, 2007; Vignier et al., 2019), hindered food assimilation efficiency (Jeong & Cho, 2007), as well as elevated respiration rates and ammonium excretion (Jeong & Cho, 2007; Kim et al., 2007; Redmond et al., 2016). Nonspecific narcotic action of petroleum hydrocarbons on ciliary activity and valve closure affects these processes (Donkin et al., 1989; Jeong & Cho, 2007; Kim et al., 2007; Schmutz, 2018; Trowbridge et al., 2001; Vignier et al., 2015; Widdows et al., 1990, 2002). Prolonged accumulation of high hydrocarbon concentrations results from impaired bivalve metabolism (Jeong & Cho, 2007; Schmutz, 2018; Widdows et al., 1990). Byssal threads, crucial for attachment and settlement, can also be affected, impacting bivalve recruitment success (Chew & Ma,

1987; Schmutz, 2018; Trowbridge et al., 2001). The impact of petroleum contaminants on bivalve physiological responses can ultimately influence their growth rate, reproductive success, and ability to combat xenobiotic stress (Capuzzo et al., 1988).

Morphological effects

The morphological effects of oil include deterioration in the shell and somatic tissue growth, along with other morphological abnormalities (Bayen et al., 2007; Mondol et al., 2015; Schrandt et al., 2018). These morphological abnormalities consist of shell chambering, where the inner valves thicken and become ball-shaped with time, impacting the health of juvenile and adult bivalves (Bayen et al., 2007). Some other morphological modifications include alterations to the shell hinge, reduction or inhibition of shell growth and formation, as well as the development of shell crystallization (Le Pennec & Le Roux, 1979; Vignier et al., 2015).

Reproductive effects

Petroleum exposure can impact the reproductive output of bivalves, as well as their population structure and dynamics (Capuzzo et al., 1988; Chu et al., 2003; Mondol et al., 2015; Onwurah et al., 2007). These impairments include direct cytotoxicity on gonads and spawned gametes, endocrine disruption, and DNA strand damage, that are also observed in future generations (Schmutz, 2018; Vignier et al., 2015). Impaired breeding processes can consist of delayed gonad maturation, sporadic gametogenesis, spawning disruption, reduced fertilization success rates, lower sperm motility, and stunted bivalve embryo development (Aarab et al., 2011; Baussant et al., 2011; González-Fernández et al., 2016; Jeong & Cho, 2005; Laramore et al., 2014; Mondol et al., 2015; Pelletier et al., 2000; Toro et al., 2003; Vignier et al., 2015). Gametogenesis impairment usually coincides with female gonad atresia, as well as a low proportion of oocyte viability (Schmutz et al., 2021). Under harsh spawning environmental conditions, gonadal atresia can be triggered after the ripening of gametes, which can result in their over-maturation and termination of gametogenic cycle, as well as the eventual degeneration and resorption of the gonadal tissue (Schmutz et al., 2021; Smolarz et al., 2017). Moreover, transgenerational effects can occur in bivalves through exposure, maternal transfer, or a combination of both (Schmutz et al., 2021). In particular, alkylated forms of petroleum hydrocarbons have been associated with chronic embryotoxicity in bivalves through direct exposure to gametes, embryos, or larvae (Schmutz et al., 2021; Vignier et al., 2015). These effects can further

influence the growth, morphogenesis, survival, viability, and settlement success of exposed larvae since they are more sensitive relative to adults given their limited capabilities for biotransformation and elimination of petroleum components (Capuzzo et al., 1988; Choy et al., 2007; Pelletier et al., 2000; Vignier et al., 2015).

Behavioral effects

Exposure to petroleum hydrocarbons can alter bivalve behavior depending on the degree of oiling in their habitats (Trowbridge et al., 2001; Schmutz, 2018). These effects may include the impaired ability of bivalves to burrow and close their valves, which can increase their likelihood of being predated (Pearson et al., 1981; Trowbridge et al., 2001).

1.3. Pacific oysters (*Crassostrea gigas*)

One bivalve species that may be impacted by the expansion of petroleum marine transportation along the Pacific Northwest coast of Canada are Pacific oysters, *Crassostrea gigas* (Bayen et al., 2007; Cassis et al., 2011; Loh et al., 2018; Luna-Acosta et al., 2011; Orban et al., 2004; Soniat et al., 2011). These economically relevant species are cultivated around the world in indigenous, commercial, and recreational fisheries for human consumption (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Orban et al., 2004; Soniat et al., 2011). On an annual basis, approximately 10,000 t of Pacific oysters are grown within Canada, with 5,000 t coming from BC while adding $41 M to the Canadian GDP (Bayen et al., 2007; Cassis et al., 2011; Noakes, 2018; Soniat et al., 2011; Statistics Canada, 2022).

These organisms were first introduced in BC from Japan for aquaculture in 1912 (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Noakes, 2018). *Crassostrea gigas* are protandry hermaphrodites and broadcast spawners, where the males initially spawn during the first reproductive season and can transition towards becoming females in the winter months (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Pauley et al., 1988). These organisms reach sexual maturity in the first year, with gonadal development initiating in March and fully developing around June before spawning occurs in September (Gillespie et al., 2012; Pauley et al., 1988). Warmer water temperatures stimulate their gonadal development, spawning success, and larvae metamorphosis (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012). The optimal water temperature and salinity for spawning are 20-25 °C and 35 ppt, however, 11-34 °C and

20-30 ppt are the optimal conditions for growth (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012). Pacific oyster larvae have a rapid development, where the veliger stage is achieved under 28 h at 22 °C, or 72 h at 14 °C (Gillespie et al., 2012; Pauley et al., 1988). The subsequent developmental stages of the D-hinge, umbo, and eye spots occur before the settlement, which can occur within 20-30 days of fertilization (Gillespie et al., 2012). Once the larvae have completely developed and settled on a substrate they undergo metamorphosis, where they lose their eyespots, foot, and anterior adductor muscles before developing gills and mantle for adulthood (Gillespie et al., 2012).

Adult Pacific oysters are sessile, and their growth rates depend on environmental factors such as temperature, substrate, and formation of population aggregates (Gillespie et al., 2012; Quayle, 1988). The diet of these obligate filter feeders constituents of bacteria, protozoan, diatom, invertebrate larvae, algae, and other organic detritus present in the water column (Gillespie et al., 2012; Quayle, 1988). *Crassostrea gigas* populations tend to reside on harder substrates in high to mid-intertidal zones that can consist of older oyster shells, bedrock, and even outcrops (Fisheries and Oceans Canada, 2023; Ruesink et al., 2005). While the growth rate of oysters is rapid in the earlier life stages, it slows down considerably with maturity and undergoes senescence at around 4 to 5 y old (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Pauley et al., 1988; Quayle, 1988). The lifespan of Pacific oysters has been known to span decades, with maximum ages of 40 years being reported at the northern latitudes (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Pauley et al., 1988). Additionally, these robust organisms can reach 30 cm in length as adults (Gillespie et al., 2012).

Pacific oysters are at constant risk of petroleum exposure released in the marine environment due to accidental spills or oil well leakages (Li et al., 2022; Mondol et al., 2015; Orban et al., 2004; Peterio et al., 2007; Soniat et al., 2011; Wade et al., 2014). Given their filter feeding and slow biotransformation capabilities, there is a slight depuration of petroleum components from the lipid-rich compartments of their tissues (Baussant et al., 2001; Capuzzo, 1996; Soniat et al., 2011). Consequently, these organisms can be utilized as a sentinel species to understand the impact of hydrocarbon pollutants in the marine environment, as well as assist with efficient *in situ* monitoring and application of appropriate remediation techniques following an oil spill (Bodin et al. 2004; Lüchmann et al., 2011; Orban et al., 2004; Soniat et al., 2011).

1.4. References

Aarab, N., Godal, B. F., & Bechmann, R. K. (2011). Seasonal Variation of Histopathological and Histochemical Markers of PAH Exposure in Blue Mussel (*Mytilus Edulis* L.). *Marine Environmental Research, 71*(3), 213–17.

Alderman, S. L., Lin, F., Farrell, A. P., Kennedy, C. J., & Gillis, T. E. (2017). Effects of diluted bitumen exposure on juvenile sockeye salmon: From cells to performance. *Environmental Toxicology and Chemistry*, *36*(2), 354–360. https://doi.org/10.1002/etc.3533

Anderson, R. S. (1988). Effects of anthropogenic agents on bivalve cellular and humoral defence mechanisms. *American Fisheries Society Special Publication, 18*, 238-242.

Andre, C., Jonsson, P. R., & Lindegarth, M. (1993). Predation on settling bivalve larvae by benthic suspension feeders: The role of hydrodynamics and larval behaviour. *Marine Ecology Progress Series*, *97*, 183–192. https://doi.org/10.3354/meps097183

Ashrafizadeh, S., & Kamran, M. (2010). Emulsification of heavy crude oil in water for pipeline transportation. *Journal of Petroleum Science and Engineering, 71*, 205-211. doi:10.1016/j.petrol.2010.02.005

Atlas, R. M. (1975). Effects of Temperature and Crude Oil Composition on Petroleum Biodegradation. *Applied Microbiology, 30*(3), 396-403. doi:10.1128/aem.30.3.396-403.1975

Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environmental Science & Technology*, *45*(16), 6709–6715. https://doi.org/10.1021/es2013227

Bado-Nilles, A., Gagnaire, B., Thomas-Guyon, H., Le Floch, S., & Renault, T. (2008). Effects of 16 pure hydrocarbons and two oils on haemocyte and haemolymphatic parameters in the Pacific oyster, Crassostrea gigas (Thunberg). *Toxicology in Vitro*, *22*(6), 1610–1617. https://doi.org/10.1016/j.tiv.2008.04.011

Baguley, J. G., Montagna, P. A., Cooksey, C., Hyland, J. L., Bang, H. W., Morrison, C., Kamikawa, A., Bennetts, P., Saiyo, G., Parsons, E., Herdener, M., & Ricci, M. (2015). Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater Horizon Blowout and oil spill. *Marine Ecology Progress Series*, *528*, 127–140. https://doi.org/10.3354/meps11290

Baussant, T., Sanni, S., Jonsson, G., Skadsheim, A., & Børseth, J. F. (2001). Bioaccumulation of polycyclic aromatic compounds: 1. Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil. *Environmental Toxicology and Chemistry, 20*(6), 1175-1184. doi:10.1002/etc.5620200606

Bayen, S., Lee, H. K., & Obbard, J. P. (2007). Exposure and response of aquacultured oysters, *Crassostrea gigas*, to marine contaminants. *Environmental Research, 103*, 375-382. doi:10.1016/j.envres.2006.06.012

Berthou, F., Balouët, G., Bodennec, G., & Marchand, M. (1987). The Occurrence of Hydrocarbons and Histopathological Abnormalities in Oysters for Seven Years Following the Wreck of the Amoco Cadiz in Brittany (France). *Marine Environmental Research, 23*(2), 103–33.

Beyer, J., Trannum, H. C., Bakke, T., Hodson, P. V., & Collier, T. K. (2016). Environmental effects of the Deepwater Horizon oil spill: A review. *Marine Pollution Bulletin*, *110*(1), 28–51. https://doi.org/https://doi.org/10.1016/j.marpolbul.2016.06.027

Bignell, J. P., Stentiford, G. D., Taylor, N. G. H., & Lyons, B. P. (2011). Histopathology of Mussels (*Mytilus* Sp.) from the Tamar Estuary, UK. *Marine Environmental Research, 72*(1–2), 25–32.

Bodin, N., Burgeot, T., Stanisière, J. Y., Bocquené, G., Menard, D., Minier, C., Boutet, I., Amat, A., Cherel, Y., & Budzinski, H. (2004). Seasonal variations of a battery of biomarkers and physiological indices for the mussel mytilus galloprovincialis transplanted into the northwest Mediterranean Sea. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *138*(4), 411–427. https://doi.org/10.1016/j.cca.2004.04.009

Cajaraville, M. P., Uranga, J. A., & Angulo, E. (1992). Comparative effects of the water accommodated fraction of three oils on mussels—3. quantitative histochemistry of enzymes related to the detoxication metabolism. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *103*(2), 369–377. https://doi.org/10.1016/0742-8413(92)90023-z

Calfee, R. D., Little, E. E., Clevel L., & Barron, M. G. (1999). Photoenhanced toxicity of a weathered oil to *Ceriodaphnia dubia* reproduction. *Environmental Science and Pollution Research, 6*, 207-212 .

Callot, H. J., & Ocampo, R. (2000). Geochemistry of porphyrins. In: Kadish K.M, Smith, K.M., Guilard, B, eds. The porphyrin handbook Vol 1, Academic, San Diego, C.A USA. 349-398.

CAPP. (2023). Canadian Association of Petroleum Producers (CAPP) Statistics: Statistical Handbook. Retrieved April 20, 2023, from https://www.capp.ca/wp-content/uploads/2023/02/Frequently-Used-Stats-Feb-2023.pdf

CAPP Extraction. (2022). *Crude oil extraction and drilling methods*. Canadian Association of Petroleum Producers (CAPP). Retrieved April 19, 2023, from https://www.capp.ca/oil/extraction/

Capuzzo, J. M., Moore, M. N., & Widdows, J. (1988). Effects of toxic chemicals in the marine environment: Predictions of impacts from laboratory studies. *Aquatic Toxicology, 11*, 303-311. doi:10.1016/0166-445x(88)90080-x

Cassis, D., Pearce, C. M., & Maldonado, M. T. (2011). Effects of the environment and culture depth on growth and mortality in juvenile Pacific oysters in the Strait of Georgia, British Columbia. *Aquaculture Environment Interactions, 1*, 259-274. doi:10.3354/aei00025

CER. (2022). *Crude Oil Pipe Transportation System*. Canada Energy Regulator. Retrieved December 15, 2022, from https://www.cer-rec.gc.ca/en/data-analysis/facilities-we-regulate/canadas-pipeline-system/2021/crude-oil-pipeline-transportation-system.html

Chew, K. K., & Ma, A. P. (1987). Species profile: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) -- common littleneck clam. U.S. Fish Wildlife Service Biological Report 82(11.78). U.S. Army Corps of Engineers, TR EL, 82-4. 22p.

Chevron. (2021). *Chevron Marine Products*. Retrieved April 20, 2023, from https://www.chevronmarineproducts.com/content/dam/chevron-marine/fuels-brochure/Chevron_Everything%20You%20Need%20To%20Know%20About%20Marine%20Fuels_v8-21_DESKTOP.pdf

Chicarelli, M. I., Eckardt, C. B., Owenn, C. R., Maxwell, J. R., Eglington, G., Hutton, R. C., & Eaton, A. N. (1990). Application of inductivity coupled plasma mass spectrometry in the determination of organometallic compounds in chromatographic fractions from organic rich shales. *Organic Geochemistry, 15*, 26-274.

Choy, E. J., Jo, Q., Moon, H. B., Kang, C. K., & Kang, J. C. (2007). Time-Course Uptake and Elimination of Benzo(a)Pyrene and Its Damage to Reproduction and Ensuing Reproductive Outputs of Pacific Oyster, *Crassostrea Gigas*. *Marine Biology, 151*(1), 157–65.

Chu, F. E., Soudant, P., & Hale, R. C. (2003). Relationship between PCB accumulation and reproductive output in conditioned oysters *Crassostrea virginica* fed a contaminated algal diet. *Aquatic Toxicology*, *65*, 293-307.

Conney, A.H., (1982). Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: GHA clowes memorial lecture. *Cancer Research*, *42*, 4875–4917.

Cripps, G., & Shears, J. (1997). The fate in the marine environment of a minor diesel fuel spill form an Arctic research station. *Environmental Monitoring and Assessment, 46*, 221-232.

Croxton, A. N., Wikfors, G. H., & Schulterbrandt-Gragg, R. D. (2012). Immunomodulation in eastern oysters, Crassostrea virginica, exposed to a PAH-contaminated, microphytobenthic diatom. *Aquatic Toxicology*, *118–119*, 27–36. https://doi.org/10.1016/j.aquatox.2012.02.023

Crude Oil. (2023). *Government of Canada*. Canadian Centre for Energy Information. Retrieved April 19, 2023, from https://energy-information.canada.ca/en/subjects/crude-oil

Dayem, A. A., Hossain, M., Lee, S., Kim, K., Saha, S., Yang, G. M., Choi, H., & Cho, S. G. (2017). The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles. *International Journal of Molecular Sciences*, *18*(1), 120. https://doi.org/10.3390/ijms18010120

Denison, M. S., & Nagy, S. R. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annual Review of Pharmacology and Toxicology*, *43*(1), 309–334. https://doi.org/10.1146/annurev.pharmtox.43.100901.135828

Di Toro, D. M., McGrath, J. A., & Stubblefield, W. A. (2007). Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. *Environmental Toxicology and Chemistry*, *26*(1), 24. https://doi.org/10.1897/06174r.1

Donaghy, L., Hong, H.-K., Lee, H.-J., Jun, J.-C., Park, Y.-J., & Choi, K.-S. (2010). Hemocyte parameters of the Pacific Oyster Crassostrea gigas a year after the Hebei spirit oil spill off the west coast of Korea. *Helgoland Marine Research*, *64*(4), 349–355. https://doi.org/10.1007/s10152-010-0190-7

Donkin, P., Widdows, J., Evans, S. V., Worrall, C. M., & Carr, M. (1989). Quantitative structure-activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (mytilus edulis). *Aquatic Toxicology*, *14*(3), 277–293. https://doi.org/10.1016/0166-445x(89)90021-0

Dos Reis, I. M. M., Siebert, M. N., Zacchi, F. L., Mattos, J. J., Flores-Nunes, F., Toledo-Silva, G. D., Piazza, C. E., Bícego, M. C., Taniguchi, S., Melo, C. M. R. D., & Bainy, A. C. D. (2020). Differential responses in the biotransformation systems of the oyster Crassostrea gigas (Thunberg, 1789) elicited by pyrene and fluorene: Molecular, biochemical and histological approach – Part II. *Aquatic Toxicology*, *226*, 105565. https://doi.org/10.1016/j.aquatox.2020.105565

Downs, C. A., Shigenaka, G., Fauth, J. E., Robinson, C. E., & Huang, A. (2002). Cellular physiological assessment of bivalves after chronic exposure to spilled *exxon valdez* crude oil using a novel molecular diagnostic biotechnology. *Environmental Science & Technology*, *36*(13), 2987–2993. https://doi.org/10.1021/es011433k

Driskell, W. B., Fukuyama, A. K., Houghton, J. P., Lees, D. C., Shigenaka, G., & Mearns, A. J. (1993). Impacts on intertidal infauna: Exxon Valdez oil spill and cleanup. *International Oil Spill Conference Proceedings*, *1993*(1), 355–361. https://doi.org/10.7901/2169-3358-1993-1-355

Dupuis, A., & Ucan-Marin, F. (2015). A literature review on the aquatic toxicology of petroleum oil: An overview of oil properties and effects to aquatic biota. DFO Canada Science Advisory Secretariat Research Document 2015/007. vi + 52 p.

Environment Canada. (2021). *Environment Canada crude oil and petroleum product database - individual report ECCC petroleum products database 2021-01-22*. Open Government Portal. Retrieved April 19, 2023, from https://open.canada.ca/data/en/dataset/53c38f91-35c8-49a6-a437-b311703db8c5/resource/d1ecec5b-f577-4edc-8f1d-57384742c391?inner_span=True

Federal Government. (2013). *Properties, Composition and Marine Spill Behaviour, Fate and Transport of Two Diluted Bitumen Products from the Canadian Oil Sands* . Federal Government Technical Report . Retrieved April 20, 2023, from https://publications.gc.ca/collections/collection_2014/ec/En84-96-2013-eng.pdf

Finch, B. E., Stefansson, E. S., Langdon, C. J., Pargee, S. M., Blunt, S. M., Gage, S. J., & Stubblefield, W. A. (2016). Photo-enhanced toxicity of two weathered Macondo crude oils to early life stages of the Eastern Oyster (*C*rassostrea virginica). *Marine Pollution Bulletin*, *113*(1-2), 316–323. https://doi.org/10.1016/j.marpolbul.2016.10.008

Fisheries and Oceans Canada. (2023). *Government of Canada*. Pacific oyster (Crassostrea gigas), 2023/2024 | Pacific Region | Fisheries and Oceans Canada. Retrieved April 20, 2023, from https://www.pac.dfo-mpo.gc.ca/fm-gp/mplans/oyster-huitre-ifmp-pgip-sm-eng.html#econ

Fleeger, J. W., Riggio, M. R., Mendelssohn, I. A., Lin, Q., Hou, A., & Deis, D. R. (2018). Recovery of saltmarsh meiofauna six years after the Deepwater Horizon Oil Spill. *Journal of Experimental Marine Biology and Ecology*, *502*, 182–190. https://doi.org/10.1016/j.jembe.2017.03.001

French-Mccay, D. P. (2004). Oil Spill Impact Modeling: Development And Validation. *Environmental Toxicology and Chemistry, 23*(10), 2441-2456. doi:10.1897/03-382

French-McCay, D., Crowley, D., & McStay, L. (2019). Sensitivity of modeled oil fate and exposure from a subsea blowout to oil droplet sizes, depth, dispersant use, and degradation rates. *Marine Pollution Bulletin*, *146*, 779–793. https://doi.org/10.1016/j.marpolbul.2019.07.038

Fukuyama, A. K., Shigenaka, G., & Hoff, R. Z. (2000). Effects of residual Exxon Valdez oil on intertidal protothaca staminea: Mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. *Marine Pollution Bulletin*, *40*(11), 1042–1050. https://doi.org/10.1016/s0025-326x(00)00055-2

Fukuyama, A. K., Shigenaka, G., & Coats, D. A. (2014). Status of intertidal infaunal communities following the Exxon Valdez oil spill in Prince William Sound, Alaska. *Marine Pollution Bulletin*, *84*(1-2), 56–69. https://doi.org/10.1016/j.marpolbul.2014.05.043

Gan, N., Martin, L., & Xu, W. (2021). Impact of Polycyclic Aromatic Hydrocarbon Accumulation on Oyster Health. *Frontiers in Physiology*, *12*, 734463. https://doi.org/10.3389/fphys.2021.734463

Gardner, G. R., Yevich, P. P., Harshbarger, J. C., & Malcom, A. R. (1991). Carcinogenicity of Black Rock Harbor sediment to the eastern oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. *Environmental Health Perspective, 90*, 53–66. https://doi.org/10.1289/ehp.901519497

Gillespie, G. E., Bower, S. A., Marcus, K. L., & Kieser, D. (2012). Biological synopsises for three exotic mollusks, Manila Clam (Venerupis philippinarum), Pacific Oyster (Crassostrea gigas) and Japanese Scallop (Mizuhopecten yessoensis) licensed for Aquaculture in British Columbia. DFO Canadian Science Advisory Secretariat Research Document, 2012/013. v + 97p.

González-Fernández, C., Albentosa, M., Campillo, J. A., Viñas, L., Franco, A., & Bellas, J. (2016). Effect of Mussel Reproductive Status on Biomarker Responses to PAHs: Implications for Large-Scale Monitoring Programs. *Aquatic Toxicology, 177*, 380–94.

Government of Canada (2022). *Canada energy regulator / Régie de l'énergie du Canada*. CER. Retrieved April 19, 2023, from https://www.cer-rec.gc.ca/en/data-analysis/energy-commodities/crude-oil-petroleum-products/report/canadian-crude-oil-exports-30-year-review/

Grabowski, J. H., Powers, S. P., Roman, H., & Rouhani, S. (2017). Potential impacts of the 2010 Deepwater Horizon oil spill on subtidal oysters in the Gulf of Mexico. *Marine Ecology Progress Series*, *576*, 163–174. https://doi.org/10.3354/meps12208

Hodson, P. V., Adams, J., & Brown, R. S. (2019). Oil toxicity test methods must be improved. *Environmental Toxicology and Chemistry, 38*(2), 302-311. doi:10.1002/etc.4303

Hounjet, L. J., Stoyanov, S. R., & Chao, D. (2018). Distributions of diluted bitumen and conventional crude oil in a range of water types. *Chemosphere*, *211*, 1212–1218. https://doi.org/10.1016/j.chemosphere.2018.08.025

Hwang, H.-M., Wade, T. L., & Sericano, J. L. (2008). Residue-response relationship between PAH body burdens and lysosomal membrane destabilization in eastern oysters (*Crassostrea virginica*) and toxicokinetics of PAHs. *Journal of Environmental Science and Health, Part A*, *43*(12), 1373–1380. https://doi.org/10.1080/10934520802232006

ITOPF. (2021). *TIP 02: Fate of marine oil spills*. International Tanker Owners Pollution Federation Limited. Retrieved November 23, 2022, from https://www.itopf.org/knowledge-resources/documents-guides/tip-02-fate-of-marine-oil-spills/

Jeong, W. G., & Cho, S. M. (2007). Long-Term Effect of Polycyclic Aromatic Hydrocarbon on Physiological Metabolisms of the Pacific Oyster, *Crassostrea Gigas*. *Aquaculture, 265*(1–4), 343–50.

Jesus, F. (2022). A review on polycyclic aromatic hydrocarbons in freshwater ecosystems and their toxicity to benthic fauna. *Science of the Total Environment*. *820*, 153-282. https://doi.org/10.1016/J.SCITOTENV.2022.153282

Jewett, S. C., Dean, T. A., Smith, R. O., & Blanchard, A. (1999). Exxon Valdez oil spill: Impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. *Marine Ecology Progress Series*, *185*, 59–83. https://doi.org/10.3354/meps185059

Jiang, M., Li, L., Li, Y., Shen, G., & Shen, X. (2017). Oxidative stress in shellfish sinonovacula constricta exposed to the water accommodated fraction of zero sulfur diesel oil and Pinghu Crude Oil. *Archives of Environmental Contamination and Toxicology*, *73*(2), 294–300. https://doi.org/10.1007/s00244-017-0391-z

Joshy, A., Sharma, S. R. K., Mini, K. G., Gangadharan, S., & Pranav, P. (2022). Histopathological evaluation of bivalves from the southwest coast of India as an indicator of Environmental Quality. *Aquatic Toxicology*, *243*, 106076. https://doi.org/10.1016/j.aquatox.2022.106076

Kang, C. K., Lee, Y. J., Han, E., Park, H. J., Yun, S. G., & Lee, W. C. (2015). Effects of temperature and body size on the physiological energetics of the stalked sea squirt *Styela clava*. *Journal of Experimental Marine Biology and Ecology*, *462*, 105-112.

Khan, M. I., Ayub, Z., & Siddiqui, G., (2015). Impact of marine pollution in green mussel, *Perna viridis*, from four coastal sites in Karachi, Pakistan, North Arabian Sea: histopathological observations. *Indian Journal of Experimental Biology, 53*, 222–227.

Kim, M., Cho, S., & Jeong, W. (2007). Short-Term Physiological Response of the Pacific Oyster, *Crassostrea Gigas*, on Exposure to Varying Levels of Polycyclic Aromatic Hydrocarbon. *Aquaculture Research, 38*(15), 1612–1618. https://doi.org/10.1111/j.1365-2109.2007.01821.x

Langdon, C. J., Stefansson, E. S., Pargee, S. M., Blunt, S. M., Gage, S. J., & Stubblefield, W. A. (2016). Chronic effects of non-weathered and weathered crude oil and dispersant associated with the deepwater horizon incident on development of larvae of the eastern oyster,*crassostrea virginica*. *Environmental Toxicology and Chemistry*, *35*(8), 2029–2040. https://doi.org/10.1002/etc.3352

Laramore, S., Krebs, W., & Garr, A. (2014). Effects of Macondo Canyon 252 oil (naturally and chemically dispersed) on larval*crassostrea virginica*(gmelin, 1791). *Journal of Shellfish Research*, *33*(3), 709–718. https://doi.org/10.2983/035.033.0305

Le Pennec, L., & Le Roux, S., (1979). Effets d'un pétrole brut sur la formation de la coquille de Mytilus edulis (L.)(Mytilidae, Bivalvia). *Revue Internationale d'Océanographie Médicale, 55*, 49–55.

Lee, R. F. (2003). Photo-oxidation and photo-toxicity of crude and refined oils. *Spill Science & Technology Bulletin*, *8*(2), 157–162. https://doi.org/10.1016/s1353-2561(03)00015-x

Lee, K., Boufadel, M., Chen, B., Foght, J., Hodson, P., Swanson, S., & Venosa, A. (2015). The Royal Society of Canada Expert Panel: The behaviour and environmental impacts of crude oil released into aqueous environments. Royal Society of Canada, Ottawa, ON. ISBN: 978-1- 928140-02-3

Lee, R. F., & Page, D. S. (1997). Petroleum hydrocarbons and their effects in subtidal region after major oil spills. *Marine Pollution Bulletin*, *34*, 928-940.

Li, L., Wang, C., Li, Z., Xv, G., Tang, B., & Jiang, M. (2022). Evaluation of toxic effects of petroleum hydrocarbon on *Mytilus coruscus* under different salinity based on an integrated Biomarker Response index. *Environmental Pollutants and Bioavailability*, *34*(1), 263–272. https://doi.org/10.1080/26395940.2022.2087740

Lima, D., Mattos, J. J., Piazza, R. S., Righetti, B. P., Monteiro, J. S., Grott, S. C., Alves, T. C., Taniguchi, S., Bícego, M. C., de Almeida, E. A., Bebianno, M. J., Medeiros, I. D., & Bainy, A. C. D. (2019). Stress responses in Crassostrea Gasar exposed to combined effects of acute ph changes and phenanthrene. *Science of The Total Environment*, *678*, 585–593. https://doi.org/10.1016/j.scitotenv.2019.04.450

Loh, A., Yim, U. H., Ha, S. Y., & An, J. G. (2018). A preliminary study on the role of suspended particulate matter in the bioavailability of oil-derived polycyclic aromatic hydrocarbons to oysters. *Science of The Total Environment*, *643*, 1084–1090. https://doi.org/10.1016/j.scitotenv.2018.06.129

Lüchmann, K. H., Mattos, J. J., Siebert, M. N., Granucci, N., Dorrington, T. S., Bícego, M. C., Taniguchi, S., Sasaki, S. T., Daura-Jorge, F. G., & Bainy, A. C. D. (2011). Biochemical biomarkers and hydrocarbons concentrations in the mangrove oyster crassostrea brasiliana following exposure to diesel fuel water-accommodated fraction. *Aquatic Toxicology*, *105*(3-4), 652–660. https://doi.org/10.1016/j.aquatox.2011.09.003

Luna-Acosta, A., Kanan, R., Le Floch, S., Huet, V., Pineau, P., Bustamante, P., & Thomas-Guyon, H. (2011). Enhanced immunological and detoxification responses in Pacific oysters, *Crassostrea gigas*, exposed to chemically dispersed oil. *Water Research*, *45*(14), 4103–4118. https://doi.org/10.1016/j.watres.2011.05.011

Mackay, D., & Mcauliffe, C. D. (1989). Fate of hydrocarbons discharged at sea. *Oil and Chemical Pollution, 5*, 1-20. doi:10.1016/s0269-8579(89)80002-4

Mackay, D., Shiu, W. Y., Chais, A., Southwood, J., & Johnson, C. I. (1985). Environmental fate of diesel fuel spills on land. Report of Department of chemical Engineering and Applied Chemistry of University of Toronto for Association of American Railroads, Washington, DC.

Madison B. N., Hodson P. V., & Langlois V. S. (2017). Cold Lake Blend diluted bitumen toxicity to the early development of Japanese medaka. *Environmental Pollution*, *225*, 579–586.

Marine Diesel Fuel Oil. (n.d.). *Minister of Environment and Climate Change*. Retrieved April 20, 2023, from https://etc-cte.ec.gc.ca/databases/OilProperties/pdf/WEB_Marine_Diesel_Fuel_Oil.pdf

McCarrick, S., Cunha, V., Zapletal, O., Vondráček, J., & Dreij, K. (2019). In vitro and in vivo genotoxicity of oxygenated polycyclic aromatic hydrocarbons. *Environmental Pollution*, *246*, 678–687. https://doi.org/10.1016/j.envpol.2018.12.092

Mondol, M. R., Keshavmurthy, S., Lee, H., Hong, H., Park, H., Park, S., . . . & Choi, K. (2015). Recovery of wild Pacific oyster, *Crassostrea gigas* in terms of reproduction and gametogenesis two-years after the Hebei Spirit Oil Spill Accident off the West Coast of Korea. *Continental Shelf Research, 111*, 333-341. doi:10.1016/j.csr.2015.08.019

Moore, M. N., Livingstone, D. R., Widdows, J., Lowe, D. M., & Pipe, R. K. (1987). Molecular, cellular, and physiological effects of oil-derived hydrocarbons on molluscs and their use in impact assessment. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *316*(1181), 603–623. https://doi.org/10.1098/rstb.1987.0041

Neff, J. M., Ostazeski, S., Gardiner, W., & Stejskal, I. (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to Marine Animals. *Environmental Toxicology and Chemistry*, *19*(7), 1809–1821. https://doi.org/10.1002/etc.5620190715

Neff, J. M., Hillman, R. E., Carr, R. S., Buhl, R. L., & Lahey, J. I. (1987). Histopathologic and biochemical responses in Arctic marine bivalve molluscs exposed to experimentally spilled oil. *ARCTIC*, *40*(5). https://doi.org/10.14430/arctic1816

Newell, R. I. E. (2004). Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research*, *23*(1), 51+.

Noakes, D. J. (2018). Oceans of opportunity: A review of Canadian aquaculture. *Marine Economics and Management*, *1*(1), 43–54. https://doi.org/10.1108/maem-06-2018-002

NRC. (2022). *Energy Fact Book 2021-2022*. Natural Resources Canada, 146pp.

Ogunola, O. S. (2017). Physiological, immunological, genotoxic, and histopathological biomarker responses of molluscs to heavy metal and water-quality parameter exposures: A critical review. *Journal of Oceanography and Marine Research*, *05*(01). https://doi.org/10.4172/2572-3103.1000158

Onwurah, I. N., Ogugua, V., Onyike, N., Ochonogor, A., & Otitoju, O. (2007). Crude Oils Spills in the Environment, Effects and Some Innovative Clean Up Biotechnologies. *International Journal of Environmental Research, 1*(4), 307-320.

Orban, E., Lena, G. D., Masci, M., Nevigato, T., Casini, I., Caproni, R., . . . & Pellizzato, M. (2004). Growth, nutritional quality, and safety of oysters(Crassostrea gigas) cultured in the lagoon of Venice (Italy). *Journal of the Science of Food and Agriculture, 84*, 1929-1938. doi:10.1002/jsfa.1896

Patel, B., & Eapen, J. T. (1989). Biochemical evaluation of naphthalene intoxication in the tropical acrid blood clam *Anadara granosa*. *Marine Biology, 103*, 203–209.

Pathak, C., & Mandalia, H. C. (2012). Petroleum Industries: Environmental Pollution Effects, Management And Treatment Methods. *International Journal of Separation for Environmental Sciences, 1*(1), 55-62.

Pauley, G. B., Van Der Ray, B., & Troutt, D., (1988). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) – Pacific oyster. U.S. Fisheries Wildlife Services Biological Report, 82(11.85). U.S. Army Corps of Engineers, TR EL-82.4. 28 p.

Pearson, W. H., Woodruff, D. L., Sugarman, P. C., & Olla, B. L. (1981). Effects of oiled sediment on predation on the littleneck clam, Protothaca staminea, by the Dungeness Crab, cancer magister. *Estuarine, Coastal and Shelf Science*, *13*(4), 445–454. https://doi.org/10.1016/s0302-3524(81)80040-0

Pelletier, M. C., Burgess, R. M., Cantwell, M. G., Serbst, J. R., Ho, K. T., & Ryba, S. A. (2000). Importance of Maternal Transfer of the Photoreactive Polycyclic Aromatic Hydrocarbon Fluoranthene from Benthic Adult Bivalves to Their Pelagic Larvae. *Environmental Toxicology and Chemistry, 19*(11), 2691–98.

Peterio, L. G., Labarta, U., & Fernandez-Reiriz, M. J. (2007). Variability in biochemical components of the mussel (*Mytilus galloprovincialis*) cultured after Prestige oil spill. *Comparative Biochemistry and Physiology Part C*, *145*, 588-594.

Peterson, C. H., Rice, S. D., Short, J. W., Esler, D., Bodkin, J. L., Ballachey, B. E., & Irons, D. B. (2003). Long-term ecosystem response to the Exxon Valdez Oil Spill. *Science*, *302*(5653), 2082–2086. https://doi.org/10.1126/science.1084282

Philibert D. A., Philibert C. P., Lewis C., Tierney K. B. (2016). Comparison of diluted bitumen (dilbit) and conventional crude oil toxicity to developing zebrafish. *Environmental Science & Technology, 50*, 6091–6098.

Piehler, M. F., & Smyth, A. R. (2011). Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. *Ecosphere*, *2*(1). https://doi.org/10.1890/es10-00082.1

Powers, S. P., Peterson, C. H., Cebrian, J., & Heck, K. L. (2017). Response of nearshore ecosystems to the Deepwater Horizon Oil Spill. *Marine Ecology Progress Series*, *576*, 107–110. https://doi.org/10.3354/meps12254

Prescott, M. L., Harley, J. P., & Klan, A. D. (1996). Industrial Microbiology and Biotechnology. In: Microbiology. 3rd Edition. Wim C Brown Publishers, Chicago, 923-927.

Proffitt, E., Coen, L., Geiger, S., Kimbro, D., Nance, H., & Weinstein, J. (2011). *The Deepwater Horizon Oil Spill: Assessing impacts on a critical habitat, oyster reefs and associated species in Florida Gulf estuaries*. Gulf of Mexico Research Initiative. Retrieved April 20, 2023, from https://www.fio.usf.edu/documents/grant-reports/gomri-yr1-deepwater-horizon-oil-spill-assessing-impacts-on-a-critical-habitat-oyster-reefs-and-associated-species.pdf

Quayle, D. B., (1988). Pacific oyster culture in British Columbia. Canadian Bulletin of Fisheries and Aquatic Sciences, 218. 241 p.

Redmond, K. J., Berry, M., Sanni, S., & Andersen, O. K. (2016). Effect of dispersed crude oil on the feeding activity, retention efficiency, and filtration rate of differently sized blue mussels (*Mytilus edulis*). *Journal of Toxicology and Environmental Health, Part A*, *79*(13-15), 658–674. https://doi.org/10.1080/15287394.2016.1171996

Refined Petroleum Products. (2023). *Government of Canada*. Canadian Centre for Energy Information. Retrieved April 19, 2023, from https://energy-information.canada.ca/en/subjects/refined-petroleum-products

Reuscher, M. G., Baguley, J. G., Conrad-Forrest, N., Cooksey, C., Hyland, J. L., Lewis, C., Montagna, P. A., Ricker, R. W., Rohal, M., & Washburn, T. (2017). Temporal Patterns of Deepwater Horizon impacts on the benthic infauna of the northern Gulf of Mexico continental slope. *PLOS ONE*, *12*(6). https://doi.org/10.1371/journal.pone.0179923

Ruesink, J. L., Lenihan, H. S., Trimble, A. C., Heiman, K. W., Micheli, F., Byers, J. E., & Kay, M. C. (2005). Introduction of non-native oysters: Ecosystem effects and restoration implications. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 643–689. https://doi.org/10.1146/annurev.ecolsys.36.102003.152638

Sarkar, A., Bhagat, J., Saha Sarker, M., Gaitonde, D. C., & Sarker, S. (2017). Evaluation of the impact of bioaccumulation of PAH from the marine environment on DNA integrity and oxidative stress in Marine Rock Oyster (*Saccostrea Cucullata*) along the Arabian Sea Coast. *Ecotoxicology*, *26*(8), 1105–1116. https://doi.org/10.1007/s10646-017-1837-9

Schmutz, A. (2018). Effets sous-létaux sur la Moule bleue (*Mytilus edulis*) et sa progéniture d'une exposition chronique à trois pétroles (classiques et bitumes dilués) en condition hivernale avec couvert de glace. [M.Sc. Thesis, Universite du Quebec a Rimouski].

Schmutz, A., Tremblay, R., Audet, C., Gagné, J.-P., Pelletier, É., & St-Louis, R. (2021). Under ice spills of conventional crude oil and diluted bitumen: Physiological resilience of the Blue Mussel and transgenerational effects. *Science of The Total Environment*, *779*, 146316. https://doi.org/10.1016/j.scitotenv.2021.146316

Schrandt, M., Powers, S., Rikard, F. S., Thongda, W., & Peatman, E. (2018). Short-term low salinity mitigates effects of oil and dispersant on Juvenile Eastern Oysters: A laboratory experiment with implications for oil spill response activities. *PLOS ONE*, *13*(9). https://doi.org/10.1371/journal.pone.0203485

Shiu, W. Y., Bobra, M., Bobra, A. M., Maijanen, A., Suntio, L., & Mackay, D. (1990). The water solubility of crude oils and petroleum products. *Oil and Chemical Pollution, 7*, 57-84. doi:10.1016/s0269-8579(05)80034-6

Smolarz, K., Hallmann, A., Zabrzańska, S., & Pietrasik, A. (2017). Elevated Gonadal Atresia as Biomarker of Endocrine Disruptors: Field and Experimental Studies Using *Mytilus Trossulus* (L.) and 17-Alpha Ethinylestradiol (EE2). *Marine Pollution Bulletin, 120*(1– 2), 58–67.

Smolders, R., Bervoets, L., De Coen, W., & Blust, R. (2004). Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. *Environmental Pollution*, *129*, 99-112.

Soniat, T. M., King, S. M., Tarr, M. A., & Thorne, M. A. (2011). Chemical and Physiological Measures on Oysters (Crassostrea virginica) from Oil-Exposed Sites in Louisiana. *Journal of Shellfish Research, 30*(3), 713-717. doi:10.2983/035.030.0311

Soon, T. K., & Ransangan, J. (2019). Extrinsic factors and marine bivalve mass mortalities: An overview. *Journal of Shellfish Research*, *38*(2), 223. https://doi.org/10.2983/035.038.0202

Speight, J. G. (2010). Handbook of industrial hydrocarbon processes. Gulf Professional, Houston, TX, USA.

Statistics Canada. (2022, November 29). Aquaculture, production, and value. Retrieved April 20, 2023, from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210010701

Stefansson, E. S., Langdon, C. J., Pargee, S. M., Blunt, S. M., Gage, S. J., & Stubblefield, W. A. (2016). Acute effects of non-weathered and weathered crude oil and dispersant associated with the Deepwater Horizon Incident on the development of marine bivalve and echinoderm larvae. *Environmental Toxicology and Chemistry*, *35*(8), 2016–2028. https://doi.org/10.1002/etc.3353

Stekoll, M. S., Clement, L. E., & Shaw, D. G. (1980). Sublethal effects of chronic oil exposure on the intertidal clam *Macoma balthica*. *Marine Biology*, *57*, 51-60.

Stoyanovich, S. S., Yang, Z., Hanson, M., Hollebone, B. P., Orihel, D. M., Palace, V., . . . Blais, J. M. (2019). Simulating a Spill of Diluted Bitumen: Environmental Weathering and Submergence in a Model Freshwater System. *Environmental Toxicology and Chemistry, 38*(12), 2621-2628. doi:10.1002/etc.4600

Sun, S., Shi, W., Tang, Y., Han, Y., Du, X., Zhou, W., Hu, Y., Zhou, C., & Liu, G. (2020). Immunotoxicity of petroleum hydrocarbons and microplastics alone or in combination to a bivalve species: Synergic impacts and potential toxication mechanisms. *Science of The Total Environment*, *728*, 138852. https://doi.org/10.1016/j.scitotenv.2020.138852

Tang, Y., Zhou, W., Sun, S., Du, X., Han, Y., Shi, W., & Liu, G. (2020). Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, Tegillarca granosa. *Environmental Pollution*, *265*, 115115. https://doi.org/10.1016/j.envpol.2020.115115

Toro, B., Navarro, J. M., & Palma-Fleming, H. (2003). Use of clearance rate in Choromytilus Chorus (Bivalvia: Mytilidae) as a non-destructive biomarker of aquatic pollution. *Revista Chilena De Historia Natural*, *76*(2). https://doi.org/10.4067/s0716-078x2003000200011

Transport Canada. (2016). *Tanker safety by the numbers infographic*. Transport Canada. Retrieved April 19, 2023, from https://tc.canada.ca/en/marine-transportation/marine-safety/tanker-safety-numbers-infographic

Trowbridge, C., Baker, T. T., & Johnson, J. D. (2001). Effects of hydrocarbons on bivalves following the *Exxon Valdez* Oil Spill, *Exxon Valdez* Oil Spill State Federal Natural Resource Damage Assessment Final Report (Fish/Shellfish Study 13), Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage, Alaska.

Vignier, J., Donaghy, L., Soudant, P., Chu, F. L. E., Morris, J. M., Carney, M. W., Lay, C., Krasnec, M., Robert, R., & Volety, A. K. (2015). Impacts of deepwater horizon oil and associated dispersant on early development of the Eastern Oyster Crassostrea virginica. *Marine Pollution Bulletin*, *100*(1), 426–437. https://doi.org/10.1016/j.marpolbul.2015.08.011

Vignier, J., Rolton, A., Soudant, P., Chu, F. L. E., Robert, R., & Volety, A. K. (2019). Interactions between Crassostrea virginica larvae and Deepwater Horizon oil: Toxic effects via dietary exposure. *Environmental Pollution*, *246*, 544–551. https://doi.org/10.1016/j.envpol.2018.12.057

Vignier, J., Rolton, A., Soudant, P., Chu, F. E., Robert, R., & Volety, A. K. (2018). Evaluation of toxicity of deepwater horizon slick oil on spat of the oyster crassostrea virginica. *Environmental Science and Pollution Research*, *25*(2), 1176–1190. https://doi.org/10.1007/s11356-017-0476-2

Vignier, J., Soudant, P., Chu, F. L. E., Morris, J. M., Carney, M. W., Lay, C. R., Krasnec, M. O., Robert, R., & Volety, A. K. (2016). Lethal and sub-lethal effects of Deepwater Horizon slick oil and dispersant on oyster (Crassostrea virginica) larvae. *Marine Environmental Research*, *120*, 20–31. https://doi.org/10.1016/j.marenvres.2016.07.006

Volety, A., Boulais, M., Donaghy, L., Vignier, J., Loh, A. N., & Soudant, P. (2016). Application of flow cytometry to assess Deepwater horizon oil toxicity on the eastern oyster *Crassostrea virginica* spermatozoa. *Journal of Shellfish Research*, *35*(1), 91–99. https://doi.org/10.2983/035.035.0111

Wade, T. L., Sweet, S. T., Sericano, J. L., DeFreitas, D. A., & Lauenstein, G. G. (2014). Polychlorinated dibenzo-dioxins and dibenzofurans detected in bivalve samples from the NOAA National Status and Trend program. *Marine Pollution Bulletin, 81*, 317-324.

Washburn, T. W., Reuscher, M. G., Montagna, P. A., Cooksey, C., & Hyland, J. L. (2017). Macrobenthic community structure in the Deep Gulf of Mexico one year after the Deepwater Horizon Blowout. *Deep Sea Research Part I: Oceanographic Research Papers*, *127*, 21–30. https://doi.org/10.1016/j.dsr.2017.06.001

Widdows, J., Bakke, T., Bayne, B. L., Donkin, P., Livingstone, D. R., Lowe, D. M., Moore, M. N., Evans, S. V., & Moore, S. L. (1982). Responses of *Mytilus Edulis* on Exposure to the Water-Accommodated Fraction of North Sea Oil. *Marine Biology, 67*(1), 15–31.

Widdows, J., Burns, K., Menon, N., Page, D., & Soria, S. (1990). Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contamination gradient in Bermuda. *Journal of Experimental Marine Biology and Ecology, 138*, 99-117. doi:10.1016/0022-0981(90)90179-g

Widdows, J., & Donkin, P. (1991). Role of physiological energetics in ecotoxicology. Mini review. *Comparative Biochemistry and Physiology*, *100*, 65-69.

Widdows, J., Donkin, P., Staff, F., Matthiessen, P., Law, R., Allen, Y., . . . Jones, B. (2002). Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. *Marine Environmental Research, 53*(4), 327-356. doi:10.1016/s0141-1136(01)00120-9

Widdows, J., & Johnson, D. (1988). Physiological energetics of mytilus edulis: Scope for growth. *Marine Ecology Progress Series*, *46*, 113–121. https://doi.org/10.3354/meps046113

Widdows, J., & Staff, F. (2006). Biological effects of contaminants: Measurement of scope for growth in mussels. *ICES Techniques in Marine Environmental Sciences*, *40*, 1-30.

Wu, Y., Gu, E., Li, H., Tian, C., & Feng, C. (2019). Oxidative stress and histological changes in *corbicula fluminea* exposed to nano-al13 and monomeric Al Coagulants. *Environmental Science: Nano*, *6*(9), 2736–2748. https://doi.org/10.1039/c9en00320g

Yang, C., Wang, Z., Yang, Z., Hollebone, B., Brown, C. E., Landriault, M., & Fieldhouse, B. (2011). Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products. *Environmental Forensics, 12*(2), 173-188. doi:10.1080/15275922.2011.574312

Yim, U. H., Oh, J. R., Hong, S. H., Lee, S. H., Shim, W. J., & Shim, J. H. (2002). Identification of PAHs Sources in Bivalves and Sediments 5 Years After the Sea Prince Oil Spill in Korea. *Environmental Forensics, 3*, 357-366. doi:10.1006/enfo.2002.0107

Zacchi, F. L., dos Reis, I. M., Siebert, M. N., Mattos, J. J., Flores-Nunes, F., Toledo-Silva, G. de, Piazza, C. E., Bícego, M. C., Taniguchi, S., & Bainy, A. C. (2019). Differential responses in the biotransformation systems of the Oyster *Crassostrea Gasar* (Adanson, 1757) elicited by Pyrene and fluorene: Molecular, biochemical and histological approach - Part I. *Aquatic Toxicology*, *216*, 105318. https://doi.org/10.1016/j.aquatox.2019.105318

Zhong, X., Li, P., Lin, X., Zhao, Z., He, Q. S., Niu, H., & Yang, J. (2022). Diluted bitumen: Physicochemical properties, Weathering Processes, emergency response, and recovery. *Frontiers in Environmental Science*, *10*. https://doi.org/10.3389/fenvs.2022.910365

Chapter 2. Effects of petroleum exposure on Pacific oysters

2.1. Abstract

With the increase in petroleum product transportation through marine habitats in the Pacific region of North America, an understanding of the hazards associated with these mixtures to bivalve species is essential. The sub-chronic effects of crude oil (CO), marine diesel (MD), and diluted bitumen (DB) water-accommodated fractions (WAFs) on the scope for growth (including oxygen consumption, food assimilation, and clearance rate), condition and health assessment indices, gonadal and digestive gland histopathology, and larval development in Pacific oysters (*Crassostrea gigas*) were examined. Initial total polycyclic aromatic compound (TPAC) concentrations in WAFs were ranked CO > MD > DB and these accumulated in oyster tissues in the same order. Sub-chronic exposures to different WAF dilutions of three petroleum products did not cause adverse effects to the measured Pacific oyster endpoints, while the polycyclic aromatic compounds (PACs) accumulated in oyster tissues had a rapid depuration after the exposure period.

Keywords: Petroleum products; Pacific oyster; scope for growth; morphometric indices; histopathology; larval development

2.2. Introduction

Canada ranks fourth in global petroleum production and third in export volume, meeting 5.9% of the global oil demand (CER, 2022; NRC, 2022). The oil and gas industry contributed $133 B to the Canadian GDP in 2021, with estimated capital expenditures of $35 B in oil and gas extraction (CAPP, 2023; Crude Oil, 2023). The Western Canada Sedimentary Basin (WCSB) contains the largest crude oil reserves in Canada, comprising almost the entirety of Alberta, with parts of Manitoba, Saskatchewan, British Columbia (BC), Yukon, and Northwestern Territories (CER, 2022). Once these crude oil products have been extracted, they are refined by altering their physiochemical properties, with around 1.33 M barrels/d being refined within Canada (NRC, 2022).

Each year, Canada transports substantial quantities of petroleum products *via* marine tankers along its coasts (CER, 2022; Transport Canada, 2016). In 2020, average daily marine crude oil exports reached 279 M t, with 2.2 M t sent to Asia and the US from western Canada ports, and 82 M t of offshore petroleum products exported to Europe and the US from Atlantic Canada ports (CER, 2022; Transport Canada, 2016). With the expansion of marine petroleum transportation routes, the potential for accidental spills of products (e.g., conventional crude oils, diluted bitumen, and marine diesel) increases substantially (CER, 2022; Crude Oil, 2023; Environment Canada, 2021; Refined Petroleum Products, 2023; Transport Canada, 2016; Zhong et al., 2022).

Marine diesel (MD) is a heavy gas distillate fuel oil used in marine engines, with a daily production of 750,000 barrels in Canada (Refined Petroleum Products, 2023). It consists of hydrocarbon classes including alkanes, polycyclic aromatic hydrocarbons (PAHs), isoprenoids, aromatics, and polar compounds containing sulfur, oxygen, or nitrogen (Mackay et al., 1985; Neff et al., 2000; Onwurah et al., 2007). Monocyclic aromatic hydrocarbons (MAHs) present in MD include benzene, toluene, xylenes, and trimethylbenzenes (Neff et al., 2000). Non-hydrocarbon components consist of porphyrins and metals such as nickel, iron, zinc, vanadium, cobalt, copper, and titanium (Callot & Ocampo, 2000; Onwurah et al., 2007; Yang et al., 2011). Conventional crude oil (CO) produced in Canada amounts to 3.3 M barrels/d (CER, 2022; Zhong et al., 2022). It contains alkyl PAH derivatives, polycyclic aromatic compounds (PACs) like anthracene, phenanthrene, chrysene, pyrene, and perylene, as well as resin and asphaltene (Yim et al., 2002; Madison et al., 2017; Philibert et al., 2016; Stoyanovich et al., 2019). Diluted

bitumen (DB) from oil sands has a daily production of 5.5 M barrels in Canada (CER, 2022; Crude Oil, 2023; NRC, 2022; Yang et al., 2011). It is refined with diluents comprised of BTEX, naphthalene, alkane hydrocarbons, metals, and naphthenic acids (Lee et al., 2015; Speight, 2010; Stoyanovich et al., 2019; Zhong et al., 2022). Major DB components are alkylated and priority PACs including naphthalene, phenanthrene, dibenzothiophene, fluorene, benzonaphthothiophene, chrysene, perylene, pyrene, biphenyl, acenaphthene, acenaphthylene, fluoranthene, and anthracene (Environment Canada, 2021).

Understanding the effects of MD, CO, and DB on bivalves such as the Pacific oyster (*Crassostrea gigas*) is of paramount importance. Pacific oysters are not only a commercially valuable species but also a key component of aquatic ecosystems, playing crucial roles in water filtration, nutrient cycling, and providing habitat for other marine organisms (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Mondol et al., 2015; Newell, 2004). Bivalves are particularly vulnerable to oil spills due to their filter-feeding behavior that exposes them to large volumes of water containing dissolved contaminants, which can accumulate to toxic thresholds (Mondol et al., 2015; Orban et al., 2004). Understanding the effects of various petroleum products on oysters can provide insights into the potential impacts on their health, survival, and reproduction, which are crucial factors for both commercial oyster farming and the maintenance of healthy marine ecosystems (Gillespie et al., 2012; Luna-Acosta et al., 2011; Mondol et al., 2015). Furthermore, Pacific oysters are a sentinel species which makes them a valuable indicator of overall marine environmental health. Changes in oyster populations, growth rates, and reproductive success can signal shifts in the ecosystem health and provide early warning signs of potential environmental stressors (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012). By studying the effects of oil spills on oysters, researchers gain a deeper understanding of the broader ecological consequences of pollution that can help inform effective mitigation strategies and resource management decisions.

In addition to their ecological significance, Pacific oysters are widely cultured and harvested from indigenous, commercial, and recreational fisheries for human consumption, making them a valuable source of revenue and livelihood around the world (Fisheries and Oceans Canada, 2023; Gillespie et al., 2012; Orban et al., 2004). On an annual basis, approximately 10,000 t of Pacific oysters are grown within Canada, with 5,000 t coming from BC while adding $41 M to the Canadian GDP (Bayen et al., 2007; Cassis et al., 2011; Noakes, 2018; Statistics Canada, 2022). The potential impacts of oil

spills on oyster populations can lead to substantial economic losses for oyster farmers and seafood industries. Understanding the effects on oysters is thus, essential for devising strategies to mitigate economic losses and ensure the safety of the seafood supply.

The current study was aimed at determining the effects of a sub-chronic exposure of Pacific oysters to water-accommodated fractions (WAFs) of marine diesel, crude oil, and diluted bitumen using a comprehensive suite of endpoints relevant to oyster fitness (e.g., scope for growth, condition and health indices, histopathology, larval development), and to assess the potential short-term recovery of oysters from any petroleum effects.

2.3. Methodology

2.3.1. Chemicals

The three petroleum products used in the WAF exposures (marine diesel [MD], Alaska North Slope [ANS] conventional crude oil [CO], and Cold Lake Winter Blend [CLWB] diluted bitumen [DB]) were obtained from the Centre for Offshore Oil, Gas and Energy Research (COOGER, Fisheries and Oceans Canada). A summary of the physical and chemical characteristics of each petroleum product is presented in Table 2.1. Sodium azide (Sigma), Remel™ Lugol's Iodine (Thermo Scientific), ammonium formate (97% anhydrous; Sigma), sodium sulfite (Sigma), formalin (37%; Sigma), sodium chloride (Sigma), disodium phosphate (99% anhydrous; Sigma), Mayer's hematoxylin (Thermo Scientific), Eosin Y (Thermo Scientific), ethanol (Sigma), and formaldehyde (37%; Sigma) were used in the assays.

Table 2.1. Chemical constituent and properties summary for the petroleum products used in this study: marine diesel (MD), Alaska North Slope (ANS) conventional crude oil (CO), and Cold Lake Winter Blend (CLWB) diluted bitumen (DB; Environment Canada, 2021).

Parameter	MD	CO	DB
Origin	Newfoundland	Alaska	Alberta
API Gravity	37.3	31.3	21.5
Density (g/mL) at 15 °C	0.832	0.864	0.922
Dynamic Viscosity (mPa.s) at 15 °C	6	10	360
Mass Loss (% after 48 h)	15.7	36.8	23
Saturated Hydrocarbons (% w/w)	88	58	46
Aromatic Hydrocarbons (% w/w)	11	32	30
Resin (% w/w)	2	6	11
Asphaltene (% w/w)	0	4	13
Waxes (% w/w)	4.9	4	3

2.3.2. Organisms

Cultivated commercial-sized adult Pacific oysters (*Crassostrea gigas*) between 2 and 3 y of age were obtained from the Deep Bay Marine Field Station (Bowser, BC), an operation of Vancouver Island University (VIU) for shellfish husbandry and research. The male population originated from a hatchery in Chile and the females were sourced from a hatchery in the USA. This segregation occurred to avoid inbreeding in this population.

Oyster sizes ranged from 21–94 mm shell length, 19–69 mm shell width, 6–51 mm shell depth, and 0.7–10.4 g tissue wet weight. Oysters were transported to Simon Fraser University (SFU) within 24 h of collection, housed inside a cooler filled with ice, and acclimated for a minimum of 7 d under laboratory conditions. They were placed in 40 L fiberglass tanks in 1 µm-filtered, UV-sterilized, and aerated seawater (28 ‰) held at 10 °C for the remainder of experiments. Tanks and other glassware were cleaned with 10% household bleach and DI water. A 100% seawater change was performed twice a week, where tanks were rinsed 3 times with seawater. Oysters were fed daily with a marine unicellular algae diet of 100 x 10^6 *Isochrysis galbana* cells/L obtained from the Canadian Center for the Culture of Microorganisms (CCCM; Gerdes, 1983; Widdows & Staff, 2006).

2.3.3. Petroleum exposures

Water-accommodated fractions (WAFs) of each product were generated according to a standardized protocol (Singer et al., 2000). Several factors were important in the preparation of consistent WAFs, including the pre-treatment of dilution seawater, oil-to-water volume ratios, vessel size, headspace height, as well as mixing speed/duration. Filtered and sterilized seawater (240 L) was obtained from the Vancouver Aquarium (Vancouver, BC) and added into 500 L fiberglass tanks, along with 8 L of oil product (1:30 v/v mixture oil:water). The oil/water mixture was covered and mixed with a voltage-regulated mechanical stainless-steel stirrer at 92 rpm for 24 h. After a 1 h settling period, the WAF mixture was pumped into each of the 9 L individual glass exposure tanks. The exposure duration was 7 d and followed by an additional 28 d recovery period in fresh uncontaminated seawater. Four replicate tanks per treatment concentration were used and each tank contained 15 oysters. The treatment groups were 0% (control), 25%, 50%, and 100% WAF dilutions. Tanks were randomly placed in water baths at 10 °C. Tanks were not aerated, oysters were not fed during the exposure, and a water change was only done at d 7 to uncontaminated water for the recovery period. Water quality parameters and oyster mortality or spawning were recorded daily. At each sampling time (d 7, 21, and 35) several endpoints were measured to determine whether sublethal effects occurred (n = 8, 2 from each replicate tank).

2.3.4. Water and tissue PAC concentrations

Water samples were taken on 0, 3, and 7 d of the exposure period, and sent to AXYS Analytical Services for individual polycyclic aromatic compound (PAC) analysis. Each water sample was collected in a 1-L amber glass bottle in duplicate with no headspace. For preservation, 0.5 g of sodium azide (Sigma) was added to each sample. Individual PAC concentrations in oyster tissue were determined by SGS AXYS Analytical Services from oysters collected at each time point. Oyster tissues from the same WAF treatment group were pooled to obtain minimal wet weight requirements for analyzing oyster tissue (without shell; 10 g). These samples were stored at -80 °C before being shipped frozen on dry ice for analysis. The individual PAC concentrations were quantified in water and tissue by low-resolution GC/MS analysis. The final chemical concentration results were blank corrected for each analyte, which were further categorized as alkylated or parent PAC compound (Hong et al., 2016).

2.3.5. Scope for growth

Scope for growth (SFG) is a value that indicates the overall growth potential of a bivalve and is calculated from measurements of several physiological processes. Theoretical SFG values were estimated from a balanced energy equation, where the obtained energy expended through respiration was deducted from the energy absorbed through acquired food with the remainder indicating the energy available for their growth potential. The methodology described in Widdows and Staff (2006) was used for all SFG calculations, including the measurement of algal clearance rate (CR), particulate absorption efficiency (AE), and oxygen consumption (MO_2).

Isochrysis galbana culture

Pacific oysters were fed a cultured marine unicellular algae, *Isochrysis galbana*, (Marcus & Wilcox, 2007). An algae stock solution was obtained from the CCCM (University of BC, BC) and maintained using a Guillard's f/2 enrichment (GE) solution (Appendix Table A.42.) at 16 °C under a 12/12 h light-dark cycle. Seed cultures were diluted every 3-4 weeks to maintain algal density using sterile techniques. A 20 mL algae seed culture was used to start the large-scale culture production in 20 L autoclavable glass containers. Twice a week, 3.5 mL of GE solution, along with 2 mL of f/2 vitamin mix (Appendix Table A.43.) and 2 mL of trace metal solution (Appendix Table A.44.) were added to each 1 L of

culture (Guillard, 1975; Guillard & Ryther, 1962). Culture flasks were placed 30 cm from a 6500 K intensity light and swirled daily to keep the algae suspended in the water column. After 2-3 weeks, the algae culture reached a peak density determined by the culture color (greenish-yellow), as well as the cell count ($7x10^6$ cells/mL) using a hemocytometer after preserving them in Remel™ Lugol's Iodine (Thermo Scientific). Algae cultures were diluted twice a week under sterile conditions to maintain their health.

Standard curves were generated that aided in the determination of cell counts in algal solutions; one was generated for low cell concentrations ($15x10^3$–$350x10^3$ cells/mL) and one for high concentrations ($1.2x10^6$–$7x10^6$ cells/mL) using a fluorescence spectrophotometer (Varian Cary Eclipse, Agilent Technologies) based on cell counts obtained with a hemocytometer. The instrument settings for both standard curves included the same emission (682 nm) and excitation (341 nm) wavelengths, but variable slit lengths (low: 20 nm; high: 10 nm) and reading times (low: 3 sec; high: 0.3 sec), due to differences in their fluorescence detection limit. PS 4-sided polystyrene BRAND® macro-cuvettes (BR759035; Sigma) were used for all the algae concentration fluorescence readings. New standard curves were prepared before each petroleum product exposure. Knowing the algal cell concentrations was integral for determining algal clearance rate values used in the SFG calculations.

Algal clearance rate

Clearance rate (CR) is defined as the volume of seawater cleared of suspended *Isochrysis galbana* cells as a function of time. These measurements were made in semi-static conditions at 10 °C. Oysters were not fed 24 h before CR measurements to ensure a maximum feeding capacity and discharge of fecal matter from the mantle cavity. An individual oyster from one of the WAF treatment groups was placed in a 1.5 L beaker filled with seawater. In addition, a negative control beaker without an oyster was used to account for algal settling. Vessels contained air stones that minimally aerated and mixed algae in seawater without impacting the filtration rate of oysters. An algae solution of 100 x $l0^6$ cells/L was added to each beaker (Gerdes, 1983) after an acclimation period of 20 min that allowed time for each oyster to open their valves and resume pumping. The volume of algae solution (~ 20 mL) added to each beaker was done using a serological pipette. After 2 min of uniform mixing (t_0), 7 ml water aliquots (in duplicate) were collected from the center of the beaker with a serological pipette for algae cell concentration

measurements using the spectrophotometric method. Two additional algae samples were collected from each beaker at 45 min intervals (t_1 and t_2), over a total period of 1.5 h to determine the overall decline in algae cell concentration due to oyster feeding.

Clearance rates (CR) were measured using Equation 1 (Coughlan, 1969):

$$CR\ (L/h) = \frac{(V) \times (\log_e C_1 - \log_e C_2)}{t} \qquad (Equation\ 1)$$

where V is the volume (L) of water in the beaker, and C_1 and C_2 are the cell concentrations (mg cells/L) at the initial and final time increment t (h).

Absorption efficiency

Absorption efficiency (AE) represents the efficiency of assimilating organic matter from ingested food material in oysters by measuring the amount of organic matter absorbed through algae consumption and subtracting the amount of organic matter excreted through their feces. Following the CR measurements, seawater remaining in the beakers was vacuum filtered through pre-rinsed Whatman® borosilicate GF/C filter papers (1.2 µm particle retention; Sigma) to collect fecal matter. Once filtered, the pre-weighed filter papers were rinsed twice with 20 mL of 0.5 M ammonium formate (97% anhydrous; Sigma) and deionized (DI) water to remove the seawater salts. The initial algae weight was determined from the culture vessel, where the same algae amount as the CR feeding volume was filtered through pre-weighed GF/C filter papers. This was done to avoid spatial and temporal variations in the quantity and quality of food used during the AE measurements (Widdows & Staff, 2006). Filters were then rinsed twice with 20 mL of 0.5 M ammonium formate (97% anhydrous; Sigma) and DI water. Filter papers containing algal and fecal samples were placed in pre-weighed aluminum dishes (HS14522; Sigma) and dried at a constant temperature of 100 °C for 48 h to obtain tissue dry weights. Upon reweighing, both samples were placed in a muffle furnace for ashing at 450 °C for 1 h to remove all organic matter. This combustion helped determine the food and feces particulate organic matter (POM) value, which was calculated based on the difference between the remaining ash (inorganic) and the total dry weight of both samples. Since the GF/C filter papers were not pre-combusted, a correction factor was applied based on the average mass of organic matter lost from 64 blank filter papers. All weight measurements (g) were done in triplicate for precision to the nearest four decimal places using an electronic microbalance. Some AE values were excluded due to the spawning of oysters,

presence of pseudo-feces, as well as extremely light algae and feces ash weights undetected by the electronic microbalance.

AE was measured using the ratio provided by Conover (1966) in Equation 2:

$$AE = \frac{(F - E)}{[(1 - E) \times F]} \qquad (Equation\ 2)$$

where F is the ash-free (organic) dry weight of algae:dry weight of algae and E is the ash-free (organic) dry weight of feces:dry weight of feces.

Oxygen consumption

The oxygen consumption (MO_2) of individual oysters was determined by measuring the oxygen partial pressure (PO_2) decline in seawater over time in a static respirometer. This was done in closed acrylic Loligo® respirometers (CH22400; large chamber) set in a temperature-controlled water bath (10 °C), where each respirometer was dedicated to an oyster from one of the treatments. The PO_2 decline was measured with the aid of temperature and oxygen equilibrated Loligo® flow-through oxygen cell (10 mm; OX11220) connected to the Witrox 4 oxygen meter (OX11875), which provided readings to AutoResp™ software version 2.3.0. These sensors were calibrated in 100% air-saturated deionized (DI) water for 15 min, and 0% air-saturated DI water (PO_2 zero solution) for 12 h, which was achieved by adding 21.0 g of sodium sulfite (Sigma) to 1 L DI water. PO_2 measurements were performed using 1 µm-filtered and UV-sterilized seawater (28 ‰) aerated for a minimum of 1 h before each run at 972.7 hPa. The air-saturated seawater was carefully added to the respirometer chambers to ensure the elimination of air bubbles. Oysters were gently placed inside a respirometer after the CR measurements were performed and allowed 30 min for acclimation. This allowed them to release deoxygenated water from their valves after opening and initiate pumping of the aerated seawater, which sometimes caused a spike in the PO_2 readings during the acclimation period due to the presence of air bubbles (Widdows & Staff, 2006). Subsequently, the PO_2 readings were obtained for 1 h, where the initial and final PO_2 concentrations were selected at 1800 and 5400 sec of the respiration measurement period to standardize the readings over a 1 h period. The individual MO_2 in oysters was calculated based on Equation 3:

$$MO_2 = \frac{[C(t_0) - C(t_1)] \times V \times 60}{(t_1 - t_0)} \quad \textit{(Equation 3)}$$

where MO_2 is the oxygen consumption (μmol O_2/h), C_t is the concentration of oxygen in the water (mg O_2/L) at time t, V is the volume (L) of water in the respirometer chamber along with tubes, and t_0 and t_1 are the initial and final times (min) of the measurement duration. Additionally, the C_t values were converted to oxygen concentrations in μmol O_2/L by multiplying a coefficient of 31.26 based on a unit conversion calculation.

Scope for growth

Physiological measurements (CR and MO_2) were normalized to oyster tissue dry weight (DW; g) to correct for variations in oyster size. Oysters were dissected, placed in pre-weighed aluminum dishes (Z154865; Sigma), and dried in an oven at 100 °C for 48 h. Individual CR (L/h) and MO_2 (μmol O_2/h) values were converted to their energy equivalents (J/h/g) to calculate SFG. Maximum clearance rate values were selected from each run for the SFG calculations to avoid periods where individuals were partially closed (Widdows & Staff, 2006). Energy consumed or ingested (C) was converted to J/h/g using Equation 4:

$$C = (\textit{maximum clearance rate: } L/g/h) \times (mg\ POM/L) \times (23\ J/mg\ POM) \quad \textit{(Equation 4)}$$

where 23 J/mg represents the energy content of the ash-free particulate organic matter (POM) in algal food (Slobodkin & Richman, 1961; Widdows et al., 1979).

The absorption energy of oysters was calculated as follows using Equation 5:

$$A = (C) \times (AE) \quad \textit{(Equation 5)}$$

The energy lost through respiration (R; MO_2/DW) was converted into energy equivalents, where the heat equivalent of oxygen uptake is 0.456 J/μmol O_2, and used as a conversion factor according to Equation 6 (Gnaiger, 1983):

$$R = (\mu mol\ O_2/h/g) \times (0.456) \quad \textit{(Equation 6)}$$

Thus, based on the calculated absorption and energy lost through respiration, the theoretical scope for growth (SFG) was estimated using the balanced energy Equation 7:

$$SFG = (A) - (R) \quad \textit{(Equation 7)}$$

where A is the absorbed food energy and R is the respiratory energy expenditure.

2.3.6. Condition index

The condition index (CI) is a comparative measure for estimating the impact of petroleum products on oyster morphology and health by estimating their tissue yield and prevalence in shell. Morphometric measurements were used to calculate the CI and included: the longest shell length (cm), width (cm), depth (cm), and dry weight (g). Dry weights were measured by removing the tissue from shells, placing them in pre-weighed aluminum dishes, and drying them in an oven at 100 °C for 48 h (Widdows & Staff, 2006). The samples were removed and reweighed. The condition index was calculated as below (Bodoy et al., 1986) in Equation 8:

$$Condition\ Index\ (g/cm^3) = \frac{Tissue\ Dry\ Weight\ (g)}{Shell\ Length\ x\ Width\ x\ Depth\ (cm^3)}\ x\ 100\% \qquad (Equation\ 8)$$

2.3.7. Health assessment index

The health assessment index (HAI) is a visual inspection of oyster tissue and organ health to assess the impact of petroleum products. The HAI criteria used were modified from one developed for teleosts (Adams et al., 1993). The variables selected for oyster HAI were mantle tentacle (papilla) responsiveness and firmness, coloration of digestive glands and gonads, prevalence of tissue in the shell, presence of parasites, and tissue abnormalities that included degeneration, inflammation, mottling, or granulation. These variables were assigned a numerical value, where the normal conditions were given a value of 0, and those that were abnormal were given a value ranging from 1–3 depending on the severity of deviation from normal conditions. HAI value for each oyster was calculated by summing the numerical values of all variables. The scoring for this assessment was subjective but performed in a standardized manner based on predetermined descriptors assigned to each variable (Table A.45.). A labeled normal oyster anatomical image is presented in the Appendix Fig. A.3.

2.3.8. Histopathology

Histopathology is an ecotoxicological tool used to assess the impact of petroleum contaminants on bivalve organs and tissues, which was performed according to standard

procedures (Howard et al., 2004), where tissue cross-sections between gill filaments and labial palps were dissected and removed. This 4-5 mm cross-section comprised of oyster gonad and digestive gland tissue. Tissues were fixed in a 10% neutral buffered formalin solution (100 mL formalin [37% formaldehyde; Sigma], 900 mL DI H_2O, 9.0 g NaCl, and 12.0 g Na_2HPO_4 [99% anhydrous; Sigma]). After 24 h, the samples were transferred to scintillation vials filled with 70% ethanol for dehydration. These samples were then embedded in paraffin wax, and histological sections (5 µm) were cut at an angle of 5° using the HistoCore BIOCUT microtome (Wetzlar, Germany). The section ribbons were placed in a warm water bath (45 °C), allowing them to relax before being mounted on glass slides. These slides were dried on a slide warmer, and treated with xylene and ethanol, before being stained with Mayer's hematoxylin (Thermo Scientific) and Eosin Y (Thermo Scientific) using the Leica ST4020 Linear Stainer (Nussloch, Germany). Prepared slides were visualized using the inverted brightfield microscope Leica DMi8 in the Leica Application Suite X (LAS X) software at 50x total magnification. A quantitative histopathological analysis was performed on these slides using a calibrated tool with a scale in µm, where length measurements were taken for the gonad and digestive gland tissue samples. Histopathological images of a healthy male (Fig. A.1.) and unhealthy female (Fig. A.2.) oyster tissue are presented in the Appendix.

The first histopathological analysis was performed on gonadal thickness (µm), which was measured in three different sections of one oyster tissue on the same slide. Five measurements were taken from different gonadal regions of each section. All the gonad thickness values were corrected to oyster wet weight and averaged.

The second histopathological analysis was performed on the oyster digestive gland tissue, where the digestive lumen and tubule thickness (µm) were measured in three different sections on the same slide. In a digestive gland, lumen tissue is the inner epithelium lining, while a tubule is the external layer. Five measurements of the digestive lumen and tubule thickness were taken per section from the same digestive gland for a consistent ratio comparison and averaged before the final analysis.

2.3.9. Larval bioassays

Healthy oyster broodstock was collected from the Deep Bay marine station (Vancouver Island, BC) to maximize fertilization and developmental success. Five female

oysters were strip spawned; small incisions were made in their gonads with a scalpel blade and gametes were released (Lafont et al., 2019; Nordio et al., 2020; Rico-Villa et al., 2009). The gametes were washed in beakers containing 1 L seawater at room temperature, 1 h before fertilization. Egg concentrations were determined by adding 1 mL of each egg solution to a Graticules S50 Sedgewick Rafter Counting Chamber and counting the number of eggs under a compound light microscope at 100x total magnification. These egg concentrations ranged between 420–8850 eggs/mL. One viable male control oyster was used to fertilize all the diluted eggs by strip spawning, where 1 mL sperm was added to each beaker containing eggs for a minimal spermatozoa:oocyte ratio of 400:1 over a gamete contact time of 10 min (Song et al., 2009).

Following this period, 1 mL of fertilized egg solution was added to a scintillation vial filled with 15 mL seawater, where eggs from each female represented a biological replicate (total n = 5/treatment). For the MD, CO, and DB exposure, 0.5 mL of diluted WAF treatment groups (100%, 75%, 50%, 25%, 0%) were added to each vial resulting in a final oil:seawater dilution of 3.2%, 2.4%, 1.6%, 0.8%, and 0%. The incubation was performed 30 min after the exposure at 24 °C for 48 h; vials were placed horizontally in an orbital shaker at 48 rpm to accelerate larval development (Nordio et al., 2020). These samples were then fixed in 1 mL formaldehyde (37%; Sigma) to determine the impact of petroleum WAF treatment groups on larval developmental success, where the trochophore and D-veliger stages were scored as successful, while unfertilized and fertilized egg stages were considered unsuccessful. Larval developmental success was assessed by pipetting 1 mL of the fixed sample into a Graticules S50 Sedgewick Rafter Counting Chamber after gently mixing the scintillation vials. These samples were observed under a light compound microscope (BA310E, Motic) at a total magnification of 100x, where the developmental success of the first 50 viable individuals was counted and scored. Decaying or lysed eggs/larvae were not included in the final count. Images for the different larvae developmental stages are provided in the Appendix Fig. A.4.

2.3.10. Statistical analysis

A linear-mixed model design was used to test for differences in mean endpoint values between WAF dilutions (0, 25, 50, 100%) for each petroleum product, at each sampling time point (d 7, 21, 35), and their interaction effects (combination of WAF dilution and time). Interaction effects tested for significant differences between the treatment

groups on any given sampling day and whether these differences changed over time. If there were differences between sampling days, there was an interaction impact between the treatment groups and sampling time. The average responses of 2 oysters per exposure tank were used to remove pseudo-replication from the analysis with a final sample size of n = 4, before any outlier exclusion. Tanks in water baths (blocks) were considered as a random factor in the model, while the dilutions, sampling time points, and their two-way interaction were considered to be fixed effect variables. Given the presence of a confounding effect caused by the placement of tanks in the water bath, a two-way ANOVA analysis was performed for that particular factor without including the random effect variable. Fixed effect tests were followed by the Tukey Kramer posthoc tests to determine whether differences in mean endpoint values for all levels of WAF dilutions, time points, and their interaction existed. The larval development analysis was performed using a one-way ANOVA, followed by Tukey Kramer posthoc test to determine if significant variation between individual petroleum WAF treatment groups (0, 25, 50, 75, 100%) existed. The normality assumption for all these models, including Shapiro-Wilk's test (p-value > 0.05) and uniform distribution within normal quantile-quantile plots were verified, where the data points were centered about zero and had constant variance. A natural logarithm transformation of the data was applied, whenever required, to satisfy model assumptions of normal distribution for those endpoints (CR and SFG). Finally, potential outliers with absolute studentized residual values greater than ± 3.5 were removed and the models were re-fit to see whether the data points were influential (Blatná, 2006). A significance level of p = 0.05 was used to determine the statistical significance of all the endpoint analyses that were expressed as mean ± SEM and carried out in the JMP® statistical software version 16.0 (SAS Institute Inc., Cary, NC).

2.4. Results

2.4.1. Water PAC concentrations

Individual PAC profile concentrations for each petroleum product WAF during the 7 d exposure period are shown in Appendix Figs. A.5–A.7, A.8–A.10, and A.11–A.13. Total, parent, and alkylated PAC concentrations in water for each WAF dilution of the three petroleum products on sampling d 0, 3, and 7 are displayed in Fig. 2.1. For each oil type, there was a decline in the individual (Table A.46.) and total (Tables A.28, A.29, A.30) PAC concentration over time. This resulted in an altered oil composition, where all three products had greater proportions of alkylated relative to parent PAC forms, which comprised of the longest hydrocarbon chains.

On d 0 of WAF exposure, CO had the highest TPAC concentrations at all dilutions (25%: 148 µg/L; 50%: 240 µg/L; 100%: 350 µg/L), followed by MD (25%: 24 µg/L; 50%: 52 µg/L; 100%: 150 µg/L) and DB (25%: 32 µg/L; 50%: 60 µg/L; 100%: 128 µg/L). Across the three oils, TPAC concentrations in control WAFs ranged from 0.001-0.49 µg/L. Over the 7 d exposure, the greatest decline in TPAC concentrations occurred in MD (99.9%), followed by CO (93.1%), and DB (78.1%), with parent PAC concentrations declining faster than the alkylated PACs.

The initial PAC composition for all three WAFs mostly comprised of low molecular weight (LMW) 2-3 ring hydrocarbons. MD WAFs had naphthalenes, biphenyls, and fluorenes; CO comprised of naphthalenes and biphenyls; DB constituents included naphthalenes, phenanthrenes, anthracenes, fluorenes, and dibenzothiophenes. After the 7 d exposure, the proportion of 3-4 ring PACs increased, while the proportions of 2-ring PACs, such as naphthalenes and biphenyls decreased in all three petroleum WAFs. This resulted in MD WAFs mainly comprising of fluorenes, with some phenanthrenes, anthracenes, and naphthalenes; CO consisting of phenanthrenes, anthracenes, dibenzothiophenes, fluorenes, and naphthalenes; DB having phenanthrenes, anthracenes, fluorenes, dibenzothiophenes, and naphthalenes at the end of the exposure period.

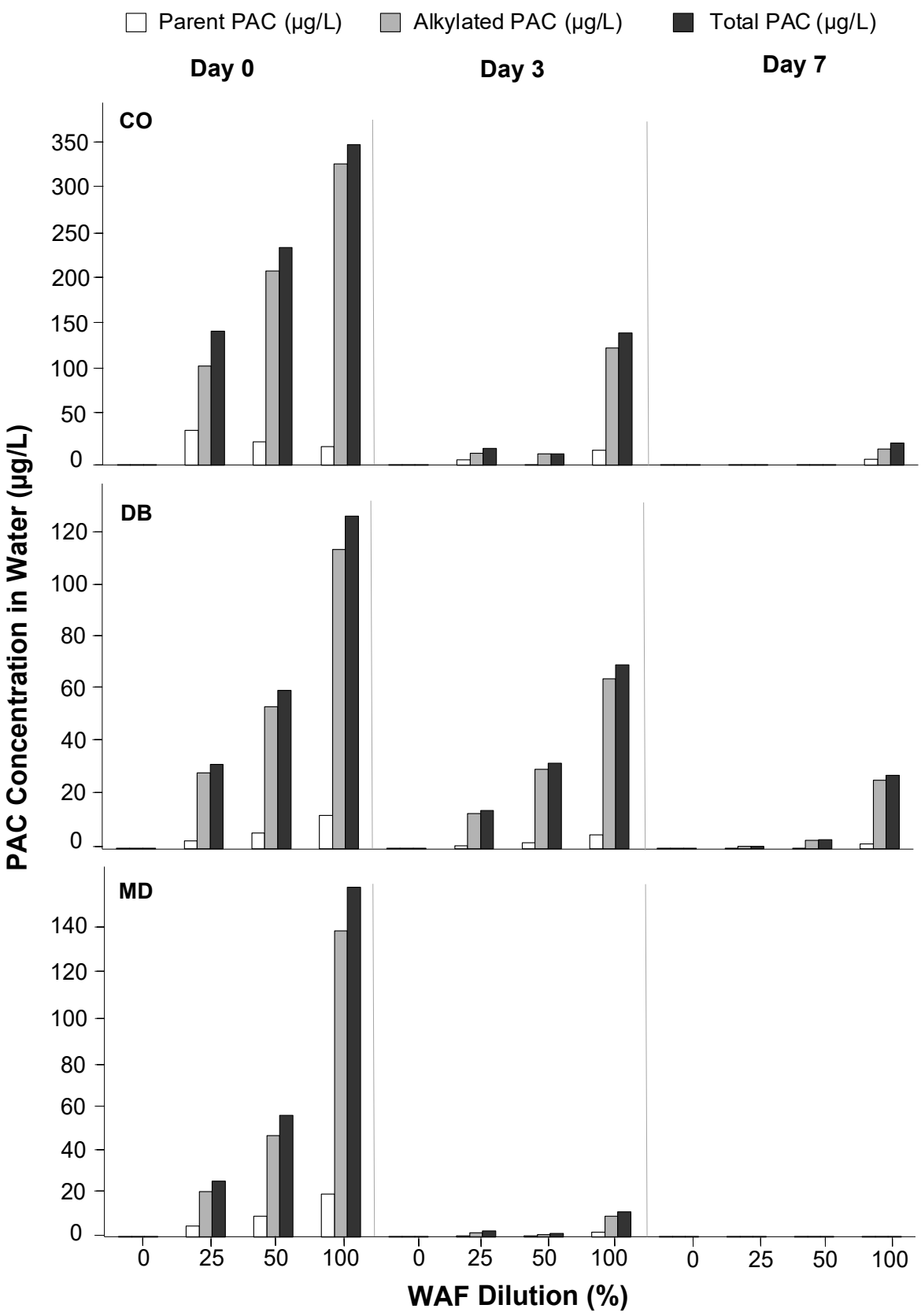
Parent PAC (µg/L)
Alkylated PAC (µg/L)
Total PAC (µg/L)
Day 0
Day 3
Day 7
CO
DB
MD
PAC Concentration in Water (µg/L)
WAF Dilution (%)
0
25
50
100

Figure 2.1. Total, parent, and alkylated polycyclic aromatic compound (PAC) concentrations in seawater during the sub-chronic exposure of oysters to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions at d 0, 3, and 7.

2.4.2. Tissue PAC concentrations

Individual PAC profile concentrations in oyster tissues exposed to the three petroleum products at each sampling time point (d 7, 21, 35) are presented in Appendix Figs. A.14–A.16, A.17–A.19, and A.20–A.22. The total, parent, and alkylated PAC concentrations in oyster tissue exposed to WAF dilutions (%) at each sampling day are shown in Fig. 2.2. There was a decline in individual (Table A.47) and total (Tables A.31, A.32, A.33) PAC concentrations in tissues for all three products over time, which corresponded with the decline in water PAC concentrations.

Post-exposure, CO had the highest accumulation of PACs in oyster tissues (25%: 4.5 µg/g; 50%: 15 µg/g; 100%: 29 µg/g), followed by MD (25%: 1 µg/g; 50%: 2.3 µg/g; 100%: 4 µg/g) and DB (25%: 1.5 µg/g; 50%: 2 µg/g; 100%: 3 µg/g). The TPAC concentrations accumulated in control oysters ranged from 0.07-0.38 µg/g. On d 7 of exposure, TPAC concentrations detected in oyster tissue compared to WAFs were much higher for MD (18 fold) and slightly greater for CO (1.2 fold), while the water TPAC concentrations were higher than tissue concentrations for DB (9 fold). The comparison between tissue and water TPAC concentrations on exposure d 7 are presented in Appendix Fig. A.23. Over the four week recovery period in uncontaminated water, TPAC concentrations in tissues declined by 97.8% (CO), 93.5% (MD), and 92.3% (DB), with parent PAC concentrations declining faster than the alkylated forms.

Following the 7 d exposures, MD exposed oysters mainly accumulated LMW PACs such as naphthalenes, biphenyls, phenanthrenes, anthracenes, and fluorenes; CO mostly accumulated naphthalenes, phenanthrenes, anthracenes, and dibenzothiophenes; DB oysters majorly consisted of naphthalenes, phenanthrenes, anthracenes, dibenzothiophenes, and fluorenes. A 4-week recovery in uncontaminated water, resulted in oysters exposed to all three products having lower proportions of 2-ring PACs, while increasing the proportions of 3-6 ring PACs in tissues. Oysters exposed to MD had higher proportions of phenanthrenes, fluoranthenes, and pyrenes, along with some anthracenes, fluorenes, dibenzothiophenes, and naphthalenes; CO tissues majorly comprised of phenanthrenes, anthracenes, dibenzothiophenes, fluorenes, and naphthalenes; DB exposed oysters accumulated phenanthrenes, anthracenes, dibenzothiophenes, naphthalenes, fluoranthenes, pyrenes, benzo[a]anthracenes, and chrysenes.

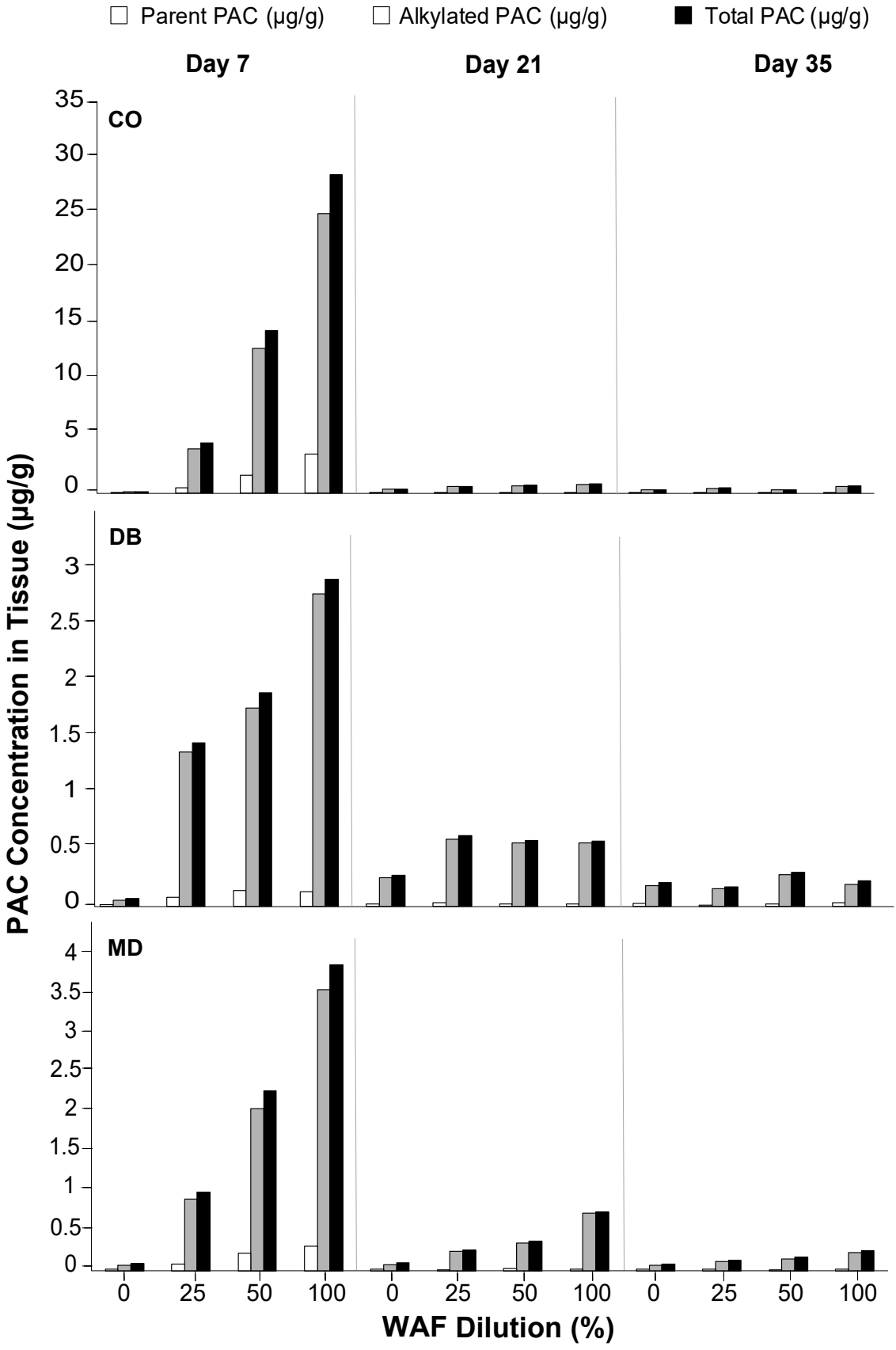
Parent PAC (µg/g)
Alkylated PAC (µg/g)
Total PAC (µg/g)
Day 7
Day 21
Day 35
CO
DB
MD
PAC Concentration in Tissue (µg/g)
WAF Dilution (%)
0
25
50
100

Figure 2.2. Total, parent, and alkylated polycyclic aromatic compound (PAC) concentrations in tissues of oysters exposed to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions at d 7, 21, and 35.

2.4.3. Mortality

The cumulative percent mortality of oysters exposed to each petroleum product over the 7 d exposure period is shown in Appendix Fig. A.24. The mortality observed in controls across all exposures were 0.6% (MD), 6.7% (CO), and 3.9% (DB). No significant differences in percent mortality between treatment groups were seen for CO ($F_{3,44}$: 1.12; p-value: 0.3511) and MD ($F_{3,44}$: 0.64; p-value: 0.5948). Although significant percent mortality differences were observed between oysters exposed to 25% and 100% DB treatment groups during the 7 d period ($F_{3,44}$: 4.79; p-value: 0.0057*), no differences relative to the control oysters and no concentration-dependent effects were observed.

2.4.4. Scope for growth

Clearance rate

Marine Diesel

The natural log-transformed CR values for control oysters ranged from 1.08-2.28 L/h/g and did not change over time (Fig. 2.3). While some significant differences were found between WAF dilutions at certain time points ($F_{6,35}$: 3.20; p-values: 0.0131*; Fig. 2.3), no concentration-dependent effects were present. At d 21, CR values for control oysters (1.08 ± 0.03 L/h/g) were significantly lower than the 25% (2.37 ± 0.19 L/h/g) and 50% (2.37 ± 0.17 L/h/g) treatment groups; at d 7, oyster CR values in the 25% (1.34 ± 0.04 L/h/g) and 50% (1.00 ± 0.21 L/h/g) WAF treatment groups were significantly lower than at d 21.

Crude Oil

The natural log-transformed CR values for control oysters ranged from 1.75–3.03 L/h/g and did not change over time (Fig. 2.3). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution CR did not change over time ($F_{6,33}$: 0.50; p-values: 0.8066; Fig. 2.3).

Diluted Bitumen

The natural log-transformed CR values for control oysters ranged from 1.53-2.91 L/h/g and did not change over time. No significant differences were found between WAF

dilutions at any time points and at any given WAF dilution CR did not change over time ($F_{6,34}$: 0.23; p-values: 0.9625; Fig. 2.3).

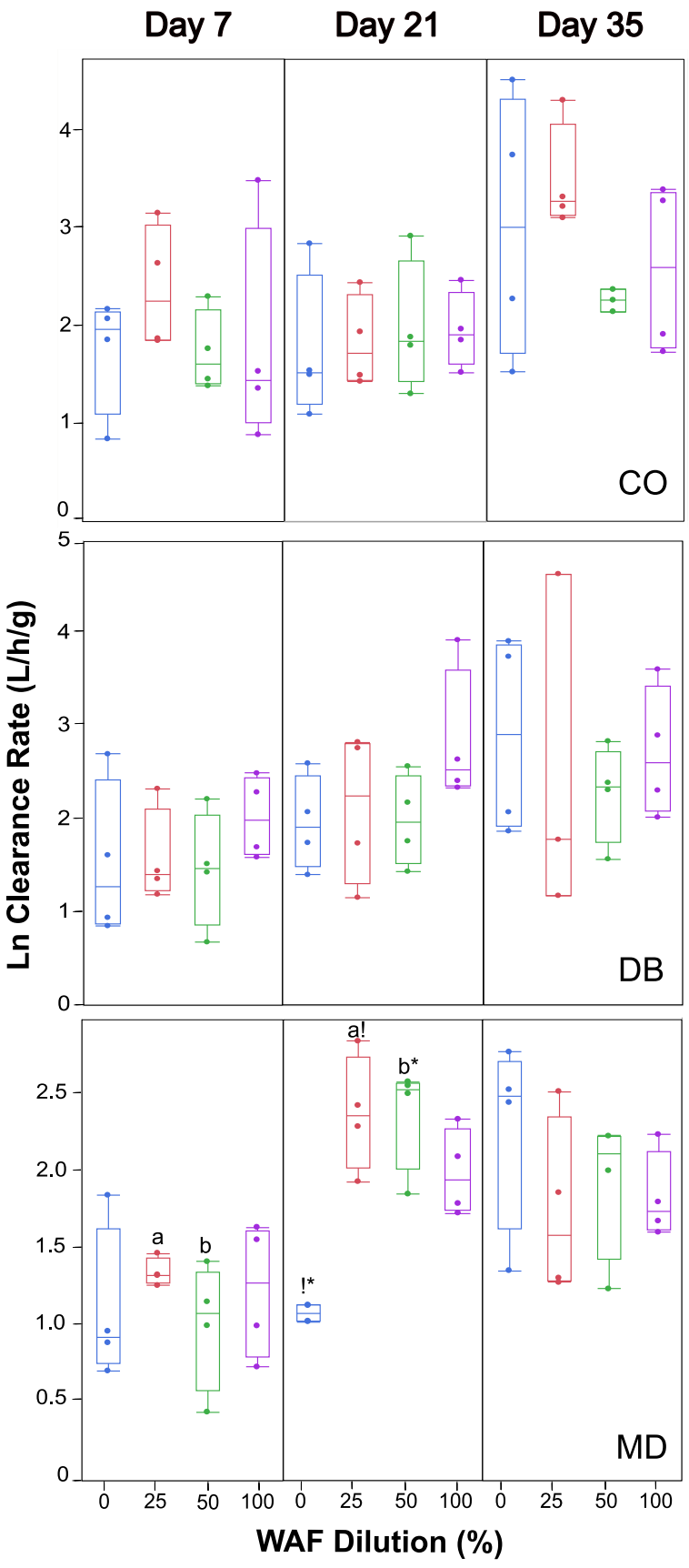
Day 7
Day 21
Day 35
CO
DB
MD
Ln Clearance Rate (L/h/g)
WAF Dilution (%)
0
25
50
100
a
b
!*
a!
b*

Figure 2.3. Mean natural log-transformed clearance rates (CR) of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through d 35. Boxplots represent median CR values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 3-4 oysters. Similar superscript symbols indicate significant differences between treatment groups at p = 0.05. Similar superscript letters indicate significant differences across time at p = 0.05.

Absorption efficiency

Marine Diesel

The AE values for control oysters ranged from 0.75-0.81 and did not change over time (Fig. 2.4). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution AE did not change over time ($F_{6,29}$: 0.11; p-values: 0.9941; Fig. 2.4).

Crude Oil

The AE values for control oysters ranged from 0.73-0.79 and did not change over time (Fig. 2.4). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution AE did not change over time ($F_{6,25}$: 1.05; p-values: 0.4200; Fig. 2.4).

Diluted Bitumen

The AE values for control oysters ranged from 0.47-0.73 and did not change over time (Fig. 2.4). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution AE did not change over time ($F_{6,23}$: 0.72; p-values: 0.6382; Fig. 2.4).

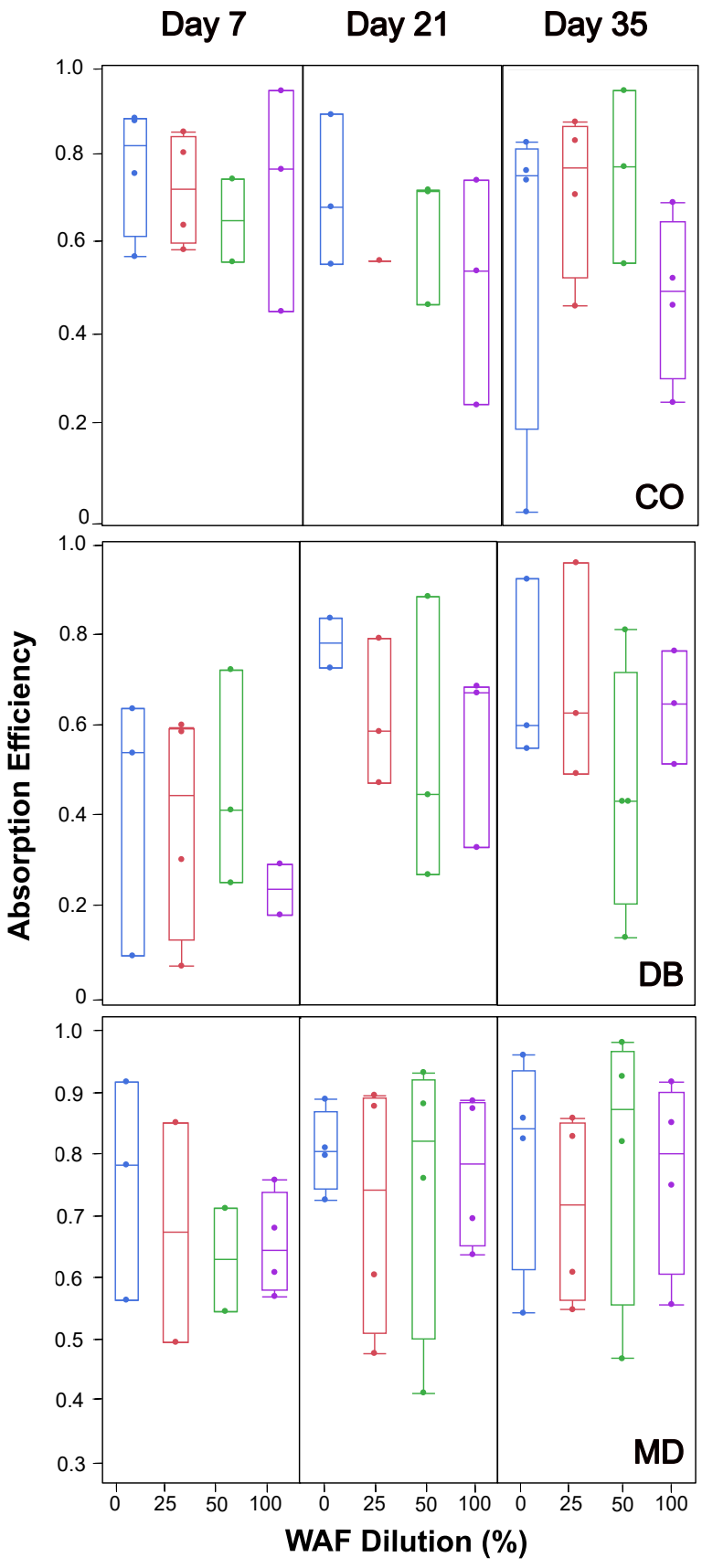
Day 7
Day 21
Day 35
CO
DB
MD
Absorption Efficiency
WAF Dilution (%)
0
25
50
100

Figure 2.4 Mean absorption efficiency (AE) values of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through d 35. Boxplots represent median AE values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 1-4 oysters. Significant differences between treatment groups across time were compared at p = 0.05.

Oxygen consumption

Marine Diesel

The MO_2 values for control oysters ranged from 14.71-21.85 µmol O_2/h/g and did not change over time (Fig. 2.5). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution MO_2 did not change over time ($F_{6,36}$: 0.80; p-values: 0.5743; Fig. 2.5).

Crude Oil

The MO_2 values for control oysters ranged from 10.86-18.98 µmol O_2/h/g and did not change over time (Fig. 2.5). While some significant differences were found between WAF dilutions at certain time points ($F_{6,36}$: 1.64; p-values: 0.1646; Fig. 2.5), no concentration-dependent effects were present. At d 35, MO_2 for the 50% oysters (25.52 ± 3.34 µmol O_2/h/g) were significantly higher than the 100% (11.32 ± 1.43 µmol O_2/h/g) WAF treatment group; at d 7, MO_2 values for oysters in the 25% (25.02 ± 1.36 µmol O_2/h/g) WAF treatment group were significantly higher than at d 21 (16.53 ± 2.59 µmol O_2/h/g).

Diluted Bitumen

The MO_2 values for control oysters ranged from 18.44-21.11 µmol O_2/h/g and did not change over time (Fig. 2.5). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution MO_2 did not change over time ($F_{6,36}$: 0.42; p-values: 0.8588; Fig. 2.5).

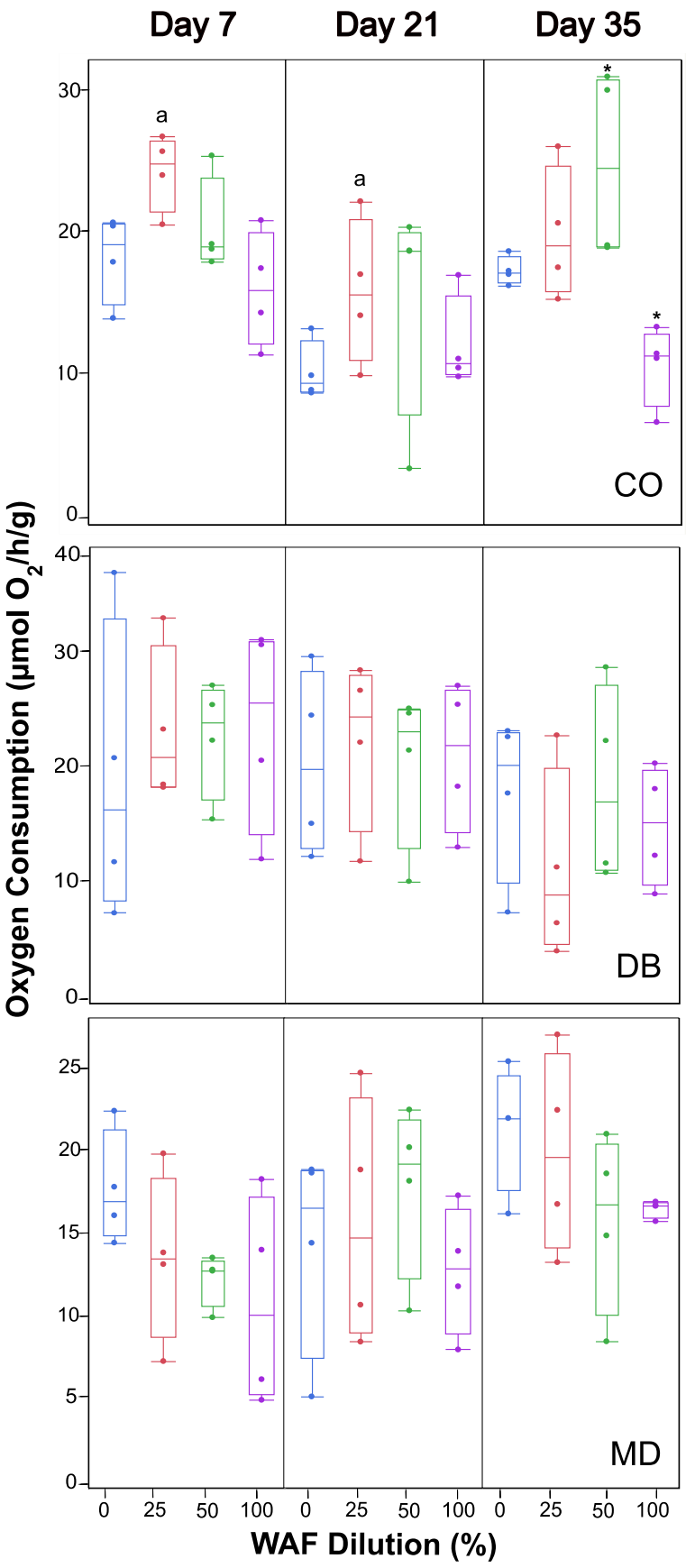
Day 7
Day 21
Day 35
a
a
*
*
CO
DB
MD
Oxygen Consumption (µmol O2/h/g)
WAF Dilution (%)
0
25
50
100

Figure 2.5. Mean oxygen consumption (MO_2) in oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through d 35. Boxplots represent median MO_2 values (lines within the box), 25^{th} and 75^{th} percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 4 oysters. Similar superscript symbols indicate significant differences between treatment groups at p = 0.05. Similar superscript letters indicate significant differences across time at p = 0.05.

Scope for growth

Marine Diesel

The natural log-transformed SFG values for control oysters ranged from 1.08-2.28 J/h/g and did not change over time (Fig. 2.6). While significant differences were found between SFG values at WAF dilutions at certain time points ($F_{6,35}$: 3.33; p-values: 0.0107*; Fig. 2.6), no concentration-dependent effects were present. At d 21, SFG for the 50% (4.16 ± 0.18 J/h/g) treatment group were significantly higher than the control oysters (2.68 ± 0.06 J/h/g); at d 7, oyster SFG values in the 25% (2.96 ± 0.08 J/h/g) treatment group were significantly lower than at d 21 (4.13 ± 0.19 J/h/g) and SFG values in the 50% (2.32 ± 0.37 J/h/g) treatment group were significantly lower than at d 21 (4.16 ± 0.18 J/h/g) and d 35 (3.74 ± 0.24 J/h/g).

Crude Oil

The natural log-transformed SFG values for control oysters ranged from 3.66-5.01 J/h/g and did not change over time (Fig. 2.6). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution SFG did not change over time ($F_{6,33}$: 0.57; p-values: 0.7532; Fig. 2.6).

Diluted Bitumen

The natural log-transformed SFG values for control oysters ranged from 2.09-4.50 J/h/g and did not change over time (Fig. 2.6). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution SFG did not change over time ($F_{6,32}$: 0.52; p-values: 0.7902; Fig. 2.6).

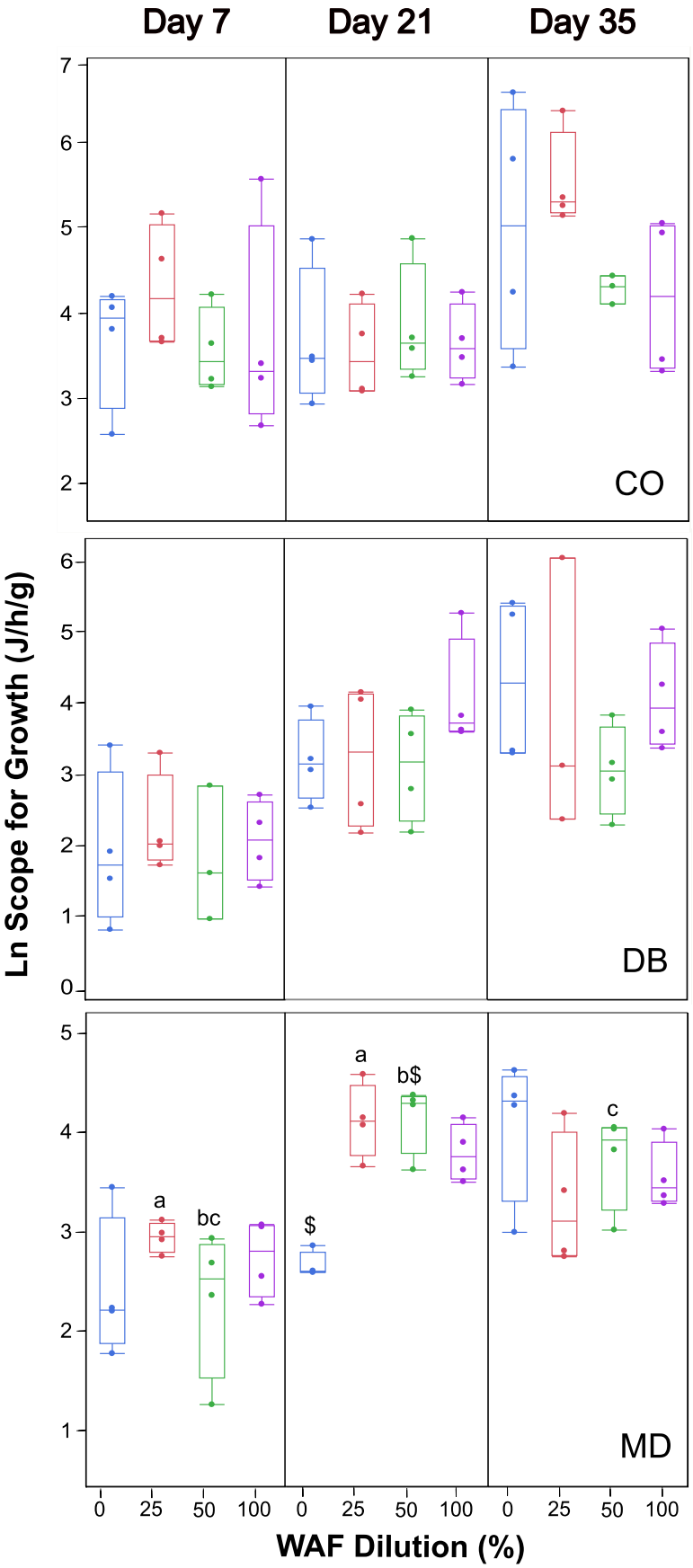
Day 7
Day 21
Day 35
CO
DB
MD
Ln Scope for Growth (J/h/g)
WAF Dilution (%)
0
25
50
100
a
bc
$
a
b$
c

Figure 2.6. Mean natural log-transformed scope for growth (SFG) of oysters following a 7 d exposure of 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through d 35. Boxplots represent median SFG values (lines within the box), 25^{th} and 75^{th} percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 3-4 oysters. Similar superscript symbols indicate significant differences between treatment groups at p = 0.05. Similar superscript letters indicate significant differences across time at p = 0.05.

2.4.5. Condition index

Marine Diesel

The CI values for control oysters ranged from 1.60-1.68 cm^3/g and did not change over time (Fig. 2.7). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution CI did not change over time ($F_{6,33}$: 0.22; p-values: 0.9668; Fig. 2.7).

Crude Oil

The CI values for control oysters ranged from 1.36-1.78 cm^3/g but did not change over time (Fig. 2.7). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution CI did not change over time ($F_{6,28}$: 1.26; p-values: 0.3096; Fig. 2.7).

Diluted Bitumen

The CI values for control oysters ranged from 0.90-1.10 cm^3/g but did not change over time (Fig. 2.7). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution CI did not change over time ($F_{6,36}$: 0.69; p-values: 0.6630; Fig. 2.7).

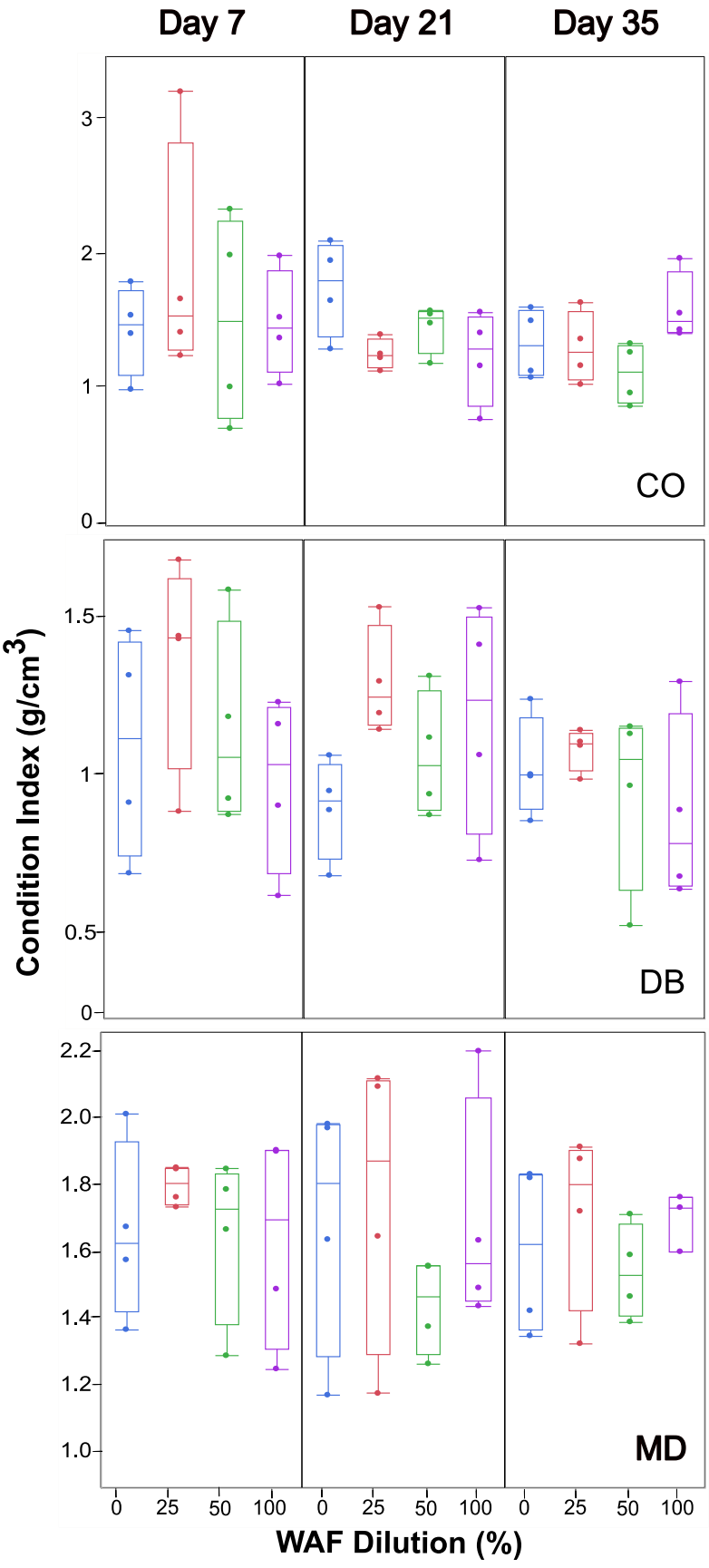
Day 7
Day 21
Day 35
CO
DB
MD
Condition Index (g/cm3)
WAF Dilution (%)
0
25
50
100

Figure 2.7. Mean condition index (CI) of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through 35 d. Boxplots represent median CI values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 4 oysters. Significant differences between treatment groups across time were compared at p = 0.05.

2.4.6. Health assessment index

Marine Diesel

The HAI values for control oysters ranged from 1.00-2.25 and did not change over time (Fig. 2.8). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution HAI did not change over time ($F_{6,35}$: 0.76; p-values: 0.6036; Fig. 2.8).

Crude Oil

The HAI values for control oysters ranged from 0.00-0.38 and did not change over time (Fig. 2.8). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution HAI did not change over time ($F_{6,21}$: 1.95; p-values: 0.1209; Fig. 2.8).

Diluted Bitumen

The HAI values for control oysters ranged from 0.50-1.13 and did not change over time (Fig. 2.8). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution HAI did not change over time ($F_{6,36}$: 0.40; p-values: 0.8725; Fig. 2.8).

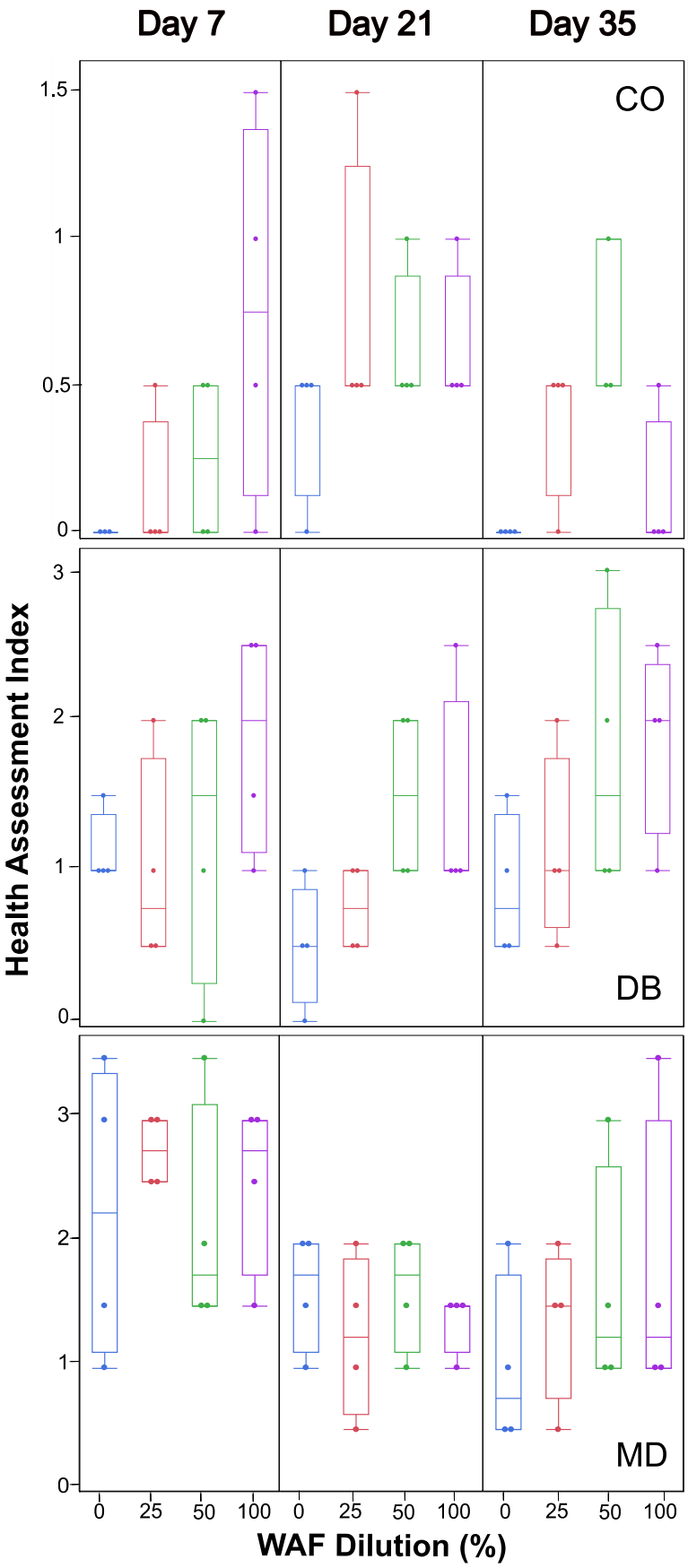
Day 7
Day 21
Day 35
CO
DB
MD
Health Assessment Index
WAF Dilution (%)
0
25
50
100
0
0.5
1
1.5
0
1
2
3
0
1
2
3

Figure 2.8. Mean health assessment index (HAI) of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through 35 d. Boxplots represent median HAI values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 3-4 oysters. Significant differences between treatment groups across time were compared at p = 0.05.

2.4.7. Gonad thickness

Marine Diesel

The wet weight corrected gonadal thickness for control oysters ranged from 291.5-376.0 µm/g and did not change over time (Fig. 2.9). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution gonad thickness did not change over time ($F_{6,35}$: 1.41; p-values: 0.2374; Fig. 2.9).

Crude Oil

The wet weight corrected gonadal thickness for control oysters ranged from 523.8-694.4 µm/g and did not change over time (Fig. 2.9). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution gonad thickness did not change over time ($F_{6,35}$: 0.83; p-values: 0.5571; Fig. 2.9).

Diluted Bitumen

The wet weight corrected gonadal thickness for control oysters ranged from 609.8-910.2 µm/g and did not change over time (Fig. 2.9). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution gonad thickness did not change over time ($F_{6,23}$: 0.63; p-values: 0.7052; Fig. 2.9).

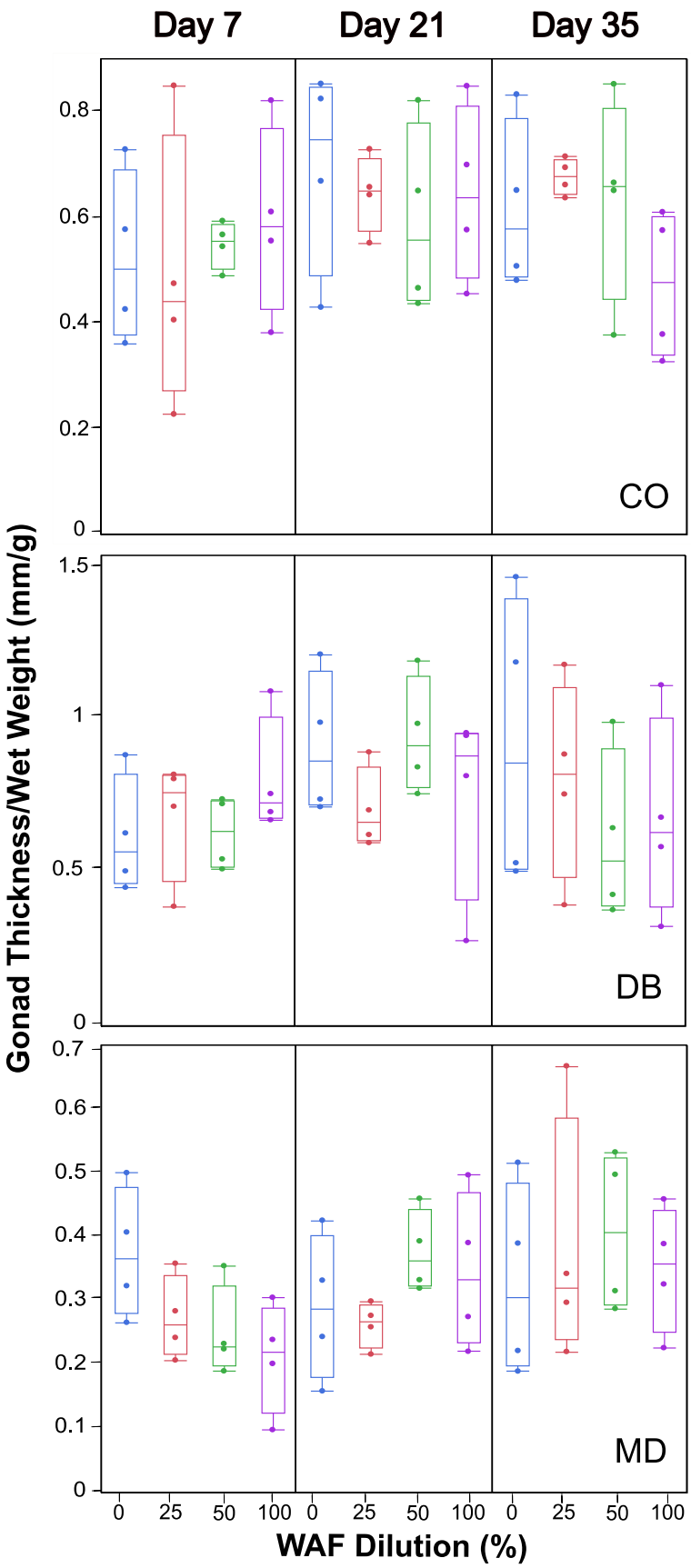
Day 7
Day 21
Day 35
Gonad Thickness/Wet Weight (mm/g)
0
0.2
0.4
0.6
0.8
CO
0
0.5
1
1.5
DB
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
MD
0
25
50
100
WAF Dilution (%)

Figure 2.9. Mean wet weight corrected gonadal thickness of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through 35 d. Boxplots represent median corrected gonadal thickness values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 4 oysters. Significant differences between treatment groups across time were compared at p = 0.05.

2.4.8. Digestive lumen to tubule thickness

Marine Diesel

The digestive lumen to tubule thickness for control oysters ranged from 0.88-1.75 and did not change over time (Fig. 2.10). While significant differences were found between WAF dilutions at certain time points ($F_{6,36}$: 1.92; p-values: 0.1039; Fig. 2.10), no concentration-dependent effects were present. At d 7, the digestive gland thickness ratio for the 25% oysters (0.35 ± 0.01) was only significantly higher than the 100% (0.27 ± 0.01) treatment group.

Crude Oil

The digestive lumen to tubule thickness for control oysters ranged from 0.50-0.75 and did not change over time (Fig. 2.10). While significant differences were found between WAF dilutions at certain time points ($F_{6,35}$: 2.08; p-values: 0.0806; Fig. 2.10), no concentration-dependent effects were present. At d 21, the digestive gland thickness ratio for control oysters (0.36 ± 0.02) was significantly higher than the 25% (0.28 ± 0.01), 50% (0.29 ± 0.00), and 100% (0.28 ± 0.01) WAF treatment groups.

Diluted Bitumen

The digestive lumen to tubule thickness for control oysters ranged from 0.63-0.88 and did not change over time (Fig. 2.10). No significant differences were found between WAF dilutions at any time points and at any given WAF dilution digestive gland thickness ratio did not change over time ($F_{6,36}$: 1.60; p-values: 0.1751; Fig. 2.10).

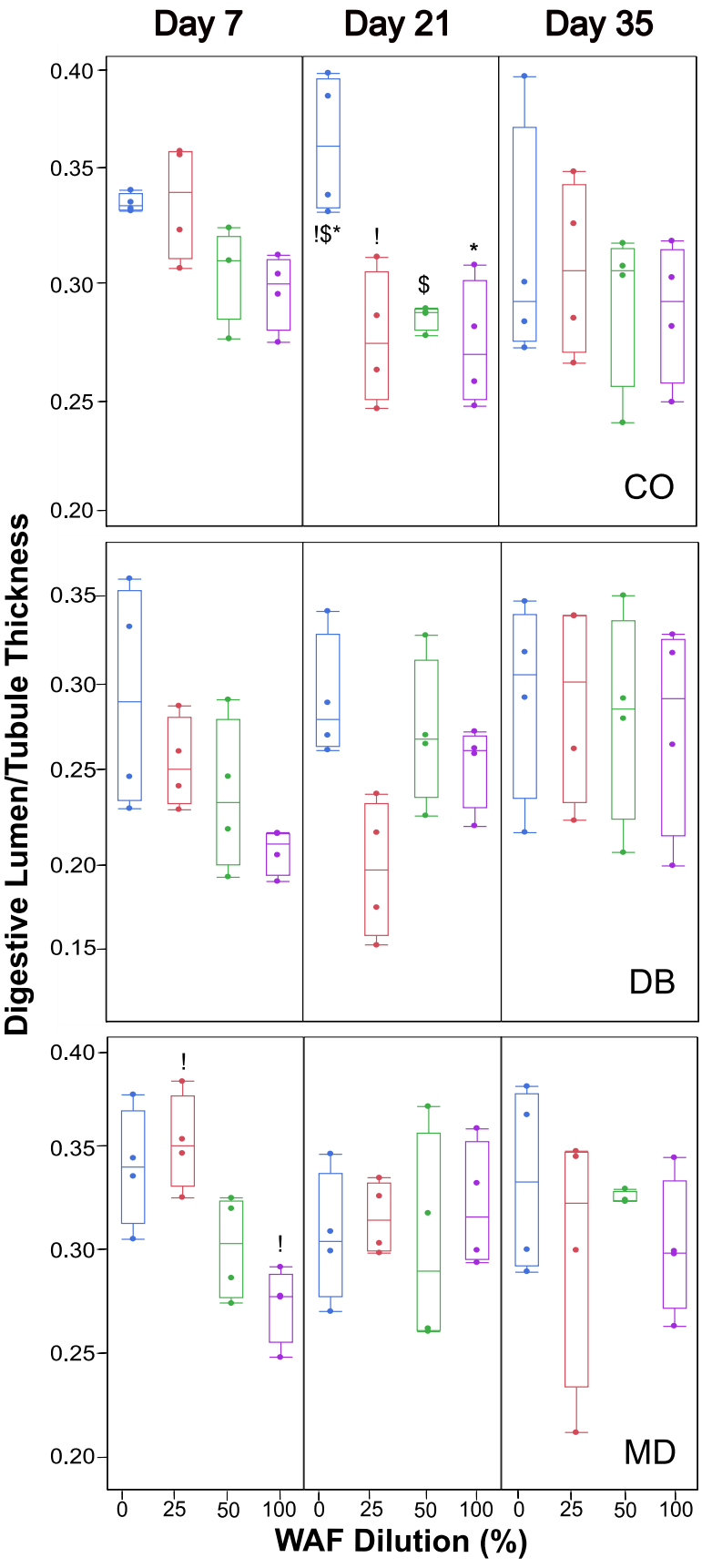
Day 7
Day 21
Day 35
Digestive Lumen/Tubule Thickness
0.40
0.35
0.30
0.25
0.20
0.15
!$*
!
$
*
CO
DB
MD
0
25
50
100
WAF Dilution (%)

Figure 2.10. Mean digestive lumen to tubule thickness ratio of oysters following a 7 d exposure to 0%, 25%, 50%, and 100% dilutions of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs) and recovery through 35 d. Boxplots represent median digestive gland thickness values (lines within the box), 25th and 75th percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 4 oysters. Similar superscript symbols indicate significant differences between treatment groups at p = 0.05.

2.4.9. Larvae developmental success

Marine Diesel

The proportion of larvae development ranged from 0.19 in the 100% treatment group to 0.40 in the control oysters (Fig. 2.11). No significant effects were observed between WAF dilutions ($F_{4,20}$: 1.66; p-value: 0.1992; Fig. 2.11).

Crude Oil

The proportion of larvae development ranged from 0.24 in the 75% treatment group to 0.49 in the 25% treatment group oysters, while the control oysters had a larval development proportion of 0.43 (Fig. 2.11). No significant effects were observed between WAF dilutions ($F_{4,20}$: 1.38; p-value: 0.2768; Fig. 2.11).

Diluted Bitumen

The proportion of larvae development ranged from 0.21 in the 100% treatment group to 0.36 in the control oysters (Fig. 2.11). No significant effects were observed between WAF dilutions ($F_{4,20}$: 1.22; p-value: 0.3330; Fig. 2.11).

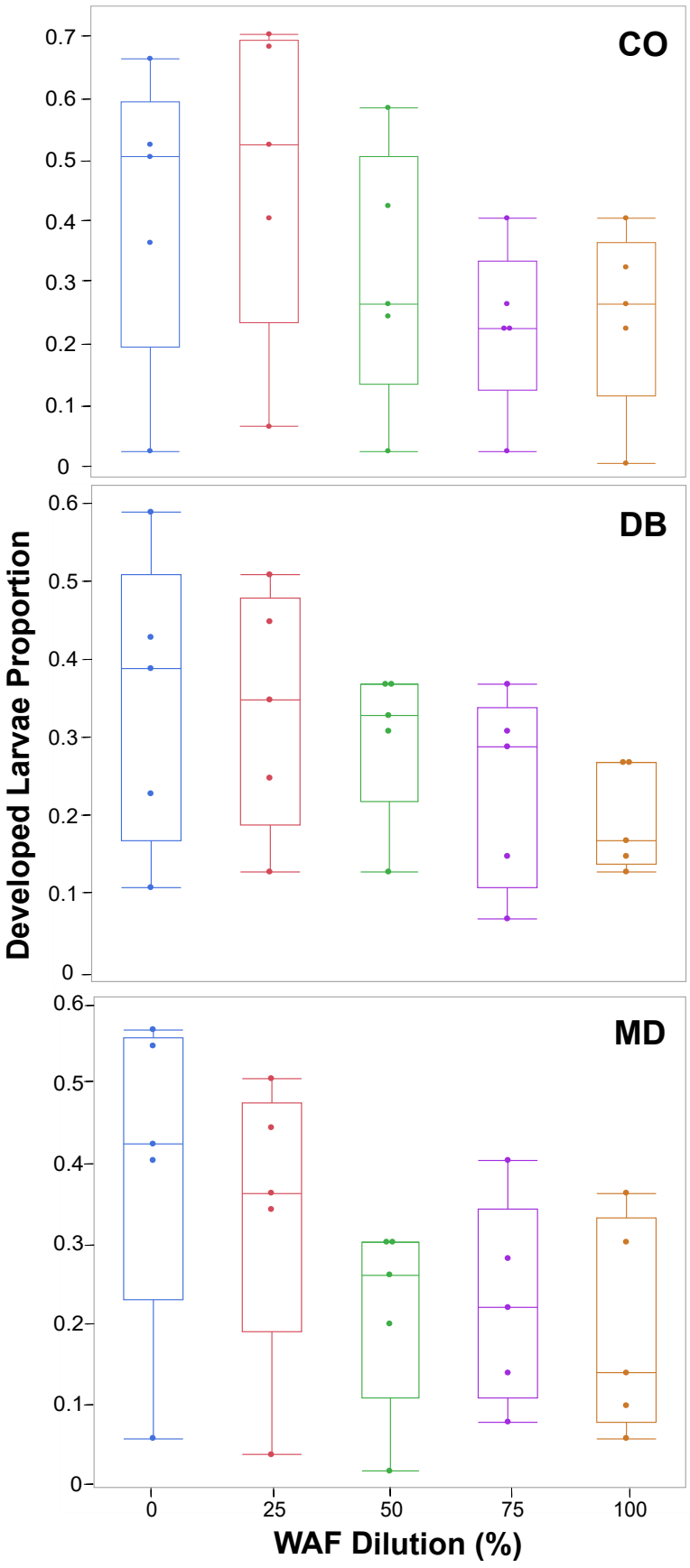
CO
DB
MD
Developed Larvae Proportion
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
25
50
75
100
WAF Dilution (%)

Figure 2.11. Mean developmental success proportion of oyster larvae following acute exposure to 0%, 25%, 50%, 75%, and 100% treatment groups of crude oil (CO), diluted bitumen (DB), and marine diesel (MD) water accommodated fractions (WAFs). Boxplots represent median larval developmental success values (lines within the box), 25^{th} and 75^{th} percentiles (boundaries of the box), as well as minimum and maximum data points (whiskers), exclusive of any outliers. n = 5 oysters. Significant differences between treatment groups were compared at p = 0.05.

2.5. Discussion

With a rise in the production and transport of petroleum products across the Pacific region of North America, there is an elevated risk of contaminating marine environments due to accidental spills. The toxicological repercussions of exposure to three petroleum products with different hydrocarbon constituents in Pacific oysters were examined to provide information regarding hazards posed to marine bivalves. In this study, a comprehensive suite of endpoints was utilized to measure toxic effects that included physiological processes, morphology, histopathology, and development. Additionally, a recovery period in uncontaminated seawater was used to determine if any observed effects were short-term in nature and whether recovery was possible.

PAC water concentrations

The three petroleum products investigated in this study have variable PAC compositions and concentrations (Environment Canada, 2021; EPA, 2003; King et al., 2017). Fresh marine diesel (MD) has TPAC concentrations ranging from 25-40 mg/g oil and is primarily comprised of naphthalenes with smaller proportions of phenanthrenes, biphenyls, and fluorenes (EPA, 2003; Neff et al., 2000; Onwurah et al., 2007). Fresh crude oil (CO) has a TPAC range between 5-15 mg/g oil, with a composition of low molecular weight (LMW; 2-3 ring) and high molecular weight (HMW; 4-6 ring) hydrocarbons that include naphthalenes, phenanthrenes, dibenzothiophenes, fluorenes, and chrysenes (EPA, 2003; Lee et al., 2015; Philibert et al., 2016; Stoyanovich et al., 2019; Yang et al., 2011). Fresh diluted bitumen (DB) has the lowest TPAC concentration range amongst the three oil products (2-6 mg/g oil) and is composed of LMW naphthalenes, phenanthrenes, fluorenes, and dibenzothiophenes, as well as HMW naphthobenzothiophenes, pyrenes, and chrysenes (Environment Canada, 2021; King et al., 2017).

Initial WAFs had lower TPAC concentrations and greater proportions of LMW PACs in their constitution, compared to the parent oil products. MD WAFs had a TPAC concentration of approximately 150 µg/L, predominantly consisting of naphthalenes, with some biphenyls and fluorenes. Similarly, CO WAFs had a TPAC concentration of around 350 µg/L, which was mostly comprised of naphthalenes. DB WAFs exhibited the lowest TPAC concentration (approximately 130 µg/L) among the three oils while having an even

distribution of naphthalenes, phenanthrenes, anthracenes, dibenzothiophenes, and fluorenes. The reduced TPAC concentrations in all three WAFs are a result of dilution with water and are influenced by factors including water solubility, oil-to-water ratios, speed and duration of mixing, settling period, as well as seawater temperature and salinity (Bilbao et al., 2022; Faksness et al., 2008; Forth et al., 2017; Singer et al., 2000). Moreover, the greater proportion of LMW PACs found in all the initial WAFs could be a result of their increased water solubility and dissolution, relative to the HMW hydrocarbons (Faksness et al., 2008; French-Mccay, 2004; Neff et al., 2000; Yim et al., 2002).

The composition and TPAC concentrations of all three WAFs generated in this study were similar to those found in the literature. Previous studies have determined that TPAC concentrations in WAFs prepared from different crude oils ranged from 0.1-390 µg/L (Couillard et al., 2015; Forth et al., 2017; Langdon et al., 2016; Loh et al., 2018; Ramachandran et al., 2004; Stefansson et al., 2016). These studies also found that naphthalenes were the most abundant semi-volatile hydrocarbons being released in WAFs due to their higher water solubility, while phenanthrenes, dibenzothiophenes, fluorenes, and fluoranthenes were present in lower proportions (Bilbao et al., 2022; Faksness et al., 2008). Additionally, in their WAFs, HMW hydrocarbons did not contribute much to the TPAC concentration, resulting in PACs with molecular weights greater than chrysene remaining undetected (Faksness et al., 2008). These findings encompass the TPAC concentrations found in the three WAFs (150-350 µg/L) while having similar proportions of individual LMW hydrocarbons in all the initial WAF compositions.

Studies following marine petroleum spills have also reported similar TPAC constituent proportions and concentrations in water. For example, TPAC concentrations measured after the Deepwater Horizon spill of crude oil in the Gulf of Mexico ranged from 0.01-150 µg/L (Boehm et al., 2011; Liu et al., 2014; Sammarco et al., 2013; Wade et al., 2011). Other spills, such as the Prestige oil spill near Spain, released 77,000 t of heavy fuel oil with an average TPAC concentration of 100 µg/L (Gonzalez et al., 2006), while the North Cape barge spill in the US resulted in an average concentration of 115 µg/L from over 2700 metric t of spilled No. 2 Fuel Oil (Reddy & Quinn, 1999). Within h or d of an oil spill there is a rapid loss of LMW and volatile PACs (e.g., naphthalenes and biphenyls), while HMW PACs (e.g., pyrene, fluoranthene, benzo[a]anthracene) tend to persist longer in seawater (Federal Government, 2013; Forth et al., 2017; Neff et al., 2000; Reddy et al., 2012). Despite having similar TPAC composition and concentration as our laboratory-

prepared WAFs, naturally occurring oil spills have some variations that can be attributed to the type, volume, and distribution of the spilled oil, oil-water mixing conditions, as well as the weathering of oil products (Forth et al., 2017).

For all the WAFs, there was a rapid reduction in the TPAC concentration over 3 d which continued to slowly decline till d 7. This was mainly due to the gradual loss of the 2-ring naphthalenes and biphenyls, with an increase in proportions of the 3-ring phenanthrenes, anthracenes, dibenzothiophenes, and fluorenes. This variation in TPAC concentration and composition of WAFs over the 7 d exposure period could be attributed to weathering processes that include evaporative loss, dissolution, photolysis, and biodegradation (Forth et al., 2017; French-Mccay, 2004; Loh et al., 2018; Uno et al., 2009). This resulted in LMW PACs having reduced persistence in water during our WAF exposures and natural oil spills due to their high rates of volatilization (Bilbao et al., 2022; Forth et al., 2017; Neff et al., 2000; Reddy et al., 2012). The loss of these hydrocarbons from water could also be a result of their potential accumulation in oyster tissues given their hydrophobicity and lipophilicity (Baussant et al., 2001; Loh et al., 2018), as well as potential binding to the exposure glass tanks.

PAC tissue concentrations

On d 7 of MD and CO exposures, greater concentrations of TPACs were detected in oyster tissues compared to water. The major PACs detected in tissues included LMW hydrocarbons such as naphthalenes, biphenyls, and fluorenes. DB-exposed oyster tissues had a lower TPAC concentration than water but a similar composition that included naphthalenes, phenanthrenes, anthracenes, dibenzothiophenes, and fluorenes. Similar PAC compositions have also been measured in the tissues of other bivalves exposed to WAFs of different crude oils (Al-Saad et al., 2011; Ito et al., 2015; Li et al., 2021; Onozato et al., 2016; Oros & Ross, 2005). The higher accumulation of LMW PACs in tissues is likely due to their greater solubility in water, which makes them more bioavailable for uptake than HMW hydrocarbons (French-Mccay et al., 2019; Hodson et al., 2019; Li et al., 2021; Loh et al., 2018; Mackay & Mcauliffe, 1989). Additionally, while lower concentrations of HMW PACs are found in petroleum WAFs, their bioavailable components tend to accumulate more in bivalve tissues due to their high K_{OW} values and lipophilicity (Baussant et al., 2001; Ito et al., 2015; Uno et al., 2009).

Numerous reports of TPAC concentrations in bivalve tissues following oil exposures are similar to those found after the oyster exposures to CO (29 µg/g), MD (4 µg/g), and DB (3 µg/g). For example, after a 24 h crude oil WAF exposure, average TPAC concentrations of 35 µg/g were measured in *C. gigas* tissues (Loh et al., 2018). Similarly, TPAC concentrations in *C. gigas* tissues after the Wu Yi San crude oil spill (China) over 60 d were 0.52-110 µg/g (Loh et al., 2017), and during the Hebei Spirit spill (South Korea) were 8-100 µg/g (Yim et al., 2012). The background TPAC concentrations detected in *C. gigas* tissues found in the San Francisco Bay were 0.2–6.9 µg/g (Oros & Ross, 2005) and the coastal areas in China were 0.05 µg/g (Li et al., 2021). TPAC concentrations measured in other bivalve species included 0.1–25 µg/g in *C. virginica* tissue after the Apex barge spill (United States) of No. 5 Fuel Oil over 110 d (Wade et al., 1993) and 4.7-234 µg/g in the *M. trossulus* tissue following the Exxon Valdez spill (Neff & Burns, 1996; Short & Babcock, 1996). These studies suggest that the TPAC concentrations detected in oyster tissues in our study mirrored the accumulation patterns of PACs in bivalve tissues during historical oil spills in a marine environment. Moreover, the accumulation pattern of PACs in bivalve tissues can be influenced by the type, extent, and duration of the oil exposure (Ito et al., 2015; Mason, 1988).

During the depuration period, TPAC concentrations in oyster tissues declined. Between d 7 and 21, there was a rapid loss of accumulated hydrocarbons, followed by a slower reduction from d 21 to 35. Similar depuration rate patterns have been found in other studies, where most of the hydrocarbons are eliminated from bivalve tissues within two weeks of the exposure period (Al-Saad et al., 2011; Baussant et al., 2001; Li et al., 2021; Mason, 1988; Schmutz, 2018). These results suggest that Pacific oysters can potentially biotransform parent PACs, which allows for easier elimination of the contaminant metabolites through diffusion or excretion (Baussant et al., 2001; Ertl et al., 2016; Gan et al., 2021; Kim et al., 2007; Loh et al., 2018). Additionally, during the depuration period, the proportions of LMW PACs decreased in oyster tissues, while the HMW hydrocarbons increased for all three petroleum types. This indicates that the biotransformation of different PACs could be based on their molecular weight and structure, which would influence the depuration rates of their metabolites from oyster tissues.

Scope for growth

Scope for growth (SFG) is a quantitative measure of contaminant-induced stress to determine the overall growth potential of oysters using a balanced energy equation (Widdows & Staff, 2006), which is calculated based on the energy difference expended through oxygen consumption (MO_2) and absorbed through acquisition (clearance rate; CR) and assimilation (absorption efficiency; AE) of organic matter from food.

The CR of algae in Pacific oysters was measured by assessing the amount of seawater cleared of suspended *Isochrysis galbana* cells as a function of time. CR values for control oysters in the current study (2.94-20.7 L/h/g) fell within the range (0.002-5.02 L/h/g) measured for control bivalves in other studies (Guzman-Aguero et al., 2012; Hutchinson & Hawkins, 1992; Jeong & Cho, 2007; Widdows et al., 1995; Widdows & Staff; 2006). In our study, WAF exposures did not impact oyster CR for any oil type. These results are contradictory to several studies which showed a negative correlation between CR values and hydrocarbon accumulation in bivalves (Guzman-Aguero et al., 2013; Hutchinson & Hawkins, 1992; Jeong & Cho, 2007; Kim et al., 2007; Schmutz, 2018; Widdows & Johnson, 1988; Widdows et al., 1982, 1985, 2002). Other studies found that reduced CR could be attributed to oyster shell closure as a biological response to minimize the intrusion of PACs during petroleum exposures, which can cause variation in their gill ciliary action to procure food particles due to narcotic effects (Donkin et al., 1989; Jeong & Cho, 2007; Kim et al., 2007; Schmutz, 2018). However, the absence of alterations in the current CR measurements could be a result of the short sub-chronic exposure duration, which did not allow high bioavailability of HMW PACs to be accumulated in oysters, which could impact their CR measurements.

AE measured the efficiency of assimilating organic matter from consumed food. The AE values for control oysters in the current study ranged between 0.47-0.81, which was similar to the range (0.24-0.59) reported for *C. gigas* and *M. edulis* (Guzman-Aguero et al., 2012; Hutchinson & Hawkins, 1992; Jeong & Cho, 2007; Kim et al., 2007; Widdows et al., 1995; Widdows & Staff, 2006). While no impacts on AE values were found in the present study, other studies have found a reduction in AE with increasing PAC concentrations (Jeong & Cho, 2007; Redmond et al., 2016; Toro et al., 2003). The decrease in AE values has been attributed to the impairment of oyster digestive gland pathology and stability of their lysosomal membranes due to petroleum exposure (Jeong

& Cho, 2007; Lowe & Clarke, 1989; Schmutz, 2018; Toro et al., 2003; Wang et al., 2005). In the current study, however, it is possible that the accumulation of PAC concentrations in oyster tissues after the three oil exposures was not high enough to induce such alterations, which would elucidate the absence of variation in AE values across the different treatment groups and sampling times.

MO_2 was used to estimate the amount of energy expended through respiration in a static respirometer. The MO_2 values for control oysters in this study ranged from 10.86-21.85 µmol O_2/h/g, which fell within the range (7.19-53.75 µmol O_2/h/g) reported for control *C. gigas* and *M. edulis* (Guzman-Aguero et al., 2012; Jeong & Cho, 2007; Widdows et al., 1995; Widdows & Staff; 2006). Oyster MO_2 values were not impacted by WAF exposure, which was contradictory to the findings of previous studies. Several studies have shown that bivalve MO_2 values can be reduced with increasing hydrocarbon body burdens (Hutchinson & Hawkins, 1992; Kim et al., 2007; Schmutz, 2018), while others have found higher MO_2 values with exposures (Jeong & Cho, 2007; Toro et al., 2003). It has been suggested that reduced MO_2 rates could be due to the narcotic impact of PACs on the ciliary activity of their gill epithelium (Donkin et al., 1989; Hutchinson & Hawkins, 1992; Schmutz, 2018), while others have suggested an increase in oxygen consumption is due to the biotransformation and elimination of PACs (Jeong & Cho, 2007; Stekoll et al., 1980; Widdows et al., 1982). While no variations were observed on oyster MO_2 in this study, it could be attributed to the short exposure duration for all three oils that did not allow HMW PACs to accumulate in high concentrations within oyster tissues due to their potentially lower bioavailability. Moreover, the concentrations of LMW PACs accumulated on d 7 for all three petroleum exposures may not have been elevated enough to induce or inhibit oxygen consumption in oysters.

SFG has been suggested as a sensitive indicator of petroleum exposure, which can be influenced by the exposure duration and contaminant concentration (Jeong & Cho, 2007; Kim et al., 2007; Schmutz, 2018; Toro et al., 2003; Widdows & Johnson, 1988; Widdows et al., 2002). In the current study, SFG values for control oysters were between 8.08-149.90 J/h/g, which were similar to literature values for control *C. gigas* and *M. edulis* that ranged from 43.63-163 J/h/g (Guzman-Aguero et al., 2012; Hutchinson & Hawkins, 1992; Jeong & Cho, 2007; Widdows et al., 1995; Widdows & Staff; 2006). Other studies have found decreased SFG values with increasing PAC concentrations in bivalve tissues (Toro et al., 2003; Widdows et al., 1987, 2002; Widdows & Johnson, 1988). However, in

the current study, oyster SFG values were not impacted by exposure to the three petroleum WAFs. Since SFG is largely dependent on the loading concentrations used during oil WAF studies, the PAC concentrations used in this study may not have been substantial enough to cause significant physiological changes to the overall oyster SFG, which uses the preserved energy for combating contamination related stress (Bayen et al., 2007; Kim et al., 2007; Schmutz, 2018). Oysters also tend to adapt to varying PAC concentrations, where they can maintain their metabolic rates at low exposure levels (Jeong & Cho, 2007). Moreover, adult bivalves have been known to show resilience to short petroleum exposures (Schmutz, 2018), which could potentially explain the absence of effects observed on oyster physiological responses after the 7 d petroleum exposures.

Condition index

Condition index (CI) is a physiological parameter for addressing potential alterations in the health status, growth, tissue yield, and sexual maturity of bivalves, due to the presence of environmental stressors (Lundebye et al., 1997; Sasikumar & Krishnakumar, 2011; Zeng & Yang, 2020). CI can be utilized for determining the efficiency of tissue growth in oysters exposed to contaminants and their ability to utilize shell space (Lawrence & Scott, 1982). The control CI values in this study ranged from 0.9-1.78 g/cm^3. Exposing oysters to the WAFs of three petroleum products did not affect their CI. As different methodologies are used for calculating bivalve CI given their non-uniform shell morphology and varied thickness, making comparisons with previous studies was not possible (Zeng & Yang, 2020). This could potentially be a result of the short 7 d exposure where minimal time had passed for the oils to cause measurable changes to the tissue or shell morphometrics. Consequently, additional studies with longer exposure periods might be required to observe the long-term impacts of petroleum products on bivalve morphology and CI values.

Health assessment index

Health assessment index (HAI) is a field necropsy method that assigns a numerical value to various oyster tissues and organs to assess their health, based on the severity of damage incurred due to environmental stressors, such as petroleum products (Adams et al., 1993; Goede & Barton, 1990). This visual inspection of gross changes occurring in the oyster mantle, digestive gland, gill, adductor muscle, and gonadal tissue can provide a

rapid and cost-effective assessment of their health (Adams et al., 1993). HAI values for control oysters in the current study ranged from 0.00-2.25. As there is limited literature available for bivalve HAI values, making comparisons with the current study was challenging. The HAI score analysis revealed no WAF-dependent differences in oysters or time-related effects. This could be a result of the short exposure duration, where the PACs did not alter the visual health of oyster tissues and organs. However, longer exposures might induce adverse impacts on the oyster HAI, which could be observed with chronic studies.

Gonad histopathology

One tool for assessing the impact of petroleum exposure on bivalve reproductive health is gonad histopathology (Aarab et al., 2011; Smolarz et al., 2017; Schmutz, 2018). This can be achieved by measuring their gonadal structures to ascertain differences in their gametogenesis and development (Aarab et al., 2011; Schmutz, 2018). Robust gonads are an indicator of a successful gametogenic cycle since oysters have a higher success rate for spawning and continuing their progeny (Joseph & Madhystha, 1982; Kang et al., 2010). In the present study, the gonadal thickness of control oysters was normalized to their wet weight and had values between 291.5-910.2 µm/g. Given the limited literature available for these values, making comparisons with the current study was not possible. Oyster gonadal thickness in this study was not influenced by all three WAF exposures. These results could potentially be due to the short exposure periods and lower accumulated PAC concentrations in their tissues, which might have prevented alterations in their gonadal thickness. Oyster gonads also have high lipid content (Wang et al., 2020) that can reduce the rate of elimination due to increased accumulation of PACs, which might take longer to elicit changes in oyster gonadal tissue.

Digestive gland histopathology

Another tool for assessing ecotoxicity due to petroleum exposure is the digestive gland histopathology of bivalves (Aarab et al., 2011; Smolarz et al., 2017; Schmutz, 2018). This can be achieved by observing and measuring the somatic tissue structures, including the digestive lumen and tubules to assess the impact of oil products on oyster metabolism and energy utilization (Schmutz, 2018). The ratio of these measurements provides information regarding oyster health, where thinner digestive lumen to tubule proportions

would indicate greater PAC-related stress due to overt utilization of stored glycogen resources (Aarab et al., 2011; Bignell et al., 2011; Kang et al., 2010; Peterio et al., 2007; Smolders et al., 2004). In this study, the digestive tubule histopathology ratios for control oysters ranged from 0.29-0.36. As limited studies have been performed for such measurements, making comparisons with the present study was challenging. However, histopathological analysis of digestive tubule to lumen (epithelial tissue) thickness revealed no significant changes in their digestive gland anatomy due to all three WAF exposures. This could be a result of the short exposure duration where not enough time had passed to drastically affect the inner epithelium layer of digestive glands. Hence, additional chronic studies might be required to assess the long-term impacts of petroleum exposure on such tissues, along with quantifying the time associated to regain used glycogen resources and rejuvenate the epithelium tissue lining.

Larval development

The developmental success of oyster larvae was selected as the criteria for determining the impact of petroleum WAFs on oyster reproduction. The proportion of developmental success for control oysters ranged from 0.36-0.43. Given the limited literature available for these values, comparisons with the current study could not be performed. After conducting the WAF exposures, no significant variations in the developmental success of oyster larvae were observed. There are a few factors that might have influenced these results. Firstly, high levels of pCO_2 could be present in the potentially hypoxic seawater used for exposures, which would cause a delay in gamete fertilization and larval development (Nordio et al., 2020; Parker et al., 2012; Ventura et al., 2016; Waldbusser et al., 2015). Since most larvae developed to the trochophore stage, where the velum had started to form, they might have required additional time and small quantities of food to help develop further (Parker et al., 2012; Ventura et al., 2016; Waldbusser et al., 2013; Widdows, 1991). However, since 48 h is an acceptable period for oyster larvae to reach the D-veliger stage given the mineral precipitation of calcium carbonate from 90% of the total larval mass (His & Maurer, 1988; Song et al., 2009; Waldbusser et al., 2013), the delayed development of oyster larvae could have been induced by other factors. These could include the low fertilization success rate due to reduced sperm motility, oocyte density, seawater volume, gamete contact time, incubation

temperature, as well as the spermatozoa-to-oocyte ratio present during the exposure (Jeong & Cho, 2005; Parker et al., 2012; Rico-Villa et al., 2009; Song et al., 2009).

Conclusion

This study assessed the effects of MD, CO, and DB WAFs on several aspects of the physiology, morphology, histopathology, and reproduction of Pacific oysters. During the exposure, CO WAFs released the highest TPAC concentrations, followed by MD and DB, which accumulated within oyster tissues in the same order. This suggested that CO spills can have a longer presence in a marine environment since their volatilization rate is not as rapid as lighter fuel oils like MD while having a higher dissolution rate compared to heavier petroleum such as DB. This can result in greater spreading of crude oil during spills while impacting the remediation strategies required to mitigate them. Moreover, after being placed in uncontaminated seawater there was a rapid decline in the oyster tissue PAC concentrations, potentially due to the biotransformation of parent hydrocarbons and elimination of their metabolites. These results were antithetical to other studies that found a slower biotransformation rate of PACs in bivalves. Although oysters have been previously used as a biomonitor species due to their ability to accumulate hydrocarbons for long periods, the current results recommend an immediate tissue sampling after being extracted from a polluted environment. This would provide environmentally realistic petroleum concentrations and be representative of bivalve responses after an oil spill.

In the present study, short-term petroleum exposures to the three petroleum products did not alter the measured endpoints in Pacific oysters. Although this species accumulated PACs initially, no adverse impacts of oil were found. This might not hold true for all bivalve species since many studies have shown the detrimental impacts of petroleum products. Consequently, in future studies, longer WAF exposure experiments with other bivalve species might be required to evaluate the ecotoxicological impacts of petroleum spills since continuous exposures to varying concentrations of PACs could potentially have detrimental impacts on their survival, growth, and reproduction. These findings can help guide targeted conservation and remediation efforts through understanding the impact of oil spills on multiple bivalve species, as well as developing appropriate monitoring and management plans to protect and restore marine habitats following petroleum spills of varying lengths under different conditions.

2.6. References

Aarab, N., Godal, B. F., & Bechmann, R. K. (2011). Seasonal Variation of Histopathological and Histochemical Markers of PAH Exposure in Blue Mussel (*Mytilus Edulis* L.). *Marine Environmental Research, 71*(3), 213–17.

Adams, S. M., Brown, A. M., & Goede, R. W. (1993). A quantitative health assessment index for rapid evaluation of fish condition in the field. *Transactions of the American Fisheries Society*, *122*(1), 63–73. https://doi.org/10.1577/1548-8659(1993)122<0063:aqhaif>2.3.co;2

Al-Saad, H. T., Farid, W. A., & Al-Adhub, A. Y. (2011). Uptake and depuration of water-soluble fractions (WSF) of crude oil by the bivalve corbicula fluminea (Müller) from Shatt al-Arab river. *Mesopotamian Journal of Marine Sciences*, *26*(2), 134–145. https://doi.org/10.58629/mjms.v26i2.180

Barillé, L., Haure, J., Pales-Espinosa, E., & Morancais, M. (2003). Finding new diatoms for intensive rearing of the Pacific oyster (*Crassostrea gigas*): energy budget as a selective tool. *Aquaculture, 217*, 501–514.

Baussant, T., Sanni, S., Jonsson, G., Skadsheim, A., & Børseth, J. F. (2001). Bioaccumulation of polycyclic aromatic compounds: 1. Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil. *Environmental Toxicology and Chemistry, 20*(6), 1175-1184. doi:10.1002/etc.5620200606

Bayen, S., Lee, H. K., & Obbard, J. P. (2007). Exposure and response of aquacultured oysters, *Crassostrea gigas*, to marine contaminants. *Environmental Research, 103*, 375-382. doi:10.1016/j.envres.2006.06.012

Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U., & Egli, T. (2007). Assessment and Interpretation of Bacterial Viability by Using the LIVE/DEAD BacLight Kit in Combination with Flow Cytometry. *Applied and Environmental Microbiology, 73*(10), 3283–3290.

Bignell, J. P., Stentiford, G. D., Taylor, N. G. H., & Lyons, B. P. (2011). Histopathology of Mussels (*Mytilus* Sp.) from the Tamar Estuary, UK. *Marine Environmental Research, 72*(1–2), 25–32.

Bilbao, D., De Miguel-Jiménez, L., Igartua, A., Olivares, M., Izagirre, U., Prieto, A., & Etxebarria, N. (2022). Chemical characterization of oil and water accommodated fraction (WAF) at different temperatures. *Results in Engineering*, *14*, 100433. https://doi.org/10.1016/j.rineng.2022.100433

Blatná, D. (2006). *Outliers in Regression*. Statistika. Retrieved April 27, 2023, from https://statistika.vse.cz/konference/amse/PDF/Blatna.pdf

Bodoy, A., Prou, J., & Berthome, J.-P. (1986). Etude comparative de différents indices de condition chez l'huître creuse (*Crassostrea gigas*). *Haliotis*, *15*, 173–182.

Boehm, P. D., Cook, L. L., Murray, K. J., & Royer, L. (2011). Distribution and fate of PAH and Chemical Dispersants in the Water Column Following the Deepwater Horizon OIl Spill. In *Society of Environmental Toxicology and Chemistry Conference, (poster)*: Boston, MA.

Bougrier, S., Geairon, P., Deslous-Paoli, J. M., Bacher, C., & Jonquières, G. (1995). Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas* (Thunberg). *Aquaculture*, *134*(1–2), 143–154. https://doi.org/10.1016/0044-8486(95)00036-2

Callot, H. J., & Ocampo, R. (2000). Geochemistry of porphyrins. In: Kadish K.M, Smith, K.M., Guilard, B, eds. The porphyrin handbook Vol 1, Academic, San Diego, CA USA. 349-398.

CAPP. (2023). Canadian Association of Petroleum Producers (CAPP) Statistics: Statistical Handbook [WWW Document].

Cassis, D., Pearce, C. M., & Maldonado, M. T. (2011). Effects of the environment and culture depth on growth and mortality in juvenile Pacific oysters in the Strait of Georgia, British Columbia. *Aquaculture Environment Interactions, 1*, 259-274. doi:10.3354/aei00025

CER. (2022). *Crude Oil Pipe Transportation System*. Canada Energy Regulator. Retrieved December 15, 2022, from https://www.cer-rec.gc.ca/en/data-analysis/facilities-we-regulate/canadas-pipeline-system/2021/crude-oil-pipeline-transportation-system.html

Conover, R. J. (1966). Assimilation of Organic Matter by Zooplankton. *Limnology and Oceanography, 11*(3), 338-345. doi:10.4319/lo.1966.11.3.0338

Coughlan, J. (1969). The estimation of filtering rate from the clearance of suspensions. *Marine Biology, 2*(4), 356-358. doi:10.1007/bf00355716

Couillard, C. M., Lee, K., Légaré, B., & King, T. L. (2005). Effect of Dispersant on the Composition of the Water- Accommodated Fraction of Crude Oil and its Toxicity to Larval Marine Fish. *Environmental Toxicology and Chemistry*, *24*(6), 1496. https://doi.org/10.1897/04-267R.1

Cripps, G., & Shears, J. (1997). The fate in the marine environment of a minor diesel fuel spill from an Arctic research station. *Environmental Monitoring and Assessment, 46*, 221-232.

Crude Oil. (2023). *Government of Canada*. Canadian Centre for Energy Information. Retrieved April 19, 2023, from https://energy-information.canada.ca/en/subjects/crude-oil

Donkin, P., Widdows, J., Evans, S. V., Worrall, C. M., & Carr, M. (1989). Quantitative Structure Activity Relationships for the Effect of Hydrophobic Organic Chemicals on Rate of Feeding by Mussels (*Mytilus edulis*). *Aquatic Toxicology, 14*(3), 277–293.

Environment Canada. (2021). *Environment canada crude oil and petroleum product database - individual report ECCC petroleum products database 2021-01-22*. Open Government Portal. Retrieved April 19, 2023, from https://open.canada.ca/data/en/dataset/53c38f91-35c8-49a6-a437-b311703db8c5/resource/d1ecec5b-f577-4edc-8f1d-57384742c391?inner_span=True

EPA. (2003). *Characteristics of Spilled Oils, Fuels, and Petroleum Products: 1. Composition and Properties of Selected Oils*. Emergencies Science and Technology Division. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1000AE6.PDF?Dockey=P1000AE6.PDF

Ertl, N. G., O'Connor, W. A., Brooks, P., Keats, M., & Elizur, A. (2016). Combined exposure to pyrene and fluoranthene and their molecular effects on the Sydney rock oyster, *Saccostrea glomerata*. *Aquatic Toxicology, 177*, 136–145. doi: 10.1016/j. aquatox.2016.05.012

Faksness, L.-G., Brandvik, P. J., & Sydnes, L. K. (2008). Composition of the water accommodated fractions as a function of exposure times and temperatures. *Marine Pollution Bulletin*, *56*(10), 1746–1754. https://doi.org/10.1016/j.marpolbul.2008.07.001

Federal Government. (2013). *Properties, Composition and Marine Spill Behaviour, Fate and Transport of Two Diluted Bitumen Products from the Canadian Oil Sands . Federal Government Technical Report* . Retrieved April 20, 2023, from https://publications.gc.ca/collections/collection_2014/ec/En84-96-2013-eng.pdf

Fisheries and Oceans Canada. (2023). *Government of Canada*. Pacific oyster (Crassostrea gigas), 2023/2024 | Pacific Region | Fisheries and Oceans Canada. Retrieved April 20, 2023, from https://www.pac.dfo-mpo.gc.ca/fm-gp/mplans/oyster-huitre-ifmp-pgip-sm-eng.html#econ

Forth, H. P., Mitchelmore, C. L., Morris, J. M., & Lipton, J. (2017). Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the *Deepwater Horizon* oil spill natural resource damage assessment: Characterization of oil and water accommodated fractions. *Environmental Toxicology and Chemistry*, *36*(6), 1450–1459. https://doi.org/10.1002/etc.3672

French-Mccay, D. P. (2004). Oil Spill Impact Modeling: Development and Validation. *Environmental Toxicology and Chemistry, 23*(10), 2441-2456. doi:10.1897/03-382

French-McCay, D., Crowley, D., & McStay, L. (2019). Sensitivity of modeled oil fate and exposure from a subsea blowout to oil droplet sizes, depth, dispersant use, and degradation rates. *Marine Pollution Bulletin*, *146*, 779–793. https://doi.org/10.1016/j.marpolbul.2019.07.038

Gan, N., Martin, L., & Xu, W. (2021). Impact of Polycyclic Aromatic Hydrocarbon Accumulation on Oyster Health. *Frontiers in Physiology*, *12*, 734463. https://doi.org/10.3389/fphys.2021.734463

Gerdes, D. (1983). The Pacific oyster *Crassostrea gigas* Part 1. Feeding Behaviour of Larvae and Adults. *Aquaculture, 31*, 195-219.

Gillespie, G. E., Bower, S. A., Marcus, K. L., & Kieser, D. (2012). Biological synopsises for three exotic mollusks, Manila Clam (*Venerupis philippinarum*), Pacific Oyster (*Crassostrea gigas*) and Japanese Scallop (*Mizuhopecten yessoensis*) licensed for Aquaculture in British Columbia. *DFO Canadian Science Advisory Secretariat Research Document*, 2012/013. v + 97p.

Gnaiger, E. (1983). Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. *Journal of Experimental Zoology, 228*, 471–490.

González, J. J., Viñas, L., Franco, M. A., Fumega, J., Soriano, J. A., Grueiro, G., Muniategui, S., López-Mahía, P., Prada, D., & Bayona, J. M. (2006). Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. *Marine Pollution Bulletine, 53* (5–7), 250–259.

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. *Culture of Marine Invertebrate Animals*, 29–60. https://doi.org/10.1007/978-1-4615-8714-9_3

Guillard, R. R., & Ryther, J. H. (1962). Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (Cleve) Gran. *Canadian Journal of Microbiology*, *8*(2), 229–239. https://doi.org/10.1139/m62-029

Guzman-Aguero, J. E., Nieves-Soto, M., Hurtado, M. Á., Piña-Valdez, P., & Garza-Aguirre, M. D. C. (2013). Feeding physiology and scope for growth of the oyster Crassostrea corteziensis (Hertlein, 1951) acclimated to different conditions of temperature and salinity. *Aquaculture International*, *21*(2), 283–297. https://doi.org/10.1007/s10499-012-9550-4

Hartwell, S. I., Wright, D. A., Takacs, R., & Hocutt, C. H. (1991) Relative respiration and feeding rates of oyster and brackish clam in variously contaminated waters. *Marine Pollution Bulletin, 22*, 191-197.

His, E., & Maurer, D. (1988). Shell growth and gross biochemical composition of oyster larvae (*Crassostrea gigas*) in the field. *Aquaculture*, *69*(1–2), 185–194. https://doi.org/10.1016/0044-8486(88)90195-0

Hodson, P. V., Adams, J., & Brown, R. S. (2019). Oil toxicity test methods must be improved. *Environmental Toxicology and Chemistry, 38*(2), 302-311. doi:10.1002/etc.4303

Hong, W. J., Jia, H., Li, Y. F., Sun, Y., Liu, X., & Wang, L. (2016). Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the coastal seawater, surface sediment and oyster from Dalian, Northeast China. *Ecotoxicology and Environmental Safety*, *128*, 11–20. https://doi.org/10.1016/j.ecoenv.2016.02.003

Howard, D. W., Lewis, E. J., Keller, B. J., & Smith, C. S. (2004). Histological techniques for marine bivalve mollusks and crustaceans. *NOAA Technical Memorandum NOS NCCOS, 5*, 218 pp.

Hutchinson, S., & Hawkins, L. E. (1992). Quantification of the Physiological Responses of the European Flat Oyster *Ostrea edulis* L. to Temperature and Salinity. *Journal of Molluscan Studies*, *58*(2), 215–226. https://doi.org/10.1093/mollus/58.2.215

Ito, K., Uno, S., Tanaka, H., Miki, S., Kokushi, E., Yamamoto, M., Koyama, J., & Añasco, N. C. (2015). Distribution of Parent and Alkylated PAHs in Bivalves Collected from Osaka Bay, Japan. *Japanese Journal of Environmental Toxicology*, *18*(1), 11–24.

ITOPF. (2021). *TIP 02: Fate of marine oil spills*. International Tanker Owners Pollution Federation Limited. Retrieved November 23, 2022, from https://www.itopf.org/knowledge-resources/documents-guides/tip-02-fate-of-marine-oil-spills/

Jeong, W. G., & Cho, S. M. (2007). Long-Term Effect of Polycyclic Aromatic Hydrocarbon on Physiological Metabolisms of the Pacific Oyster, *Crassostrea Gigas*. *Aquaculture, 265*(1–4), 343–50.

Joseph, M., & Madhystha, M. N. (1982). Gametogenesis and somatic versus gonadal growth in the oyster *Crassostrea madrasensis* (Preston). *Indian Journal of Marine Sciences*, *11*, 303–310.

Joshy, A., Sharma, S. R. K., Mini, K. G., Gangadharan, S., & Pranav, P. (2022). Histopathological evaluation of bivalves from the southwest coast of India as an indicator of Environmental Quality. *Aquatic Toxicology*, *243*, 106076. https://doi.org/10.1016/j.aquatox.2022.106076

Kang, D.-H., Chu, F.-L. E., Yang, H.-S., Lee, C.-H., Koh, H.-B., & Choi, K.-S. (2010). Growth, Reproductive Condition, and Digestive Tubule Atrophy of Pacific Oyster *Crassostrea gigas* in Gamakman Bay off the Southern Coast of Korea. *Journal of Shellfish Research*, *29*(4), 839–845. https://doi.org/10.2983/035.029.0418

Kesarcodi-Watson, A., Klumpp, D. W., & Lucas, J. S. (2001) Comparative feeding and physiological energetics of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* II. Influences of food concentration and tissue energy distribution. *Aquaculture, 203*, 195-216.

Kim, M., Cho, S., & Jeong, W. (2007). Short-Term Physiological Response of the Pacific Oyster, *Crassostrea Gigas*, on Exposure to Varying Levels of Polycyclic Aromatic Hydrocarbon. *Aquaculture Research, 38*(15), 1612–1618. https://doi.org/10.1111/j.1365-2109.2007.01821.x

King, T. L., Robinson, B., Cui, F., Boufadel, M., Lee, K., & Clyburne, J. A. (2017). An oil spill decision matrix in response to surface spills of various bitumen blends. *Environmental Science: Processes & Impacts*, *19*(7), 928–938. https://doi.org/10.1039/c7em00118e

Lafont, M., Goncalves, P., Guo, X., Montagnani, C., Raftos, D., & Green, T. (2019). Transgenerational plasticity and antiviral immunity in the Pacific oyster (*Crassostrea gigas*) against Ostreid herpesvirus 1 (OsHV-1). *Developmental & Comparative Immunology, 91*, 17-25. doi:10.1016/j.dci.2018.09.022

Langdon, C. J., Stefansson, E. S., Pargee, S. M., Blunt, S. M., Gage, S. J., & Stubblefield, W. A. (2016). Chronic effects of non-weathered and weathered crude oil and dispersant associated with the Deepwater horizon incident on development of larvae of the eastern oyster, *crassostrea virginica*. *Environmental Toxicology and Chemistry*, *35*(8), 2029–2040. https://doi.org/10.1002/etc.3352

Law, R. J., Kelly, C. A., & Nicholson, M. D. (1999). Polycyclic Aromatic Hydrocarbons (PAH) in Shellfish Affected by the *Sea Empress* Oil Spill in Wales in 1996. *Polycyclic Aromatic Compounds*, *17*(1–4), 229–239. https://doi.org/10.1080/10406639908020618

Lawrence, D.R., & Scott, G.I. (1982). The determination and use of condition index of oysters. *Estuaries, 5*, 23-27.

Lee, K., Boufadel, M., Chen, B., Foght, J., Hodson, P., Swanson, S., & Venosa, A. (2015). The Royal Society of Canada Expert Panel: The behaviour and environmental impacts of crude oil released into aqueous environments. Royal Society of Canada, Ottawa, ON. ISBN: 978-1- 928140-02-3

Li, L., Li, Z., Xv, G., Wang, C., & Jiang, M. (2021). *Accumulation of PAH in Bivalves (Crassostrea Gigas Andmytilus Coruscus) from Zhejiang Coastal, China, and Associated Human Health Risk Assessment*. https://doi.org/10.21203/rs.3.rs-189664/v1

Liu, Z., Liu, J., Gardner, W. S., Shank, G. C., & Ostrom, N. E. (2014). The impact of Deepwater Horizon oil spill on petroleum hydrocarbons in surface waters of the northern Gulf of Mexico. *Deep Sea Research Part II: Topical Studies in Oceanography*, *129*, 292–300. https://doi.org/10.1016/j.dsr2.2014.01.013

Loh, A., Yim, U. H., Ha, S. Y., An, J. G., & Kim, M. (2017). Contamination and Human Health Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Oysters After the Wu Yi San Oil Spill in Korea. *Archives of Environmental Contamination and Toxicology*, *73*(1), 103–117. https://doi.org/10.1007/s00244-017-0394-9

Loh, A., Yim, U. H., Ha, S. Y., & An, J. G. (2018). A preliminary study on the role of suspended particulate matter in the bioavailability of oil-derived polycyclic aromatic hydrocarbons to oysters. *Science of The Total Environment*, *643*, 1084–1090. https://doi.org/10.1016/j.scitotenv.2018.06.129

Lowe, D. M., & Clarke, K. R. (1989). Contaminant-Induced Changes in the Structure of the Digestive Epithelium of *Mytilus edulis*. *Aquatic Toxicology, 15*(4), 345–358.

Luna-Acosta, A., Kanan, R., Le Floch, S., Huet, V., Pineau, P., Bustamante, P., & Thomas-Guyon, H. (2011). Enhanced immunological and detoxification responses in Pacific oysters, *Crassostrea gigas*, exposed to chemically dispersed oil. *Water Research*, *45*(14), 4103–4118. https://doi.org/10.1016/j.watres.2011.05.011

Lundebye, A. K., Langston, W., & Depledge, M. (1997). Stress proteins and condition index as biomarkers of tributyltin exposure and effect in mussels. *Ecotoxicology, 6*, 127–136. https://doi.org/10.1023/A:1018662324296

Mackay, D., & Mcauliffe, C. D. (1989). Fate of hydrocarbons discharged at sea. *Oil and Chemical Pollution, 5*, 1-20. doi:10.1016/s0269-8579(89)80002-4

Mackay, D., Shiu, W. Y., Chais, A., Southwood, J., & Johnson, C. I. (1985). Environmental fate of diesel fuel spills on land. Report of Department of chemical Engineering and Applied Chemistry of University of Toronto for Association of American Railroads, Washington, DC.

Madison B. N., Hodson P. V., & Langlois V. S. (2017). Cold Lake Blend diluted bitumen toxicity to the early development of Japanese medaka. *Environmental Pollution*, *225*, 579–586.

Mao, Y., Zhou, Y., Yang, H., & Wang, R., (2006). Seasonal variation in metabolism of cultured Pacific oyster, *Crassostrea gigas*, in Sanggou Bay, China. *Aquaculture, 253* (1–4), 322–333.

Marcus, N. H., & Wilcox, J. A. (2007). *Guide to the Meso-scale production of the copepod acartia tonsa*. Aquatic Commons. https://core.ac.uk/reader/11017950

Mason, R. P. (1988). Accumulation and depuration of petroleum hydrocarbons by black mussels. 1. Laboratory Exposure Trials. *South African Journal of Marine Science*, *6*(1), 143–153. https://doi.org/10.2989/025776188784480582

Mondol, M. R., Keshavmurthy, S., Lee, H., Hong, H., Park, H., Park, S., . . . & Choi, K. (2015). Recovery of wild Pacific oyster, *Crassostrea gigas* in terms of reproduction and gametogenesis two-years after the Hebei Spirit Oil Spill Accident off the West Coast of Korea. *Continental Shelf Research, 111*, 333-341. doi:10.1016/j.csr.2015.08.019

Moore, M. (1987). Molecular, cellular, and physiological effects of oil-derived hydrocarbons on molluscs and their use in impact assessment. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *316*(1181), 603–623. https://doi.org/10.1098/rstb.1987.0041

Moore, M. (1988). Cytochemical Responses of the Lysosomal System and NADPH-Ferrihemoprotein Reductase in Molluscan Digestive Cells to Environmental and Experimental Exposure to Xenobiotics. *Marine Ecology Progress Series, 46*, 81–89.

Neff, J. M., Ostazeski, S., Gardiner, W., & Stejskal, I. (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to Marine Animals. *Environmental Toxicology and Chemistry*, *19*(7), 1809–1821. https://doi.org/10.1002/etc.5620190715

Neff, J. M., & Burns, W. A. (1996). Estimation of polycyclic aromatic hydrocarbon concentrations in the water column based on tissue residues in mussels and salmon: An equilibrium partitioning approach. *Environmental Toxicology and Chemistry, 15* (12), 2240−2253.

Newell, R. I. E. (2004). Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research*, *23*(1), 51+.

NRC. (2022). *Energy Fact Book 2021-2022*. Natural Resources Canada, 146pp.

Noakes, D. J. (2018). Oceans of opportunity: A review of Canadian aquaculture. *Marine Economics and Management*, *1*(1), 43–54. https://doi.org/10.1108/maem-06-2018-002

Nordio, D., Khtikian, N., Andrews, S., Bertotto, D., Leask, K., & Green, T. (2020). Adaption potential of *Crassostrea gigas* to ocean acidification and disease caused by Vibrio harveyi. *ICES Journal of Marine Science,* 1-8. doi:10.1093/icesjms/fsaa080

Onozato, M., Nishigaki, A., & Okoshi, K. (2016). Polycyclic aromatic hydrocarbons in sediments and bivalves on the Pacific coast of Japan: Influence of tsunami and fire. *PLOS ONE*, *11*(5). https://doi.org/10.1371/journal.pone.0156447

Onwurah, I. N., Ogugua, V., Onyike, N., Ochonogor, A., & Otitoju, O. (2007). Crude Oils Spills in the Environment, Effects and Some Innovative Clean Up Biotechnologies. *International Journal of Environmental Research, 1*(4), 307-320.

Orban, E., Lena, G. D., Masci, M., Nevigato, T., Casini, I., Caproni, R., . . . & Pellizzato, M. (2004). Growth, nutritional quality, and safety of oysters(Crassostrea gigas) cultured in the lagoon of Venice (Italy). *Journal of the Science of Food and Agriculture, 84*, 1929-1938. doi:10.1002/jsfa.1896

Oros, D. R., & Ross, J. R. M. (2005). Polycyclic aromatic hydrocarbons in bivalves from the San Francisco estuary: Spatial Distributions, temporal trends, and sources (1993–2001). *Marine Environmental Research*, *60*(4), 466–488. https://doi.org/10.1016/j.marenvres.2005.02.001

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., & Pörtner, H. O. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, *18*(1), 82–92. https://doi.org/10.1111/j.1365-2486.2011.02520.x

Patel, B., & Eapen, J. T. (1989). Biochemical evaluation of naphthalene intoxication in the tropical acrid blood clam *Anadara granosa*. *Marine Biology, 103*, 203–209.

Pelletier, M. C., Burgess, R. M., Cantwell, M. G., Serbst, J. R., Ho, K. T., & Ryba, S. A. (2000). Importance of Maternal Transfer of the Photoreactive Polycyclic Aromatic Hydrocarbon Fluoranthene from Benthic Adult Bivalves to Their Pelagic Larvae. *Environmental Toxicology and Chemistry, 19*(11), 2691–98.

Peterio, L. G., Labarta, U., & Fernandez-Reiriz, M. J. (2007). Variability in biochemical components of the mussel (*Mytilus galloprovincialis*) cultured after Prestige oil spill. *Comparative Biochemistry and Physiology Part C*, *145*, 588-594.

Philibert D. A., Philibert C. P., Lewis C., Tierney K. B. (2016). Comparison of diluted bitumen (dilbit) and conventional crude oil toxicity to developing zebrafish. *Environmental Science & Technology, 50*, 6091–6098.

Ramachandran, S. D., Hodson, P. V., Khan, C. W., & Lee, K. (2004). Oil dispersant increases PAH uptake by fish exposed to crude oil. *Ecotoxicology and Environmental Safety*, *59*(3), 300–308. https://doi.org/10.1016/j.ecoenv.2003.08.018

Reddy, C. M., & Quinn, J. G. (1999). GC-MS analysis of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in seawater samples after the North Cape oil spill. *Marine Pollution Bulletin*, *38*(2), 126–135. https://doi.org/10.1016/S0025-326X(98)00106-4

Reddy, C. M., Arey, J. S., Seewald, J. S., Sylva, S. P., Lemkau, K. L., Nelson, R. K., Carmichael, C. A., McIntyre, C. P., Fenwick, J., Ventura, G. T., Van Mooy, B. A., & Camilli, R. (2011). Composition and fate of gas and oil released to The water column during the *Deepwater horizon* oil spill. *Proceedings of the National Academy of Sciences*, *109*(50), 20229–20234. https://doi.org/10.1073/pnas.1101242108

Redmond, K. J., Berry, M., Sanni, S., & Andersen, O. K. (2016). Effect of dispersed crude oil on the feeding activity, retention efficiency, and filtration rate of differently sized blue mussels (*mytilus edulis*). *Journal of Toxicology and Environmental Health, Part A*, *79*(13–15), 658–674. https://doi.org/10.1080/15287394.2016.1171996

Refined Petroleum Products. (2023). *Government of Canada*. Canadian Centre for Energy Information. Retrieved April 19, 2023, from https://energy-information.canada.ca/en/subjects/refined-petroleum-products

Ren, J., Ross, A., & Schiel, D. (2000). Functional descriptions of feeding and energetics of the Pacific Oyster Crassostrea gigas in New Zealand. *Marine Ecology Progress Series*, *208*, 119–130. https://doi.org/10.3354/meps208119

Rico-Villa, B., Pouvreau, S., & Robert, R. (2009). Influence of food density and temperature on ingestion, growth, and settlement of Pacific oyster larvae, *Crassostrea gigas*. *Aquaculture*, *287*(3-4), 395–401. https://doi.org/10.1016/j.aquaculture.2008.10.054

Sammarco, P. W., Kolian, S. R., Warby, R. A. F., Bouldin, J. L., Subra, W. A., & Porter, S. A. (2013). Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil Spill, Gulf of Mexico. *Marine Pollution Bulletin*, *73*(1), 129–143. https://doi.org/10.1016/j.marpolbul.2013.05.029

Schmutz, A. (2018). Effets sous-létaux sur la Moule bleue (*Mytilus edulis*) et sa progéniture d'une exposition chronique à trois pétroles (classiques et bitumes dilués) en condition hivernale avec couvert de glace. [M.Sc. Thesis, Universite du Quebec a Rimouski].

Short, J. W., & Babcock, M. M. (1996) Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. In: Proceedings of the Exxon Valdez oil spill symposium, American Fisheries Society Symposium 18, Maryland, USA

Singer, M., Aurand, D., Bragin, G., Clark, J., Coelho, G., Sowby, M., & Tjeerdema, R. (2000). Standardization of the Preparation and Quantitation of Water-accommodated Fractions of Petroleum for Toxicity Testing. *Marine Pollution Bulletin, 40*(11), 1007-1016. doi:10.1016/s0025-326x(00)00045-x

Slobodkin, L. B., & Richman, S. (1961). Calories/gram in species of animals. *Nature*, *191*, 299.

Smolders, R., Bervoets, L., De Coen, W., & Blust, R. (2004). Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. *Environmental Pollution*, *129*, 99-112.

Song, Y. P., Suquet, M., Quéau, I., & Lebrun, L. (2009). Setting of a procedure for experimental fertilisation of Pacific oyster (*Crassostrea gigas*) oocytes. *Aquaculture*, *287*(3-4), 311–314. https://doi.org/10.1016/j.aquaculture.2008.10.018

Soniat, T. M., King, S. M., Tarr, M. A., & Thorne, M. A. (2011). Chemical and Physiological Measures on Oysters (Crassostrea virginica) from Oil-Exposed Sites in Louisiana. *Journal of Shellfish Research, 30*(3), 713-717. doi:10.2983/035.030.0311

Speight, J. G. (2010). Handbook of industrial hydrocarbon processes. Gulf Professional, Houston, TX, USA.

Statistics Canada. (2022, November 29). Aquaculture, production and value. Retrieved April 20, 2023, from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210010701

Stefansson, E. S., Langdon, C. J., Pargee, S. M., Blunt, S. M., Gage, S. J., & Stubblefield, W. A. (2016). Acute effects of non-weathered and weathered crude oil and dispersant associated with the Deepwater Horizon Incident on the development of marine bivalve and echinoderm larvae. *Environmental Toxicology and Chemistry*, *35*(8), 2016–2028. https://doi.org/10.1002/etc.3353

Stoyanovich, S. S., Yang, Z., Hanson, M., Hollebone, B. P., Orihel, D. M., Palace, V., . . . Blais, J. M. (2019). Simulating a Spill of Diluted Bitumen: Environmental Weathering and Submergence in a Model Freshwater System. *Environmental Toxicology and Chemistry, 38*(12), 2621-2628. doi:10.1002/etc.4600

Toro, B., Navarro, J. M., & Palma-Fleming, H. (2003). Relationship between Bioenergetics Responses and Organic Pollutants in the Giant Mussel, *Choromytilus chorus* (*Mollusca: Mytilidae*). *Aquatic Toxicology, 63*(3), 257–269.

Transport Canada. (2016). *Tanker safety by the numbers infographic*. Transport Canada. Retrieved April 19, 2023, from https://tc.canada.ca/en/marine-transportation/marine-safety/tanker-safety-numbers-infographic

Uno, S., Koyama, J., Kokushi, E., Monteclaro, H., Santander, S., Cheikyula, J. O., Miki, S., Añasco, N., Pahila, I. G., Taberna, H. S., & Matsuoka, T. (2009). Monitoring of PAHs and alkylated PAHs in aquatic organisms after 1 month from the Solar I oil spill off the coast of Guimaras Island, Philippines. *Environmental Monitoring and Assessment*, *165*(1–4), 501–515. https://doi.org/10.1007/s10661-009-0962-1

Ventura, A., Schulz, S., & Dupont, S. (2016). Maintained larval growth in mussel larvae exposed to acidified under-saturated seawater. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep23728

Vignier, J., Rolton, A., Soudant, P., Chu, F. E., Robert, R., & Volety, A. K. (2018). Evaluation of toxicity of deepwater horizon slick oil on spat of the oyster crassostrea virginica. *Environmental Science and Pollution Research*, *25*(2), 1176–1190. https://doi.org/10.1007/s11356-017-0476-2

Wade, T. L., Jackson, T. J., McDonald, T. J., Wilkinson, D. L., & Brooks, J. M. (1993). Oysters as Biomonitors of the Apex Barge Oil Spill. *International Oil Spill Conference Proceedings*, *1993*(1), 313–317. https://doi.org/10.7901/2169-3358-1993-1-313

Wade, T. L., Sweet, S. T., Sericano, J. L., Guinasso, N. L., Diercks, A.-R., Highsmith, R. C., Asper, V. L., Joung, D., Shiller, A. M., Lohrenz, S. E., & Joye, S. B. (2011). Analyses of Water Samples From the Deepwater Horizon Oil Spill: Documentation of the Subsurface Plume. In Y. Liu, A. MacFadyen, Z.-G. Ji, & R. H. Weisberg (Eds.), *Geophysical Monograph Series* (Vol. 195, pp. 77–82). American Geophysical Union. https://doi.org/10.1029/2011GM001103

Wang, H., Huang, W., Gong, Y., Chen, C., Zhang, T., & Diao, X. (2020). Occurrence and potential health risks assessment of polycyclic aromatic hydrocarbons (PAHs) in different tissues of bivalves from Hainan Island, China. *Food and Chemical Toxicology, 136*, 111108. doi: 10.1016/j.fct.2019.111108

Waldbusser, G. G., Brunner, E. L., Haley, B. A., Hales, B., Langdon, C. J., & Prahl, F. G. (2013). A developmental and energetic basis linking larval oyster shell formation to acidification sensitivity. *Geophysical Research Letters*, *40*(10), 2171–2176. https://doi.org/10.1002/grl.50449

Waldbusser, G. G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., . . . Gimenez, I. (2015). Saturation-state sensitivity of marine bivalve larvae to ocean acidification. *Nature Climate Change, 5*(3), 273-280. doi:10.1038/nclimate2479

Widdows, J. (1991). Physiological ecology of mussel larvae. *Aquaculture*, *94*(2–3), 147–163. https://doi.org/10.1016/0044-8486(91)90115-n

Widdows, J., Bakke, T., Bayne, B. L., Donkin, P., Livingstone, D. R., Lowe, D. M., Moore, M. N., Evans, S. V., & Moore, S. L. (1982). Responses of *Mytilus Edulis* on Exposure to the Water-Accommodated Fraction of North Sea Oil. *Marine Biology, 67*(1), 15–31.

Widdows, J., Burns, K., Menon, N., Page, D., & Soria, S. (1990). Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contamination gradient in Bermuda. *Journal of Experimental Marine Biology and Ecology, 138*, 99-117. doi:10.1016/0022-0981(90)90179-g

Widdows, J., & Donkin, P. (1991). Role of physiological energetics in ecotoxicology. Mini review. *Comparative Biochemistry and Physiology*, *100*, 65–69.

Widdows, J., Donkin, P., & Evans, S. V. (1985). Recovery of *Mytilus edulis* L. from Chronic Oil Exposure. *Marine Environmental Research, 17*(2–4), 250–253.

Widdows, J., Donkin, P., Salkeld, P. N., & Evans, S. V. (1987). Measurement of scope for growth and tissue concentrations of mussels *Mytilus edulis* at sites in the vicinity of Sullom Voe: a case study. *In* Fate and Effects of Oil in the Marine Ecosystems, pp. 269– 277. Ed. by J. Kuiper and W. J. van den Brink. Nijhoff, Dordrecht.

Widdows, J, Donkin, P., Brinsley, M., Evans, S., Salkeld, P., Franklin, A., Law, R., & Waldock, M. (1995). Scope for growth and contaminant levels in North Sea mussels mytilus edulis. *Marine Ecology Progress Series*, *127*, 131–148. https://doi.org/10.3354/meps127131

Widdows, J., Donkin, P., Staff, F., Matthiessen, P., Law, R., Allen, Y., . . . Jones, B. (2002). Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. *Marine Environmental Research, 53*(4), 327-356. doi:10.1016/s0141-1136(01)00120-9

Widdows, J., & Johnson, D. (1988). Physiological Energetics of *Mytilus edulis*: Scope for Growth. *Marine Ecology, 46*, 113–121.

Widdows, J., Moore, M. N., Lowe, D. M., & Salkeld, P. N. (1979). Some effects of a dinoflagellate bloom *Gyrodinium aureolum* on the mussel *Mytilus edulis*. *Journal of the Marine Biological Association UK, 59*, 522–524.

Widdows, J., & Staff, F. (2006). Biological effects of contaminants: Measurement of scope for growth in mussels. *ICES Techniques in Marine Environmental Sciences*, *40*, 1-30.

Yang, C., Wang, Z., Yang, Z., Hollebone, B., Brown, C. E., Landriault, M., & Fieldhouse, B. (2011). Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products. *Environmental Forensics, 12*(2), 173-188. doi:10.1080/15275922.2011.574312

Yim, U. H., Kim, M., Ha, S. Y., Kim, S., & Shim, W. J. (2012) Oil spill environmental forensics: the Hebei Spirit oil spill case. *Environmental Science and Technology*, *46*(12), 6431–6437

Yim, U. H., Oh, J. R., Hong, S. H., Lee, S. H., Shim, W. J., & Shim, J. H. (2002). Identification of PAHs Sources in Bivalves and Sediments 5 Years After the Sea Prince Oil Spill in Korea. *Environmental Forensics, 3*, 357-366. doi:10.1006/enfo.2002.0107

Zeng, Y., & Yang, H. (2020). Review of molluscan bivalve condition index calculations and application in Northern Quahogs *Mercenaria*. *Aquaculture Research*, *52*(1), 23–36. https://doi.org/10.1111/are.14866

Zhong, X., Li, P., Lin, X., Zhao, Z., He, Q. S., Niu, H., & Yang, J. (2022). Diluted bitumen: Physicochemical properties, Weathering Processes, emergency response, and recovery. *Frontiers in Environmental Science*, *10*. https://doi.org/10.3389/fenvs.2022.910365

Chapter 3. General Conclusions and Future Recommendations

3.1. General Conclusion

Petroleum product spills in the environment have been extensively studied and are known to cause lethal and sublethal effects on exposed bivalves due to the presence of PACs, alongside other contaminants. Consequently, this study was designed to assess the ecotoxicological impact of three petroleum product (MD, CO, and DB) sub-chronic exposures, followed by a four-week recovery period. Endpoints assessed in this study included SFG, inclusive of CR, AE, and MO_2 measurements, gonad and digestive gland histopathology, larval developmental success, along with the CI and HAI of Pacific oysters (*Crassostrea gigas*). After analyzing the WAFs and oyster tissues exposed to the three products, the highest TPAC concentrations were detected in the CO exposure, followed by MD and DB. However, these exposures did not induce any significant impacts on the physiology, morphology, histopathology, or development of Pacific oysters over time.

The current study suggested that during the 7 d exposure period, oysters were able to retain bioavailable PACs in their tissues. However, after being placed in uncontaminated water, TPAC concentration in their tissues rapidly declined. One potential reason for this could be the biotransformation of parent PACs resulting in their fast depuration through gut egestion or passive diffusion from gills. Another possible reason could be that after being removed from petroleum WAFs, there was an absence of continuous PAC exposure that reduced the tissue TPAC concentrations over time and prevented adverse toxicological impacts.

Over the years, a multitude of studies have used bivalves as biomonitors to inform decisions regarding the monitoring, regulation, and remediation of oil spills. Previous studies have stated that the sedentary lifestyle, robust size, filter-feeding ability, and slow biotransformation of hydrocarbons within this species make them viable candidates to study the impact of petroleum in the marine environment. However, given the results from the current study, it is pertinent that this species may be used for testing the mechanisms of hydrocarbon uptake, depuration rates, and biotransformation capacity in bivalves, but within a time-sensitive manner of being removed from a marine oil spill.

3.2. Future Recommendations

Given the present results, some additional research might be required to ascertain the use of Pacific oysters as biomonitors for assessing the environmental impact of petroleum products. Alterations in their populations, growth rates, and reproductive success are indicators of ecological health that can assist with the proper management of these contaminants in a marine environment. While the current study did not provide evidence regarding the detrimental effects of the three petroleum products on Pacific oysters, extensive research is still required, with additional resources and endpoints being allocated to the experimental system. Based on their economic and cultural significance, caution needs to be taken while establishing acceptable daily human intake concentrations by federal and provincial agencies. Moreover, with the global expansion of petroleum mining and transportation, it is important to understand the ecotoxicological ramifications of oil spills. While some PACs have high volatilization rates, significant portions of the chemicals can remain in the environment for long durations and have adverse impacts on the exposed biota. Hence, additional chronic studies with different bivalve species should be utilized to determine the long-term exposure effects of such contaminants, especially on endpoints related to their morphology, such as CI, HAI, and histopathology that might require continuous exposure to demonstrate substantial alterations. These studies could utilize different oil types and concentrations, exposure durations and conditions, while also assessing the effects of weathering on petroleum toxicity. Information obtained from these experiments can be utilized for developing oil spill models and response strategies, environmental risk assessments, and monitoring plans for managing marine organisms in the event of potential spills that can allow the protection of sites and organisms more prone to petroleum exposure over a long duration.

Appendix. Supplemental information

Additional results

Algal clearance rate

Algal concentration in the negative control did not change over time and therefore a standard rate of decline was not needed to calculate clearance rate (CR) values. To meet the normality assumptions for oysters exposed to CO, one outlier (S4-50%-D35) was omitted from the dataset. There was also a random effect of 2.76% produced because of the placement of exposure tanks in the waterbaths. Similarly, for DB, one outlier (S1-25%-D35) was omitted from the dataset and there was a random effect of 6.09% produced due to the placement of exposure tanks in the waterbaths. Finally, for MD no outliers had to be omitted from the dataset but there was a random effect of 38.55% produced because of the placement of exposure tanks in the waterbaths.

Absorption efficiency

Based on the average algae ash-free (organic) weights, the same particulate organic matter (POM) concentrations were used for all oysters exposed to a petroleum product, where values for DB, CO, and MD were 0.3277 mg/L, 0.4667 mg/L, and 0.3896 mg/L, respectively. For all biological replicates, the same mean absorption efficiency (AE) values per petroleum treatment group were used in the SFG calculations to remove influential data noise. To meet the normality assumptions for oysters exposed to CO, 10 outliers were omitted from the dataset. There was also a random effect of 23.41% produced because of the placement of exposure tanks in the waterbaths. Similarly, for DB, 12 outliers were omitted from the dataset and there was a random effect of 13.03% produced due to the placement of exposure tanks in the waterbaths. Finally, for MD, 5 outliers had to be omitted from the dataset and there was a random effect of 12.49% produced because of the placement of exposure tanks in the waterbaths.

Oxygen consumption

For the oysters exposed to CO, DB, and MD no outliers were required to be excluded for meeting the model normality assumptions. There was also a random effect

of 0.00% produced due to the placement of the exposure tanks in the waterbaths for all three products.

Scope for growth

One outlier (S4-50%-D35) was excluded from the dataset to meet the model normality assumptions for oysters exposed to CO, while there was also a 3.65% random effect produced due to the placement of exposure tanks in waterbaths. Similarly, no outliers were removed for MD, which had a 45.54% random effect produced due to the experimental tank placement, while two outliers (S1-50%-D7, S1-25%-D35) were excluded from the dataset for oysters exposed to DB, with a 3.01% random effect produced due to the tank placement.

Condition index

No outliers were excluded from the dataset to meet the model normality assumptions for oysters exposed to all three petroleum products. Also, there was a random effect of 0.00% produced due to the placement of the exposure tanks in the waterbaths for CO and DB, while there was a 33.37% effect produced for MD.

Health assessment index

Two outliers (S2-0%-D7, S1-50%-D35) were excluded from the dataset to meet the model normality assumptions for oysters exposed to CO, while no random effect was produced due to the exposure tank placement in the waterbaths. For oysters exposed to DB, no outliers were required to be excluded and 0.00% tank random effects were produced. Similarly, no outliers were excluded for MD oysters, but there was a 32.49% random effect because of the tank placement in the waterbaths.

Gonad thickness/oyster wet weight

For oysters exposed to CO, no outliers were excluded for meeting the model normality assumptions, and there was a random effect of 4.29% produced due to the placement of exposure tanks in the waterbaths. Similarly for DB exposed oysters, no outliers were excluded from the dataset, but there was a 0.00% random effect induced due to the waterbath tank placement. Finally, for oysters exposed to MD, no outliers were removed but there was a 33.35% random effect produced because of the exposure tank placement in the waterbaths.

Digestive lumen/digestive tubule thickness

No outliers were excluded from the CO dataset to meet the model normality assumptions but there were 7.17% random effects produced due to the placement of exposure tanks in the waterbaths. For DB and MD, no outliers were excluded, while producing a 0.00% random effect due to the placement of exposure tanks in the waterbaths.

Larvae developmental success

This analysis was performed on an average of five biological replicates (prior to the outlier exclusion) per petroleum product WAF concentration (0%, 25%, 50%, 75%, 100%). The normality assumption of this model was achieved with no data transformation required. No outliers were required to be excluded for meeting the model normality assumptions in oysters exposed to all three petroleum products.

Figures

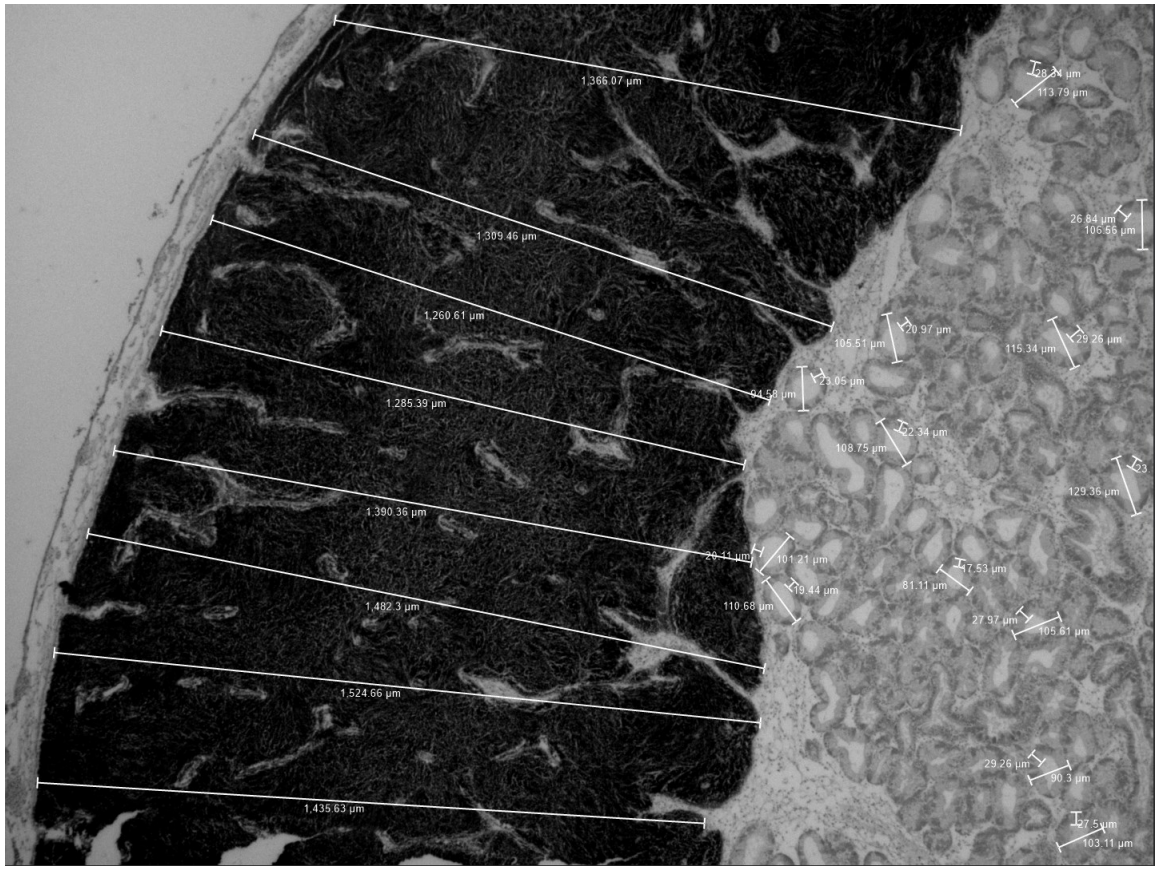

Figure A.1. Gonad, digestive lumen, and digestive tubule thickness (µm) measurement in a healthy control (0%) male oyster on day 7 of the diluted bitumen exposure at 50x total magnification with the inverted brightfield microscope Leica DMi8. Micrograph taken of a 5 µm section stained with hematoxylin and eosin.

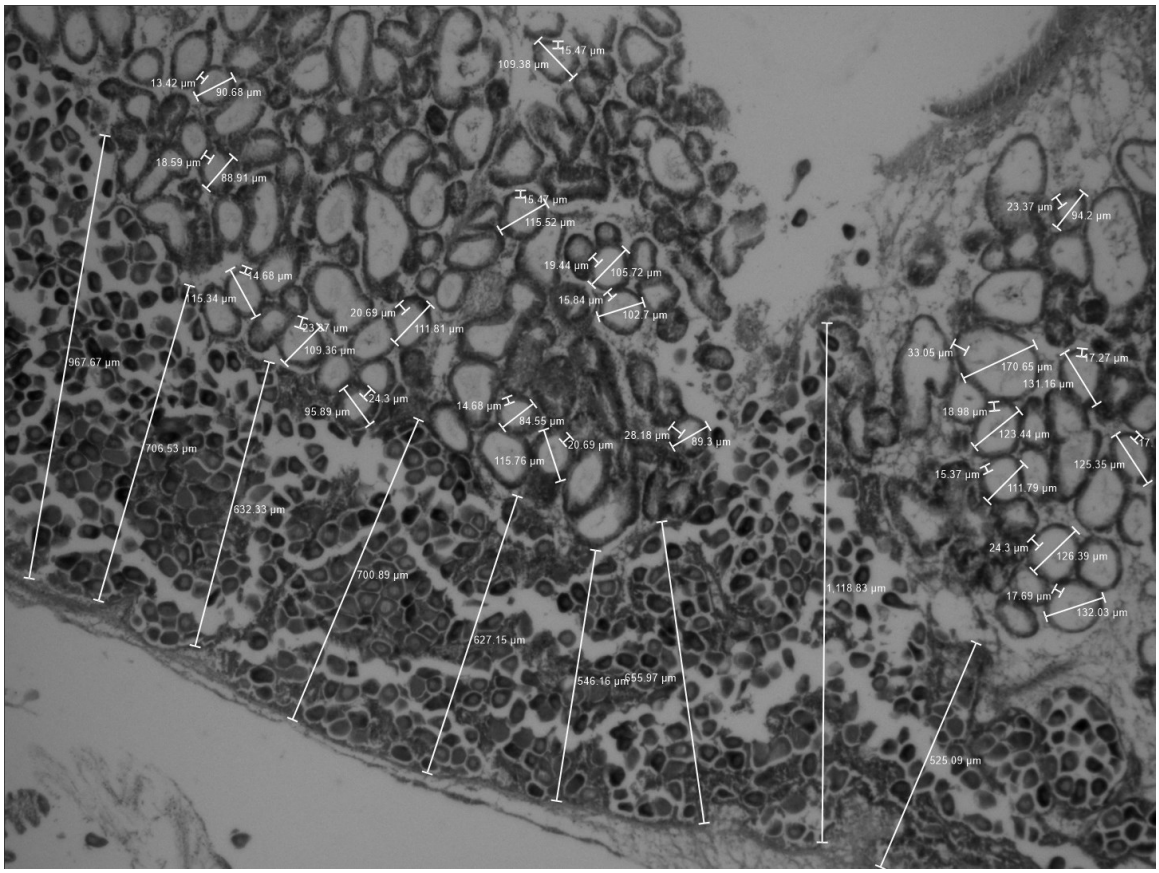

Figure A.2. Gonad, digestive lumen, and digestive tubule thickness (µm) measurement in an unhealthy 100% female oyster on day 7 of the diluted bitumen exposure at 50x total magnification with the inverted brightfield microscope Leica DMi8. Micrograph taken of a 5 µm section stained with hematoxylin and eosin.

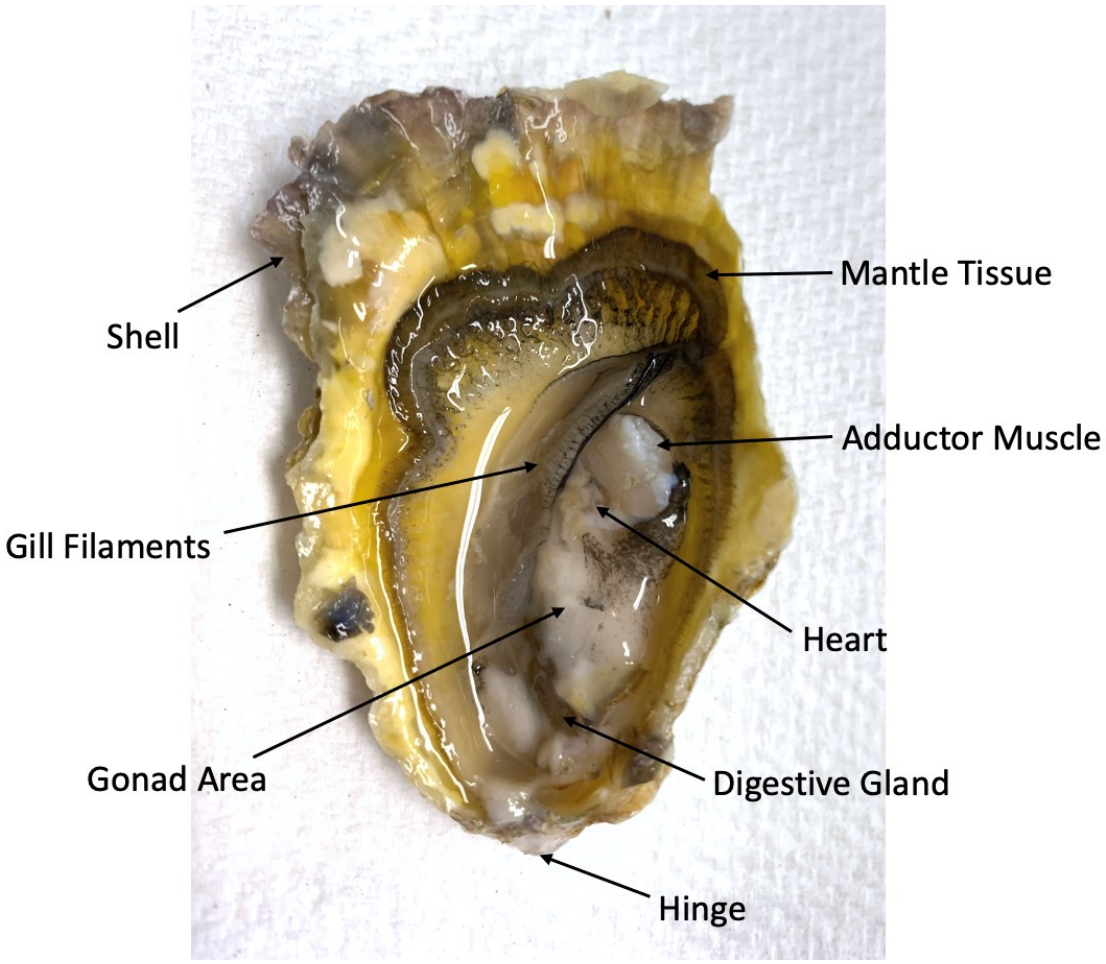

Figure A.3. Oyster anatomy with labels used for the health assessment index.

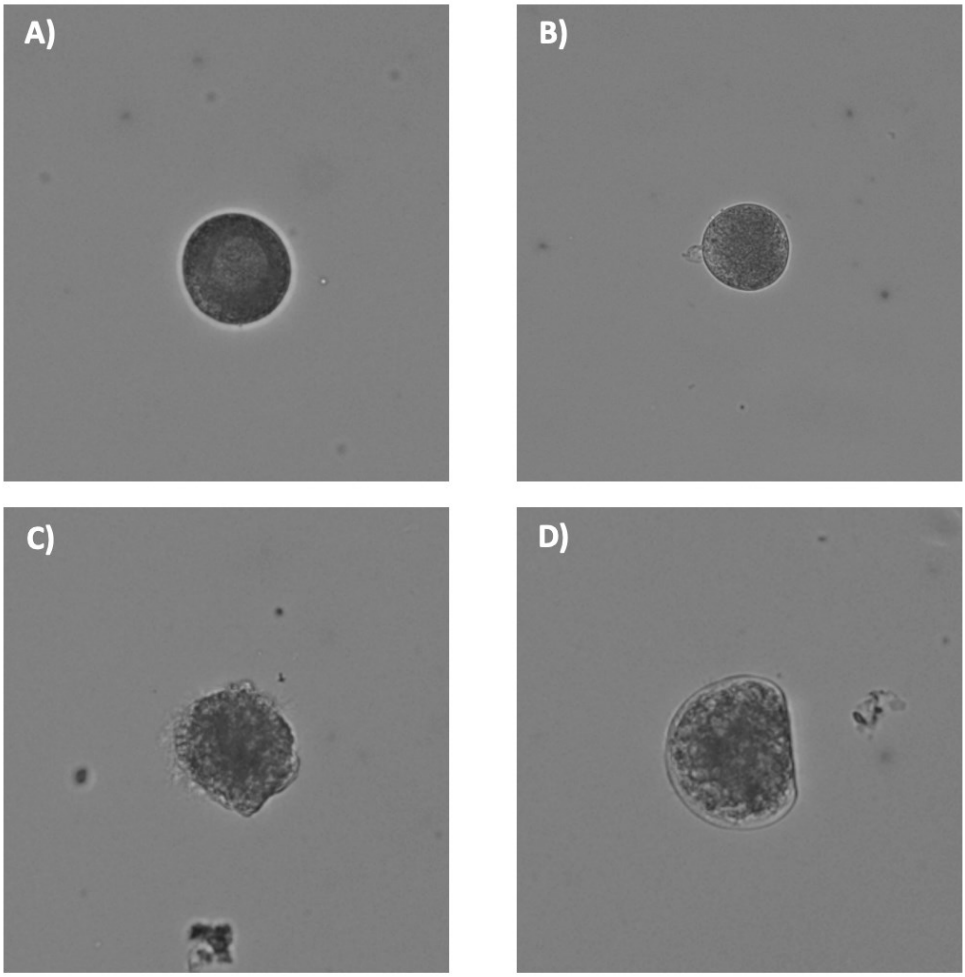

Figure A.4. **Different larval developmental stages (A) Unfertilized egg; (B) Fertilized egg; (C) Trochophore; (D) D-veliger larvae at 100x total magnification with the inverted brightfield microscope Leica DMi8.**

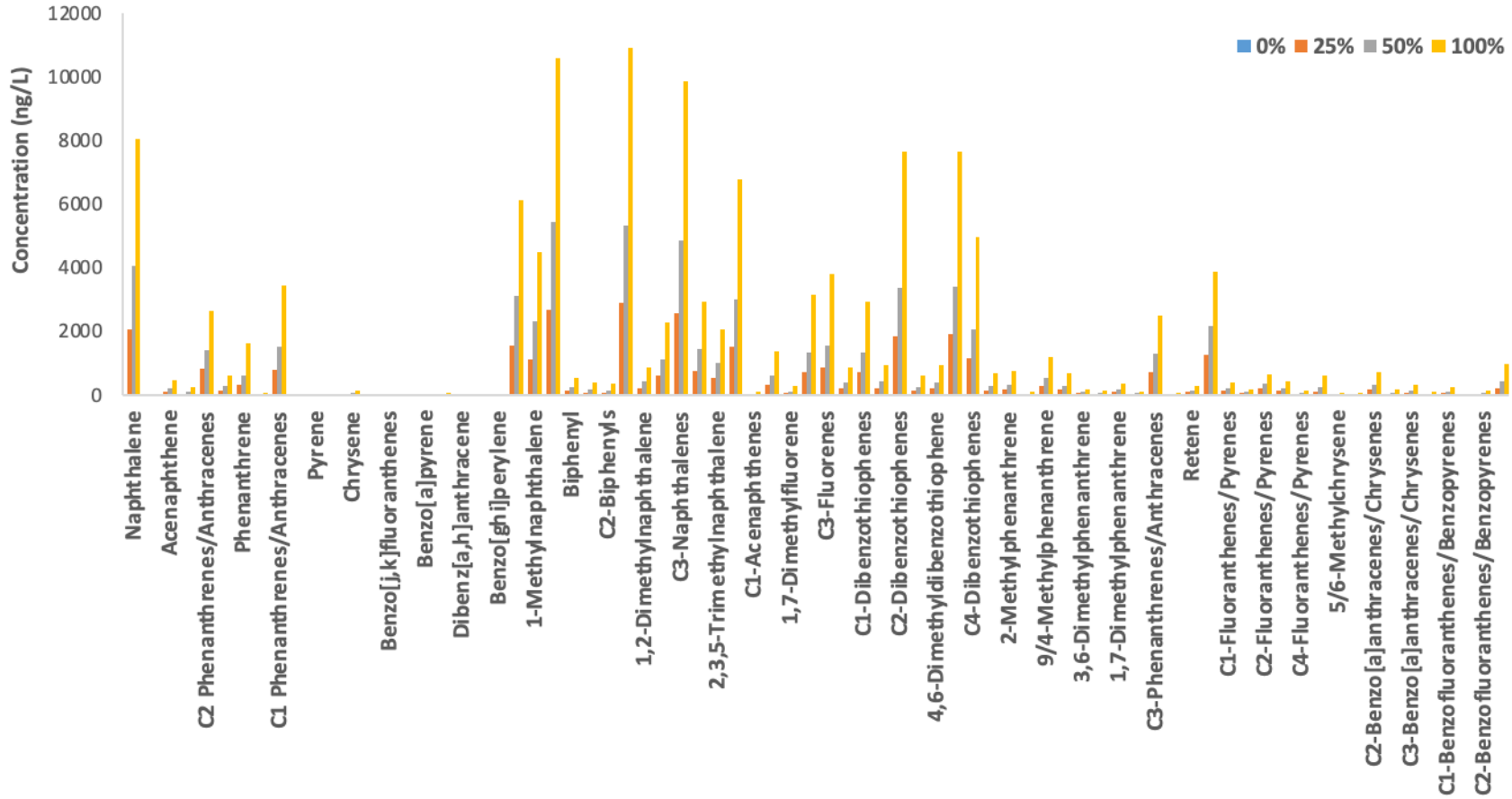

Figure A.5. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-diluted bitumen exposure on Day 0.

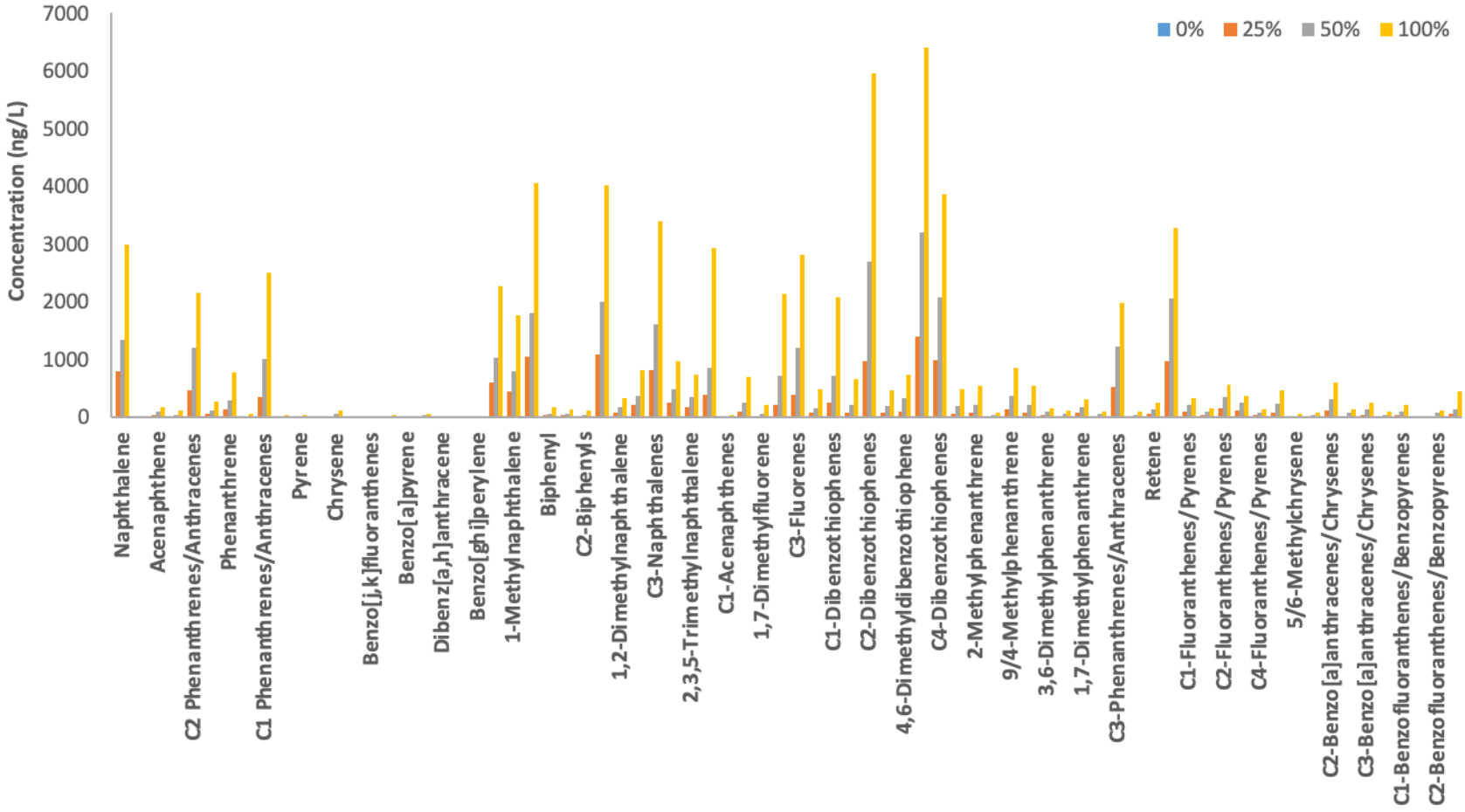

Figure A.6. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-diluted bitumen exposure on Day 3.

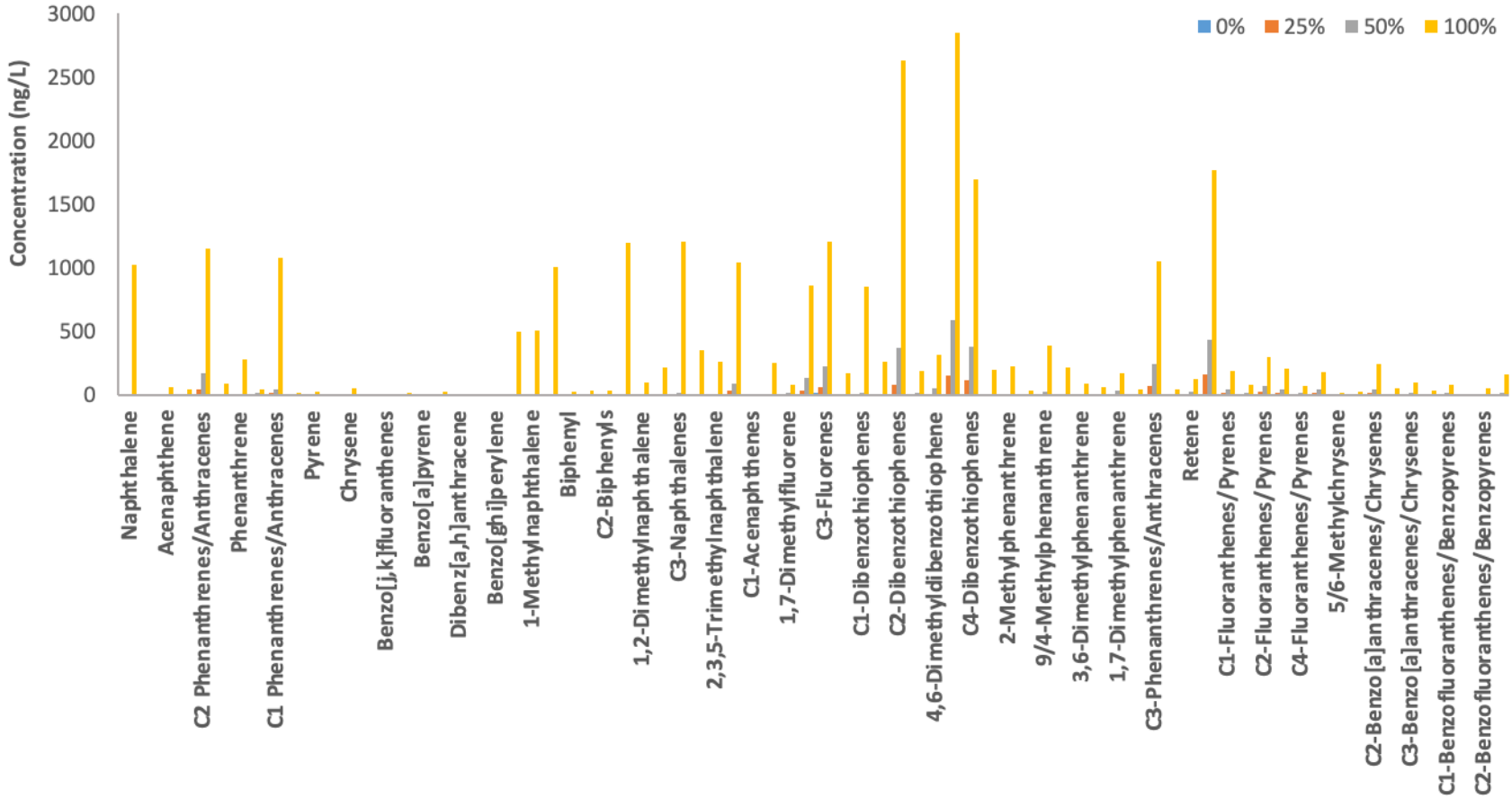

Figure A.7. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-diluted bitumen exposure on Day 7.

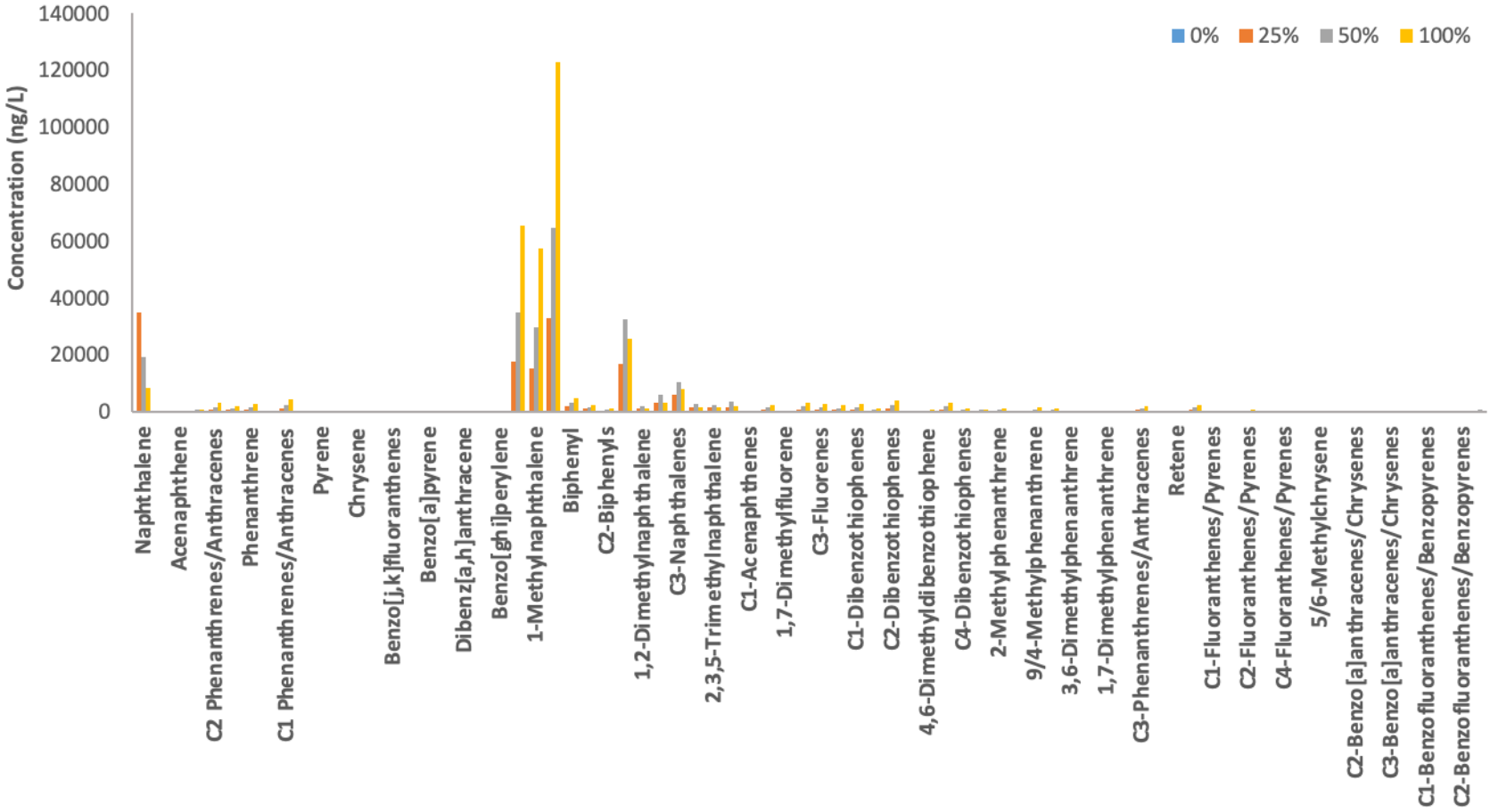

Figure A.8. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-crude oil exposure on Day 0.

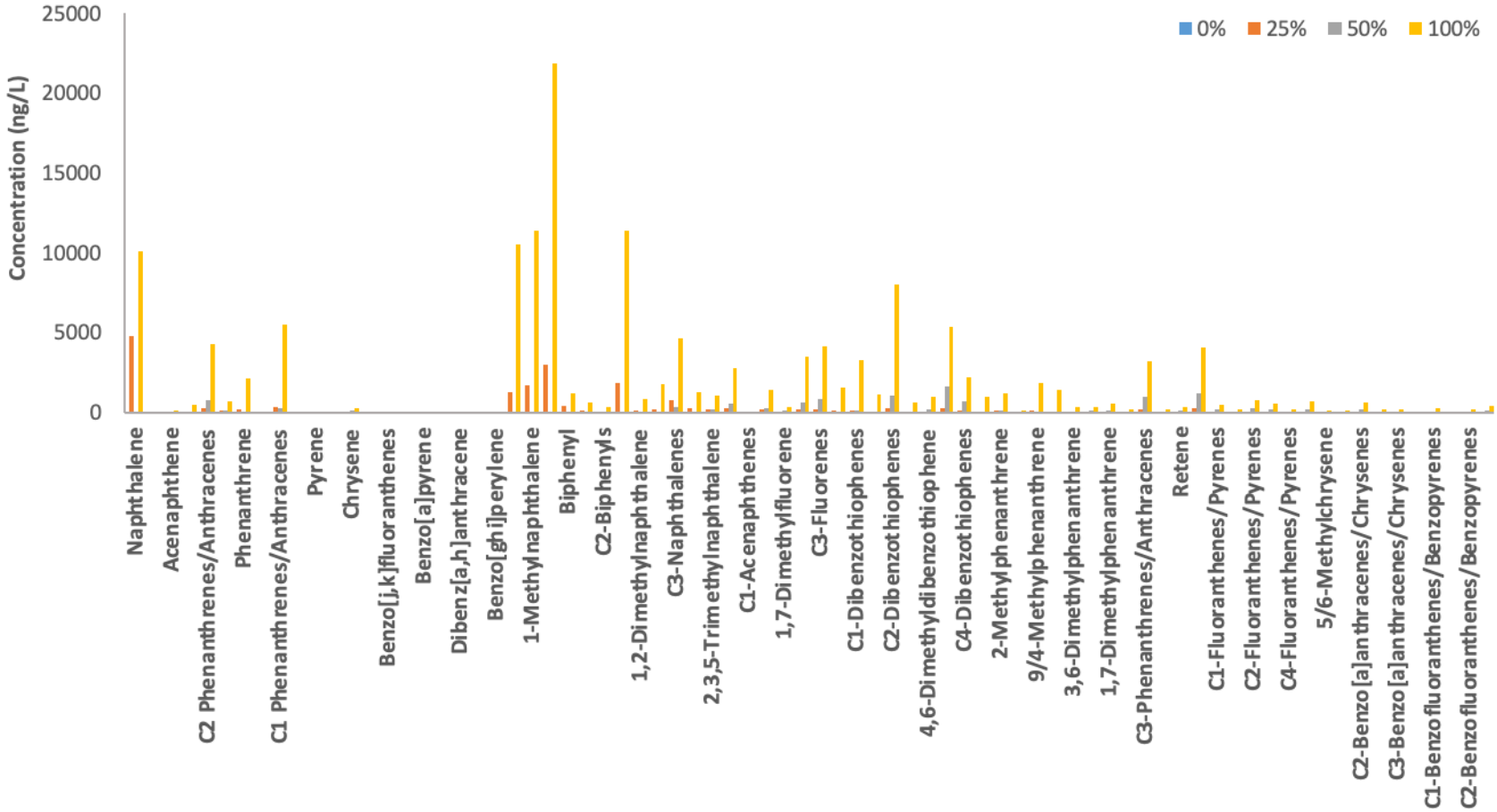

Figure A.9. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-crude oil exposure on Day 3.

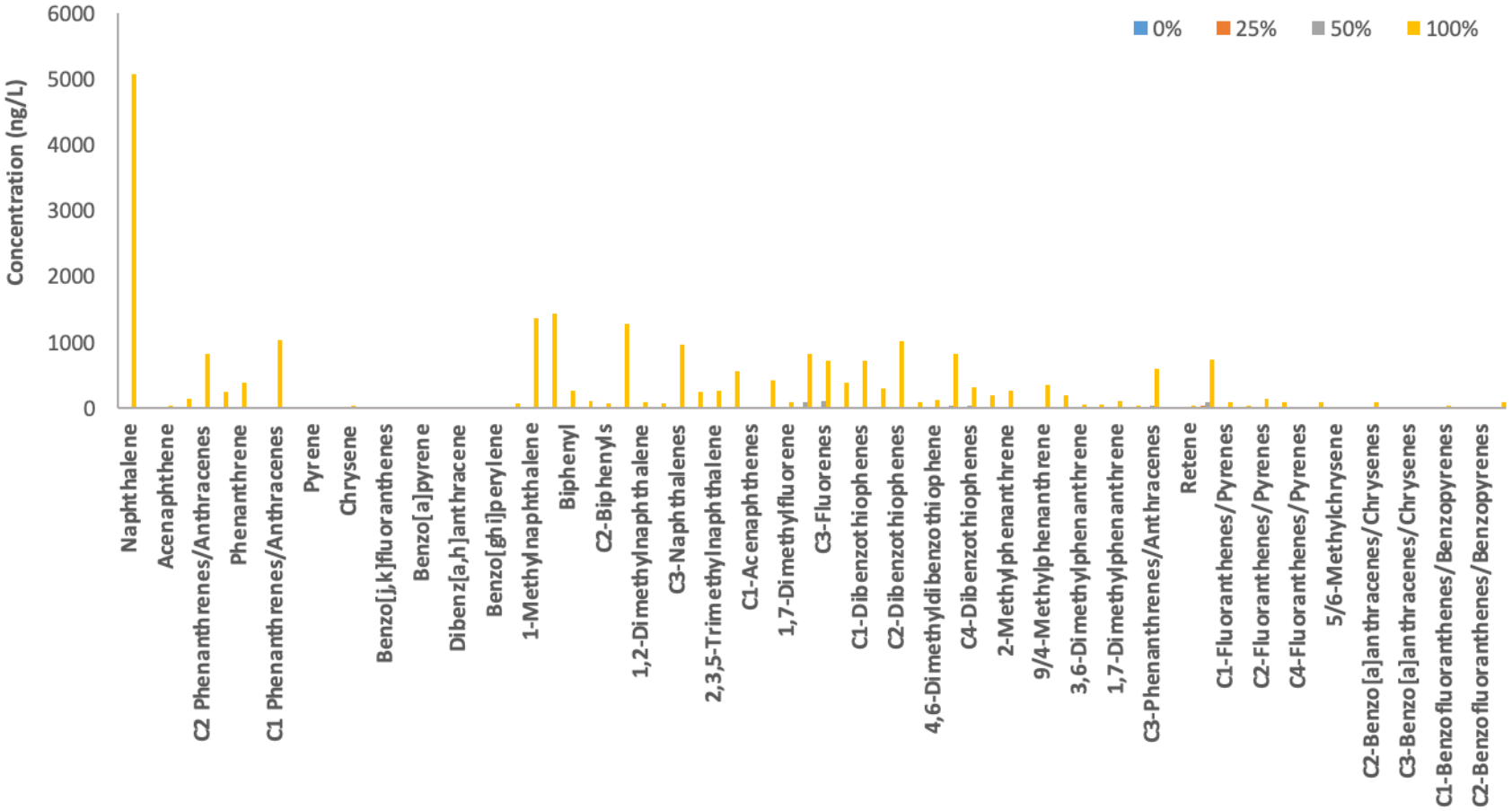

Figure A.10. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-crude oil exposure on Day 7.

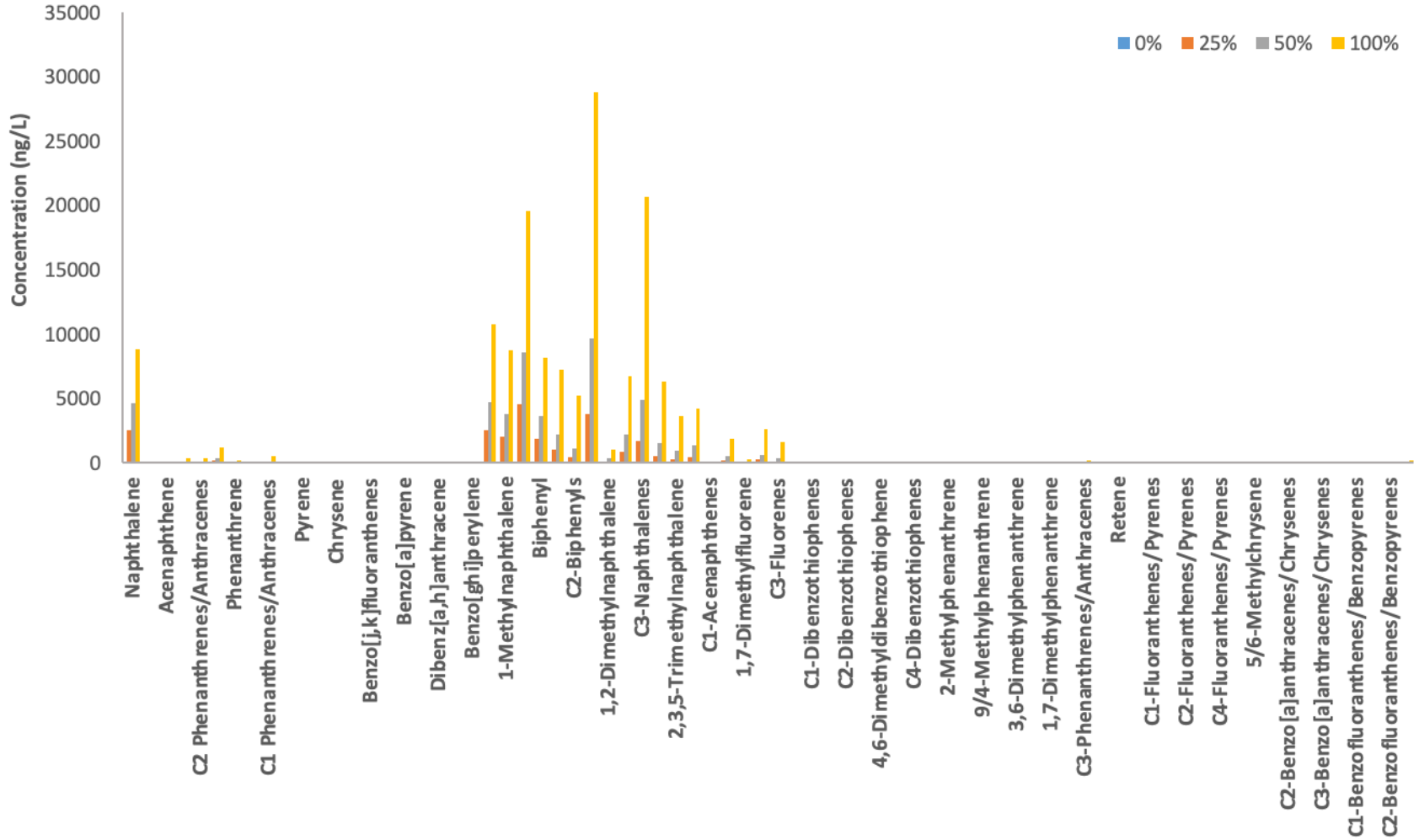

Figure A.11. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-marine diesel exposure on Day 0.

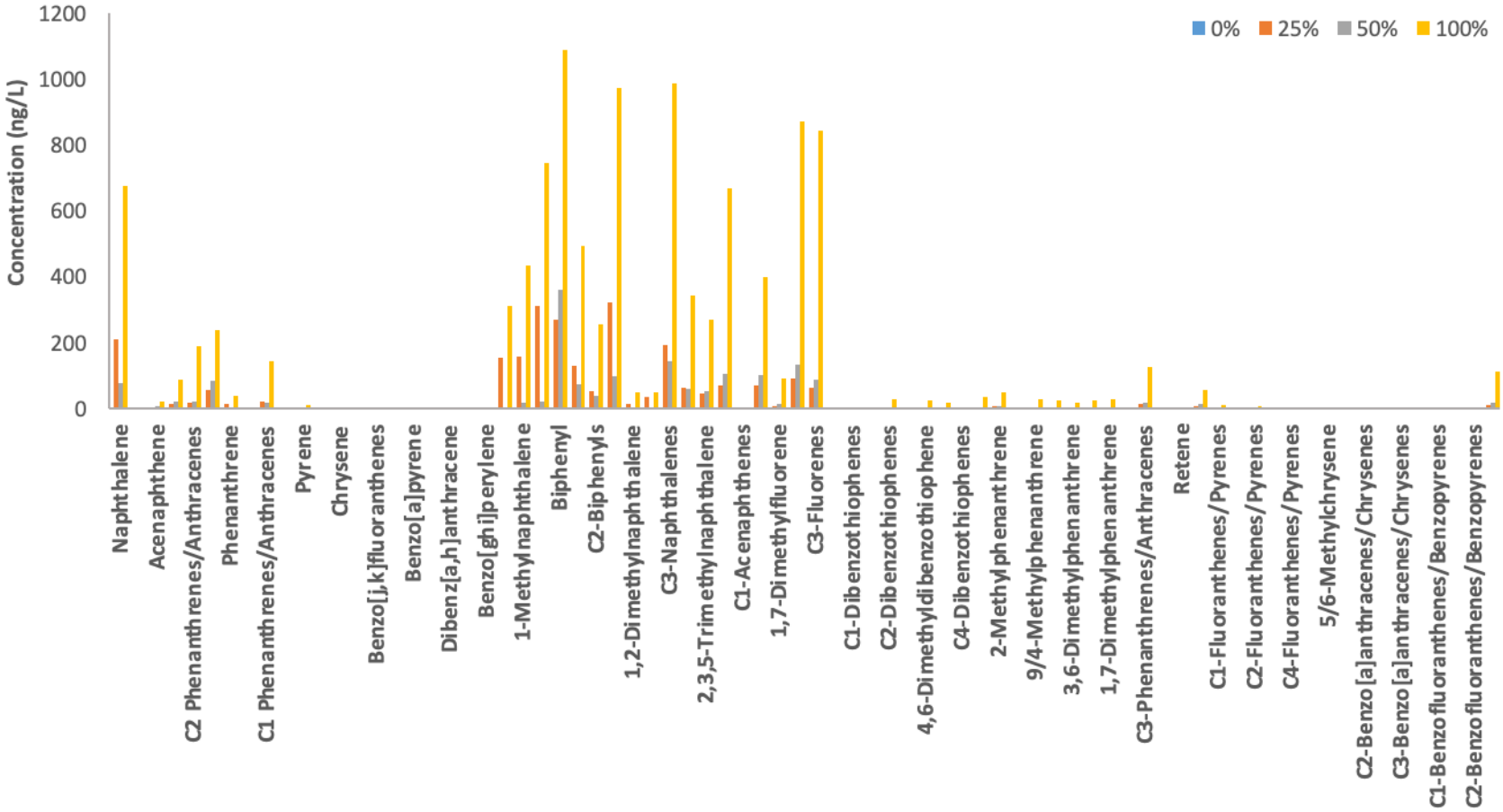

Figure A.12. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-marine diesel exposure on Day 3.

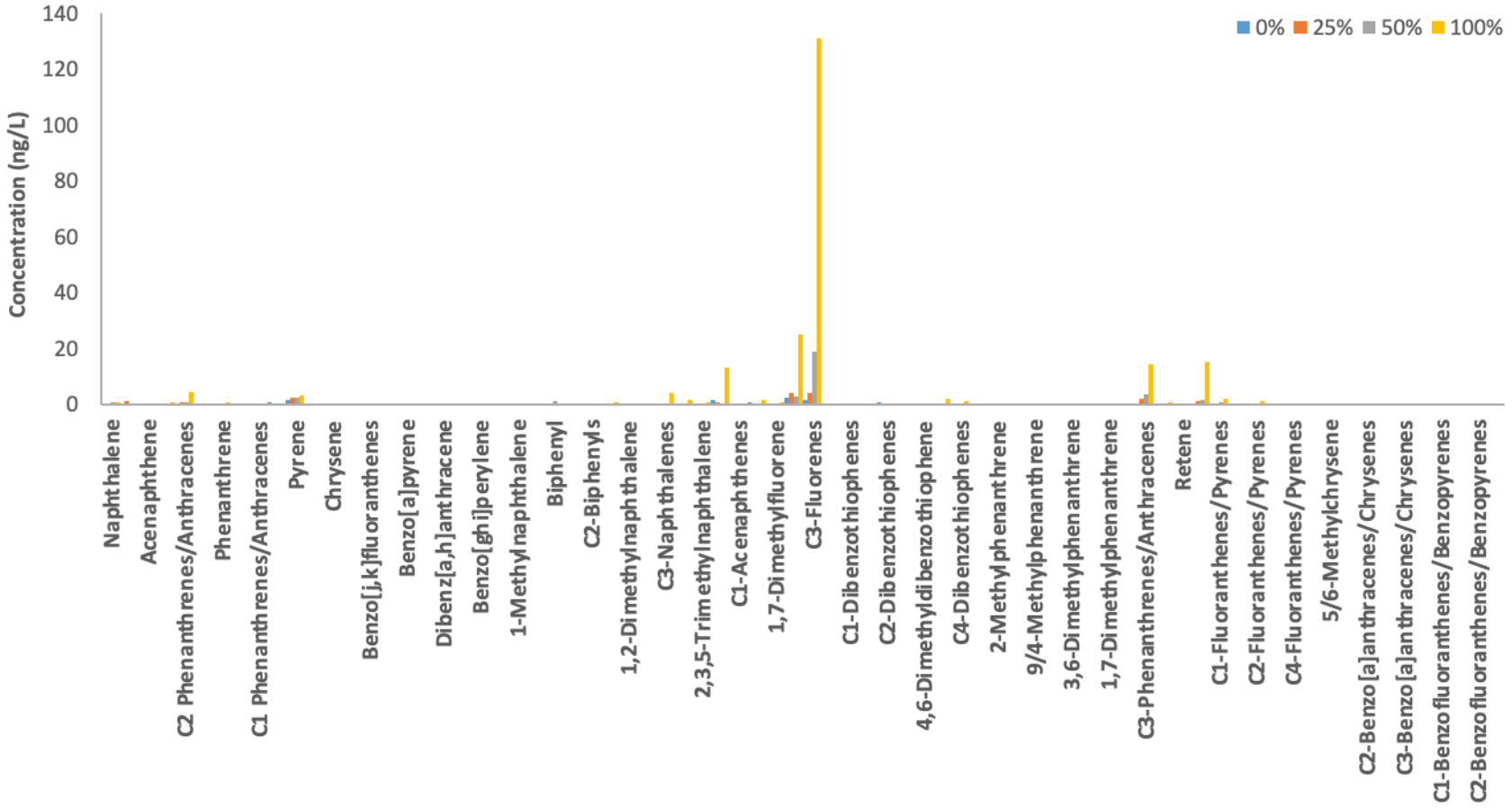

Figure A.13. PAC profile of water concentrations (0%, 25%, 50%, 100%) in ng/L post-marine diesel exposure on Day 7.

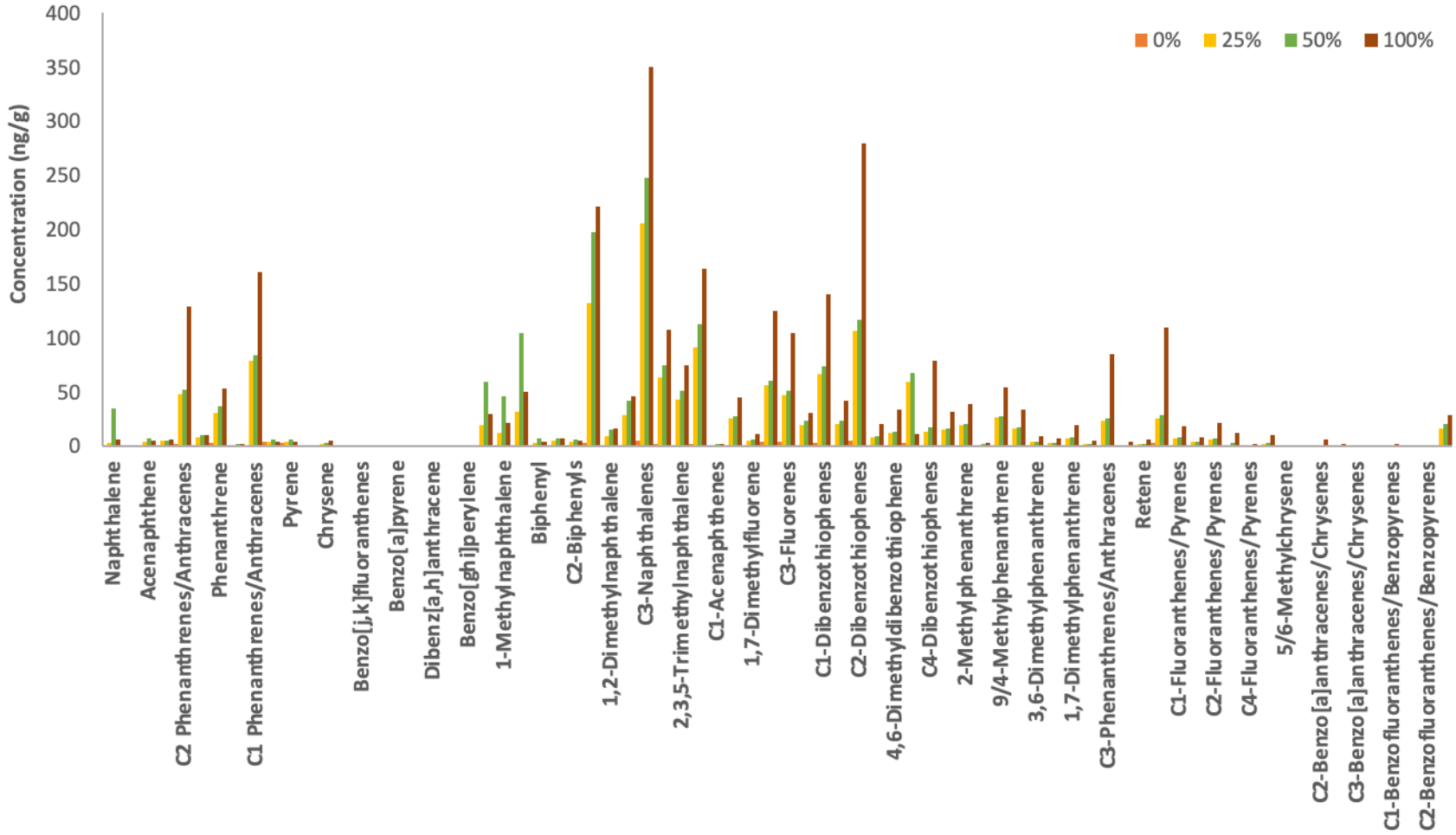

Figure A.14. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-diluted bitumen exposed oysters on Day 7.

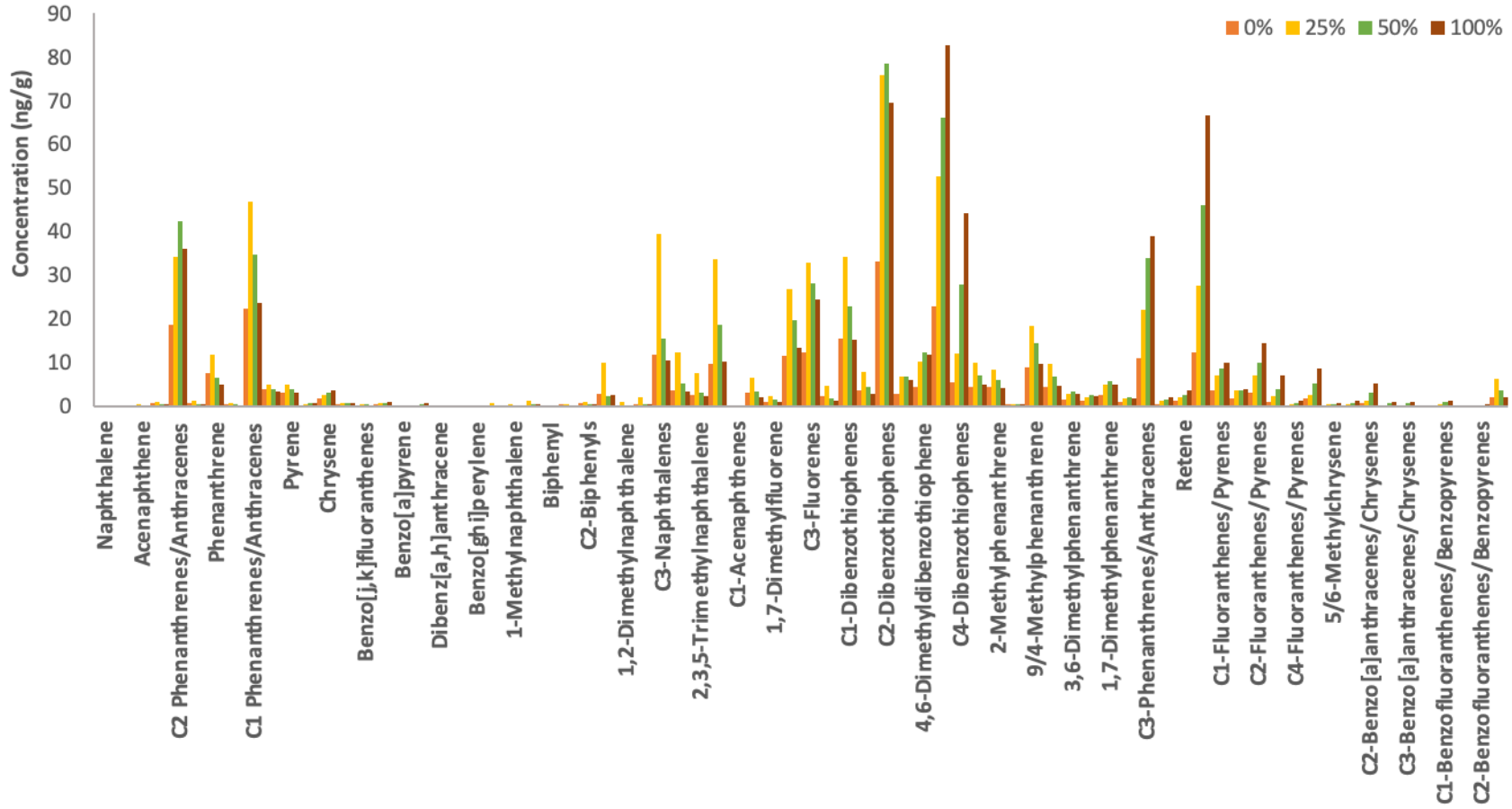

Figure A.15. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-diluted bitumen exposed oysters on Day 21.

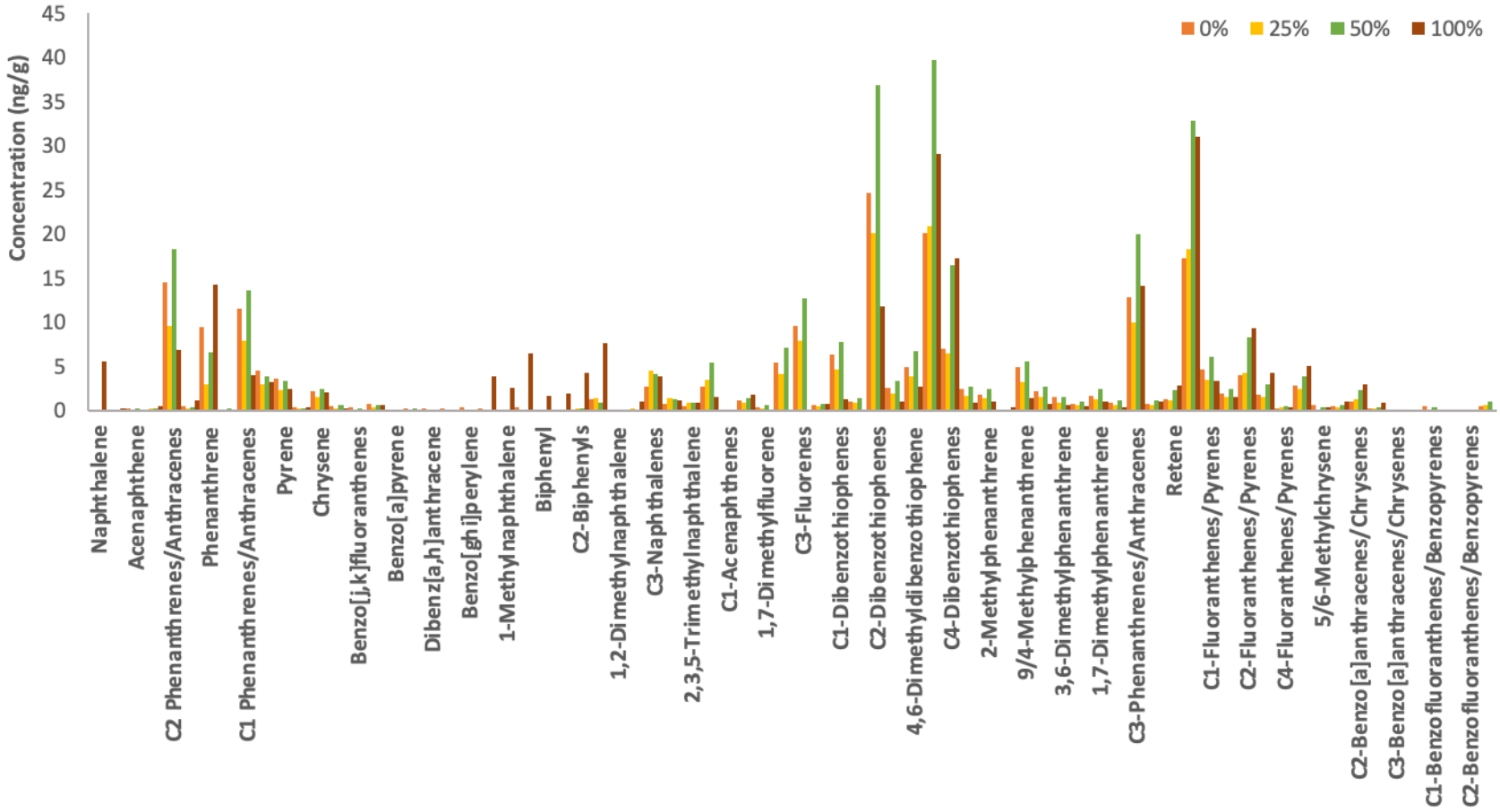

Figure A.16. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-diluted bitumen exposed oysters on Day 35.

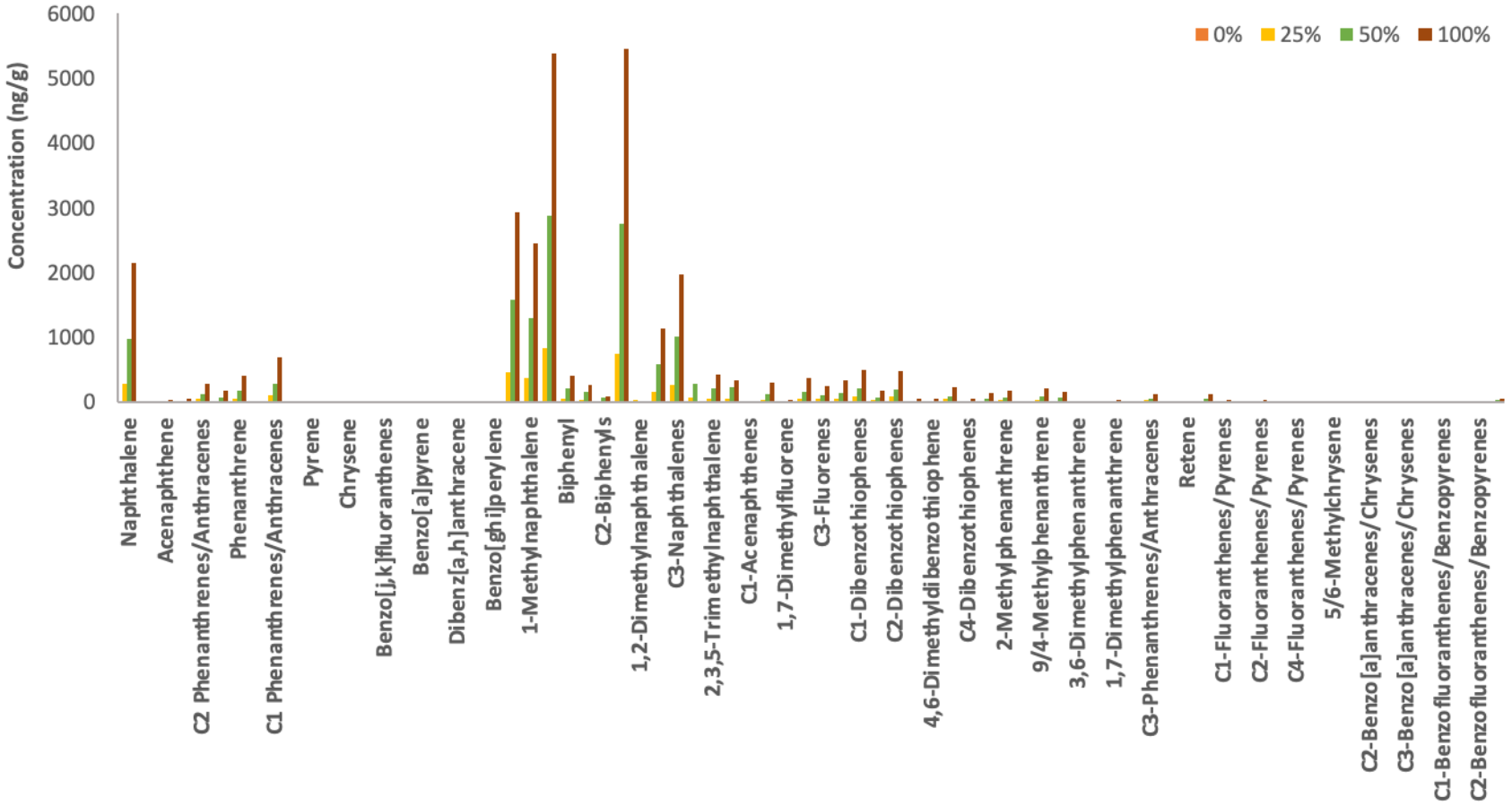

Figure A.17. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-crude oil exposed oysters on Day 7.

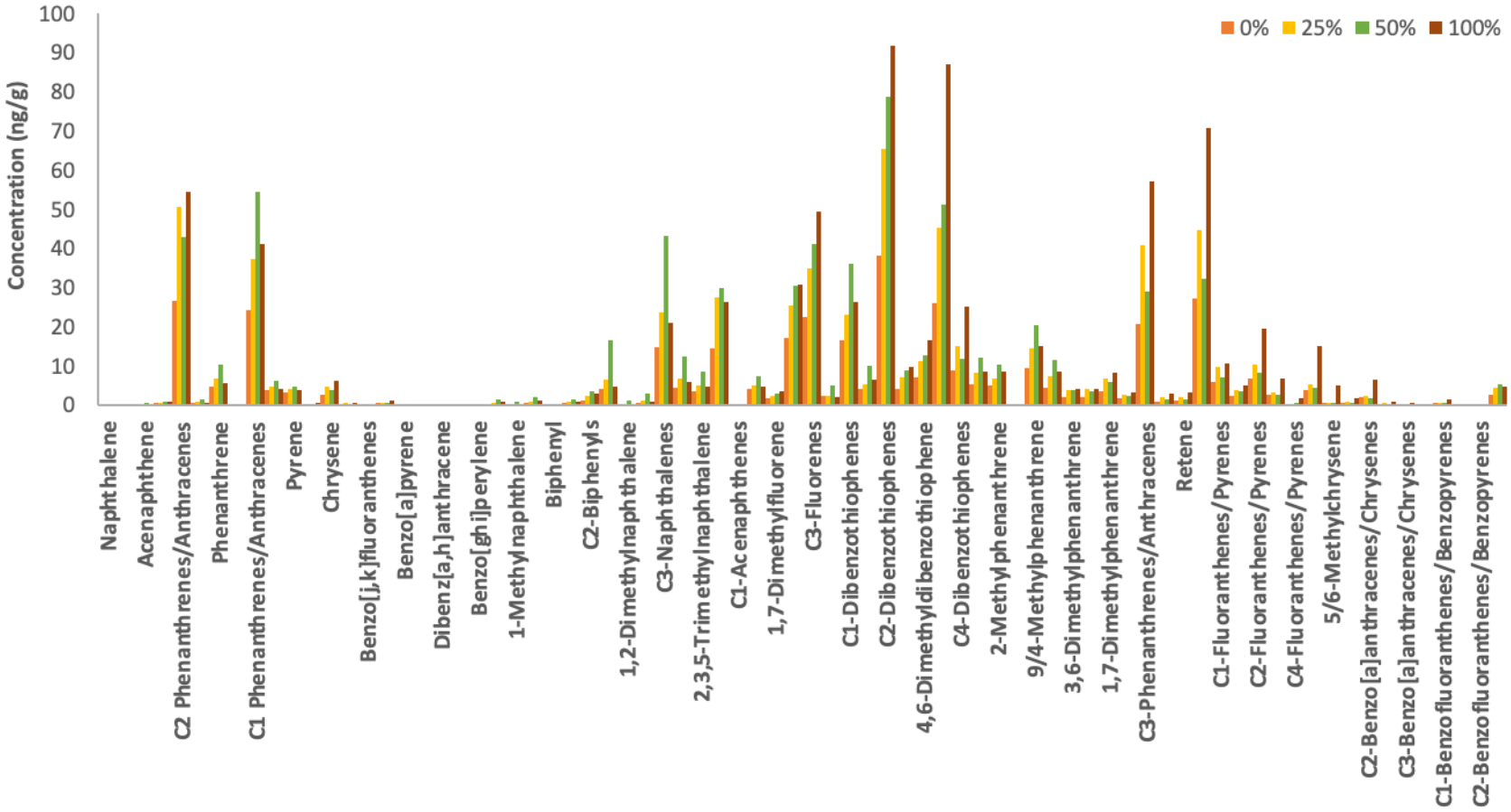

Figure A.18. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-crude oil exposed oysters on Day 21.

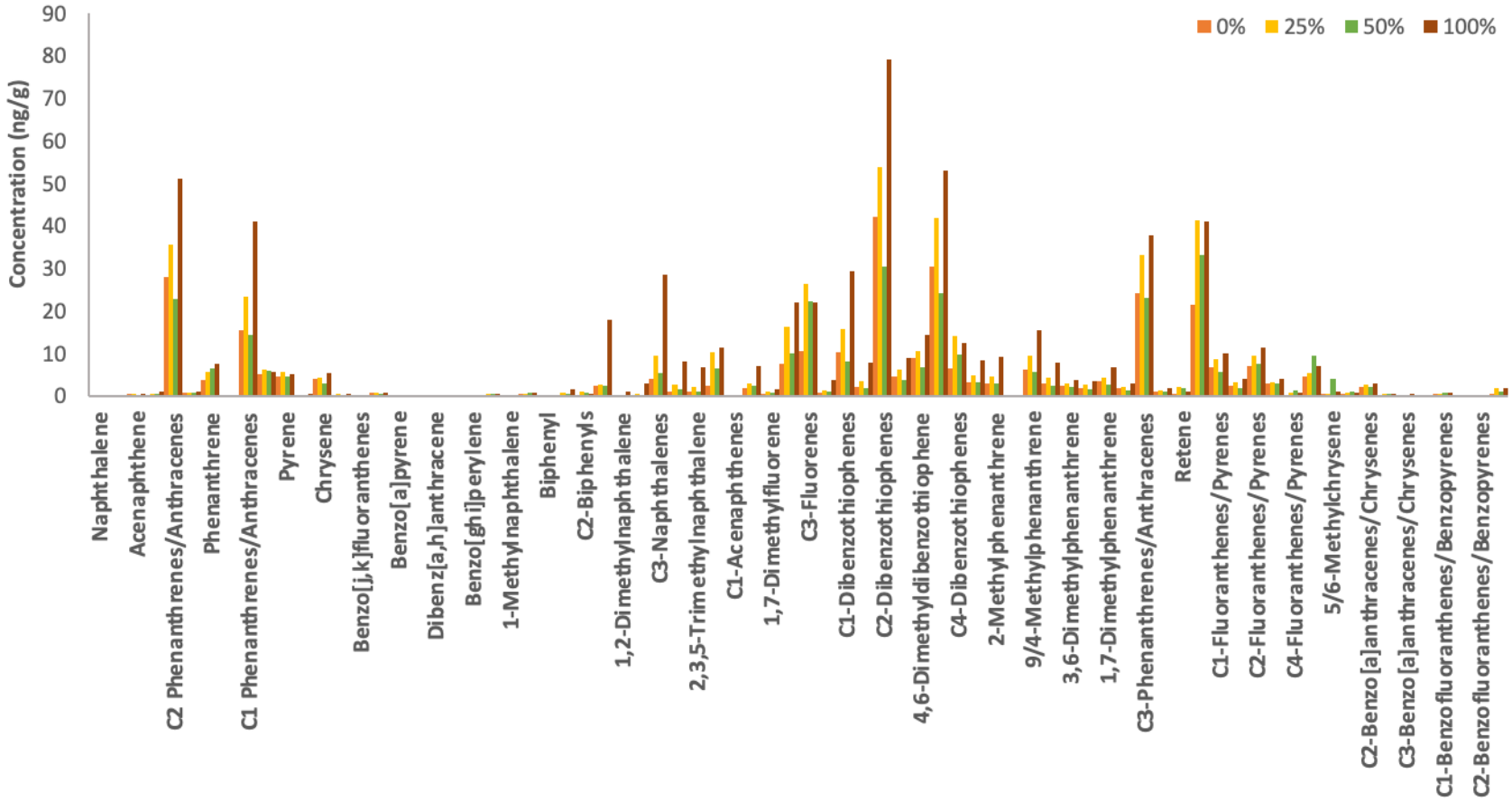

Figure A.19. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-crude oil exposed oysters on Day 35.

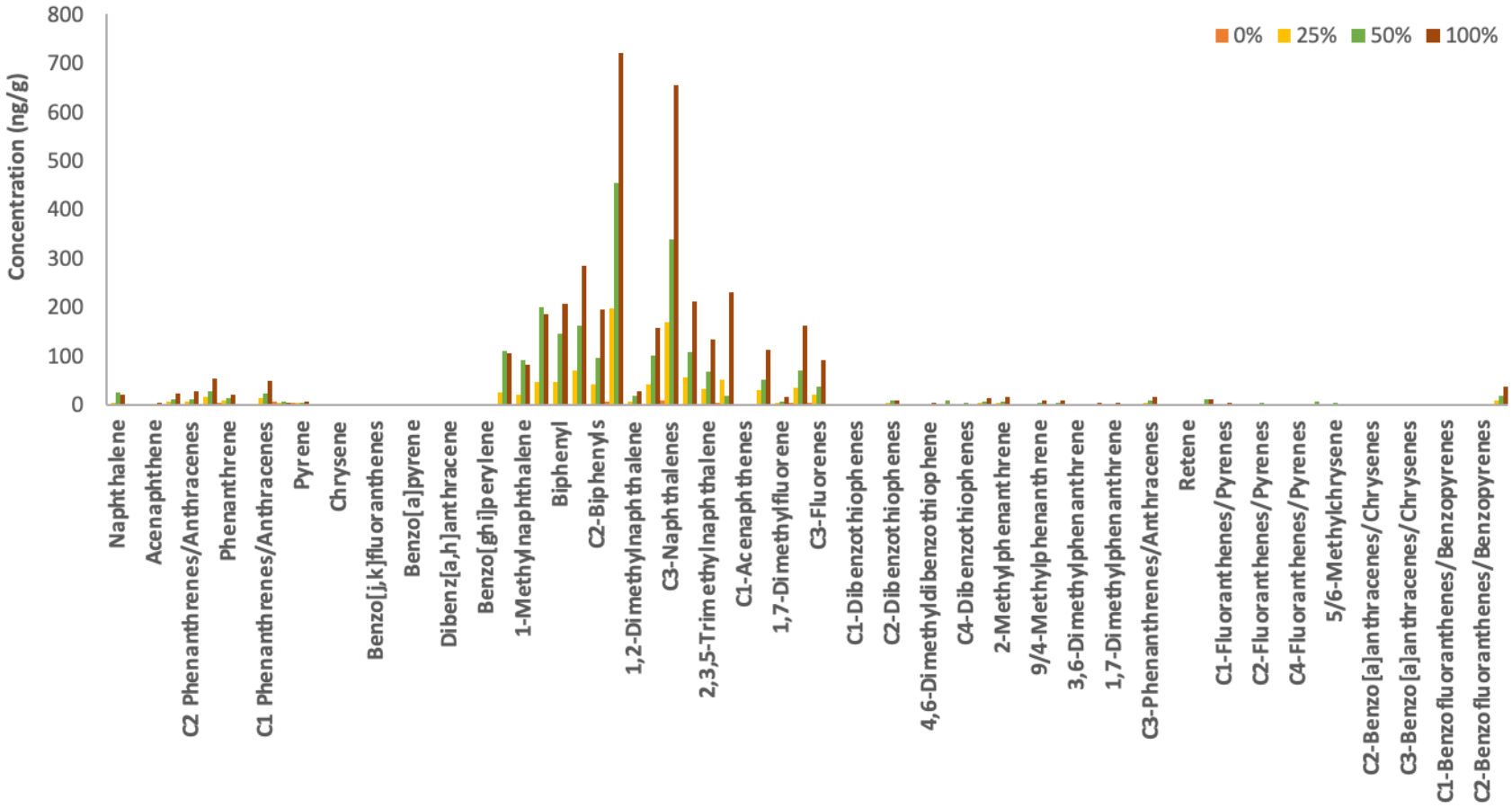

Figure A.20. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-marine diesel exposed oysters on Day 7.

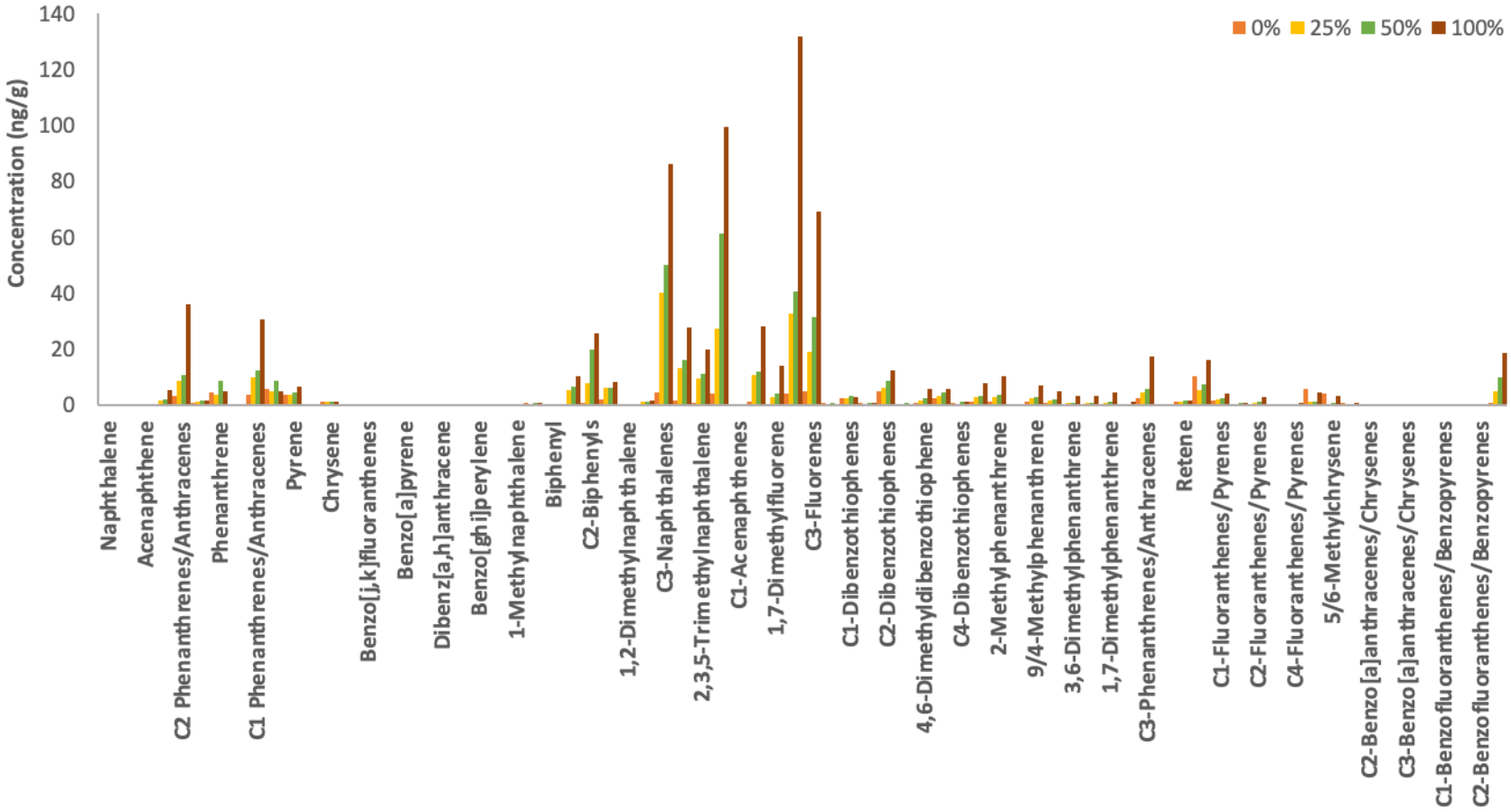

Figure A.21. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-marine diesel exposed oysters on Day 21.

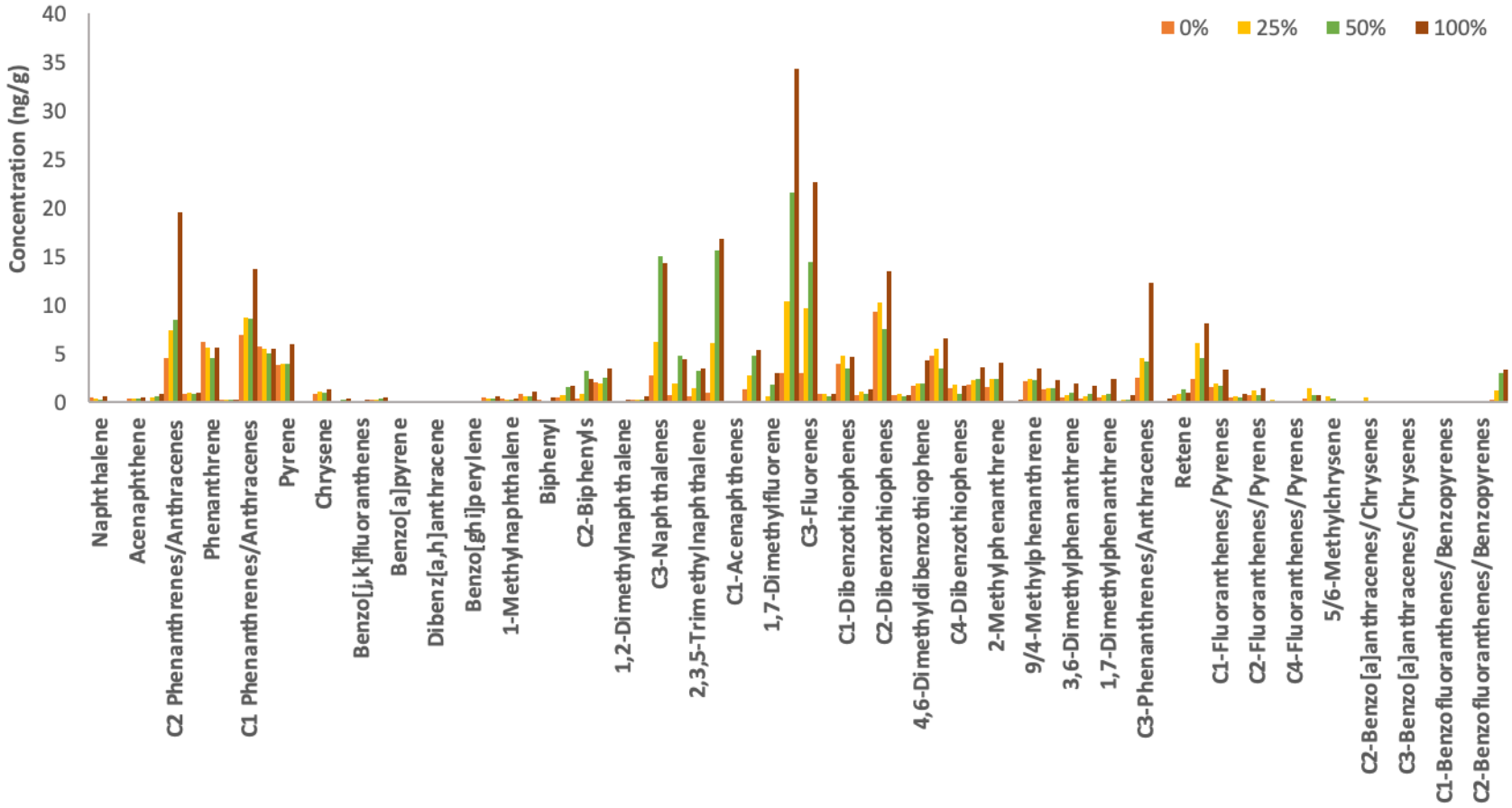

Figure A.22. PAC profile of tissue concentrations (0%, 25%, 50%, 100%) in ng/g post-marine diesel exposed oysters on Day 35.

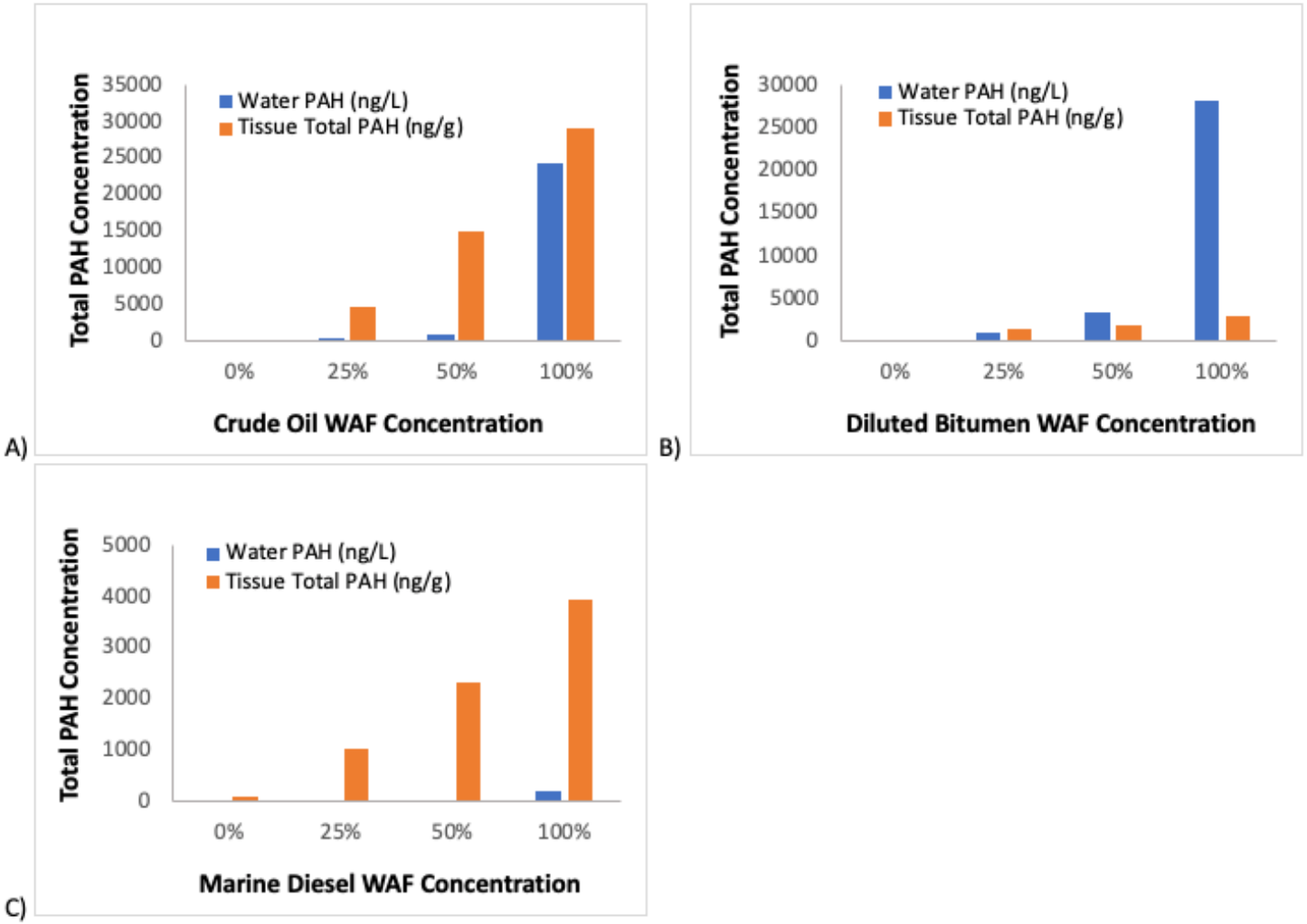

Figure A.23. The bioconcentration of PAH in oyster tissue (ng/g) based on the water concentration (ng/L) on day 7 of the exposure to A) crude oil, B) diluted bitumen, and C) marine diesel.

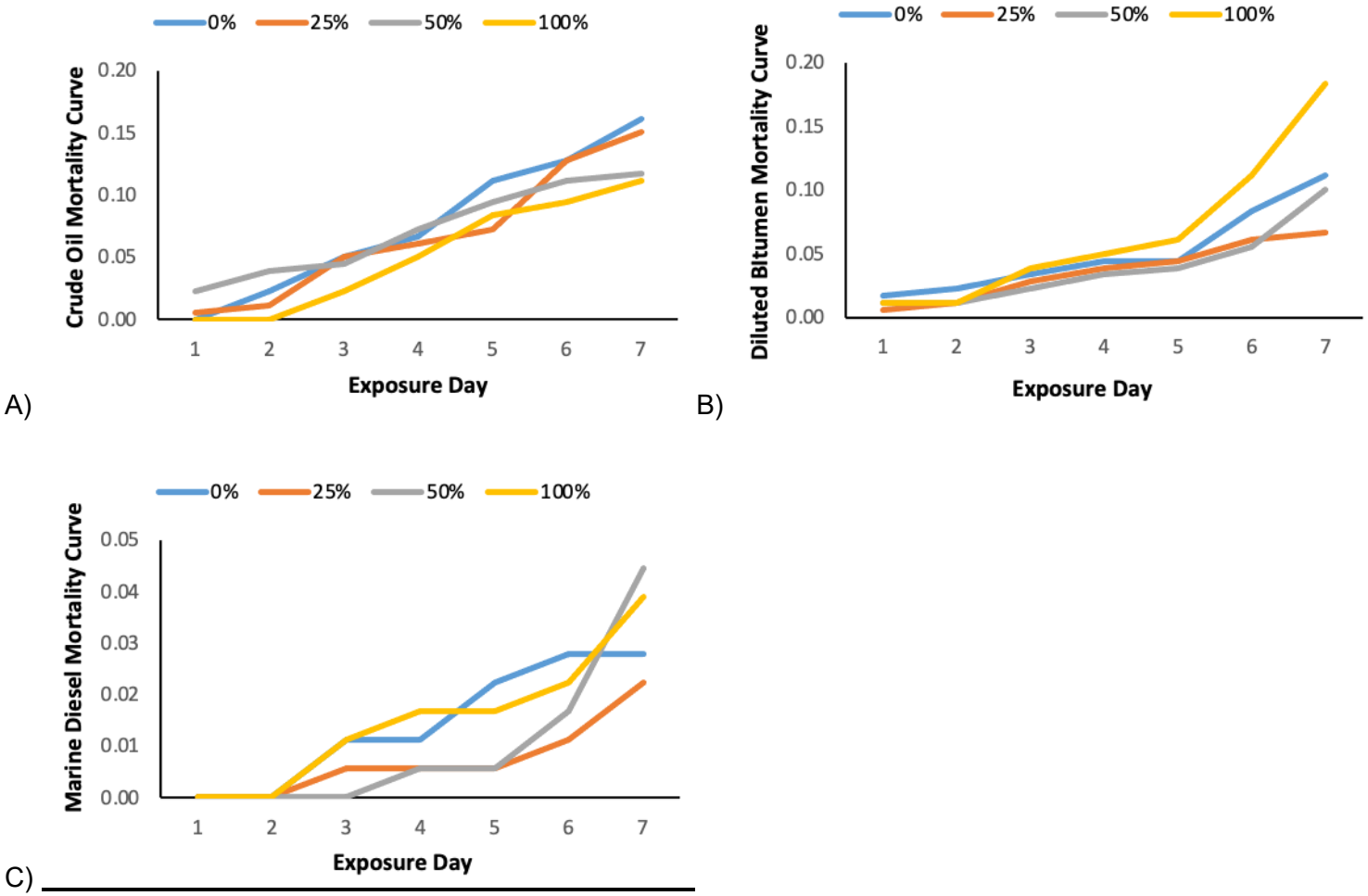

Figure A.24. Cumulative curve for percent mortality of Pacific oysters exposed to 0%, 25%, 50%, and 100% A) crude oil, B) diluted bitumen, and C) marine diesel WAF concentrations during a 7-d exposure.

Tables

Table A.1. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed clearance rate (L/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.75	0.61	4	0.31
25% - 7d	2.39	0.64	4	0.32
50% - 7d	1.74	0.42	4	0.21
100% - 7d	1.83	1.15	4	0.58
0% - 21d	1.76	0.76	4	0.38
25% - 21d	1.84	0.47	4	0.24
50% - 21d	1.99	0.68	4	0.34
100% - 21d	1.97	0.39	4	0.20
0% - 35d	3.03	1.37	4	0.68
25% - 35d	3.51	0.56	4	0.28
50% - 35d	2.27	0.11	3	0.07
100% - 35d	2.60	0.88	4	0.44

Table A.2. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed clearance rate (L/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21,and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.53	0.85	4	0.43
25% - 7d	1.58	0.51	4	0.25
50% - 7d	1.46	0.63	4	0.32
100% - 7d	2.02	0.44	4	0.22
0% - 21d	1.96	0.51	4	0.26
25% - 21d	2.13	0.82	4	0.41
50% - 21d	1.99	0.49	4	0.25
100% - 21d	2.84	0.74	4	0.37
0% - 35d	2.91	1.08	4	0.54
25% - 35d	2.54	1.85	3	1.07
50% - 35d	2.28	0.53	4	0.26
100% - 35d	2.72	0.70	4	0.35

Table A.3. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed clearance rate (L/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.10	0.51	4	0.25
25% - 7d	1.34	0.09	4	0.04
50% - 7d	1.00	0.41	4	0.21
100% - 7d	1.23	0.44	4	0.22
0% - 21d	1.08	0.06	4	0.03
25% - 21d	2.37	0.38	4	0.19
50% - 21d	2.37	0.35	4	0.17
100% - 21d	1.99	0.28	4	0.14
0% - 35d	2.28	0.63	4	0.31
25% - 35d	1.74	0.58	4	0.29
50% - 35d	1.92	0.47	4	0.24
100% - 35d	1.83	0.28	4	0.14

Table A.4. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average oxygen consumption (µmol O_2/h/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	18.98	3.15	4	1.57
25% - 7d	25.02	2.73	4	1.36
50% - 7d	21.07	3.44	4	1.72
100% - 7d	16.73	4.09	4	2.05
0% - 21d	10.86	2.11	4	1.05
25% - 21d	16.53	5.18	4	2.59
50% - 21d	15.99	8.04	4	4.02
100% - 21d	12.78	3.32	4	1.66
0% - 35d	18.03	1.01	4	0.51
25% - 35d	20.62	4.69	4	2.34
50% - 35d	25.52	6.68	4	3.34
100% - 35d	11.32	2.86	4	1.43

Table A.5. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average oxygen consumption (µmol O_2/h/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	19.93	13.19	4	6.60
25% - 7d	24.02	6.94	4	3.47
50% - 7d	23.35	5.18	4	2.59
100% - 7d	24.34	9.18	4	4.59
0% - 21d	21.11	8.18	4	4.09
25% - 21d	23.03	7.51	4	3.76
50% - 21d	21.07	7.10	4	3.55
100% - 21d	21.72	6.58	4	3.29
0% - 35d	18.44	7.38	4	3.69
25% - 35d	11.79	8.41	4	4.20
50% - 35d	19.09	8.72	4	4.36
100% - 35d	15.63	5.26	4	2.63

Table A.6. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average oxygen consumption (µmol O_2/h/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	18.14	3.44	4	1.72
25% - 7d	13.96	5.15	4	2.57
50% - 7d	12.68	1.60	4	0.80
100% - 7d	11.28	6.40	4	3.20
0% - 21d	14.71	6.44	4	3.22
25% - 21d	16.12	7.52	4	3.76
50% - 21d	18.25	5.29	4	2.65
100% - 21d	13.18	3.92	4	1.96
0% - 35d	21.85	3.83	4	1.92
25% - 35d	20.33	6.11	4	3.05
50% - 35d	16.19	5.48	4	2.74
100% - 35d	16.94	0.53	4	0.26

Table A.7. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed scope for growth (J/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	3.66	0.74	4	0.37
25% - 7d	4.30	0.74	4	0.37
50% - 7d	3.56	0.50	4	0.25
100% - 7d	3.73	1.27	4	0.64
0% - 21d	3.69	0.83	4	0.41
25% - 21d	3.54	0.55	4	0.28
50% - 21d	3.86	0.71	4	0.35
100% - 21d	3.65	0.46	4	0.23
0% - 35d	5.01	1.46	4	0.73
25% - 35d	5.54	0.57	4	0.28
50% - 35d	4.29	0.17	3	0.10
100% - 35d	4.20	0.94	4	0.47

Table A.8. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed scope for growth (J/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	2.09	1.09	4	0.55
25% - 7d	2.44	0.70	4	0.35
50% - 7d	1.98	0.95	3	0.55
100% - 7d	2.24	0.57	4	0.28
0% - 21d	3.37	0.59	4	0.29
25% - 21d	3.42	1.01	4	0.51
50% - 21d	3.29	0.78	4	0.39
100% - 21d	4.26	0.80	4	0.40
0% - 35d	4.50	1.16	4	0.58
25% - 35d	4.02	1.94	3	1.12
50% - 35d	3.23	0.64	4	0.32
100% - 35d	4.24	0.76	4	0.38

Table A.9. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for natural log-transformed scope for growth (J/hr/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	2.43	0.72	4	0.36
25% - 7d	2.96	0.15	4	0.08
50% - 7d	2.32	0.74	4	0.37
100% - 7d	2.75	0.39	4	0.20
0% - 21d	2.68	0.13	4	0.06
25% - 21d	4.13	0.38	4	0.19
50% - 21d	4.16	0.35	4	0.18
100% - 21d	3.81	0.29	4	0.14
0% - 35d	4.08	0.73	4	0.36
25% - 35d	3.30	0.67	4	0.34
50% - 35d	3.74	0.49	4	0.24
100% - 35d	3.56	0.34	4	0.17

Table A.10. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average condition index (g/cm^3) values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.46	0.34	4	0.168
25% - 7d	1.91	0.90	4	0.451
50% - 7d	1.54	0.78	4	0.390
100% - 7d	1.51	0.40	4	0.198
0% - 21d	1.78	0.36	4	0.179
25% - 21d	1.28	0.11	4	0.056
50% - 21d	1.48	0.18	4	0.090
100% - 21d	1.26	0.35	4	0.174
0% - 35d	1.36	0.26	4	0.131
25% - 35d	1.33	0.27	4	0.133
50% - 35d	1.14	0.23	4	0.114
100% - 35d	1.62	0.26	4	0.129

Table A.11. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average condition index (g/cm^3) values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.10	0.35	4	0.177
25% - 7d	1.37	0.34	4	0.168
50% - 7d	1.15	0.32	4	0.162
100% - 7d	0.99	0.28	4	0.139
0% - 21d	0.90	0.16	4	0.080
25% - 21d	1.30	0.17	4	0.086
50% - 21d	1.07	0.20	4	0.099
100% - 21d	1.19	0.36	4	0.180
0% - 35d	1.03	0.16	4	0.080
25% - 35d	1.09	0.07	4	0.034
50% - 35d	0.95	0.29	4	0.146
100% - 35d	0.88	0.30	4	0.150

Table A.12. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average condition index (g/cm^3) values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.65	0.27	4	0.135
25% - 7d	1.79	0.06	4	0.030
50% - 7d	1.64	0.25	4	0.126
100% - 7d	1.63	0.32	4	0.162
0% - 21d	1.68	0.38	4	0.191
25% - 21d	1.75	0.45	4	0.223
50% - 21d	1.43	0.14	4	0.072
100% - 21d	1.68	0.35	4	0.175
0% - 35d	1.60	0.26	4	0.128
25% - 35d	1.70	0.27	4	0.135
50% - 35d	1.53	0.14	4	0.071
100% - 35d	1.69	0.09	3	0.050

Table A.13. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for mean health assessment index (scope for growth analysis) values of Pacific oysters used for scope for growth analysis exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.00	0.00	3	0.00
25% - 7d	0.13	0.25	4	0.13
50% - 7d	0.25	0.29	4	0.14
100% - 7d	0.75	0.65	4	0.32
0% - 21d	0.38	0.25	4	0.13
25% - 21d	0.75	0.50	4	0.25
50% - 21d	0.63	0.25	4	0.13
100% - 21d	0.63	0.25	4	0.13
0% - 35d	0.00	0.00	4	0.00
25% - 35d	0.38	0.25	4	0.13
50% - 35d	0.67	0.29	3	0.17
100% - 35d	0.13	0.25	4	0.13

Table A.14. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average health assessment index (scope for growth analysis) values of Pacific oysters used for scope for growth analysis exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.13	0.25	4	0.13
25% - 7d	1.00	0.71	4	0.35
50% - 7d	1.25	0.96	4	0.48
100% - 7d	1.88	0.75	4	0.38
0% - 21d	0.50	0.41	4	0.20
25% - 21d	0.75	0.29	4	0.14
50% - 21d	1.50	0.58	4	0.29
100% - 21d	1.38	0.75	4	0.38
0% - 35d	0.88	0.48	4	0.24
25% - 35d	1.13	0.63	4	0.31
50% - 35d	1.75	0.96	4	0.48
100% - 35d	1.88	0.63	4	0.31

Table A.15. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average health assessment index (scope for growth analysis) values of Pacific oysters used for scope for growth analysis exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	2.25	1.19	4	0.60
25% - 7d	2.75	0.29	4	0.14
50% - 7d	2.13	0.95	4	0.47
100% - 7d	2.50	0.71	4	0.35
0% - 21d	1.63	0.48	4	0.24
25% - 21d	1.25	0.65	4	0.32
50% - 21d	1.63	0.48	4	0.24
100% - 21d	1.38	0.25	4	0.13
0% - 35d	1.00	0.71	4	0.35
25% - 35d	1.38	0.63	4	0.31
50% - 35d	1.63	0.95	4	0.47
100% - 35d	1.75	1.19	4	0.60

Table A.16. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average gonadal thickness/wet weight (µm/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	523.80	163.95	4	81.98
25% - 7d	489.74	261.75	4	130.87
50% - 7d	549.05	44.24	4	22.12
100% - 7d	592.80	181.16	4	90.58
0% - 21d	694.38	193.81	4	96.90
25% - 21d	645.59	73.03	4	36.52
50% - 21d	594.24	178.97	4	89.49
100% - 21d	645.36	168.53	4	84.27
0% - 35d	618.55	161.53	4	80.77
25% - 35d	677.45	34.64	4	17.32
50% - 35d	636.89	195.78	4	97.89
100% - 35d	473.36	140.56	4	70.28

Table A.17. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average gonadal thickness/wet weight (µm/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	609.82	189.94	4	94.97
25% - 7d	674.55	198.16	4	99.08
50% - 7d	622.31	117.25	4	58.62
100% - 7d	794.75	192.14	4	96.07
0% - 21d	902.53	231.50	4	115.75
25% - 21d	695.89	132.01	4	66.00
50% - 21d	933.04	187.22	4	93.61
100% - 21d	739.68	316.36	4	158.18
0% - 35d	910.17	473.63	4	236.81
25% - 35d	793.80	320.71	4	160.36
50% - 35d	603.83	275.74	4	137.87
100% - 35d	666.75	323.51	4	161.76

Table A.18. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average gonadal thickness/wet weight (µm/g) values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	375.98	102.53	4	51.26
25% - 7d	274.33	65.36	4	32.68
50% - 7d	251.70	72.13	4	36.07
100% - 7d	212.10	86.97	4	43.49
0% - 21d	291.48	115.25	4	57.63
25% - 21d	264.17	35.27	4	17.64
50% - 21d	378.20	64.64	4	32.32
100% - 21d	347.45	123.61	4	61.80
0% - 35d	331.07	153.03	4	76.51
25% - 35d	383.67	197.70	4	98.85
50% - 35d	410.22	125.11	4	62.56
100% - 35d	351.79	99.25	4	49.62

Table A.19. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average lumen/digestive tubule thickness values of Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.34	0.004	4	0.002
25% - 7d	0.34	0.025	4	0.012
50% - 7d	0.31	0.020	4	0.010
100% - 7d	0.30	0.016	4	0.008
0% - 21d	0.36	0.030	4	0.015
25% - 21d	0.28	0.028	4	0.014
50% - 21d	0.29	0.005	4	0.003
100% - 21d	0.28	0.027	4	0.013
0% - 35d	0.32	0.053	4	0.026
25% - 35d	0.31	0.037	4	0.019
50% - 35d	0.30	0.035	4	0.018
100% - 35d	0.29	0.030	4	0.015

Table A.20. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average lumen/digestive tubule thickness values of Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration % - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.29	0.063	4	0.032
25% - 7d	0.26	0.025	4	0.013
50% - 7d	0.24	0.043	4	0.021
100% - 7d	0.21	0.013	4	0.006
0% - 21d	0.29	0.035	4	0.018
25% - 21d	0.20	0.039	4	0.019
50% - 21d	0.27	0.042	4	0.021
100% - 21d	0.26	0.024	4	0.012
0% - 35d	0.29	0.056	4	0.028
25% - 35d	0.29	0.057	4	0.029
50% - 35d	0.28	0.059	4	0.030
100% - 35d	0.28	0.059	4	0.030

Table A.21. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for average lumen/digestive tubule thickness values of Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.34	0.029	4	0.014
25% - 7d	0.35	0.023	4	0.012
50% - 7d	0.30	0.025	4	0.013
100% - 7d	0.27	0.018	4	0.009
0% - 21d	0.30	0.032	4	0.016
25% - 21d	0.31	0.018	4	0.009
50% - 21d	0.30	0.052	4	0.026
100% - 21d	0.32	0.030	4	0.015
0% - 35d	0.33	0.046	4	0.023
25% - 35d	0.30	0.064	4	0.032
50% - 35d	0.32	0.003	4	0.001
100% - 35d	0.30	0.034	4	0.017

Table A.22. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for mean health assessment index (gonad histopathological analysis) values of Pacific oysters used for histopathological analysis exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.63	0.48	4	0.24
25% - 7d	0.75	0.65	4	0.32
50% - 7d	0.88	0.48	4	0.24
100% - 7d	1.00	0.82	4	0.41
0% - 21d	0.50	0.41	4	0.20
25% - 21d	0.88	0.25	4	0.13
50% - 21d	1.13	0.63	4	0.31
100% - 21d	1.13	0.85	4	0.43
0% - 35d	0.75	0.29	4	0.14
25% - 35d	0.50	0.41	4	0.20
50% - 35d	1.25	0.87	4	0.43
100% - 35d	0.88	0.85	4	0.43

Table A.23. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for mean health assessment index (gonad histopathological analysis) values of Pacific oysters used for histopathological analysis exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.75	0.50	4	0.25
25% - 7d	1.38	0.48	4	0.24
50% - 7d	1.13	1.03	4	0.52
100% - 7d	1.75	0.87	4	0.43
0% - 21d	0.63	0.63	4	0.31
25% - 21d	0.88	0.25	4	0.13
50% - 21d	1.88	0.48	4	0.24
100% - 21d	1.63	0.95	4	0.47
0% - 35d	0.88	0.75	4	0.38
25% - 35d	1.13	0.25	4	0.13
50% - 35d	1.50	0.41	4	0.20
100% - 35d	1.75	0.65	4	0.32

Table A.24. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for mean health assessment index (gonad histopathological analysis) values of Pacific oysters used for histopathological analysis exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) - Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	1.75	0.96	4	0.48
25% - 7d	1.63	0.75	4	0.38
50% - 7d	1.75	0.65	4	0.32
100% - 7d	2.88	1.18	4	0.59
0% - 21d	1.63	0.48	4	0.24
25% - 21d	1.25	0.65	4	0.32
50% - 21d	1.63	0.48	4	0.24
100% - 21d	1.38	0.25	4	0.13
0% - 35d	0.88	0.63	4	0.31
25% - 35d	1.25	0.50	4	0.25
50% - 35d	1.38	0.48	4	0.24
100% - 35d	1.25	1.04	4	0.52

Table A.25. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for development proportion of Pacific oyster larvae exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration (%)	Mean	Standard Deviation	Sample Size	SEM
0%	0.43	0.24	5	0.11
25%	0.49	0.26	5	0.12
50%	0.32	0.21	5	0.09
75%	0.24	0.14	5	0.06
100%	0.26	0.15	5	0.07

Table A.26. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for development proportion of Pacific oyster larvae exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration (%)	Mean	Standard Deviation	Sample Size	SEM
0%	0.36	0.19	5	0.08
25%	0.35	0.15	5	0.07
50%	0.31	0.10	5	0.04
75%	0.25	0.12	5	0.06
100%	0.21	0.07	5	0.03

Table A.27. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for development proportion of Pacific oyster larvae exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%)	Mean	Standard Deviation	Sample Size	SEM
0%	0.40	0.20	5	0.09
25%	0.34	0.18	5	0.08
50%	0.22	0.12	5	0.05
75%	0.22	0.12	5	0.06
100%	0.19	0.13	5	0.06

Table A.28. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/L) in the water samples collected from 0%, 25%, 50%, 100% crude oil WAFs on sampling days 0, 3, and 7 of the exposure period are presented.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/L)	$\sum_{PARENT}$PAH (ng/L)	$\sum_{ALKYL}$PAH (ng/L)	$\sum_{DBT}$PAH (ng/L)
0	0 day	485.46	55.591	429.869	66.31
25	0 day	147684.536	38618.401	109066.135	3780.1
50	0 day	240165.356	26016.141	214149.215	8347
100	0 day	353620.476	20684.901	332935.575	15266
0	3 day	89.69	8.925	80.765	21.844
25	3 day	12537.266	420.511	12116.755	3707.6
50	3 day	18587.171	5664.776	12922.395	944.1
100	3 day	145821.896	16434.421	129387.475	23080
0	7 day	59.366	4.761	54.605	6.577
25	7 day	282.253	14.626	267.627	55.2
50	7 day	625.639	22.714	602.925	105.45
100	7 day	24182.856	6520.311	17662.545	3844

Table A.29. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/L) in the water samples collected from 0%, 25%, 50%, 100% diluted bitumen WAFs on sampling days 0, 3, and 7 of the exposure period are presented.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/L)	$\sum_{PARENT}$PAH (ng/L)	$\sum_{ALKYL}$PAH (ng/L)	$\sum_{DBT}$PAH (ng/L)
0	0 day	58.961	9.377	49.584	2.376
25	0 day	32374.557	3155.877	29218.68	6430.866
50	0 day	60733.637	6133.557	54600.08	11703.866
100	0 day	127609.457	12790.427	114819.03	26561.866
0	3 day	390.19	59.85	330.34	59.616
25	3 day	14800.969	1252.489	13548.48	3940.666
50	3 day	32787.797	2323.997	30463.8	9574.866
100	3 day	70549.417	5346.087	65203.33	20657.866
0	7 day	96.843	6.019	90.824	20.112
25	7 day	1060.694	25.968	1034.726	382.639
50	7 day	3467.022	65.205	3401.817	1433.967
100	7 day	28132.807	1896.157	26236.65	8981.866

Table A.30. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/L) in the water samples collected from 0%, 25%, 50%, 100% marine diesel WAFs on sampling days 0, 3, and 7 of the exposure period are presented.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/L)	$\sum_{PARENT}$PAH (ng/L)	$\sum_{ALKYL}$PAH (ng/L)	$\sum_{DBT}$PAH (ng/L)
0	0 day	99.116	24.008	75.108	3.418
25	0 day	24207.33	4703.178	19504.152	12.401
50	0 day	52612.516	8863.853	43748.663	34.811
100	0 day	150880.268	18621.541	132258.727	174.291
0	3 day	1.506	0.572	0.934	0.567
25	3 day	1675.248	545.253	1129.995	8.354
50	3 day	2502.339	562.733	1939.606	14.669
100	3 day	10940.349	2076.465	8863.884	81.851
0	7 day	8.323	3.707	4.616	0.836
25	7 day	14.951	3.662	11.289	0.967
50	7 day	29.883	4.765	25.118	0
100	7 day	224.698	5.738	218.96	3.091

Table A.31. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/g) in the pooled oyster tissue samples exposed to 0%, 25%, 50%, 100% crude oil WAFs on sampling days 7, 21, and 35 post-exposure are presented. The samples were corrected based on the tissue wet weight.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/g)	$\sum_{PARENT}$PAH (ng/g)	$\sum_{ALKYL}$PAH (ng/g)	$\sum_{DBT}$PAH (ng/g)
0	7 day	117.924	21.788	96.136	21.85
25	7 day	4517.453	502.922	4014.531	350.40
50	7 day	14720.817	1639.078	13081.739	787.00
100	7 day	28792.06	3551.37	25240.69	1877.30
0	21 day	381.745	19.797	361.948	107.43
25	21 day	620.003	26.458	593.545	175.41
50	21 day	721.341	35.23	686.111	214.96
100	21 day	821.792	26.501	795.291	265.55
0	35 day	307.445	21.777	285.668	105.38
25	35 day	463.731	27.059	436.672	147.76
50	35 day	325.615	25.038	300.577	86.22
100	35 day	652.031	32.242	619.789	209.20

Table A.32. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/g) in the pooled oyster tissue samples exposed to 0%, 25%, 50%, 100% diluted bitumen WAFs on sampling days 7, 21, and 35 post-exposure are presented. The samples were corrected based on the tissue wet weight.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/g)	$\sum_{PARENT}$PAH (ng/g)	$\sum_{ALKYL}$PAH (ng/g)	$\sum_{DBT}$PAH (ng/g)
0	7 day	67.96	14.28	53.68	14.73
25	7 day	1470.00	82.74	1387.26	304.31
50	7 day	1921.33	139.88	1781.45	345.85
100	7 day	2936.34	130.83	2805.50	635.63
0	21 day	278.00	21.09	256.91	90.06
25	21 day	636.37	33.45	602.92	203.94
50	21 day	593.27	23.01	570.25	220.68
100	21 day	588.60	20.47	568.13	233.42
0	35 day	212.13	25.06	187.07	67.34
25	35 day	172.78	12.17	160.61	59.36
50	35 day	305.82	20.34	285.48	113.18
100	35 day	229.58	32.79	196.80	63.94

Table A.33. The total, parent, alkylated, and dibenzothiophene PAH concentrations (ng/g) in the pooled oyster tissue samples exposed to 0%, 25%, 50%, 100% marine diesel WAFs on sampling days 7, 21, and 35 post-exposure are presented. The samples were corrected based on the tissue wet weight.

WAF (%)	Time	$\sum_{TOTAL}$PAH (ng/g)	$\sum_{PARENT}$PAH (ng/g)	$\sum_{ALKYL}$PAH (ng/g)	$\sum_{DBT}$PAH (ng/g)
0	7 day	94.14	20.28	73.86	7.82
25	7 day	1020.62	91.00	929.62	13.37
50	7 day	2322.36	231.84	2090.52	27.97
100	7 day	3949.80	319.58	3630.22	18.98
0	21 day	102.47	19.40	83.07	14.01
25	21 day	267.79	17.47	250.32	16.08
50	21 day	387.75	28.46	359.29	22.81
100	21 day	766.00	22.16	743.84	29.62
0	35 day	90.88	20.91	69.96	23.75
25	35 day	138.99	20.28	118.71	27.19
50	35 day	174.52	18.14	156.38	19.38
100	35 day	258.65	23.82	234.83	33.59

Table A.34. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for absorption efficiency values for Pacific oysters exposed to 0%, 25%, 50%, 100% crude oil concentrations at 7, 21, and 35 recovery days.

Concentration (%) – Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.7895	0.1484	8	0.05
25% - 7d	0.7397	0.1794	8	0.06
50% - 7d	0.7368	0.3143	6	0.13
100% - 7d	0.7655	0.2533	7	0.10
0% - 21d	0.7317	0.2330	7	0.09
25% - 21d	0.6310	0.2997	5	0.13
50% - 21d	0.6960	0.2516	7	0.10
100% - 21d	0.5842	0.3509	7	0.13
0% - 35d	0.7377	0.2029	7	0.08
25% - 35d	0.7377	0.2223	8	0.08
50% - 35d	0.8032	0.1993	7	0.08
100% - 35d	0.5033	0.2708	8	0.10

Table A.35. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for absorption efficiency values for Pacific oysters exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations at 7, 21, and 35 recovery days.

Concentration (%) – Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.4708	0.3190	6	0.13
25% - 7d	0.6149	0.3264	6	0.13
50% - 7d	0.4771	0.2460	6	0.10
100% - 7d	0.3535	0.2076	6	0.08
0% - 21d	0.7211	0.2228	6	0.09
25% - 21d	0.6734	0.2502	7	0.09
50% - 21d	0.7028	0.3206	6	0.13
100% - 21d	0.6321	0.2552	7	0.10
0% - 35d	0.7291	0.3468	7	0.13
25% - 35d	0.6483	0.3586	7	0.14
50% - 35d	0.4661	0.3143	8	0.11
100% - 35d	0.6818	0.2185	7	0.08

Table A.36. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for absorption efficiency values for Pacific oysters exposed to 0%, 25%, 50%, 100% marine diesel concentrations at 7, 21, and 35 recovery days.

Concentration (%) – Recovery Day	Mean	Standard Deviation	Sample Size	SEM
0% - 7d	0.7464	0.2712	7	0.10
25% - 7d	0.7483	0.2401	6	0.10
50% - 7d	0.6905	0.2003	6	0.08
100% - 7d	0.6598	0.2025	8	0.07
0% - 21d	0.8110	0.1478	8	0.05
25% - 21d	0.7192	0.2540	8	0.09
50% - 21d	0.7524	0.2296	8	0.08
100% - 21d	0.7785	0.2616	8	0.09
0% - 35d	0.8017	0.2489	8	0.09
25% - 35d	0.7164	0.3031	8	0.11
50% - 35d	0.8041	0.2332	8	0.08
100% - 35d	0.7739	0.2863	8	0.10

Table A.37. Results summary (p-values) of the concentration, recovery period, and interaction effects for clearance rate, rate of oxygen uptake, scope for growth, condition index, health assessment index of Pacific oysters (*Crassostrea gigas*) exposed to crude oil, diluted bitumen, and marine diesel over recovery period days 7, 21, and 35.

Petroleum	Endpoint	Concentration	Recovery Period	Interaction Effect
Crude Oil	Clearance Rate	0.3010	**0.0015***	0.8066
Diluted Bitumen	Clearance Rate	0.3162	**0.0089***	0.9625
Marine Diesel	Clearance Rate	0.1561	**<0.0001***	**0.0131***
Crude Oil	Rate of Oxygen Uptake	**<0.0001***	**0.0002***	0.1646
Diluted Bitumen	Rate of Oxygen Uptake	0.9701	0.0595	0.8588
Marine Diesel	Rate of Oxygen Uptake	0.1965	**0.0320***	0.5743
Crude Oil	Scope for Growth	0.2779	**0.0018***	0.7532
Diluted Bitumen	Scope for Growth	0.3092	**<0.0001***	0.7902
Marine Diesel	Scope for Growth	0.0979	**<0.0001***	**0.0107***
Crude Oil	Condition Index	0.7898	0.2796	0.3096
Diluted Bitumen	Condition Index	0.1108	0.2080	0.6630
Marine Diesel	Condition Index	0.6357	0.8857	0.9668
Crude Oil	Health Assessment Index	0.1371	**0.0216***	0.1209
Diluted Bitumen	Health Assessment Index	**0.0054***	0.2533	0.8725
Marine Diesel	Health Assessment Index	0.7172	**0.0006***	0.6036

Table A.38. Results summary (p-values) of the concentration, recovery period, and interaction effects for gonad thickness relative to wet weight, digestive lumen relative to tubule thickness, health assessment index, and larvae development success (one-way ANOVA) of Pacific oysters (*Crassostrea gigas*) exposed to crude oil, diluted bitumen, and marine diesel over recovery period days 7, 21, and 35.

Petroleum	Endpoint	Concentration	Recovery Period	Interaction Effect
Crude Oil	Gonad/Wet Weight	0.9679	0.2011	0.5571
Diluted Bitumen	Gonad/Wet Weight	0.8742	0.3790	0.7052
Marine Diesel	Gonad/Wet Weight	0.6235	0.2534	0.2374
Crude Oil	Digestive Lumen/Tubule	**0.0045***	0.1057	0.0806
Diluted Bitumen	Digestive Lumen/Tubule	0.0627	0.0516	0.1751
Marine Diesel	Digestive Lumen/Tubule	**0.0225***	0.8961	0.1039
Crude Oil	Health Assessment Index	0.2297	0.9209	0.9375
Diluted Bitumen	Health Assessment Index	**0.0043***	0.9484	0.6732
Marine Diesel	Health Assessment Index	0.3790	**0.0130***	0.5755
Crude Oil	Larvae Development	0.2768	-	-
Diluted Bitumen	Larvae Development	0.3330	-	-
Marine Diesel	Larvae Development	0.1992	-	-

Table A.39. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for proportion of Pacific oyster mortality exposed to 0%, 25%, 50%, 100% crude oil concentrations.

CO WAF (%)	Mean	Standard Dev	Sample Size	Standard Error of Mean
0	0.16	0.08	12	0.02
25	0.15	0.11	12	0.03
50	0.12	0.06	12	0.02
100	0.11	0.07	12	0.02

Table A.40. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for proportion of Pacific oyster mortality exposed to 0%, 25%, 50%, 100% diluted bitumen concentrations.

DB WAF (%)	Mean	Standard Dev	Sample Size	Standard Error of Mean
0	0.11	0.07	12	0.02
25	0.07	0.05	12	0.01
50	0.10	0.08	12	0.02
100	0.18	0.11	12	0.03

Table A.41. The mean, standard deviation, standard error of means, and sample size (exclusive of outliers) presented for proportion of Pacific oyster mortality exposed to 0%, 25%, 50%, 100% marine diesel concentrations.

MD WAF (%)	Mean	Standard Dev	Sample Size	Standard Error of Mean
0	0.03	0.04	12	0.01
25	0.02	0.03	12	0.01
50	0.04	0.05	12	0.01
100	0.04	0.04	12	0.01

Table A.42. The recipe for Guillard's f/2 enrichment solution for growth of the *Isochyrisis galbana* culture.

Component	Stock Solution	Quantity	Molar Concentration
$NaNO_3$	75 g/L dH_2O	1 mL	8.82×10^{-4} M
$NaH_2PO_4.H_2O$	5 g/L dH_20	1 mL	3.62×10^{-5} M
$Na_2SiO_3.5H_2O$	22.4 g/L dH_2O	1 mL	1.06×10^{-4} M
Trace metal solution	Recipe Below	1 mL	-
Vitamin solution	Recipe Below	0.5 mL	-

Table A.43. The recipe for f/2 vitamin solution for growth of the *Isochyrisis galbana* culture.

Component	Stock Solution	Quantity	Molar Concentration
Thiamine HCl (Vit. B_1)	-	200 mg	2.96×10^{-7} M
Biotin (Vit. H)	0.1 g/L dH_2O	10 mL	2.05×10^{-9} M
Cyanocobalamin (Vit. B_{12})	1.0 g/L dH_2O	1 mL	3.69×10^{-10} M

Table A.44. The recipe for the trace metal solution for growth of the *Isochyrisis galbana* culture.

Component	Stock Solution	Quantity	Molar Concentration
FeCl3	-	1.89 g	1.17×10^{-5} M
$Na_2EDTA.2H_2O$	-	4.36 g	1.17×10^{-5} M
$CuSO_4.5H_2O$	9.8 g/L dH_2O	1 mL	3.93×10^{-8} M
$Na_2MoO_4.2H_2O$	6.3 g/L dH_2O	1 mL	2.60×10^{-8} M
$ZnSO_4.7H_2O$	22.0 g/L dH_2O	1 mL	7.65×10^{-8} M
$CoCl_2.6H_2O$	10.0 g/L dH_2O	1 mL	4.20×10^{-8} M
$MnCl_2$	114.5 g/L dH_2O	1 mL	9.10×10^{-7} M

Table A.45. Scoring criteria for the oyster health assessment index of each selected variable based on the severity of abnormality.

Variable	Variable Condition	HAI Score
Mantle Tentacle (Papilla) Responsiveness	Quick movement upon stimulus	0
	Slight movement upon stimulus	1
	Slow movement upon stimulus	2
	No movement - Discolored	3
Mantle Tissue Firmness	Mantle Tissue is Firm and Dark Colored	0
	Mantle Tissue is Thin and Colored	1
	Mantle Tissue is Thinner and Opaque	2
	Mantle Tissue is Absent and Translucent	3
Digestive Glands	Brown Reddish Color – Oval Shaped	0
	Light Brown Colored	1
	Cream Colored	2
	Irregular Shaped - Discolored	3
Gonads	Large white tissue mass	0
	Gonadal Tissue with slight discoloration (Darker)	1
	Gonadal Tissue with prevalent discoloration (Darker)	2
	Irregular with extreme discoloration/translucent	3
Tissue Prevalence in Shell Cavity	Large tissue inside shell	0
	Slightly reduced tissue inside shell	1
	Extremely reduced tissue inside shell	2
	Non-existent tissue inside shell	3
Parasite Load	No observed parasites	0
	Few observed parasites	1
	Moderate parasite infestation	2
	Numerous parasites	3
Mottled or Granular Tissue	Normal tissue with no spots or granular texture	0
	Tissue with few light spots or granular texture	1
	Tissue with prevalent darker spots or granular texture	2
	Tissue completely covered with spots or granular texture	3
Tissue Degeneration or Inflammation	Normal tissue with no degeneration or swelling	0
	Tissue with slight degeneration or swelling	1
	Tissue with prevalent degeneration or swelling	2
	Tissue with extreme degeneration or swelling	3

Table A.46. The concentration (ng/L) for all the PAH components for the 0%, 25%, 50%, 100% WAF samples of DB, CO, and MD measured on exposure day 0, 3, and 7.

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Naphthalene	0	0	1.61	10.86	22.4
Acenaphthylene	0	0	0.314	0.239	1.28
Acenaphthene	0	0	0.482	1.03	1.53
2-Methylfluorene	0	0	0.189	0.262	2.62
C2 Phenanthrenes/Anthracenes	0	0	0.204	0.625	15.6
Fluorene	0	0	0.809	1.65	3.45
Phenanthrene	0	0	2.544	2.939	11.733
Anthracene	0	0	0.166	0	0
C1 Phenanthrenes/Anthracenes	0	0	0.986	1.16	17.8
Fluoranthene	0	0	0.642	0.685	1.129
Pyrene	0	0	0.848	0.448	1.567
Benz[a]anthracene	0	0	0.081	0	0
Chrysene	0	0	0.149	0.175	0.822
Benzo[b]fluoranthene	0	0	0	0	0
Benzo[j,k]fluoranthenes	0	0	0	0	0
Benzo[e]pyrene	0	0	0	0	0
Benzo[a]pyrene	0	0	0	0	0
Perylene	0	0	0	0	0
Dibenz[a,h]anthracene	0	0	0	0	0
Indeno[1,2,3-cd]pyrene	0	0	0.246	0	0
Benzo[ghi]perylene	0	0	0.266	0	0
2-Methylnaphthalene	0	0	1.22	6.64	23.46

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
1-Methylnaphthalene	0	0	0.851	6.725	20.725
C1-Naphthalenes	0	0	2.08	13.39	44.19
Biphenyl	0	0	0.774	5.33	5.22
C1-Biphenyls	0	0	1.13	3.44	4.55
C2-Biphenyls	0	0	0.82	1.44	2.94
C2-Naphthalenes	0	0	3.66	13.81	60.7
1,2-Dimethylnaphthalene	0	0	0.29	1.13	4.49
2,6-Dimethylnaphthalene	0	0	0.815	3.064	11.5
C3-Naphthalenes	0	0	2.03	8.939	34.9
2,3,6-Trimethylnaphthalene	0	0	0.622	2.549	10.5
2,3,5-Trimethylnaphthalene	0	0	0.426	1.716	8.05
C4-Naphthalenes	0	0	0.971	0.332	12.8
C1-Acenaphthenes	0	0	0	0	0
C1-Fluorenes	0	0	0.688	1.36	7.7
1,7-Dimethylfluorene	0	0	0	0	1.28
C2-Fluorenes	0	0	0.895	2.33	12.2
C3-Fluorenes	0	0	24.5	0.586	12.4
Dibenzothiophene	0	0	0.446	0.652	6.46
C1-Dibenzothiophenes	0	0	0.456	0.895	12.1
2/3-Methyldibenzothiophenes	0	0	0.161	0.247	3.84
C2-Dibenzothiophenes	0	0	0.577	1.04	21.3
2,4-Dimethyldibenzothiophene	0	0	0.09	0	1.51
4,6-Dimethyldibenzothiophene	0	0	0.068	0.168	2.77
C3-Dibenzothiophenes	0	0	0.393	0.416	14.1
C4-Dibenzothiophenes	0	0	0.185	0	4.23

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
3-Methylphenanthrene	0	0	3.7	0.365	3.63
2-Methylphenanthrene	0	0	0.406	0.283	4.19
2-Methylanthracene	0	0	0	0.007	0
9/4-Methylphenanthrene	0	0	0.333	0.316	5.68
1-Methylphenanthrene	0	0	0.247	0.199	4.27
3,6-Dimethylphenanthrene	0	0	0.059	0	1.13
2,6-Dimethylphenanthrene	0	0	0.053	0	1.06
1,7-Dimethylphenanthrene	0	0	0.066	0	1.65
1,8-Dimethylphenanthrene	0	0	0	0	0.649
C3-Phenanthrenes/Anthracenes	0	0	0.039	0.494	10.6
1,2,6-Trimethylphenanthrene	0	0	0	0	0.394
Retene	0	0	0	0	0.969
C4-Phenanthrenes/Anthracenes	0	0	0	0.504	10.7
C1-Fluoranthenes/Pyrenes	0	0	-0.018	0	1.68
3-Methylfluoranthene/Benzo[a]fluorene	0	0	0.058	0.15	0.704
C2-Fluoranthenes/Pyrenes	0	0	0.228	0.151	2.93
C3-Fluoranthenes/Pyrenes	0	0	0	0	0
C4-Fluoranthenes/Pyrenes	0	0	0	0	0
C1-Benzo[a]anthracenes/Chrysenes	0	0	0	0	1.54
5/6-Methylchrysene	0	0	0	0	0.256
1-Methylchrysene	0	0	0	0	0.241
C2-Benzo[a]anthracenes/Chrysenes	0	0	0	0	1.42
5,9-Dimethylchrysene	0	0	0	0	0.557
C3-Benzo[a]anthracenes/Chrysenes	0	0	0	0	0.524
C4-Benzo[a]anthracenes/Chrysenes	0	0	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Benzofluoranthenes/Benzopyrenes	0	0	0	0	2.15
7-Methylbenzo[a]pyrene	0	0	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	0	0	0	0	2.53
1,4,6,7-Tetramethylnaphthalene	0	0	0.106	0.375	2.16
Naphthalene	25	0	2057.89	2538.16	34797.6
Acenaphthylene	25	0	1.38	-0.119	63.7
Acenaphthene	25	0	115	18.2	118
2-Methylfluorene	25	0	45.3	44.5	200
C2 Phenanthrenes/Anthracenes	25	0	841.665	37.6	776
Fluorene	25	0	156.679	209	484
Phenanthrene	25	0	319.394	41.769	768.333
Anthracene	25	0	19.7	3.4	5.35
C1 Phenanthrenes/Anthracenes	25	0	813	62.9	1130
Fluoranthene	25	0	13.67	0.643	5.619
Pyrene	25	0	18.238	2.935	14.397
Benz[a]anthracene	25	0	10.2	0.132	4.22
Chrysene	25	0	35.016	0.187	34.422
Benzo[b]fluoranthene	25	0	6.74	0	3.28
Benzo[j,k]fluoranthenes	25	0	3.49	0	0
Benzo[e]pyrene	25	0	10.2	0	9.13
Benzo[a]pyrene	25	0	6.54	0	0
Perylene	25	0	20	0	0
Dibenz[a,h]anthracene	25	0	1.32	0	0
Indeno[1,2,3-cd]pyrene	25	0	2.74	0	0
Benzo[ghi]perylene	25	0	5.43	0	2.62

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2-Methylnaphthalene	25	0	1548.97	2557.75	17698.56
1-Methylnaphthalene	25	0	1139.301	2029.535	15199.325
C1-Naphthalenes	25	0	2688.28	4587.29	32897.89
Biphenyl	25	0	140.384	1888.98	1688.73
C1-Biphenyls	25	0	88.14	1049.1	898
C2-Biphenyls	25	0	77.19	468.95	339
C2-Naphthalenes	25	0	2908.04	3796.91	16797.4
1,2-Dimethylnaphthalene	25	0	228	137	1010
2,6-Dimethylnaphthalene	25	0	611.795	846.694	3100
C3-Naphthalenes	25	0	2568.77	1679.469	5820
2,3,6-Trimethylnaphthalene	25	0	761.771	534.859	1630
2,3,5-Trimethylnaphthalene	25	0	535.834	320.856	1280
C4-Naphthalenes	25	0	1530	485	1590
C1-Acenaphthenes	25	0	24.1	0	19.8
C1-Fluorenes	25	0	320.498	244	619
1,7-Dimethylfluorene	25	0	65.7	31.6	84.9
C2-Fluorenes	25	0	729.595	281	800
C3-Fluorenes	25	0	861	154	634
Dibenzothiophene	25	0	211.866	-0.109	619
C1-Dibenzothiophenes	25	0	713	0	775
2/3-Methyldibenzothiophenes	25	0	219	0	264
C2-Dibenzothiophenes	25	0	1840	4.78	939
2,4-Dimethyldibenzothiophene	25	0	141	0	91.1
4,6-Dimethyldibenzothiophene	25	0	226	4.96	132
C3-Dibenzothiophenes	25	0	1910	2.77	699

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Dibenzothiophenes	25	0	1170	0	261
3-Methylphenanthrene	25	0	159	17.7	238
2-Methylphenanthrene	25	0	176	20	269
2-Methylanthracene	25	0	24.6	-0.199	8
9/4-Methylphenanthrene	25	0	286	12.1	366
1-Methylphenanthrene	25	0	167	13	253
3,6-Dimethylphenanthrene	25	0	61.2	4	58.1
2,6-Dimethylphenanthrene	25	0	45.3	4.79	55
1,7-Dimethylphenanthrene	25	0	120	4.46	102
1,8-Dimethylphenanthrene	25	0	34.3	1.09	31.2
C3-Phenanthrenes/Anthracenes	25	0	730.789	20.6	504
1,2,6-Trimethylphenanthrene	25	0	40.2	0.816	26.2
Retene	25	0	94.2	0	43.3
C4-Phenanthrenes/Anthracenes	25	0	1280	10.2	640
C1-Fluoranthenes/Pyrenes	25	0	133.705	2.37	77.5
3-Methylfluoranthene/Benzo[a]fluorene	25	0	57.587	0.296	32
C2-Fluoranthenes/Pyrenes	25	0	211	0.806	118
C3-Fluoranthenes/Pyrenes	25	0	142	0	56.2
C4-Fluoranthenes/Pyrenes	25	0	50.2	0	19.1
C1-Benzo[a]anthracenes/Chrysenes	25	0	123	0	75.8
5/6-Methylchrysene	25	0	14.8	0	9.48
1-Methylchrysene	25	0	20.8	0	10.6
C2-Benzo[a]anthracenes/Chrysenes	25	0	176	0	74.7
5,9-Dimethylchrysene	25	0	42.7	0	15.3
C3-Benzo[a]anthracenes/Chrysenes	25	0	67.6	0	18.7

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Benzo[a]anthracenes/Chrysenes	25	0	30.5	0	2.67
C1-Benzofluoranthenes/Benzopyrenes	25	0	59.1	0	30
7-Methylbenzo[a]pyrene	25	0	3.65	0	4.11
C2-Benzofluoranthenes/Benzopyrenes	25	0	34.5	0	17.2
1,4,6,7-Tetramethylnaphthalene	25	0	227	30.6	226
Naphthalene	50	0	4057.89	4658.16	18997.6
Acenaphthylene	50	0	1.33	-0.119	0
Acenaphthene	50	0	229	27.3	234
2-Methylfluorene	50	0	102	93.7	446
C2 Phenanthrenes/Anthracenes	50	0	1399.665	87.2	1600
Fluorene	50	0	296.679	412	919
Phenanthrene	50	0	601.394	81.769	1489.333
Anthracene	50	0	41.9	0	14.8
C1 Phenanthrenes/Anthracenes	50	0	1530	132	2440
Fluoranthene	50	0	20.57	0.568	11.669
Pyrene	50	0	28.238	5.155	31.097
Benz[a]anthracene	50	0	16.7	0	9.11
Chrysene	50	0	63.816	0.149	79.922
Benzo[b]fluoranthene	50	0	10.5	0	7.64
Benzo[j,k]fluoranthenes	50	0	5.87	0	0
Benzo[e]pyrene	50	0	19.2	0	22.2
Benzo[a]pyrene	50	0	11.1	0	0
Perylene	50	0	36.7	0	5.69
Dibenz[a,h]anthracene	50	0	2.33	0	0
Indeno[1,2,3-cd]pyrene	50	0	4.89	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[ghi]perylene	50	0	10.2	0	5.35
2-Methylnaphthalene	50	0	3108.97	4757.75	34798.56
1-Methylnaphthalene	50	0	2319.301	3799.535	29699.325
C1-Naphthalenes	50	0	5428.28	8557.29	64397.89
Biphenyl	50	0	270.384	3678.98	2998.73
C1-Biphenyls	50	0	179.84	2259.1	1540
C2-Biphenyls	50	0	156.69	1137.95	650
C2-Naphthalenes	50	0	5348.04	9696.91	32297.4
1,2-Dimethylnaphthalene	50	0	432	372	2000
2,6-Dimethylnaphthalene	50	0	1119.795	2199.694	5910
C3-Naphthalenes	50	0	4868.77	4899.469	10400
2,3,6-Trimethylnaphthalene	50	0	1439.771	1549.859	2850
2,3,5-Trimethylnaphthalene	50	0	1029.834	928.856	2340
C4-Naphthalenes	50	0	3010	1390	3520
C1-Acenaphthenes	50	0	47.5	0	41.8
C1-Fluorenes	50	0	613.498	522	1280
1,7-Dimethylfluorene	50	0	112	63.1	195
C2-Fluorenes	50	0	1359.595	600	1780
C3-Fluorenes	50	0	1560	339	1440
Dibenzothiophene	50	0	404.866	-0.109	1190
C1-Dibenzothiophenes	50	0	1330	0	1640
2/3-Methyldibenzothiophenes	50	0	418	0	563
C2-Dibenzothiophenes	50	0	3380	15.7	2150
2,4-Dimethyldibenzothiophene	50	0	265	0	227
4,6-Dimethyldibenzothiophene	50	0	416	11.3	301

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Dibenzothiophenes	50	0	3420	5.92	1670
C4-Dibenzothiophenes	50	0	2070	2	606
3-Methylphenanthrene	50	0	299	38.6	526
2-Methylphenanthrene	50	0	331	39	565
2-Methylanthracene	50	0	44.3	1.941	15.1
9/4-Methylphenanthrene	50	0	548	25.6	772
1-Methylphenanthrene	50	0	305	26.2	560
3,6-Dimethylphenanthrene	50	0	107	9	127
2,6-Dimethylphenanthrene	50	0	77.8	11.1	129
1,7-Dimethylphenanthrene	50	0	199	12.5	212
1,8-Dimethylphenanthrene	50	0	57	2.84	64.3
C3-Phenanthrenes/Anthracenes	50	0	1309.789	46.6	1100
1,2,6-Trimethylphenanthrene	50	0	33.8	1.86	60.3
Retene	50	0	156	0	90.2
C4-Phenanthrenes/Anthracenes	50	0	2180	25.5	1340
C1-Fluoranthenes/Pyrenes	50	0	215.705	5.1	170
3-Methylfluoranthene/Benzo[a]fluorene	50	0	94.787	0.469	72.3
C2-Fluoranthenes/Pyrenes	50	0	345	2.12	237
C3-Fluoranthenes/Pyrenes	50	0	231	0	169
C4-Fluoranthenes/Pyrenes	50	0	80.8	0	47.4
C1-Benzo[a]anthracenes/Chrysenes	50	0	244	0	183
5/6-Methylchrysene	50	0	29.2	0	25.1
1-Methylchrysene	50	0	38.2	0	28
C2-Benzo[a]anthracenes/Chrysenes	50	0	319	0	167
5,9-Dimethylchrysene	50	0	75.1	0	39.4

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Benzo[a]anthracenes/Chrysenes	50	0	129	0	52.5
C4-Benzo[a]anthracenes/Chrysenes	50	0	50.8	0	9.44
C1-Benzofluoranthenes/Benzopyrenes	50	0	115	0	73.9
7-Methylbenzo[a]pyrene	50	0	8.15	0	10.2
C2-Benzofluoranthenes/Benzopyrenes	50	0	72.1	0	45.1
1,4,6,7-Tetramethylnaphthalene	50	0	440	79.9	476
Naphthalene	100	0	8047.89	8858.16	8167.6
Acenaphthylene	100	0	1.8	45.581	0
Acenaphthene	100	0	475	107	413
2-Methylfluorene	100	0	245	338	828
C2 Phenanthrenes/Anthracenes	100	0	2649.665	394	2870
Fluorene	100	0	626.679	1210	1920
Phenanthrene	100	0	1629.394	243.469	2779.333
Anthracene	100	0	72.5	0	23.7
C1 Phenanthrenes/Anthracenes	100	0	3440	512	4280
Fluoranthene	100	0	36.67	-0.345	19.669
Pyrene	100	0	50.638	18.625	51.897
Benz[a]anthracene	100	0	42.1	0	18.3
Chrysene	100	0	149.916	0.18	143.822
Benzo[b]fluoranthene	100	0	28.3	0	14.1
Benzo[j,k]fluoranthenes	100	0	10.5	0	0
Benzo[e]pyrene	100	0	45.6	0	41.4
Benzo[a]pyrene	100	0	28.3	0	0
Perylene	100	0	90.2	0	0
Dibenz[a,h]anthracene	100	0	5.89	0	3.15

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Indeno[1,2,3-cd]pyrene	100	0	14.2	0	0
Benzo[ghi]perylene	100	0	27.6	0	10.2
2-Methylnaphthalene	100	0	6118.97	10797.75	65398.56
1-Methylnaphthalene	100	0	4509.301	8739.535	57299.325
C1-Naphthalenes	100	0	10598.28	19597.29	122997.89
Biphenyl	100	0	551.384	8138.98	4788.73
C1-Biphenyls	100	0	393.84	7219.1	2400
C2-Biphenyls	100	0	369.69	5217.95	976
C2-Naphthalenes	100	0	10898.04	28796.91	25397.4
1,2-Dimethylnaphthalene	100	0	861	1050	1050
2,6-Dimethylnaphthalene	100	0	2289.795	6769.694	3220
C3-Naphthalenes	100	0	9878.77	20699.469	7700
2,3,6-Trimethylnaphthalene	100	0	2929.771	6339.859	1620
2,3,5-Trimethylnaphthalene	100	0	2059.834	3629.856	1270
C4-Naphthalenes	100	0	6790	4250	1740
C1-Acenaphthenes	100	0	103	25.8	71.6
C1-Fluorenes	100	0	1369.498	1870	2100
1,7-Dimethylfluorene	100	0	284	253	379
C2-Fluorenes	100	0	3149.595	2650	3180
C3-Fluorenes	100	0	3790	1640	2520
Dibenzothiophene	100	0	855.866	-0.109	2290
C1-Dibenzothiophenes	100	0	2950	0	2630
2/3-Methyldibenzothiophenes	100	0	934	0	1080
C2-Dibenzothiophenes	100	0	7660	69.2	4020
2,4-Dimethyldibenzothiophene	100	0	599	0	425

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
4,6-Dimethyldibenzothiophene	100	0	953	56	561
C3-Dibenzothiophenes	100	0	7650	38.7	3080
C4-Dibenzothiophenes	100	0	4960	10.5	1180
3-Methylphenanthrene	100	0	680	146	699
2-Methylphenanthrene	100	0	763	155	1020
2-Methylanthracene	100	0	96	-0.199	225
9/4-Methylphenanthrene	100	0	1200	107	1280
1-Methylphenanthrene	100	0	700	104	1030
3,6-Dimethylphenanthrene	100	0	197	38.5	216
2,6-Dimethylphenanthrene	100	0	146	51.5	236
1,7-Dimethylphenanthrene	100	0	372	53.4	373
1,8-Dimethylphenanthrene	100	0	107	12.5	113
C3-Phenanthrenes/Anthracenes	100	0	2509.789	223	2030
1,2,6-Trimethylphenanthrene	100	0	64.7	8.12	106
Retene	100	0	284	0	169
C4-Phenanthrenes/Anthracenes	100	0	3880	142	2410
C1-Fluoranthenes/Pyrenes	100	0	410.705	20	305
3-Methylfluoranthene/Benzo[a]fluorene	100	0	178.887	1.99	129
C2-Fluoranthenes/Pyrenes	100	0	669	9.49	458
C3-Fluoranthenes/Pyrenes	100	0	444	2.65	328
C4-Fluoranthenes/Pyrenes	100	0	160	0	110
C1-Benzo[a]anthracenes/Chrysenes	100	0	603	0.163	347
5/6-Methylchrysene	100	0	69	0	44.8
1-Methylchrysene	100	0	90.1	0	49.7
C2-Benzo[a]anthracenes/Chrysenes	100	0	732	0	304

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
5,9-Dimethylchrysene	100	0	185	0	98.2
C3-Benzo[a]anthracenes/Chrysenes	100	0	317	0	90.2
C4-Benzo[a]anthracenes/Chrysenes	100	0	117	0	24.5
C1-Benzofluoranthenes/Benzopyrenes	100	0	254	0	141
7-Methylbenzo[a]pyrene	100	0	16.8	0	19.5
C2-Benzofluoranthenes/Benzopyrenes	100	0	160	0	80.9
1,4,6,7-Tetramethylnaphthalene	100	0	979	219	225
Naphthalene	0	3	19.49	-0.53	0.52
Acenaphthylene	0	3	1.4	0.247	1.24
Acenaphthene	0	3	2.53	0	0.819
2-Methylfluorene	0	3	1.33	0	0.736
C2 Phenanthrenes/Anthracenes	0	3	12.665	0	6.11
Fluorene	0	3	4.989	0	1.39
Phenanthrene	0	3	13.394	-0.068	0.703
Anthracene	0	3	0.508	0	0
C1 Phenanthrenes/Anthracenes	0	3	15.2	0	4.23
Fluoranthene	0	3	2.27	0.522	0.689
Pyrene	0	3	2.138	0.27	1.227
Benz[a]anthracene	0	3	0.123	0	0
Chrysene	0	3	2.856	0.11	0.397
Benzo[b]fluoranthene	0	3	0	0	0
Benzo[j,k]fluoranthenes	0	3	0	0	0
Benzo[e]pyrene	0	3	0.152	0	0
Benzo[a]pyrene	0	3	0	0	0
Perylene	0	3	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Dibenz[a,h]anthracene	0	3	0	0	0
Indeno[1,2,3-cd]pyrene	0	3	0.085	0	0
Benzo[ghi]perylene	0	3	0.085	0	0.17
2-Methylnaphthalene	0	3	11.77	-1.574	0.27
1-Methylnaphthalene	0	3	10.301	-0.028	0.625
C1-Naphthalenes	0	3	22.08	-1.6	0.9
Biphenyl	0	3	2.754	0.13	0.35
C1-Biphenyls	0	3	2.83	-0.029	0.79
C2-Biphenyls	0	3	2.43	-0.58	1.01
C2-Naphthalenes	0	3	26.54	-1.61	2.32
1,2-Dimethylnaphthalene	0	3	2.44	0	0
2,6-Dimethylnaphthalene	0	3	4.825	-0.306	0.726
C3-Naphthalenes	0	3	28.27	0.314	7
2,3,6-Trimethylnaphthalene	0	3	8.171	-0.141	2.25
2,3,5-Trimethylnaphthalene	0	3	6.664	-0.144	1.73
C4-Naphthalenes	0	3	13.6	1.91	2.48
C1-Acenaphthenes	0	3	0.41	0	0
C1-Fluorenes	0	3	8.268	0	2.29
1,7-Dimethylfluorene	0	3	1.1	0	0.66
C2-Fluorenes	0	3	12.295	2.32	3.74
C3-Fluorenes	0	3	19.1	1.2	5.8
Dibenzothiophene	0	3	7.076	-0.109	1.42
C1-Dibenzothiophenes	0	3	11.7	0	2.81
2/3-Methyldibenzothiophenes	0	3	3.9	0	0.843
C2-Dibenzothiophenes	0	3	16.3	0.486	8.11

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2,4-Dimethyldibenzothiophene	0	3	1.39	0	0.657
4,6-Dimethyldibenzothiophene	0	3	1.62	0.19	0.924
C3-Dibenzothiophenes	0	3	12.3	0	5.35
C4-Dibenzothiophenes	0	3	5.33	0	1.73
3-Methylphenanthrene	0	3	3.06	0	0.719
2-Methylphenanthrene	0	3	3.7	0	0.917
2-Methylanthracene	0	3	0.203	-0.199	0
9/4-Methylphenanthrene	0	3	5	0	1.39
1-Methylphenanthrene	0	3	3.2	0	1.2
3,6-Dimethylphenanthrene	0	3	0.883	0	0.397
2,6-Dimethylphenanthrene	0	3	0.742	0	0.364
1,7-Dimethylphenanthrene	0	3	1.76	0	0.776
1,8-Dimethylphenanthrene	0	3	0.62	0	0
C3-Phenanthrenes/Anthracenes	0	3	11.389	0.317	4.36
1,2,6-Trimethylphenanthrene	0	3	0.649	0	0
Retene	0	3	0.533	0	0
C4-Phenanthrenes/Anthracenes	0	3	17.6	0	4.17
C1-Fluoranthenes/Pyrenes	0	3	3.585	0	0.817
3-Methylfluoranthene/Benzo[a]fluorene	0	3	1.437	0	0.416
C2-Fluoranthenes/Pyrenes	0	3	4.37	0	0
C3-Fluoranthenes/Pyrenes	0	3	1.91	0	0
C4-Fluoranthenes/Pyrenes	0	3	0.682	0	0
C1-Benzo[a]anthracenes/Chrysenes	0	3	2.36	0	0.599
5/6-Methylchrysene	0	3	0.203	0	0
1-Methylchrysene	0	3	0.39	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Benzo[a]anthracenes/Chrysenes	0	3	0.98	0	0
5,9-Dimethylchrysene	0	3	0.215	0	0
C3-Benzo[a]anthracenes/Chrysenes	0	3	0	0	0
C4-Benzo[a]anthracenes/Chrysenes	0	3	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	0	3	0	0	0
7-Methylbenzo[a]pyrene	0	3	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	0	3	0	0	0
1,4,6,7-Tetramethylnaphthalene	0	3	2.04	0.408	0.549
Naphthalene	25	3	802.89	211.16	4777.6
Acenaphthylene	25	3	1.39	0.723	2.67
Acenaphthene	25	3	45	4.94	29.3
2-Methylfluorene	25	3	17.2	14.5	53.8
C2 Phenanthrenes/Anthracenes	25	3	462.665	19.9	239
Fluorene	25	3	55.879	56.4	128
Phenanthrene	25	3	126.394	14.269	177.333
Anthracene	25	3	12.6	0	3.14
C1 Phenanthrenes/Anthracenes	25	3	359	23.3	349
Fluoranthene	25	3	8.96	0.945	3.539
Pyrene	25	3	11.838	2.085	7.727
Benz[a]anthracene	25	3	6.47	0	1.79
Chrysene	25	3	24.616	0.11	14.722
Benzo[b]fluoranthene	25	3	4.4	0	1.11
Benzo[j,k]fluoranthenes	25	3	2.07	0	0
Benzo[e]pyrene	25	3	7.27	0	3.27
Benzo[a]pyrene	25	3	3.08	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Perylene	25	3	11.5	0	0
Dibenz[a,h]anthracene	25	3	0.932	0	0
Indeno[1,2,3-cd]pyrene	25	3	2.31	0	0
Benzo[ghi]perylene	25	3	3.94	0	0.845
2-Methylnaphthalene	25	3	592.97	153.75	1278.56
1-Methylnaphthalene	25	3	455.301	157.535	1679.325
C1-Naphthalenes	25	3	1048.28	311.29	2957.89
Biphenyl	25	3	40.384	270.98	405.73
C1-Biphenyls	25	3	30.64	130.1	141
C2-Biphenyls	25	3	21.09	54.25	44.6
C2-Naphthalenes	25	3	1078.04	322.91	1857.4
1,2-Dimethylnaphthalene	25	3	86.5	14.2	136
2,6-Dimethylnaphthalene	25	3	214.795	36.594	180
C3-Naphthalenes	25	3	811.77	193.469	784
2,3,6-Trimethylnaphthalene	25	3	242.771	64.359	225
2,3,5-Trimethylnaphthalene	25	3	171.834	46.356	212
C4-Naphthalenes	25	3	392	72.6	258
C1-Acenaphthenes	25	3	8.05	0.44	5
C1-Fluorenes	25	3	104.498	71.3	163
1,7-Dimethylfluorene	25	3	22.1	8.66	19.3
C2-Fluorenes	25	3	218.595	93.5	213
C3-Fluorenes	25	3	391	64	183
Dibenzothiophene	25	3	80.566	1.121	108
C1-Dibenzothiophenes	25	3	255	2.13	148
2/3-Methyldibenzothiophenes	25	3	80.3	0.902	45.8

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Dibenzothiophenes	25	3	975	4.98	255
2,4-Dimethyldibenzothiophene	25	3	69.8	0.383	22.8
4,6-Dimethyldibenzothiophene	25	3	103	1.9	32.1
C3-Dibenzothiophenes	25	3	1390	2.41	233
C4-Dibenzothiophenes	25	3	987	0.843	99.4
3-Methylphenanthrene	25	3	65.6	6.24	66.6
2-Methylphenanthrene	25	3	72.4	7.02	83.3
2-Methylanthracene	25	3	11.5	-0.199	4.81
9/4-Methylphenanthrene	25	3	131	5.18	122
1-Methylphenanthrene	25	3	78.4	4.82	72.9
3,6-Dimethylphenanthrene	25	3	32.4	2	16.7
2,6-Dimethylphenanthrene	25	3	26.5	2.43	17
1,7-Dimethylphenanthrene	25	3	68.3	3.06	34.4
1,8-Dimethylphenanthrene	25	3	19.2	0.792	10.8
C3-Phenanthrenes/Anthracenes	25	3	522.789	15.9	168
1,2,6-Trimethylphenanthrene	25	3	12.7	0.616	9.29
Retene	25	3	64.3	0	12.8
C4-Phenanthrenes/Anthracenes	25	3	965	9.6	226
C1-Fluoranthenes/Pyrenes	25	3	93.905	1.79	35.9
3-Methylfluoranthene/Benzo[a]fluorene	25	3	41.087	0.224	15.3
C2-Fluoranthenes/Pyrenes	25	3	163	0.872	49.3
C3-Fluoranthenes/Pyrenes	25	3	110	0	31.2
C4-Fluoranthenes/Pyrenes	25	3	38.5	0	10.3
C1-Benzo[a]anthracenes/Chrysenes	25	3	84.7	0	28.8
5/6-Methylchrysene	25	3	10.4	0	3.33

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
1-Methylchrysene	25	3	14.1	0	4.04
C2-Benzo[a]anthracenes/Chrysenes	25	3	118	0	23.7
5,9-Dimethylchrysene	25	3	28.3	0	5.84
C3-Benzo[a]anthracenes/Chrysenes	25	3	43.7	0	4.13
C4-Benzo[a]anthracenes/Chrysenes	25	3	15.3	0	0
C1-Benzofluoranthenes/Benzopyrenes	25	3	39.3	0	8.43
7-Methylbenzo[a]pyrene	25	3	2.9	0	1.16
C2-Benzofluoranthenes/Benzopyrenes	25	3	26.3	0	4.59
1,4,6,7-Tetramethylnaphthalene	25	3	59.7	12.7	36.8
Naphthalene	50	3	1347.89	77.96	5.9
Acenaphthylene	50	3	1.73	0.71	2.13
Acenaphthene	50	3	94.1	9.37	26.7
2-Methylfluorene	50	3	41	21.3	78.6
C2 Phenanthrenes/Anthracenes	50	3	1199.665	22.8	790
Fluorene	50	3	125.679	86	134
Phenanthrene	50	3	289.394	4.709	6.983
Anthracene	50	3	29	0	2.79
C1 Phenanthrenes/Anthracenes	50	3	1010	17.9	224
Fluoranthene	50	3	18.67	0.735	5.989
Pyrene	50	3	26.038	3.295	23.697
Benz[a]anthracene	50	3	15.1	0.122	10.1
Chrysene	50	3	59.916	0.195	82.922
Benzo[b]fluoranthene	50	3	12.7	0.11	9.36
Benzo[j,k]fluoranthenes	50	3	4.49	0	0
Benzo[e]pyrene	50	3	17.6	0.176	21.8

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[a]pyrene	50	3	9.06	0	0
Perylene	50	3	32.3	0	0
Dibenz[a,h]anthracene	50	3	2.46	0	1.87
Indeno[1,2,3-cd]pyrene	50	3	5.52	0	0
Benzo[ghi]perylene	50	3	10.7	0	6.34
2-Methylnaphthalene	50	3	1018.97	4.33	5.41
1-Methylnaphthalene	50	3	790.301	17.435	3.565
C1-Naphthalenes	50	3	1808.28	21.79	8.99
Biphenyl	50	3	67.784	361.98	61.83
C1-Biphenyls	50	3	62.04	74.6	62.1
C2-Biphenyls	50	3	44.69	39.35	30.5
C2-Naphthalenes	50	3	1998.04	99.91	21.8
1,2-Dimethylnaphthalene	50	3	166	4.22	1.93
2,6-Dimethylnaphthalene	50	3	370.795	-0.306	2.66
C3-Naphthalenes	50	3	1618.77	145.469	341
2,3,6-Trimethylnaphthalene	50	3	480.771	60.659	54.1
2,3,5-Trimethylnaphthalene	50	3	352.834	54.656	169
C4-Naphthalenes	50	3	846	108	514
C1-Acenaphthenes	50	3	17.9	1.22	8.85
C1-Fluorenes	50	3	243.498	103	240
1,7-Dimethylfluorene	50	3	67.4	13.7	82.7
C2-Fluorenes	50	3	718.595	136	596
C3-Fluorenes	50	3	1210	88.2	840
Dibenzothiophene	50	3	153.866	-0.109	18.1
C1-Dibenzothiophenes	50	3	711	1.38	93.5

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2/3-Methyldibenzothiophenes	50	3	223	0	24.7
C2-Dibenzothiophenes	50	3	2690	1.45	1030
2,4-Dimethyldibenzothiophene	50	3	193	0	77.3
4,6-Dimethyldibenzothiophene	50	3	324	2.27	172
C3-Dibenzothiophenes	50	3	3210	2.42	1600
C4-Dibenzothiophenes	50	3	2070	0.943	692
3-Methylphenanthrene	50	3	188	3.69	17.2
2-Methylphenanthrene	50	3	218	8.07	139
2-Methylanthracene	50	3	29.3	-0.199	5.18
9/4-Methylphenanthrene	50	3	363	3.74	61.6
1-Methylphenanthrene	50	3	213	2.37	6.76
3,6-Dimethylphenanthrene	50	3	88.6	1.69	52
2,6-Dimethylphenanthrene	50	3	65.9	3.1	79.9
1,7-Dimethylphenanthrene	50	3	170	3.86	144
1,8-Dimethylphenanthrene	50	3	49.9	1	45.6
C3-Phenanthrenes/Anthracenes	50	3	1229.789	20.4	997
1,2,6-Trimethylphenanthrene	50	3	30.8	0.878	55.6
Retene	50	3	144	0	81.5
C4-Phenanthrenes/Anthracenes	50	3	2060	14.8	1210
C1-Fluoranthenes/Pyrenes	50	3	213.705	2.46	159
3-Methylfluoranthene/Benzo[a]fluorene	50	3	89.487	0.38	61.1
C2-Fluoranthenes/Pyrenes	50	3	345	1.26	244
C3-Fluoranthenes/Pyrenes	50	3	246	0	189
C4-Fluoranthenes/Pyrenes	50	3	87.1	0	66.6
C1-Benzo[a]anthracenes/Chrysenes	50	3	224	0	199

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
5/6-Methylchrysene	50	3	27.3	0	25
1-Methylchrysene	50	3	35.8	0	29.9
C2-Benzo[a]anthracenes/Chrysenes	50	3	307	0	168
5,9-Dimethylchrysene	50	3	69.5	0	48.9
C3-Benzo[a]anthracenes/Chrysenes	50	3	129	0	40.8
C4-Benzo[a]anthracenes/Chrysenes	50	3	47.3	0	9.71
C1-Benzofluoranthenes/Benzopyrenes	50	3	99.4	0	68.1
7-Methylbenzo[a]pyrene	50	3	7.67	0	7.7
C2-Benzofluoranthenes/Benzopyrenes	50	3	69.7	0	34.9
1,4,6,7-Tetramethylnaphthalene	50	3	129	19.8	105
Naphthalene	100	3	2987.89	678.16	10097.6
Acenaphthylene	100	3	1.35	-0.119	2.25
Acenaphthene	100	3	184	21.4	132
2-Methylfluorene	100	3	114	87.9	492
C2 Phenanthrenes/Anthracenes	100	3	2149.665	192	4290
Fluorene	100	3	272.679	238	720
Phenanthrene	100	3	767.394	39.669	2129.333
Anthracene	100	3	55.1	0	16.7
C1 Phenanthrenes/Anthracenes	100	3	2500	144	5490
Fluoranthene	100	3	30.67	-0.345	27.169
Pyrene	100	3	42.338	10.725	72.597
Benz[a]anthracene	100	3	29.1	0	35.9
Chrysene	100	3	117.916	0.104	294.822
Benzo[b]fluoranthene	100	3	22.7	0	30.4
Benzo[j,k]fluoranthenes	100	3	8.38	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[e]pyrene	100	3	34.8	0	76.7
Benzo[a]pyrene	100	3	21	0	0
Perylene	100	3	67.6	0	21.2
Dibenz[a,h]anthracene	100	3	4.62	0	6.12
Indeno[1,2,3-cd]pyrene	100	3	10.2	0	0
Benzo[ghi]perylene	100	3	21.1	0	22.9
2-Methylnaphthalene	100	3	2278.97	312.75	10498.56
1-Methylnaphthalene	100	3	1759.301	433.535	11399.325
C1-Naphthalenes	100	3	4048.28	746.29	21897.89
Biphenyl	100	3	179.384	1088.98	1198.73
C1-Biphenyls	100	3	126.84	494.1	601
C2-Biphenyls	100	3	107.69	256.95	310
C2-Naphthalenes	100	3	4018.04	972.91	11397.4
1,2-Dimethylnaphthalene	100	3	332	50.9	832
2,6-Dimethylnaphthalene	100	3	810.795	51.694	1740
C3-Naphthalenes	100	3	3398.77	987.469	4630
2,3,6-Trimethylnaphthalene	100	3	977.771	343.859	1240
2,3,5-Trimethylnaphthalene	100	3	733.834	270.856	1070
C4-Naphthalenes	100	3	2930	669	2740
C1-Acenaphthenes	100	3	34.2	2.98	26.7
C1-Fluorenes	100	3	695.498	401	1430
1,7-Dimethylfluorene	100	3	217	92.5	353
C2-Fluorenes	100	3	2139.595	873	3520
C3-Fluorenes	100	3	2820	843	4160
Dibenzothiophene	100	3	487.866	-0.109	1550

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Dibenzothiophenes	100	3	2070	0	3270
2/3-Methyldibenzothiophenes	100	3	658	3.59	1150
C2-Dibenzothiophenes	100	3	5950	29.9	8020
2,4-Dimethyldibenzothiophene	100	3	469	0	608
4,6-Dimethyldibenzothiophene	100	3	743	24.6	982
C3-Dibenzothiophenes	100	3	6410	19.8	5320
C4-Dibenzothiophenes	100	3	3870	4.07	2180
3-Methylphenanthrene	100	3	485	37.4	996
2-Methylphenanthrene	100	3	545	49.9	1170
2-Methylanthracene	100	3	71.2	5.251	107
9/4-Methylphenanthrene	100	3	856	29.3	1810
1-Methylphenanthrene	100	3	544	27.5	1410
3,6-Dimethylphenanthrene	100	3	165	20.1	326
2,6-Dimethylphenanthrene	100	3	120	27	355
1,7-Dimethylphenanthrene	100	3	309	29.2	580
1,8-Dimethylphenanthrene	100	3	87.8	5.9	178
C3-Phenanthrenes/Anthracenes	100	3	1979.789	128	3230
1,2,6-Trimethylphenanthrene	100	3	103	4.43	175
Retene	100	3	248	0	304
C4-Phenanthrenes/Anthracenes	100	3	3280	57.5	4060
C1-Fluoranthenes/Pyrenes	100	3	333.705	11.6	485
3-Methylfluoranthene/Benzo[a]fluorene	100	3	147.887	1.06	208
C2-Fluoranthenes/Pyrenes	100	3	562	6.69	731
C3-Fluoranthenes/Pyrenes	100	3	369	1.27	539
C4-Fluoranthenes/Pyrenes	100	3	131	0	185

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Benzo[a]anthracenes/Chrysenes	100	3	470	0.13	722
5/6-Methylchrysene	100	3	53.4	0	93
1-Methylchrysene	100	3	68.4	0	104
C2-Benzo[a]anthracenes/Chrysenes	100	3	601	0	634
5,9-Dimethylchrysene	100	3	143	0	202
C3-Benzo[a]anthracenes/Chrysenes	100	3	261	0	182
C4-Benzo[a]anthracenes/Chrysenes	100	3	92.9	0	38.3
C1-Benzofluoranthenes/Benzopyrenes	100	3	219	0	277
7-Methylbenzo[a]pyrene	100	3	15	0	36.3
C2-Benzofluoranthenes/Benzopyrenes	100	3	125	0	192
1,4,6,7-Tetramethylnaphthalene	100	3	455	113	410
Naphthalene	0	7	-0.45	0.05	0.12
Acenaphthylene	0	7	1.22	0.423	1.58
Acenaphthene	0	7	0.198	0	0
2-Methylfluorene	0	7	0.491	0	0.537
C2 Phenanthrenes/Anthracenes	0	7	3.875	0.401	2.52
Fluorene	0	7	0.072	0.373	0.459
Phenanthrene	0	7	0.252	0.11	0.228
Anthracene	0	7	0.127	0	0
C1 Phenanthrenes/Anthracenes	0	7	0.958	0	0
Fluoranthene	0	7	1.12	0.685	0.719
Pyrene	0	7	1.368	1.595	0.402
Benz[a]anthracene	0	7	0	0	0.109
Chrysene	0	7	1.636	0.16	0.255
Benzo[b]fluoranthene	0	7	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[j,k]fluoranthenes	0	7	0	0	0
Benzo[e]pyrene	0	7	0.25	0	0
Benzo[a]pyrene	0	7	0	0	0
Perylene	0	7	0	0	0
Dibenz[a,h]anthracene	0	7	0	0	0
Indeno[1,2,3-cd]pyrene	0	7	0	0	0
Benzo[ghi]perylene	0	7	0.183	0	0.359
2-Methylnaphthalene	0	7	0.07	-1.443	0.09
1-Methylnaphthalene	0	7	-0.101	-0.012	0.278
C1-Naphthalenes	0	7	-0.02	-1.45	0.38
Biphenyl	0	7	-0.09	0.42	0.15
C1-Biphenyls	0	7	0.12	0.14	0.438
C2-Biphenyls	0	7	0.62	0.31	0.436
C2-Naphthalenes	0	7	0.37	-1.7	0.27
1,2-Dimethylnaphthalene	0	7	0	0	0
2,6-Dimethylnaphthalene	0	7	0.106	-0.306	0.328
C3-Naphthalenes	0	7	1.37	0.448	2.48
2,3,6-Trimethylnaphthalene	0	7	0.464	0.172	1.06
2,3,5-Trimethylnaphthalene	0	7	0.543	0.235	0.506
C4-Naphthalenes	0	7	4.42	1.77	1.64
C1-Acenaphthenes	0	7	0.196	0	0
C1-Fluorenes	0	7	1.628	0.844	2.13
1,7-Dimethylfluorene	0	7	0.5	0	0
C2-Fluorenes	0	7	5.305	2.33	6.57
C3-Fluorenes	0	7	17.9	1.46	23.2

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Dibenzothiophene	0	7	0.133	-0.109	0.38
C1-Dibenzothiophenes	0	7	1.25	0	0.531
2/3-Methyldibenzothiophenes	0	7	0.457	0	0
C2-Dibenzothiophenes	0	7	6.67	0.945	3.58
2,4-Dimethyldibenzothiophene	0	7	0.377	0	0
4,6-Dimethyldibenzothiophene	0	7	0.835	0	0.466
C3-Dibenzothiophenes	0	7	6.82	0	1.62
C4-Dibenzothiophenes	0	7	3.57	0	0
3-Methylphenanthrene	0	7	1.53	0	0
2-Methylphenanthrene	0	7	0.339	0	0
2-Methylanthracene	0	7	0.138	-0.199	0
9/4-Methylphenanthrene	0	7	0.436	0	0
1-Methylphenanthrene	0	7	0.184	0	0
3,6-Dimethylphenanthrene	0	7	0.313	0	0.385
2,6-Dimethylphenanthrene	0	7	0.223	0	0.3
1,7-Dimethylphenanthrene	0	7	0.572	0	0.229
1,8-Dimethylphenanthrene	0	7	0.32	0	0
C3-Phenanthrenes/Anthracenes	0	7	5.739	0.305	1.65
1,2,6-Trimethylphenanthrene	0	7	0.151	0	0
Retene	0	7	0.421	0	0
C4-Phenanthrenes/Anthracenes	0	7	10.8	0	0
C1-Fluoranthenes/Pyrenes	0	7	2.395	0	0
3-Methylfluoranthene/Benzo[a]fluorene	0	7	0.847	0	0
C2-Fluoranthenes/Pyrenes	0	7	2.9	0	0
C3-Fluoranthenes/Pyrenes	0	7	0.715	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Fluoranthenes/Pyrenes	0	7	0.331	0	0
C1-Benzo[a]anthracenes/Chrysenes	0	7	1.49	0	0
5/6-Methylchrysene	0	7	0.163	0	0
1-Methylchrysene	0	7	0.271	0	0
C2-Benzo[a]anthracenes/Chrysenes	0	7	0.643	0	0
5,9-Dimethylchrysene	0	7	0	0	0
C3-Benzo[a]anthracenes/Chrysenes	0	7	0.244	0	0.391
C4-Benzo[a]anthracenes/Chrysenes	0	7	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	0	7	0	0	1.07
7-Methylbenzo[a]pyrene	0	7	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	0	7	0	0	1.17
1,4,6,7-Tetramethylnaphthalene	0	7	0.865	0.366	0.35
Naphthalene	25	7	-0.29	-0.17	0.71
Acenaphthylene	25	7	2.08	1.161	2.74
Acenaphthene	25	7	1.83	0	0
2-Methylfluorene	25	7	1.36	0	0
C2 Phenanthrenes/Anthracenes	25	7	45.865	0.616	7.55
Fluorene	25	7	0.429	0.219	0.373
Phenanthrene	25	7	0.424	0.028	0.157
Anthracene	25	7	4.4	0	0
C1 Phenanthrenes/Anthracenes	25	7	15.9	0	0
Fluoranthene	25	7	1.82	-0.009	0.064
Pyrene	25	7	3.618	2.245	2.707
Benz[a]anthracene	25	7	0.867	0.079	0
Chrysene	25	7	5.376	0.018	4.942

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[b]fluoranthene	25	7	0.896	0	0.431
Benzo[j,k]fluoranthenes	25	7	0.395	0	0
Benzo[e]pyrene	25	7	1.44	0	1.27
Benzo[a]pyrene	25	7	0	0	0
Perylene	25	7	1.16	0	0
Dibenz[a,h]anthracene	25	7	0	0	0
Indeno[1,2,3-cd]pyrene	25	7	0.474	0	0
Benzo[ghi]perylene	25	7	0.85	0	0.395
2-Methylnaphthalene	25	7	0.27	-1.4	0.84
1-Methylnaphthalene	25	7	0.132	0.063	0.655
C1-Naphthalenes	25	7	0.41	-1.33	1.5
Biphenyl	25	7	0.04	0.2	-0.05
C1-Biphenyls	25	7	0.71	-0.146	0.546
C2-Biphenyls	25	7	1.67	0.15	1.36
C2-Naphthalenes	25	7	1.69	-1.47	1.41
1,2-Dimethylnaphthalene	25	7	0.32	0	0
2,6-Dimethylnaphthalene	25	7	0.274	-0.306	0.608
C3-Naphthalenes	25	7	8.08	0.223	2.29
2,3,6-Trimethylnaphthalene	25	7	0.871	0.3	0.838
2,3,5-Trimethylnaphthalene	25	7	2.884	0.114	0.908
C4-Naphthalenes	25	7	34.6	0.884	8.13
C1-Acenaphthenes	25	7	0.957	0	0
C1-Fluorenes	25	7	4.548	0.473	1.55
1,7-Dimethylfluorene	25	7	4.01	0	0
C2-Fluorenes	25	7	34.395	4	22.7

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Fluorenes	25	7	65.4	4.25	33.1
Dibenzothiophene	25	7	0.159	-0.109	0.887
C1-Dibenzothiophenes	25	7	2.98	0	0
2/3-Methyldibenzothiophenes	25	7	1.09	0	1.66
C2-Dibenzothiophenes	25	7	83.4	0.383	5.55
2,4-Dimethyldibenzothiophene	25	7	3.81	0	0
4,6-Dimethyldibenzothiophene	25	7	11.2	0.196	0.703
C3-Dibenzothiophenes	25	7	158	0.497	27.1
C4-Dibenzothiophenes	25	7	122	0	19.3
3-Methylphenanthrene	25	7	0.741	0	0
2-Methylphenanthrene	25	7	1	0	0
2-Methylanthracene	25	7	4.46	-0.199	0
9/4-Methylphenanthrene	25	7	0.748	0	0
1-Methylphenanthrene	25	7	8.98	0	0
3,6-Dimethylphenanthrene	25	7	1.89	0	0
2,6-Dimethylphenanthrene	25	7	2.21	0	0
1,7-Dimethylphenanthrene	25	7	10.6	0	0.642
1,8-Dimethylphenanthrene	25	7	3.41	0	0.848
C3-Phenanthrenes/Anthracenes	25	7	76.189	2.04	25.8
1,2,6-Trimethylphenanthrene	25	7	4.49	0.116	1.43
Retene	25	7	10.6	0	2.63
C4-Phenanthrenes/Anthracenes	25	7	167	1.1	43.7
C1-Fluoranthenes/Pyrenes	25	7	18.105	0.419	8.97
3-Methylfluoranthene/Benzo[a]fluorene	25	7	7.497	0.183	2.7
C2-Fluoranthenes/Pyrenes	25	7	29.3	0.133	12.7

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Fluoranthenes/Pyrenes	25	7	15.5	0	6.93
C4-Fluoranthenes/Pyrenes	25	7	4.37	0	1.04
C1-Benzo[a]anthracenes/Chrysenes	25	7	14.4	0	7.74
5/6-Methylchrysene	25	7	1.98	0	1.07
1-Methylchrysene	25	7	2.43	0	0.98
C2-Benzo[a]anthracenes/Chrysenes	25	7	15.5	0	7.3
5,9-Dimethylchrysene	25	7	3.91	0	1.71
C3-Benzo[a]anthracenes/Chrysenes	25	7	5.87	0	0.829
C4-Benzo[a]anthracenes/Chrysenes	25	7	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	25	7	5.66	0	2.31
7-Methylbenzo[a]pyrene	25	7	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	25	7	3.06	0	0
1,4,6,7-Tetramethylnaphthalene	25	7	8	0	0
Naphthalene	50	7	-0.04	0.63	0.69
Acenaphthylene	50	7	1.55	0.551	1.41
Acenaphthene	50	7	4.8	0	0
2-Methylfluorene	50	7	4.01	0	0.861
C2 Phenanthrenes/Anthracenes	50	7	174.665	0.752	15.8
Fluorene	50	7	1.239	0	0.752
Phenanthrene	50	7	1.464	0.309	0.573
Anthracene	50	7	14	0	0
C1 Phenanthrenes/Anthracenes	50	7	41.3	0	0
Fluoranthene	50	7	3.53	-0.044	0.12
Pyrene	50	7	6.658	2.265	6.567
Benz[a]anthracene	50	7	2.75	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Chrysene	50	7	12.916	0.053	8.372
Benzo[b]fluoranthene	50	7	2.12	0	0.7
Benzo[j,k]fluoranthenes	50	7	0.805	0	0
Benzo[e]pyrene	50	7	3.27	0	1.97
Benzo[a]pyrene	50	7	1.2	0	0
Perylene	50	7	5.36	0	0
Dibenz[a,h]anthracene	50	7	0	0	0
Indeno[1,2,3-cd]pyrene	50	7	0.954	0	0
Benzo[ghi]perylene	50	7	1.85	0	0
2-Methylnaphthalene	50	7	0.7	-1.404	0.21
1-Methylnaphthalene	50	7	0.481	0.23	0.385
C1-Naphthalenes	50	7	1.19	-1.17	0.6
Biphenyl	50	7	0.262	1.11	0.32
C1-Biphenyls	50	7	1.72	0.23	0.588
C2-Biphenyls	50	7	3.21	0.38	2.22
C2-Naphthalenes	50	7	4.42	-1.46	0.91
1,2-Dimethylnaphthalene	50	7	0.781	0	0
2,6-Dimethylnaphthalene	50	7	0.536	-0.306	0
C3-Naphthalenes	50	7	17.97	-0.531	5.33
2,3,6-Trimethylnaphthalene	50	7	2.781	0.386	1.59
2,3,5-Trimethylnaphthalene	50	7	6.114	-0.144	0.916
C4-Naphthalenes	50	7	91	0	27.6
C1-Acenaphthenes	50	7	1.86	0	0
C1-Fluorenes	50	7	12.498	0	3.02
1,7-Dimethylfluorene	50	7	17.6	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Fluorenes	50	7	131.595	2.74	86.8
C3-Fluorenes	50	7	229	19	108
Dibenzothiophene	50	7	0.517	-0.109	1.24
C1-Dibenzothiophenes	50	7	14.1	0	1.36
2/3-Methyldibenzothiophenes	50	7	5.15	0	1.36
C2-Dibenzothiophenes	50	7	374	0	9.79
2,4-Dimethyldibenzothiophene	50	7	20.6	0	0
4,6-Dimethyldibenzothiophene	50	7	53.6	0	0
C3-Dibenzothiophenes	50	7	589	0	50.5
C4-Dibenzothiophenes	50	7	377	0	41.2
3-Methylphenanthrene	50	7	1.18	0	0
2-Methylphenanthrene	50	7	5.59	0	0
2-Methylanthracene	50	7	11.6	-0.199	0
9/4-Methylphenanthrene	50	7	24.1	0	0
1-Methylphenanthrene	50	7	0.955	0	0
3,6-Dimethylphenanthrene	50	7	9.39	0	0
2,6-Dimethylphenanthrene	50	7	10	0	0
1,7-Dimethylphenanthrene	50	7	34.5	0	0.845
1,8-Dimethylphenanthrene	50	7	10.6	0	1.28
C3-Phenanthrenes/Anthracenes	50	7	245.789	3.62	45.4
1,2,6-Trimethylphenanthrene	50	7	6.42	0	2.15
Retene	50	7	29.2	0	4.03
C4-Phenanthrenes/Anthracenes	50	7	437	1.78	91.5
C1-Fluoranthenes/Pyrenes	50	7	44.605	0.956	18.6
3-Methylfluoranthene/Benzo[a]fluorene	50	7	19.987	0	4.48

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Fluoranthenes/Pyrenes	50	7	76.4	0.258	25.6
C3-Fluoranthenes/Pyrenes	50	7	49.4	0	13.5
C4-Fluoranthenes/Pyrenes	50	7	15.5	0	2.64
C1-Benzo[a]anthracenes/Chrysenes	50	7	40.9	0	12.7
5/6-Methylchrysene	50	7	4.77	0	1.4
1-Methylchrysene	50	7	6.12	0	1.54
C2-Benzo[a]anthracenes/Chrysenes	50	7	49.3	0	11.6
5,9-Dimethylchrysene	50	7	12.9	0	2.8
C3-Benzo[a]anthracenes/Chrysenes	50	7	19.5	0	0
C4-Benzo[a]anthracenes/Chrysenes	50	7	7.75	0	0
C1-Benzofluoranthenes/Benzopyrenes	50	7	17.2	0	3.82
7-Methylbenzo[a]pyrene	50	7	1.48	0	0
C2-Benzofluoranthenes/Benzopyrenes	50	7	11.9	0	0
1,4,6,7-Tetramethylnaphthalene	50	7	20.9	0	0
Naphthalene	100	7	1027.89	0.68	5077.6
Acenaphthylene	100	7	1.11	0.558	2.61
Acenaphthene	100	7	64	0	43.8
2-Methylfluorene	100	7	45	0.813	142
C2 Phenanthrenes/Anthracenes	100	7	1149.665	4.34	836
Fluorene	100	7	95.079	0	245
Phenanthrene	100	7	282.394	0.619	385.333
Anthracene	100	7	42.8	0	7
C1 Phenanthrenes/Anthracenes	100	7	1080	0	1040
Fluoranthene	100	7	17.67	-0.011	6.419
Pyrene	100	7	23.738	3.445	15.497

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benz[a]anthracene	100	7	12	0	4.61
Chrysene	100	7	50.416	0.046	42.622
Benzo[b]fluoranthene	100	7	8.95	0	3.99
Benzo[j,k]fluoranthenes	100	7	3.28	0	0
Benzo[e]pyrene	100	7	14.5	0	11.2
Benzo[a]pyrene	100	7	7.68	0	0
Perylene	100	7	27.6	0	0
Dibenz[a,h]anthracene	100	7	1.82	0	0
Indeno[1,2,3-cd]pyrene	100	7	3.83	0	0
Benzo[ghi]perylene	100	7	8.45	0	2.9
2-Methylnaphthalene	100	7	498.97	-1.4	73.76
1-Methylnaphthalene	100	7	509.301	0.079	1369.325
C1-Naphthalenes	100	7	1008.28	-1.32	1447.89
Biphenyl	100	7	29.084	0.51	274.73
C1-Biphenyls	100	7	36.24	0.056	113
C2-Biphenyls	100	7	34.39	0.5	68.4
C2-Naphthalenes	100	7	1198.04	0.83	1277.4
1,2-Dimethylnaphthalene	100	7	100	0.431	102
2,6-Dimethylnaphthalene	100	7	215.795	-0.026	81.9
C3-Naphthalenes	100	7	1208.77	3.969	970
2,3,6-Trimethylnaphthalene	100	7	354.771	1.709	258
2,3,5-Trimethylnaphthalene	100	7	259.834	0.789	261
C4-Naphthalenes	100	7	1040	13	570
C1-Acenaphthenes	100	7	11.9	0	8.1
C1-Fluorenes	100	7	253.498	1.74	426

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
1,7-Dimethylfluorene	100	7	80.8	0.627	89.1
C2-Fluorenes	100	7	864.595	24.9	826
C3-Fluorenes	100	7	1210	131	724
Dibenzothiophene	100	7	173.866	-0.109	397
C1-Dibenzothiophenes	100	7	849	0	719
2/3-Methyldibenzothiophenes	100	7	267	0	298
C2-Dibenzothiophenes	100	7	2630	0	1030
2,4-Dimethyldibenzothiophene	100	7	194	0	100
4,6-Dimethyldibenzothiophene	100	7	318	0	138
C3-Dibenzothiophenes	100	7	2850	2.16	832
C4-Dibenzothiophenes	100	7	1700	1.04	330
3-Methylphenanthrene	100	7	200	0	196
2-Methylphenanthrene	100	7	231	0	272
2-Methylanthracene	100	7	38.3	-0.199	13
9/4-Methylphenanthrene	100	7	391	0	355
1-Methylphenanthrene	100	7	222	0	202
3,6-Dimethylphenanthrene	100	7	88.3	0	61.8
2,6-Dimethylphenanthrene	100	7	64.3	0	63.6
1,7-Dimethylphenanthrene	100	7	171	0.546	118
1,8-Dimethylphenanthrene	100	7	46.6	0	36.9
C3-Phenanthrenes/Anthracenes	100	7	1049.789	14.4	599
1,2,6-Trimethylphenanthrene	100	7	48.3	0.606	32.3
Retene	100	7	127	0	48.3
C4-Phenanthrenes/Anthracenes	100	7	1770	15.1	738
C1-Fluoranthenes/Pyrenes	100	7	187.705	2.13	94.7

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
3-Methylfluoranthene/Benzo[a]fluorene	100	7	81.187	0	39.8
C2-Fluoranthenes/Pyrenes	100	7	300	1.14	144
C3-Fluoranthenes/Pyrenes	100	7	205	0	90.3
C4-Fluoranthenes/Pyrenes	100	7	69.6	0	25.9
C1-Benzo[a]anthracenes/Chrysenes	100	7	183	0	93.6
5/6-Methylchrysene	100	7	21.9	0	13.1
1-Methylchrysene	100	7	27.4	0	14.6
C2-Benzo[a]anthracenes/Chrysenes	100	7	248	0	92.5
5,9-Dimethylchrysene	100	7	55.8	0	18.4
C3-Benzo[a]anthracenes/Chrysenes	100	7	97.9	0	18.5
C4-Benzo[a]anthracenes/Chrysenes	100	7	36.2	0	4.88
C1-Benzofluoranthenes/Benzopyrenes	100	7	84.5	0	34.2
7-Methylbenzo[a]pyrene	100	7	6.12	0	4.49
C2-Benzofluoranthenes/Benzopyrenes	100	7	55.9	0	20.5
1,4,6,7-Tetramethylnaphthalene	100	7	161	0	86.3

Table A.47. The concentration (ng/L) for all the PAH components measured for tissue samples from the *C. gigas* exposed to 0%, 25%, 50%, 100% WAF samples of DB, CO, and MD on sampling day 7, 21, and 35.

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Naphthalene	0	7	0.495	0.785	1.385
Acenaphthylene	0	7	0.023	0.087	0.075
Acenaphthene	0	7	0.704	0.475	0.538
2-Methylfluorene	0	7	0.293	0.358	0.421
C2 Phenanthrenes/Anthracenes	0	7	1.973	1.663	5.773
Fluorene	0	7	0.781	1.199	1.189
Phenanthrene	0	7	3.092	3.322	5.392
Anthracene	0	7	0	0.209	0.252
C1 Phenanthrenes/Anthracenes	0	7	0.752	3.127	7.427
Fluoranthene	0	7	3.613	6.613	4.873
Pyrene	0	7	2.953	3.413	2.833
Benz[a]anthracene	0	7	0	0.178	0.227
Chrysene	0	7	1.128	1.098	1.338
Benzo[b]fluoranthene	0	7	0.385	0.314	0.556
Benzo[j,k]fluoranthenes	0	7	0	0.177	0.304
Benzo[e]pyrene	0	7	0	0.48	0.415
Benzo[a]pyrene	0	7	0	0	0
Perylene	0	7	0	0	0
Dibenz[a,h]anthracene	0	7	0	0	0
Indeno[1,2,3-cd]pyrene	0	7	0	0	0
Benzo[ghi]perylene	0	7	0	0.084	0.09
2-Methylnaphthalene	0	7	0.662	1.45	2.18
1-Methylnaphthalene	0	7	0.575	1.248	2.008

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Naphthalenes	0	7	1.239	2.699	4.189
Biphenyl	0	7	0.229	1.236	0.664
C1-Biphenyls	0	7	0.194	1.551	0.664
C2-Biphenyls	0	7	0.116	1.855	0.401
C2-Naphthalenes	0	7	3.155	7.135	6.555
1,2-Dimethylnaphthalene	0	7	0	0.517	0.548
2,6-Dimethylnaphthalene	0	7	0.453	0.895	1.035
C3-Naphthalenes	0	7	5.51	9.59	5.49
2,3,6-Trimethylnaphthalene	0	7	1.79	3.15	1.46
2,3,5-Trimethylnaphthalene	0	7	1.23	1.96	1.15
C4-Naphthalenes	0	7	2.556	4.376	2.176
C1-Acenaphthenes	0	7	0.062	0	0.041
C1-Fluorenes	0	7	1.392	2.172	1.792
1,7-Dimethylfluorene	0	7	0.194	0.424	0.312
C2-Fluorenes	0	7	3.72	4.29	3.76
C3-Fluorenes	0	7	3.69	3.87	5
Dibenzothiophene	0	7	0.878	0.611	1.657
C1-Dibenzothiophenes	0	7	2.618	1.568	3.988
2/3-Methyldibenzothiophenes	0	7	0.623	0.428	1.252
C2-Dibenzothiophenes	0	7	5.089	2.599	7.029
2,4-Dimethyldibenzothiophene	0	7	0.433	0.344	0.694
4,6-Dimethyldibenzothiophene	0	7	0.715	0.538	0.898
C3-Dibenzothiophenes	0	7	3.3	1.41	4.69
C4-Dibenzothiophenes	0	7	1.07	0.322	1.64
3-Methylphenanthrene	0	7	-0.025	0.857	1.635

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2-Methylphenanthrene	0	7	-0.037	0.747	1.653
2-Methylanthracene	0	7	-0.026	0.121	0.029
9/4-Methylphenanthrene	0	7	-0.028	0.885	2.372
1-Methylphenanthrene	0	7	0.842	0.494	1.757
3,6-Dimethylphenanthrene	0	7	0.272	0.274	0.501
2,6-Dimethylphenanthrene	0	7	0.194	0.181	0.472
1,7-Dimethylphenanthrene	0	7	0.263	0.159	0.705
1,8-Dimethylphenanthrene	0	7	0	0.061	0.237
C3-Phenanthrenes/Anthracenes	0	7	1.52	1.05	4.02
1,2,6-Trimethylphenanthrene	0	7	0.071	0	0.191
Retene	0	7	0.501	1.32	0.842
C4-Phenanthrenes/Anthracenes	0	7	3.45	3.03	4.15
C1-Fluoranthenes/Pyrenes	0	7	1.03	1.41	1.51
3-Methylfluoranthene/Benzo[a]fluorene	0	7	0.431	0.577	0.709
C2-Fluoranthenes/Pyrenes	0	7	0.664	0.461	1.07
C3-Fluoranthenes/Pyrenes	0	7	0.28	0	0.225
C4-Fluoranthenes/Pyrenes	0	7	0.041	0.197	0
C1-Benzo[a]anthracenes/Chrysenes	0	7	0	0.935	0.802
5/6-Methylchrysene	0	7	0.042	0.581	0.094
1-Methylchrysene	0	7	0	0.142	0.098
C2-Benzo[a]anthracenes/Chrysenes	0	7	0.257	0	0.14
5,9-Dimethylchrysene	0	7	0	0	0
C3-Benzo[a]anthracenes/Chrysenes	0	7	0	0	0
C4-Benzo[a]anthracenes/Chrysenes	0	7	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	0	7	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
7-Methylbenzo[a]pyrene	0	7	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	0	7	0	0	0
1,4,6,7-Tetramethylnaphthalene	0	7	0.535	0.837	0.351
Naphthalene	25	7	2.905	5.555	282.455
Acenaphthylene	25	7	-0.014	-0.014	0.071
Acenaphthene	25	7	4.32	1.16	4.96
2-Methylfluorene	25	7	4.75	6.03	7.48
C2 Phenanthrenes/Anthracenes	25	7	48.423	6.643	57.023
Fluorene	25	7	8.469	15.369	25.169
Phenanthrene	25	7	31.012	9.112	61.912
Anthracene	25	7	1.44	0.317	0.574
C1 Phenanthrenes/Anthracenes	25	7	79.077	13.477	116.877
Fluoranthene	25	7	4.033	5.263	4.193
Pyrene	25	7	4.003	3.783	3.693
Benz[a]anthracene	25	7	0.414	0.21	0.344
Chrysene	25	7	2.228	1.168	3.378
Benzo[b]fluoranthene	25	7	0.551	0.278	0.387
Benzo[j,k]fluoranthenes	25	7	0.383	0.127	0.176
Benzo[e]pyrene	25	7	0.545	0.468	0.457
Benzo[a]pyrene	25	7	0	0	0
Perylene	25	7	0.17	0	0
Dibenz[a,h]anthracene	25	7	0	0	0
Indeno[1,2,3-cd]pyrene	25	7	0	0	0
Benzo[ghi]perylene	25	7	0.104	0	0.08
2-Methylnaphthalene	25	7	19.21	25.51	458.71

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
1-Methylnaphthalene	25	7	12.788	21.288	378.788
C1-Naphthalenes	25	7	31.999	46.799	837.499
Biphenyl	25	7	3.216	47.116	62.616
C1-Biphenyls	25	7	4.631	69.351	45.451
C2-Biphenyls	25	7	4.515	43.225	17.925
C2-Naphthalenes	25	7	131.565	196.565	751.565
1,2-Dimethylnaphthalene	25	7	9.58	7.62	41.6
2,6-Dimethylnaphthalene	25	7	28.505	40.905	155.905
C3-Naphthalenes	25	7	205.85	168.85	270.85
2,3,6-Trimethylnaphthalene	25	7	63.6	55.2	75.8
2,3,5-Trimethylnaphthalene	25	7	43.1	33.7	58.2
C4-Naphthalenes	25	7	90.946	51.846	54.746
C1-Acenaphthenes	25	7	1.45	0.234	1.25
C1-Fluorenes	25	7	25.902	29.902	41.802
1,7-Dimethylfluorene	25	7	5.02	3.61	6.14
C2-Fluorenes	25	7	56.7	36	56.7
C3-Fluorenes	25	7	47.6	21.8	52.5
Dibenzothiophene	25	7	18.957	1.087	52.457
C1-Dibenzothiophenes	25	7	66.578	2.398	87.178
2/3-Methyldibenzothiophenes	25	7	20.082	0.749	33.082
C2-Dibenzothiophenes	25	7	105.979	4.839	96.779
2,4-Dimethyldibenzothiophene	25	7	8.41	0.399	10.2
4,6-Dimethyldibenzothiophene	25	7	11.8	1.21	10.4
C3-Dibenzothiophenes	25	7	59.1	2.02	49
C4-Dibenzothiophenes	25	7	13.4	0.666	11.3

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
3-Methylphenanthrene	25	7	15.175	3.685	24.175
2-Methylphenanthrene	25	7	19.063	4.123	28.263
2-Methylanthracene	25	7	1.314	0.173	0.346
9/4-Methylphenanthrene	25	7	26.972	2.802	37.272
1-Methylphenanthrene	25	7	16.467	2.637	26.767
3,6-Dimethylphenanthrene	25	7	3.7	0.816	4.45
2,6-Dimethylphenanthrene	25	7	2.59	0.714	4.13
1,7-Dimethylphenanthrene	25	7	7.245	0.808	7.885
1,8-Dimethylphenanthrene	25	7	2.07	0.249	2.6
C3-Phenanthrenes/Anthracenes	25	7	23.9	3.23	28.8
1,2,6-Trimethylphenanthrene	25	7	1.25	0	1.35
Retene	25	7	1.87	1.26	1.26
C4-Phenanthrenes/Anthracenes	25	7	25.6	2.73	26.1
C1-Fluoranthenes/Pyrenes	25	7	7.24	1.49	7.81
3-Methylfluoranthene/Benzo[a]fluorene	25	7	3.76	0.584	3.94
C2-Fluoranthenes/Pyrenes	25	7	6.54	1.67	7.85
C3-Fluoranthenes/Pyrenes	25	7	1.47	0	2.08
C4-Fluoranthenes/Pyrenes	25	7	0.235	0.055	0.485
C1-Benzo[a]anthracenes/Chrysenes	25	7	2.22	1.89	3.08
5/6-Methylchrysene	25	7	0.283	1.44	0.429
1-Methylchrysene	25	7	0.33	0.139	0.409
C2-Benzo[a]anthracenes/Chrysenes	25	7	0.876	0.146	1.26
5,9-Dimethylchrysene	25	7	0.19	0	0.252
C3-Benzo[a]anthracenes/Chrysenes	25	7	0	0	0.044
C4-Benzo[a]anthracenes/Chrysenes	25	7	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Benzofluoranthenes/Benzopyrenes	25	7	0.14	0	0.374
7-Methylbenzo[a]pyrene	25	7	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	25	7	0	0	0
1,4,6,7-Tetramethylnaphthalene	25	7	16.2	8.14	8.37
Naphthalene	50	7	34.755	26.055	982.455
Acenaphthylene	50	7	-0.014	-0.014	0.162
Acenaphthene	50	7	7.5	2.12	15.6
2-Methylfluorene	50	7	5.29	10.5	24.4
C2 Phenanthrenes/Anthracenes	50	7	51.823	12.223	128.923
Fluorene	50	7	10.369	27.469	78.169
Phenanthrene	50	7	37.312	12.812	181.912
Anthracene	50	7	1.87	0.34	1.42
C1 Phenanthrenes/Anthracenes	50	7	83.477	22.877	285.877
Fluoranthene	50	7	6.063	5.643	4.723
Pyrene	50	7	6.643	4.543	5.603
Benz[a]anthracene	50	7	0.6	0.277	0.534
Chrysene	50	7	2.658	2.008	6.018
Benzo[b]fluoranthene	50	7	0.711	0.643	0.476
Benzo[j,k]fluoranthenes	50	7	0.385	0.353	0.108
Benzo[e]pyrene	50	7	0.734	0.701	0.884
Benzo[a]pyrene	50	7	0	0.085	0
Perylene	50	7	0.268	0	0
Dibenz[a,h]anthracene	50	7	0	0	0
Indeno[1,2,3-cd]pyrene	50	7	0	0.048	0
Benzo[ghi]perylene	50	7	0.103	0.139	0.141

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2-Methylnaphthalene	50	7	59.21	110.71	1579.71
1-Methylnaphthalene	50	7	45.788	90.488	1289.788
C1-Naphthalenes	50	7	104.499	200.499	2879.499
Biphenyl	50	7	6.666	146.916	208.916
C1-Biphenyls	50	7	6.761	161.851	153.851
C2-Biphenyls	50	7	6.175	96.025	66.125
C2-Naphthalenes	50	7	197.565	454.565	2749.565
1,2-Dimethylnaphthalene	50	7	15.3	17.8	0
2,6-Dimethylnaphthalene	50	7	41.905	100.905	591.905
C3-Naphthalenes	50	7	247.85	339.85	1009.85
2,3,6-Trimethylnaphthalene	50	7	74.7	108	282
2,3,5-Trimethylnaphthalene	50	7	51.2	67.1	215
C4-Naphthalenes	50	7	112.946	17.646	224.946
C1-Acenaphthenes	50	7	1.78	0.381	3.68
C1-Fluorenes	50	7	28.002	51.202	133.902
1,7-Dimethylfluorene	50	7	5.83	7	16.9
C2-Fluorenes	50	7	60.3	69.4	155
C3-Fluorenes	50	7	51.3	38.3	109
Dibenzothiophene	50	7	23.257	1.697	151.957
C1-Dibenzothiophenes	50	7	73.978	2.348	212.978
2/3-Methyldibenzothiophenes	50	7	23.282	1.192	78.082
C2-Dibenzothiophenes	50	7	116.979	7.969	190.979
2,4-Dimethyldibenzothiophene	50	7	9.45	0.682	20.2
4,6-Dimethyldibenzothiophene	50	7	13.5	2.03	23.6
C3-Dibenzothiophenes	50	7	67.6	8.05	88.3

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Dibenzothiophenes	50	7	17.8	4	20.9
3-Methylphenanthrene	50	7	16.375	6.285	60.175
2-Methylphenanthrene	50	7	20.263	7.163	70.863
2-Methylanthracene	50	7	1.684	0.319	1.154
9/4-Methylphenanthrene	50	7	27.972	4.692	89.272
1-Methylphenanthrene	50	7	17.167	4.427	64.167
3,6-Dimethylphenanthrene	50	7	4.03	1.22	10.3
2,6-Dimethylphenanthrene	50	7	3.15	1.48	8.82
1,7-Dimethylphenanthrene	50	7	7.825	1.755	17.745
1,8-Dimethylphenanthrene	50	7	2.22	0.449	5.7
C3-Phenanthrenes/Anthracenes	50	7	25.4	8.44	57.1
1,2,6-Trimethylphenanthrene	50	7	1.42	0.365	2.84
Retene	50	7	1.96	2.19	2.09
C4-Phenanthrenes/Anthracenes	50	7	28.4	10.3	59.8
C1-Fluoranthenes/Pyrenes	50	7	7.77	2.9	14.9
3-Methylfluoranthene/Benzo[a]fluorene	50	7	3.95	1.15	7.24
C2-Fluoranthenes/Pyrenes	50	7	7.28	4.44	15.8
C3-Fluoranthenes/Pyrenes	50	7	2.86	0.447	4.7
C4-Fluoranthenes/Pyrenes	50	7	0.498	0.192	2.11
C1-Benzo[a]anthracenes/Chrysenes	50	7	3.24	5.77	8.16
5/6-Methylchrysene	50	7	0.759	4.13	1.95
1-Methylchrysene	50	7	0.47	0.367	1.15
C2-Benzo[a]anthracenes/Chrysenes	50	7	1.48	0.641	3.5
5,9-Dimethylchrysene	50	7	0.276	0.107	0.592
C3-Benzo[a]anthracenes/Chrysenes	50	7	0	0	0.336

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Benzo[a]anthracenes/Chrysenes	50	7	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	50	7	0.209	0	1.16
7-Methylbenzo[a]pyrene	50	7	0	0	0.12
C2-Benzofluoranthenes/Benzopyrenes	50	7	0	0	0.335
1,4,6,7-Tetramethylnaphthalene	50	7	20.5	17.7	34.7
Naphthalene	100	7	6.199	19.955	2149.455
Acenaphthylene	100	7	-0.045	-0.014	0.234
Acenaphthene	100	7	5.311	3.23	32.7
2-Methylfluorene	100	7	6.553	23.5	52.7
C2 Phenanthrenes/Anthracenes	100	7	128.953	29.023	281.923
Fluorene	100	7	10.462	52.969	171.969
Phenanthrene	100	7	52.995	21.412	413.912
Anthracene	100	7	2.037	0.71	3.59
C1 Phenanthrenes/Anthracenes	100	7	161	50.077	699.877
Fluoranthene	100	7	4.139	4.893	6.313
Pyrene	100	7	4.528	5.573	9.263
Benz[a]anthracene	100	7	1.17	0.236	0
Chrysene	100	7	5.11	1.258	12.888
Benzo[b]fluoranthene	100	7	0.668	0.493	0.868
Benzo[j,k]fluoranthenes	100	7	0.421	0.277	0.19
Benzo[e]pyrene	100	7	0.956	0.591	1.73
Benzo[a]pyrene	100	7	0.333	0	0
Perylene	100	7	1	0	0.184
Dibenz[a,h]anthracene	100	7	0	0	0
Indeno[1,2,3-cd]pyrene	100	7	0.178	0.042	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[ghi]perylene	100	7	0.206	0.088	0.201
2-Methylnaphthalene	100	7	29.427	104.71	2929.71
1-Methylnaphthalene	100	7	21.067	81.488	2449.788
C1-Naphthalenes	100	7	50.493	186.499	5379.499
Biphenyl	100	7	4.576	206.916	411.916
C1-Biphenyls	100	7	7.153	284.851	273.851
C2-Biphenyls	100	7	5.552	194.625	85.225
C2-Naphthalenes	100	7	220.881	720.565	5459.565
1,2-Dimethylnaphthalene	100	7	16.5	27.1	0
2,6-Dimethylnaphthalene	100	7	46.209	157.905	1129.905
C3-Naphthalenes	100	7	349.875	654.85	1969.85
2,3,6-Trimethylnaphthalene	100	7	107.952	211	0
2,3,5-Trimethylnaphthalene	100	7	74.739	134	429
C4-Naphthalenes	100	7	164	229.946	338.946
C1-Acenaphthenes	100	7	1.99	0.971	7.73
C1-Fluorenes	100	7	44.697	112.902	305.902
1,7-Dimethylfluorene	100	7	11.1	15.5	39.1
C2-Fluorenes	100	7	124.841	163	382
C3-Fluorenes	100	7	104	91.7	249
Dibenzothiophene	100	7	30.59	0.949	335.957
C1-Dibenzothiophenes	100	7	140	0.587	496.978
2/3-Methyldibenzothiophenes	100	7	42	0.656	179.982
C2-Dibenzothiophenes	100	7	279	8.469	478.979
2,4-Dimethyldibenzothiophene	100	7	20.9	0	49.8
4,6-Dimethyldibenzothiophene	100	7	33.5	4.15	60.7

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Dibenzothiophenes	100	7	11	2.93	228
C4-Dibenzothiophenes	100	7	78.637	1.24	46.9
3-Methylphenanthrene	100	7	31.7	13.875	144.975
2-Methylphenanthrene	100	7	38.5	16.363	180.963
2-Methylanthracene	100	7	3.06	0.373	3.524
9/4-Methylphenanthrene	100	7	54.3	9.892	218.972
1-Methylphenanthrene	100	7	33.8	9.617	152.967
3,6-Dimethylphenanthrene	100	7	9.168	3.09	21.6
2,6-Dimethylphenanthrene	100	7	6.75	3.65	19.5
1,7-Dimethylphenanthrene	100	7	19.6	4.195	38.445
1,8-Dimethylphenanthrene	100	7	5.09	1.05	12.6
C3-Phenanthrenes/Anthracenes	100	7	84.9	15.5	127
1,2,6-Trimethylphenanthrene	100	7	3.72	0.67	6.31
Retene	100	7	5.89	1.71	3.26
C4-Phenanthrenes/Anthracenes	100	7	110	10.9	121
C1-Fluoranthenes/Pyrenes	100	7	18.9	3.42	31.4
3-Methylfluoranthene/Benzo[a]fluorene	100	7	8.55	0.806	16.1
C2-Fluoranthenes/Pyrenes	100	7	21.3	1.78	35.4
C3-Fluoranthenes/Pyrenes	100	7	11.9	0.086	10.8
C4-Fluoranthenes/Pyrenes	100	7	2.15	0.244	2.72
C1-Benzo[a]anthracenes/Chrysenes	100	7	10.4	1.36	15.5
5/6-Methylchrysene	100	7	1.36	0.795	2.24
1-Methylchrysene	100	7	1.48	0.23	2.21
C2-Benzo[a]anthracenes/Chrysenes	100	7	6.59	0.169	7.75
5,9-Dimethylchrysene	100	7	1.56	0	1.67

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Benzo[a]anthracenes/Chrysenes	100	7	1.5	0	1.04
C4-Benzo[a]anthracenes/Chrysenes	100	7	0.125	0	0
C1-Benzofluoranthenes/Benzopyrenes	100	7	1.99	0	2.41
7-Methylbenzo[a]pyrene	100	7	0.122	0	0.314
C2-Benzofluoranthenes/Benzopyrenes	100	7	0.777	0	0.61
1,4,6,7-Tetramethylnaphthalene	100	7	28.3	38.2	54.5
Naphthalene	0	21	-0.046	0.526	0.216
Acenaphthylene	0	21	0.028	0.024	0.004
Acenaphthene	0	21	0.297	0.392	0.267
2-Methylfluorene	0	21	0.571	0.246	0.563
C2 Phenanthrenes/Anthracenes	0	21	18.623	3.223	26.653
Fluorene	0	21	0.716	0.757	0.682
Phenanthrene	0	21	7.482	4.715	4.735
Anthracene	0	21	0.359	0.241	0.172
C1 Phenanthrenes/Anthracenes	0	21	22.277	3.77	24.4
Fluoranthene	0	21	3.813	5.649	3.819
Pyrene	0	21	3.123	3.618	3.258
Benz[a]anthracene	0	21	0.284	0.138	0.226
Chrysene	0	21	1.808	1.1	2.78
Benzo[b]fluoranthene	0	21	0.33	0.441	0.324
Benzo[j,k]fluoranthenes	0	21	0	0.194	0.158
Benzo[e]pyrene	0	21	0.479	0.526	0.591
Benzo[a]pyrene	0	21	0	0	0
Perylene	0	21	0	0	0
Dibenz[a,h]anthracene	0	21	0	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Indeno[1,2,3-cd]pyrene	0	21	0	0	0
Benzo[ghi]perylene	0	21	0.049	0.108	0.053
2-Methylnaphthalene	0	21	0.108	0.6	0.425
1-Methylnaphthalene	0	21	0.074	0.396	0.324
C1-Naphthalenes	0	21	0.184	0.993	0.753
Biphenyl	0	21	0.111	0.2	0.142
C1-Biphenyls	0	21	0.325	0.658	0.627
C2-Biphenyls	0	21	0.586	1.012	1.172
C2-Naphthalenes	0	21	2.715	2.071	4.211
1,2-Dimethylnaphthalene	0	21	0.273	0.135	0.291
2,6-Dimethylnaphthalene	0	21	0.401	0.204	0.491
C3-Naphthalenes	0	21	11.65	4.515	14.875
2,3,6-Trimethylnaphthalene	0	21	3.59	1.632	4.352
2,3,5-Trimethylnaphthalene	0	21	2.45	0.883	3.479
C4-Naphthalenes	0	21	9.666	4.18	14.6
C1-Acenaphthenes	0	21	0.056	0	0.076
C1-Fluorenes	0	21	3.032	1.397	4.087
1,7-Dimethylfluorene	0	21	0.991	0.209	1.67
C2-Fluorenes	0	21	11.5	4.071	17.241
C3-Fluorenes	0	21	12.2	5.05	22.6
Dibenzothiophene	0	21	2.257	0.771	2.37
C1-Dibenzothiophenes	0	21	15.578	2.73	16.5
2/3-Methyldibenzothiophenes	0	21	3.582	0.662	4.13
C2-Dibenzothiophenes	0	21	33.079	5.02	38.3
2,4-Dimethyldibenzothiophene	0	21	2.79	0.535	4.07

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
4,6-Dimethyldibenzothiophene	0	21	4.49	0.881	7.03
C3-Dibenzothiophenes	0	21	22.8	2.66	26
C4-Dibenzothiophenes	0	21	5.48	0.746	9.027
3-Methylphenanthrene	0	21	4.315	1.31	5.27
2-Methylphenanthrene	0	21	4.483	1.09	5.01
2-Methylanthracene	0	21	0.33	0.111	0.195
9/4-Methylphenanthrene	0	21	8.752	1.38	9.41
1-Methylphenanthrene	0	21	4.337	0.737	4.48
3,6-Dimethylphenanthrene	0	21	1.52	0.369	2.088
2,6-Dimethylphenanthrene	0	21	1.09	0.247	2.07
1,7-Dimethylphenanthrene	0	21	2.575	0.305	3.72
1,8-Dimethylphenanthrene	0	21	0.956	0.077	1.68
C3-Phenanthrenes/Anthracenes	0	21	11	2.34	20.8
1,2,6-Trimethylphenanthrene	0	21	0.493	0.081	1.05
Retene	0	21	1.18	1.2	1.23
C4-Phenanthrenes/Anthracenes	0	21	12.3	10.5	27.4
C1-Fluoranthenes/Pyrenes	0	21	3.57	1.87	5.88
3-Methylfluoranthene/Benzo[a]fluorene	0	21	1.69	0.564	2.44
C2-Fluoranthenes/Pyrenes	0	21	3.15	0.394	6.71
C3-Fluoranthenes/Pyrenes	0	21	0.905	0.094	2.76
C4-Fluoranthenes/Pyrenes	0	21	0	0	0.445
C1-Benzo[a]anthracenes/Chrysenes	0	21	1.84	5.91	3.75
5/6-Methylchrysene	0	21	0.265	4.08	0.708
1-Methylchrysene	0	21	0.255	0.929	0.528
C2-Benzo[a]anthracenes/Chrysenes	0	21	0.722	0.277	2.12

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
5,9-Dimethylchrysene	0	21	0.184	0	0.397
C3-Benzo[a]anthracenes/Chrysenes	0	21	0	0	0.377
C4-Benzo[a]anthracenes/Chrysenes	0	21	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	0	21	0	0	0.618
7-Methylbenzo[a]pyrene	0	21	0	0	0.067
C2-Benzofluoranthenes/Benzopyrenes	0	21	0	0	0.088
1,4,6,7-Tetramethylnaphthalene	0	21	1.93	0.721	2.71
Naphthalene	25	21	-0.067	0.326	0.291
Acenaphthylene	25	21	0.013	0.014	0
Acenaphthene	25	21	0.5	0.194	0.271
2-Methylfluorene	25	21	0.857	1.643	0.577
C2 Phenanthrenes/Anthracenes	25	21	34.123	8.703	50.553
Fluorene	25	21	1.299	1.422	0.788
Phenanthrene	25	21	11.812	3.695	6.765
Anthracene	25	21	0.57	0.178	0.239
C1 Phenanthrenes/Anthracenes	25	21	46.877	10.1	37.3
Fluoranthene	25	21	4.833	4.949	4.699
Pyrene	25	21	4.903	3.888	4.218
Benz[a]anthracene	25	21	0.471	0.17	0.353
Chrysene	25	21	2.418	1.1	4.66
Benzo[b]fluoranthene	25	21	0.595	0.224	0.489
Benzo[j,k]fluoranthenes	25	21	0.365	0.131	0.181
Benzo[e]pyrene	25	21	0.588	0.432	0.737
Benzo[a]pyrene	25	21	0.053	0	0
Perylene	25	21	0.21	0	0.063

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Dibenz[a,h]anthracene	25	21	0	0	0
Indeno[1,2,3-cd]pyrene	25	21	0.063	0	0
Benzo[ghi]perylene	25	21	0.073	0.053	0.059
2-Methylnaphthalene	25	21	0.72	0.397	0.657
1-Methylnaphthalene	25	21	0.444	0.253	0.34
C1-Naphthalenes	25	21	1.169	0.649	0.993
Biphenyl	25	21	0.218	0.217	0.135
C1-Biphenyls	25	21	0.539	5.463	1.003
C2-Biphenyls	25	21	1.045	8.082	2.312
C2-Naphthalenes	25	21	9.965	6.191	6.551
1,2-Dimethylnaphthalene	25	21	0.851	0.289	0.421
2,6-Dimethylnaphthalene	25	21	1.965	1.119	1.099
C3-Naphthalenes	25	21	39.55	40.175	23.575
2,3,6-Trimethylnaphthalene	25	21	12.4	13.352	6.802
2,3,5-Trimethylnaphthalene	25	21	7.68	9.399	4.919
C4-Naphthalenes	25	21	33.646	27.3	27.6
C1-Acenaphthenes	25	21	0.162	0.111	0.072
C1-Fluorenes	25	21	6.622	10.897	4.947
1,7-Dimethylfluorene	25	21	2.22	3.11	2.48
C2-Fluorenes	25	21	26.7	32.741	25.541
C3-Fluorenes	25	21	32.9	19.3	35
Dibenzothiophene	25	21	4.537	0.48	2.51
C1-Dibenzothiophenes	25	21	34.078	2.6	23
2/3-Methyldibenzothiophenes	25	21	7.812	0.651	5.42
C2-Dibenzothiophenes	25	21	75.779	6.34	65.5

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2,4-Dimethyldibenzothiophene	25	21	6.63	0.463	7.24
4,6-Dimethyldibenzothiophene	25	21	10.3	1.75	11.3
C3-Dibenzothiophenes	25	21	52.7	3.23	45.3
C4-Dibenzothiophenes	25	21	12.1	0.563	15.137
3-Methylphenanthrene	25	21	9.835	2.84	8.19
2-Methylphenanthrene	25	21	8.463	2.92	6.96
2-Methylanthracene	25	21	0.529	0	0.19
9/4-Methylphenanthrene	25	21	18.472	2.51	14.6
1-Methylphenanthrene	25	21	9.547	1.82	7.33
3,6-Dimethylphenanthrene	25	21	2.81	0.913	3.918
2,6-Dimethylphenanthrene	25	21	1.97	0.873	4.27
1,7-Dimethylphenanthrene	25	21	4.905	0.991	6.77
1,8-Dimethylphenanthrene	25	21	1.86	0.308	2.81
C3-Phenanthrenes/Anthracenes	25	21	22.1	4.39	40.9
1,2,6-Trimethylphenanthrene	25	21	1.19	0.141	2.04
Retene	25	21	1.97	1.09	2.01
C4-Phenanthrenes/Anthracenes	25	21	27.7	5.47	44.7
C1-Fluoranthenes/Pyrenes	25	21	7.15	1.93	9.74
3-Methylfluoranthene/Benzo[a]fluorene	25	21	3.47	0.625	3.98
C2-Fluoranthenes/Pyrenes	25	21	6.95	0.936	10.4
C3-Fluoranthenes/Pyrenes	25	21	2.3	0.041	3.38
C4-Fluoranthenes/Pyrenes	25	21	0.406	0.216	0.415
C1-Benzo[a]anthracenes/Chrysenes	25	21	2.66	1.11	5.27
5/6-Methylchrysene	25	21	0.336	0.645	0.737
1-Methylchrysene	25	21	0.365	0.168	0.776

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Benzo[a]anthracenes/Chrysenes	25	21	1.17	0.124	2.54
5,9-Dimethylchrysene	25	21	0.265	0	0.499
C3-Benzo[a]anthracenes/Chrysenes	25	21	0	0	0.309
C4-Benzo[a]anthracenes/Chrysenes	25	21	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	25	21	0.31	0.245	0.564
7-Methylbenzo[a]pyrene	25	21	0	0	0.077
C2-Benzofluoranthenes/Benzopyrenes	25	21	0	0	0.131
1,4,6,7-Tetramethylnaphthalene	25	21	6.35	5.14	4.4
Naphthalene	50	21	0.194	0.52	0.287
Acenaphthylene	50	21	-0.021	0.024	0.048
Acenaphthene	50	21	0.226	0.211	0.536
2-Methylfluorene	50	21	0.353	2.133	1.04
C2 Phenanthrenes/Anthracenes	50	21	42.353	10.753	42.823
Fluorene	50	21	0.437	1.562	1.379
Phenanthrene	50	21	6.475	8.535	10.412
Anthracene	50	21	0.299	0.265	0.468
C1 Phenanthrenes/Anthracenes	50	21	34.7	12.3	54.577
Fluoranthene	50	21	3.819	8.889	6.213
Pyrene	50	21	3.738	4.698	4.733
Benz[a]anthracene	50	21	0.566	0.218	0.36
Chrysene	50	21	2.99	1.37	3.778
Benzo[b]fluoranthene	50	21	0.607	0.327	0.47
Benzo[j,k]fluoranthenes	50	21	0.388	0.177	0.211
Benzo[e]pyrene	50	21	0.777	0.604	0.692
Benzo[a]pyrene	50	21	0.125	0	0

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Perylene	50	21	0.406	0	0
Dibenz[a,h]anthracene	50	21	0	0	0
Indeno[1,2,3-cd]pyrene	50	21	0.062	0	0
Benzo[ghi]perylene	50	21	0.096	0.071	0.076
2-Methylnaphthalene	50	21	0.283	0.495	1.37
1-Methylnaphthalene	50	21	0.121	0.302	0.828
C1-Naphthalenes	50	21	0.403	0.793	2.199
Biphenyl	50	21	0.038	0.293	0.45
C1-Biphenyls	50	21	0.253	6.473	1.361
C2-Biphenyls	50	21	0.468	20.072	3.495
C2-Naphthalenes	50	21	2.171	6.411	16.465
1,2-Dimethylnaphthalene	50	21	0.205	0.464	1.08
2,6-Dimethylnaphthalene	50	21	0.39	1.109	3.095
C3-Naphthalenes	50	21	15.575	50.075	43.25
2,3,6-Trimethylnaphthalene	50	21	5.122	16.152	12.6
2,3,5-Trimethylnaphthalene	50	21	3.109	11.239	8.72
C4-Naphthalenes	50	21	18.5	61.5	29.946
C1-Acenaphthenes	50	21	0.054	0.094	0.125
C1-Fluorenes	50	21	3.367	12.097	7.472
1,7-Dimethylfluorene	50	21	1.53	4.05	3.04
C2-Fluorenes	50	21	19.741	40.541	30.5
C3-Fluorenes	50	21	28	31.5	41.3
Dibenzothiophene	50	21	1.79	0.696	5.117
C1-Dibenzothiophenes	50	21	22.8	3.27	36.078
2/3-Methyldibenzothiophenes	50	21	4.5	0.942	10.082

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Dibenzothiophenes	50	21	78.5	8.93	78.879
2,4-Dimethyldibenzothiophene	50	21	6.85	0.687	8.9
4,6-Dimethyldibenzothiophene	50	21	12.3	2.32	12.8
C3-Dibenzothiophenes	50	21	66.1	4.53	51.2
C4-Dibenzothiophenes	50	21	27.837	1.437	11.9
3-Methylphenanthrene	50	21	6.97	3.34	12.075
2-Methylphenanthrene	50	21	6.04	3.68	10.463
2-Methylanthracene	50	21	0.437	0.082	0.199
9/4-Methylphenanthrene	50	21	14.5	3.12	20.372
1-Methylphenanthrene	50	21	6.73	2.17	11.467
3,6-Dimethylphenanthrene	50	21	3.398	1.028	3.8
2,6-Dimethylphenanthrene	50	21	2.42	1.07	3.5
1,7-Dimethylphenanthrene	50	21	5.76	1.26	5.975
1,8-Dimethylphenanthrene	50	21	2.04	0.377	2.37
C3-Phenanthrenes/Anthracenes	50	21	33.9	5.71	29.1
1,2,6-Trimethylphenanthrene	50	21	1.41	0.195	1.45
Retene	50	21	2.66	1.59	1.6
C4-Phenanthrenes/Anthracenes	50	21	46.1	7.37	32.3
C1-Fluoranthenes/Pyrenes	50	21	8.73	2.44	7.02
3-Methylfluoranthene/Benzo[a]fluorene	50	21	3.62	0.815	3.45
C2-Fluoranthenes/Pyrenes	50	21	9.98	1.32	8.31
C3-Fluoranthenes/Pyrenes	50	21	3.85	0.119	2.7
C4-Fluoranthenes/Pyrenes	50	21	0.621	0.376	0.538
C1-Benzo[a]anthracenes/Chrysenes	50	21	5.05	1.36	4.47
5/6-Methylchrysene	50	21	0.511	0.756	0.689

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
1-Methylchrysene	50	21	0.77	0.204	0.586
C2-Benzo[a]anthracenes/Chrysenes	50	21	3.11	0.142	1.91
5,9-Dimethylchrysene	50	21	0.625	0	0.401
C3-Benzo[a]anthracenes/Chrysenes	50	21	0.625	0	0.206
C4-Benzo[a]anthracenes/Chrysenes	50	21	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	50	21	0.833	0	0.529
7-Methylbenzo[a]pyrene	50	21	0.066	0	0.046
C2-Benzofluoranthenes/Benzopyrenes	50	21	0.282	0	0
1,4,6,7-Tetramethylnaphthalene	50	21	3.63	10.1	5.46
Naphthalene	100	21	0.117	0.469	0.203
Acenaphthylene	100	21	-0.045	0.015	-0.014
Acenaphthene	100	21	0.195	0.264	0.305
2-Methylfluorene	100	21	0.332	5.413	0.961
C2 Phenanthrenes/Anthracenes	100	21	36.153	36.153	54.423
Fluorene	100	21	0.373	1.692	0.753
Phenanthrene	100	21	4.845	4.955	5.722
Anthracene	100	21	0.215	0.442	0.209
C1 Phenanthrenes/Anthracenes	100	21	23.7	30.6	41.177
Fluoranthene	100	21	3.399	5.099	4.203
Pyrene	100	21	3.058	6.568	3.903
Benz[a]anthracene	100	21	0.667	0.219	0.609
Chrysene	100	21	3.7	1.23	6.178
Benzo[b]fluoranthene	100	21	0.624	0.246	0.612
Benzo[j,k]fluoranthenes	100	21	0.265	0.173	0.082
Benzo[e]pyrene	100	21	0.932	0.508	1.14

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[a]pyrene	100	21	0.167	0	0
Perylene	100	21	0.614	0	0.129
Dibenz[a,h]anthracene	100	21	0	0	0
Indeno[1,2,3-cd]pyrene	100	21	0.062	0	0
Benzo[ghi]perylene	100	21	0.115	0.07	0.108
2-Methylnaphthalene	100	21	0.252	0.566	0.85
1-Methylnaphthalene	100	21	0.139	0.314	0.367
C1-Naphthalenes	100	21	0.39	0.883	1.219
Biphenyl	100	21	0.034	0.272	0.192
C1-Biphenyls	100	21	0.221	10.323	0.84
C2-Biphenyls	100	21	0.335	25.872	2.855
C2-Naphthalenes	100	21	2.581	8.301	4.825
1,2-Dimethylnaphthalene	100	21	0.215	0.442	0.392
2,6-Dimethylnaphthalene	100	21	0.396	1.849	1.045
C3-Naphthalenes	100	21	10.375	86.275	20.95
2,3,6-Trimethylnaphthalene	100	21	3.312	27.952	5.94
2,3,5-Trimethylnaphthalene	100	21	2.169	20.039	4.71
C4-Naphthalenes	100	21	10.1	99.5	26.446
C1-Acenaphthenes	100	21	0.044	0.106	0.061
C1-Fluorenes	100	21	2.137	28.397	4.872
1,7-Dimethylfluorene	100	21	1.07	14.2	3.54
C2-Fluorenes	100	21	13.341	131.841	30.7
C3-Fluorenes	100	21	24.3	69.1	49.5
Dibenzothiophene	100	21	1.13	-0.058	2.167
C1-Dibenzothiophenes	100	21	15.1	3.07	26.278

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
2/3-Methyldibenzothiophenes	100	21	2.89	0.82	6.542
C2-Dibenzothiophenes	100	21	69.6	12.6	91.879
2,4-Dimethyldibenzothiophene	100	21	6.06	0.567	9.78
4,6-Dimethyldibenzothiophene	100	21	11.8	5.73	16.7
C3-Dibenzothiophenes	100	21	82.7	5.67	87
C4-Dibenzothiophenes	100	21	44.137	1.217	25.2
3-Methylphenanthrene	100	21	4.83	7.91	8.685
2-Methylphenanthrene	100	21	4.11	10.6	8.713
2-Methylanthracene	100	21	0.362	0.256	0.21
9/4-Methylphenanthrene	100	21	9.69	7.04	15.072
1-Methylphenanthrene	100	21	4.67	5.08	8.487
3,6-Dimethylphenanthrene	100	21	2.838	3.418	4.15
2,6-Dimethylphenanthrene	100	21	2.18	3.4	4.31
1,7-Dimethylphenanthrene	100	21	4.94	4.68	8.345
1,8-Dimethylphenanthrene	100	21	1.85	1.32	3.29
C3-Phenanthrenes/Anthracenes	100	21	38.9	17.6	57.1
1,2,6-Trimethylphenanthrene	100	21	1.89	0.566	2.91
Retene	100	21	3.63	1.53	3.17
C4-Phenanthrenes/Anthracenes	100	21	66.5	16.4	70.7
C1-Fluoranthenes/Pyrenes	100	21	9.82	4.14	10.6
3-Methylfluoranthene/Benzo[a]fluorene	100	21	3.91	0.866	5.18
C2-Fluoranthenes/Pyrenes	100	21	14.5	2.75	19.7
C3-Fluoranthenes/Pyrenes	100	21	7.08	0.228	6.97
C4-Fluoranthenes/Pyrenes	100	21	1.13	0.981	1.95
C1-Benzo[a]anthracenes/Chrysenes	100	21	8.71	4.64	15.1

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
5/6-Methylchrysene	100	21	0.777	3.16	5.1
1-Methylchrysene	100	21	1.26	0.682	1.92
C2-Benzo[a]anthracenes/Chrysenes	100	21	5.09	0.191	6.45
5,9-Dimethylchrysene	100	21	1.03	0	1.03
C3-Benzo[a]anthracenes/Chrysenes	100	21	0.875	0	0.509
C4-Benzo[a]anthracenes/Chrysenes	100	21	0.066	0	0
C1-Benzofluoranthenes/Benzopyrenes	100	21	1.28	0	1.41
7-Methylbenzo[a]pyrene	100	21	0.088	0	0.129
C2-Benzofluoranthenes/Benzopyrenes	100	21	0.388	0	0.249
1,4,6,7-Tetramethylnaphthalene	100	21	1.89	18.6	4.8
Naphthalene	0	35	0.161	0.549	0.146
Acenaphthylene	0	35	0.007	0.092	-0.014
Acenaphthene	0	35	0.214	0.45	0.43
2-Methylfluorene	0	35	0.175	0.207	0.361
C2 Phenanthrenes/Anthracenes	0	35	14.553	4.573	28.053
Fluorene	0	35	0.459	0.915	0.719
Phenanthrene	0	35	9.515	6.175	3.845
Anthracene	0	35	0.147	0.281	0.23
C1 Phenanthrenes/Anthracenes	0	35	11.6	6.95	15.5
Fluoranthene	0	35	4.509	5.789	5.079
Pyrene	0	35	3.648	3.808	4.598
Benz[a]anthracene	0	35	0.383	0.129	0.332
Chrysene	0	35	2.21	0.905	4.1
Benzo[b]fluoranthene	0	35	0.553	0.18	0.38
Benzo[j,k]fluoranthenes	0	35	0.437	0.146	0.136

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[e]pyrene	0	35	0.788	0.334	0.728
Benzo[a]pyrene	0	35	0.136	0	0
Perylene	0	35	0.271	0	0
Dibenz[a,h]anthracene	0	35	0.275	0	0
Indeno[1,2,3-cd]pyrene	0	35	0.292	0	0
Benzo[ghi]perylene	0	35	0.366	0.051	0
2-Methylnaphthalene	0	35	0.211	0.496	0.383
1-Methylnaphthalene	0	35	0.157	0.385	0.258
C1-Naphthalenes	0	35	0.367	0.883	0.64
Biphenyl	0	35	0.071	0.218	0.118
C1-Biphenyls	0	35	0.187	0.506	0.186
C2-Biphenyls	0	35	0.111	0.34	-0.087
C2-Naphthalenes	0	35	1.301	2.021	2.461
1,2-Dimethylnaphthalene	0	35	0.125	0	0.208
2,6-Dimethylnaphthalene	0	35	0.119	0.22	0.23
C3-Naphthalenes	0	35	2.745	2.835	4.205
2,3,6-Trimethylnaphthalene	0	35	0.81	0.721	1.212
2,3,5-Trimethylnaphthalene	0	35	0.558	0.595	1.089
C4-Naphthalenes	0	35	2.69	0.995	2.52
C1-Acenaphthenes	0	35	0.028	0	0
C1-Fluorenes	0	35	1.117	1.337	1.917
1,7-Dimethylfluorene	0	35	0.321	0	0.474
C2-Fluorenes	0	35	5.451	2.971	7.681
C3-Fluorenes	0	35	9.63	2.99	10.7
Dibenzothiophene	0	35	0.618	0.89	0.95

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Dibenzothiophenes	0	35	6.37	3.99	10.3
2/3-Methyldibenzothiophenes	0	35	1.02	0.811	2.09
C2-Dibenzothiophenes	0	35	24.7	9.38	42.2
2,4-Dimethyldibenzothiophene	0	35	2.58	0.785	4.65
4,6-Dimethyldibenzothiophene	0	35	4.88	1.65	9.07
C3-Dibenzothiophenes	0	35	20.1	4.83	30.4
C4-Dibenzothiophenes	0	35	7.067	1.417	6.667
3-Methylphenanthrene	0	35	2.49	1.78	3.25
2-Methylphenanthrene	0	35	1.83	1.56	2.88
2-Methylanthracene	0	35	0.161	0.103	0.103
9/4-Methylphenanthrene	0	35	4.91	2.15	6.38
1-Methylphenanthrene	0	35	2.26	1.36	2.91
3,6-Dimethylphenanthrene	0	35	1.498	0.522	2.428
2,6-Dimethylphenanthrene	0	35	0.827	0.37	1.9
1,7-Dimethylphenanthrene	0	35	1.72	0.493	3.49
1,8-Dimethylphenanthrene	0	35	0.845	0.122	1.85
C3-Phenanthrenes/Anthracenes	0	35	12.9	2.48	24.3
1,2,6-Trimethylphenanthrene	0	35	0.815	0.081	1.15
Retene	0	35	1.34	0.749	0.664
C4-Phenanthrenes/Anthracenes	0	35	17.2	2.46	21.6
C1-Fluoranthenes/Pyrenes	0	35	4.71	1.54	6.72
3-Methylfluoranthene/Benzo[a]fluorene	0	35	1.91	0.545	2.39
C2-Fluoranthenes/Pyrenes	0	35	3.99	0.761	7.14
C3-Fluoranthenes/Pyrenes	0	35	1.76	0.103	3.03
C4-Fluoranthenes/Pyrenes	0	35	0.3	0	0.239

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C1-Benzo[a]anthracenes/Chrysenes	0	35	2.81	0.411	4.62
5/6-Methylchrysene	0	35	0.609	0.1	0.488
1-Methylchrysene	0	35	0.52	0.053	0.553
C2-Benzo[a]anthracenes/Chrysenes	0	35	1.08	0.11	2.05
5,9-Dimethylchrysene	0	35	0.315	0	0.406
C3-Benzo[a]anthracenes/Chrysenes	0	35	0	0	0.393
C4-Benzo[a]anthracenes/Chrysenes	0	35	0.06	0	0
C1-Benzofluoranthenes/Benzopyrenes	0	35	0.453	0	0.667
7-Methylbenzo[a]pyrene	0	35	0.111	0	0
C2-Benzofluoranthenes/Benzopyrenes	0	35	0.171	0	0.222
1,4,6,7-Tetramethylnaphthalene	0	35	0.504	0.223	0.477
Naphthalene	25	35	-0.092	0.385	0.267
Acenaphthylene	25	35	-0.014	0.068	-0.002
Acenaphthene	25	35	0.181	0.45	0.472
2-Methylfluorene	25	35	0.188	0.488	0.468
C2 Phenanthrenes/Anthracenes	25	35	9.563	7.393	35.753
Fluorene	25	35	0.293	0.992	0.803
Phenanthrene	25	35	2.992	5.605	5.705
Anthracene	25	35	0.135	0.334	0.283
C1 Phenanthrenes/Anthracenes	25	35	7.957	8.73	23.3
Fluoranthene	25	35	2.923	5.519	6.129
Pyrene	25	35	2.313	3.988	5.708
Benz[a]anthracene	25	35	0.221	0.14	0.388
Chrysene	25	35	1.578	1.13	4.26
Benzo[b]fluoranthene	25	35	0.308	0.147	0.479

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[j,k]fluoranthenes	25	35	0.13	0.102	0.195
Benzo[e]pyrene	25	35	0.444	0.332	0.745
Benzo[a]pyrene	25	35	0	0	0
Perylene	25	35	0.148	0	0
Dibenz[a,h]anthracene	25	35	0	0	0
Indeno[1,2,3-cd]pyrene	25	35	0	0	0
Benzo[ghi]perylene	25	35	0.044	0.064	0.07
2-Methylnaphthalene	25	35	0.06	0.403	0.437
1-Methylnaphthalene	25	35	0.034	0.261	0.243
C1-Naphthalenes	25	35	0.095	0.662	0.679
Biphenyl	25	35	0.047	0.209	0.147
C1-Biphenyls	25	35	0.14	0.792	0.715
C2-Biphenyls	25	35	0.235	0.852	1.092
C2-Naphthalenes	25	35	1.375	1.941	2.731
1,2-Dimethylnaphthalene	25	35	0.143	0.14	0.186
2,6-Dimethylnaphthalene	25	35	0.249	0.227	0.415
C3-Naphthalenes	25	35	4.55	6.205	9.565
2,3,6-Trimethylnaphthalene	25	35	1.47	1.952	2.762
2,3,5-Trimethylnaphthalene	25	35	0.944	1.439	2.229
C4-Naphthalenes	25	35	3.436	6.06	10.4
C1-Acenaphthenes	25	35	0	0	0
C1-Fluorenes	25	35	0.932	2.727	3.107
1,7-Dimethylfluorene	25	35	0.285	0.651	1.14
C2-Fluorenes	25	35	4.18	10.441	16.341
C3-Fluorenes	25	35	7.88	9.65	26.5

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Dibenzothiophene	25	35	0.517	0.815	1.41
C1-Dibenzothiophenes	25	35	4.638	4.74	15.9
2/3-Methyldibenzothiophenes	25	35	0.859	1.11	3.62
C2-Dibenzothiophenes	25	35	20.079	10.3	54
2,4-Dimethyldibenzothiophene	25	35	1.94	0.921	6.19
4,6-Dimethyldibenzothiophene	25	35	3.89	2	10.5
C3-Dibenzothiophenes	25	35	20.9	5.46	41.9
C4-Dibenzothiophenes	25	35	6.54	1.847	14.237
3-Methylphenanthrene	25	35	1.625	2.3	4.86
2-Methylphenanthrene	25	35	1.393	2.37	4.58
2-Methylanthracene	25	35	0.108	0.147	0.13
9/4-Methylphenanthrene	25	35	3.232	2.47	9.52
1-Methylphenanthrene	25	35	1.567	1.44	4.25
3,6-Dimethylphenanthrene	25	35	0.897	0.769	3.058
2,6-Dimethylphenanthrene	25	35	0.61	0.611	2.8
1,7-Dimethylphenanthrene	25	35	1.285	0.77	4.41
1,8-Dimethylphenanthrene	25	35	0.655	0.26	2.11
C3-Phenanthrenes/Anthracenes	25	35	10	4.6	33.1
1,2,6-Trimethylphenanthrene	25	35	0.579	0.123	1.37
Retene	25	35	1.15	0.876	2.05
C4-Phenanthrenes/Anthracenes	25	35	18.3	6.09	41.3
C1-Fluoranthenes/Pyrenes	25	35	3.55	1.9	8.61
3-Methylfluoranthene/Benzo[a]fluorene	25	35	1.54	0.622	3.25
C2-Fluoranthenes/Pyrenes	25	35	4.34	1.28	9.58
C3-Fluoranthenes/Pyrenes	25	35	1.52	0.256	3.27

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C4-Fluoranthenes/Pyrenes	25	35	0.326	0.204	0.71
C1-Benzo[a]anthracenes/Chrysenes	25	35	2.45	1.43	5.49
5/6-Methylchrysene	25	35	0.187	0.682	0.544
1-Methylchrysene	25	35	0.368	0.173	0.749
C2-Benzo[a]anthracenes/Chrysenes	25	35	1.27	0.557	2.73
5,9-Dimethylchrysene	25	35	0.231	0.084	0.589
C3-Benzo[a]anthracenes/Chrysenes	25	35	0	0	0.4
C4-Benzo[a]anthracenes/Chrysenes	25	35	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	25	35	0.171	0	0.674
7-Methylbenzo[a]pyrene	25	35	0	0	0.077
C2-Benzofluoranthenes/Benzopyrenes	25	35	0	0.079	0.241
1,4,6,7-Tetramethylnaphthalene	25	35	0.694	1.22	1.81
Naphthalene	50	35	-0.09	0.229	0.391
Acenaphthylene	50	35	0.025	0.042	0.005
Acenaphthene	50	35	0.219	0.336	0.395
2-Methylfluorene	50	35	0.205	0.679	0.538
C2 Phenanthrenes/Anthracenes	50	35	18.323	8.523	22.953
Fluorene	50	35	0.438	0.911	0.895
Phenanthrene	50	35	6.632	4.545	6.555
Anthracene	50	35	0.289	0.222	0.327
C1 Phenanthrenes/Anthracenes	50	35	13.577	8.55	14.4
Fluoranthene	50	35	3.833	5.049	6.029
Pyrene	50	35	3.383	3.948	4.668
Benz[a]anthracene	50	35	0.304	0.142	0.261
Chrysene	50	35	2.448	1.04	3.09

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Benzo[b]fluoranthene	50	35	0.613	0.254	0.306
Benzo[j,k]fluoranthenes	50	35	0.294	0.147	0.17
Benzo[e]pyrene	50	35	0.652	0.42	0.655
Benzo[a]pyrene	50	35	0.072	0	0
Perylene	50	35	0.283	0	0
Dibenz[a,h]anthracene	50	35	0	0	0
Indeno[1,2,3-cd]pyrene	50	35	0.049	0	0
Benzo[ghi]perylene	50	35	0.093	0.048	0
2-Methylnaphthalene	50	35	0.068	0.359	0.621
1-Methylnaphthalene	50	35	0.044	0.228	0.337
C1-Naphthalenes	50	35	0.113	0.585	0.953
Biphenyl	50	35	0.071	0.16	0.161
C1-Biphenyls	50	35	0.166	1.603	0.59
C2-Biphenyls	50	35	0.274	3.282	0.852
C2-Naphthalenes	50	35	0.915	2.541	2.441
1,2-Dimethylnaphthalene	50	35	0.151	0.171	0.225
2,6-Dimethylnaphthalene	50	35	0.1	0.334	0.346
C3-Naphthalenes	50	35	4.2	15.075	5.385
2,3,6-Trimethylnaphthalene	50	35	1.28	4.812	1.512
2,3,5-Trimethylnaphthalene	50	35	0.84	3.289	1.179
C4-Naphthalenes	50	35	5.436	15.6	6.64
C1-Acenaphthenes	50	35	0	0	0
C1-Fluorenes	50	35	1.362	4.817	2.447
1,7-Dimethylfluorene	50	35	0.59	1.8	0.723
C2-Fluorenes	50	35	7.1	21.641	10.141

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Fluorenes	50	35	12.7	14.4	22.3
Dibenzothiophene	50	35	0.733	0.646	1.13
C1-Dibenzothiophenes	50	35	7.768	3.48	8.18
2/3-Methyldibenzothiophenes	50	35	1.442	0.908	1.82
C2-Dibenzothiophenes	50	35	36.879	7.55	30.6
2,4-Dimethyldibenzothiophene	50	35	3.41	0.579	3.71
4,6-Dimethyldibenzothiophene	50	35	6.75	1.95	6.77
C3-Dibenzothiophenes	50	35	39.7	3.44	24.3
C4-Dibenzothiophenes	50	35	16.5	0.828	9.707
3-Methylphenanthrene	50	35	2.665	2.4	3.21
2-Methylphenanthrene	50	35	2.473	2.44	2.91
2-Methylanthracene	50	35	0.171	0.064	0.107
9/4-Methylphenanthrene	50	35	5.512	2.3	5.6
1-Methylphenanthrene	50	35	2.737	1.41	2.55
3,6-Dimethylphenanthrene	50	35	1.59	0.94	2.158
2,6-Dimethylphenanthrene	50	35	1.05	0.818	1.64
1,7-Dimethylphenanthrene	50	35	2.405	0.902	2.71
1,8-Dimethylphenanthrene	50	35	1.13	0.282	1.33
C3-Phenanthrenes/Anthracenes	50	35	20	4.22	23
1,2,6-Trimethylphenanthrene	50	35	1.13	0.095	0.966
Retene	50	35	2.29	1.34	1.93
C4-Phenanthrenes/Anthracenes	50	35	32.8	4.57	33.3
C1-Fluoranthenes/Pyrenes	50	35	6.11	1.75	5.76
3-Methylfluoranthene/Benzo[a]fluorene	50	35	2.46	0.568	1.8
C2-Fluoranthenes/Pyrenes	50	35	8.26	0.803	7.6

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C3-Fluoranthenes/Pyrenes	50	35	2.94	0.077	2.87
C4-Fluoranthenes/Pyrenes	50	35	0.518	0.069	1.42
C1-Benzo[a]anthracenes/Chrysenes	50	35	3.94	0.727	9.54
5/6-Methylchrysene	50	35	0.435	0.398	4.17
1-Methylchrysene	50	35	0.616	0.095	1.21
C2-Benzo[a]anthracenes/Chrysenes	50	35	2.32	0.107	2.27
5,9-Dimethylchrysene	50	35	0.436	0	0.457
C3-Benzo[a]anthracenes/Chrysenes	50	35	0.149	0	0.262
C4-Benzo[a]anthracenes/Chrysenes	50	35	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	50	35	0.4	0	0.691
7-Methylbenzo[a]pyrene	50	35	0	0	0
C2-Benzofluoranthenes/Benzopyrenes	50	35	0	0	0.406
1,4,6,7-Tetramethylnaphthalene	50	35	1.05	2.98	1.04
Naphthalene	100	35	5.555	0.669	0.215
Acenaphthylene	100	35	0.251	0.088	-0.008
Acenaphthene	100	35	0	0.486	0.45
2-Methylfluorene	100	35	0.448	0.906	1.043
C2 Phenanthrenes/Anthracenes	100	35	6.863	19.553	51.153
Fluorene	100	35	1.129	0.955	1.202
Phenanthrene	100	35	14.312	5.675	7.615
Anthracene	100	35	0	0.29	0.351
C1 Phenanthrenes/Anthracenes	100	35	4.017	13.7	41.1
Fluoranthene	100	35	3.273	5.569	5.679
Pyrene	100	35	2.413	5.948	5.308
Benz[a]anthracene	100	35	0.394	0.174	0.492

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
Chrysene	100	35	2.088	1.38	5.35
Benzo[b]fluoranthene	100	35	0.31	0.38	0.437
Benzo[j,k]fluoranthenes	100	35	0	0.228	0.191
Benzo[e]pyrene	100	35	0.681	0.548	0.827
Benzo[a]pyrene	100	35	0	0	0
Perylene	100	35	0	0	0.091
Dibenz[a,h]anthracene	100	35	0	0	0.032
Indeno[1,2,3-cd]pyrene	100	35	0	0	0
Benzo[ghi]perylene	100	35	0	0.081	0.069
2-Methylnaphthalene	100	35	3.86	0.678	0.569
1-Methylnaphthalene	100	35	2.628	0.447	0.271
C1-Naphthalenes	100	35	6.489	1.123	0.843
Biphenyl	100	35	1.636	0.528	0.231
C1-Biphenyls	100	35	1.921	1.713	1.623
C2-Biphenyls	100	35	4.335	2.472	0.582
C2-Naphthalenes	100	35	7.655	3.501	18.081
1,2-Dimethylnaphthalene	100	35	0	0.232	1.18
2,6-Dimethylnaphthalene	100	35	0.975	0.622	3.009
C3-Naphthalenes	100	35	3.83	14.275	28.475
2,3,6-Trimethylnaphthalene	100	35	1.19	4.452	8.242
2,3,5-Trimethylnaphthalene	100	35	0.955	3.499	6.929
C4-Naphthalenes	100	35	1.546	16.8	11.4
C1-Acenaphthenes	100	35	0	0	0.097
C1-Fluorenes	100	35	1.752	5.367	6.967
1,7-Dimethylfluorene	100	35	0	3.01	1.74

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Fluorenes	100	35	0	34.341	21.941
C3-Fluorenes	100	35	0	22.6	22.1
Dibenzothiophene	100	35	0.743	0.817	3.71
C1-Dibenzothiophenes	100	35	1.318	4.7	29.3
2/3-Methyldibenzothiophenes	100	35	-0.018	1.3	7.9
C2-Dibenzothiophenes	100	35	11.779	13.5	79.3
2,4-Dimethyldibenzothiophene	100	35	0.996	0.744	8.95
4,6-Dimethyldibenzothiophene	100	35	2.72	4.29	14.4
C3-Dibenzothiophenes	100	35	29.1	6.53	53
C4-Dibenzothiophenes	100	35	17.3	1.707	12.637
3-Methylphenanthrene	100	35	0.854	3.58	8.35
2-Methylphenanthrene	100	35	0.973	4.03	9.3
2-Methylanthracene	100	35	0.356	0.237	0.19
9/4-Methylphenanthrene	100	35	1.452	3.51	15.4
1-Methylphenanthrene	100	35	0.733	2.31	7.85
3,6-Dimethylphenanthrene	100	35	0.661	1.938	3.818
2,6-Dimethylphenanthrene	100	35	0.576	1.75	3.53
1,7-Dimethylphenanthrene	100	35	0.995	2.37	6.89
1,8-Dimethylphenanthrene	100	35	0.419	0.729	2.99
C3-Phenanthrenes/Anthracenes	100	35	14.1	12.3	37.8
1,2,6-Trimethylphenanthrene	100	35	0.971	0.381	1.84
Retene	100	35	2.81	0.993	1.16
C4-Phenanthrenes/Anthracenes	100	35	31	8.15	41.2
C1-Fluoranthenes/Pyrenes	100	35	3.37	3.41	10
3-Methylfluoranthene/Benzo[a]fluorene	100	35	1.51	0.846	4.08

Analyte	WAF (%)	Sampling (d)	DB PAH Conc (ng/L)	MD PAH Conc (ng/L)	CO PAH Conc (ng/L)
C2-Fluoranthenes/Pyrenes	100	35	9.34	1.49	11.3
C3-Fluoranthenes/Pyrenes	100	35	4.32	0.177	4.14
C4-Fluoranthenes/Pyrenes	100	35	0.347	0.105	0.742
C1-Benzo[a]anthracenes/Chrysenes	100	35	5.06	0.721	7.04
5/6-Methylchrysene	100	35	0.431	0.184	1.21
1-Methylchrysene	100	35	1.02	0.075	0.896
C2-Benzo[a]anthracenes/Chrysenes	100	35	2.93	0.143	3.12
5,9-Dimethylchrysene	100	35	0.91	0	0.575
C3-Benzo[a]anthracenes/Chrysenes	100	35	0	0	0.464
C4-Benzo[a]anthracenes/Chrysenes	100	35	0	0	0
C1-Benzofluoranthenes/Benzopyrenes	100	35	0	0	0.829
7-Methylbenzo[a]pyrene	100	35	0	0	0.103
C2-Benzofluoranthenes/Benzopyrenes	100	35	0	0	0.21
1,4,6,7-Tetramethylnaphthalene	100	35	0	3.34	1.93