FINAL STUDY REPORT

HUNTSMAN STUDY NUMBER:	HMSC-169
SPONSOR:	ITOPF
PROJECT NAME:	**P**hotomodification **O**f **L**ow-sulfur-fuel-oils **I**nvestigations of **T**oxic **E**ffects (POLITE).
TEST MATERIAL:	U/VLSFO
LOCATION:	Huntsman Marine Science Centre St. Andrews, NB Canada
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PROJECT COMPLETION DATE:	May 1st, 2024

Executive Summary

The following report outlines the activities from the entire project that began May 1st 2022 and ended May 1st, 2024. During this project we completed work that addressed aspects of all three of our goals;

1. How toxic are U/VLS fuel oils compared to conventional fuel oils?
2. How does photomodification change toxicity?
3. Can we effectively predict the toxicity photomodified and non-photomodified U/VLS fuel oils?

In this study we conducted 31 distinct toxicity bioassays (Figure 1) with American lobster (*Homarus americanus*), Atlantic cod (*Gadus morhua*), and green sea urchin (*Strongylocentrotus droebachiensis*) using a conventional heavy crude oil (CONV), an offshore Newfoundland crude oil (ESRF), an ultra-low sulfur fuel oil (ULSFO), and 14 very low sulfur fuel oils (VLSFO) provided by the Australian Maritime Safety Authority (AMSA). The bioassays were conducted with exposure media generated from paired water accommodated fractions (WAFs) of the oil and seawater, with one WAF prepared in the dark (e.g., standard preparation) and one prepared under a UV light (e.g., irradiated) to induce photomodification.

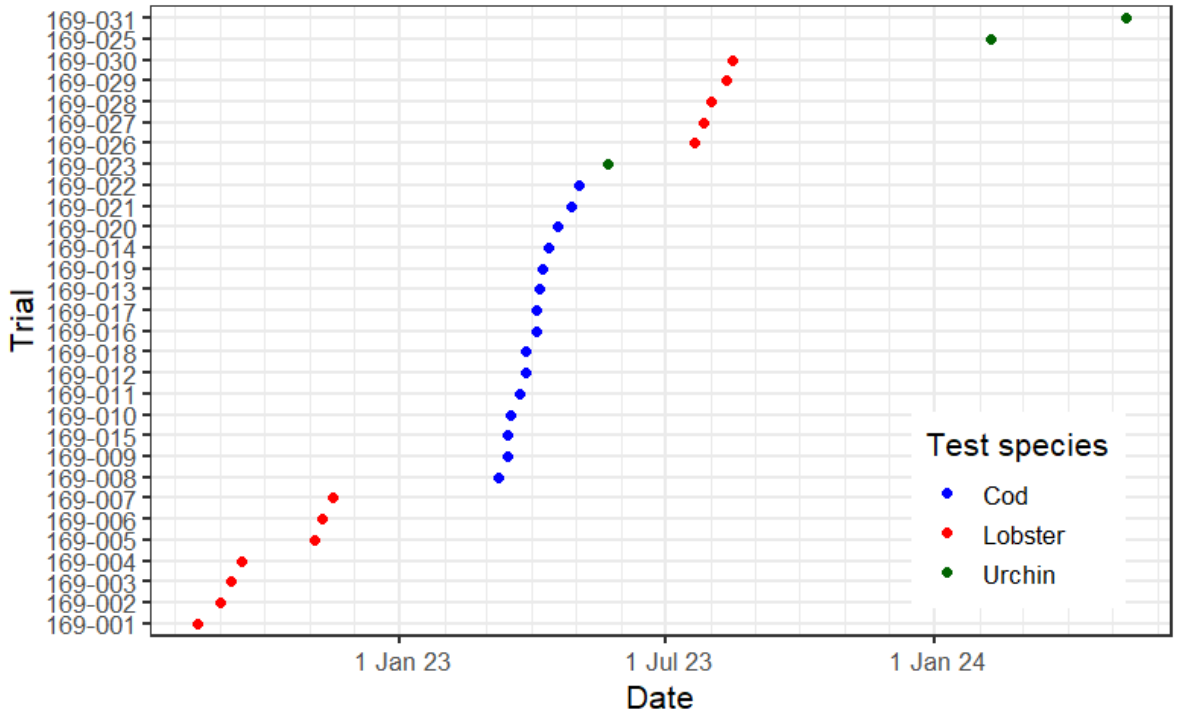


Figure 1: Timeline of the bioassays conducted over the course of this study.

Following irradiation, the exposure metrics (e.g., fluorometry units (RFU), total organic carbon (TOC), biomimetic extraction solid phase microextraction (BE-SPME), and polycyclic aromatic compounds (PACs)) for many of the products tested significantly increased, suggesting that there were photoproducts being formed. The amount of photoproducts formed varied between oil samples, with some of the tested VLSFOs not showing any detectable changes following irradiation. In nearly all cases the observed toxicity in the UV treated WAF was equal or greater than the WAF prepared in the dark. Larval lobster immobilization was the most sensitive endpoint across the tested species, and it showed a full range of responses with the tested products varying from 10 - 100% immobilization (Figure 2).

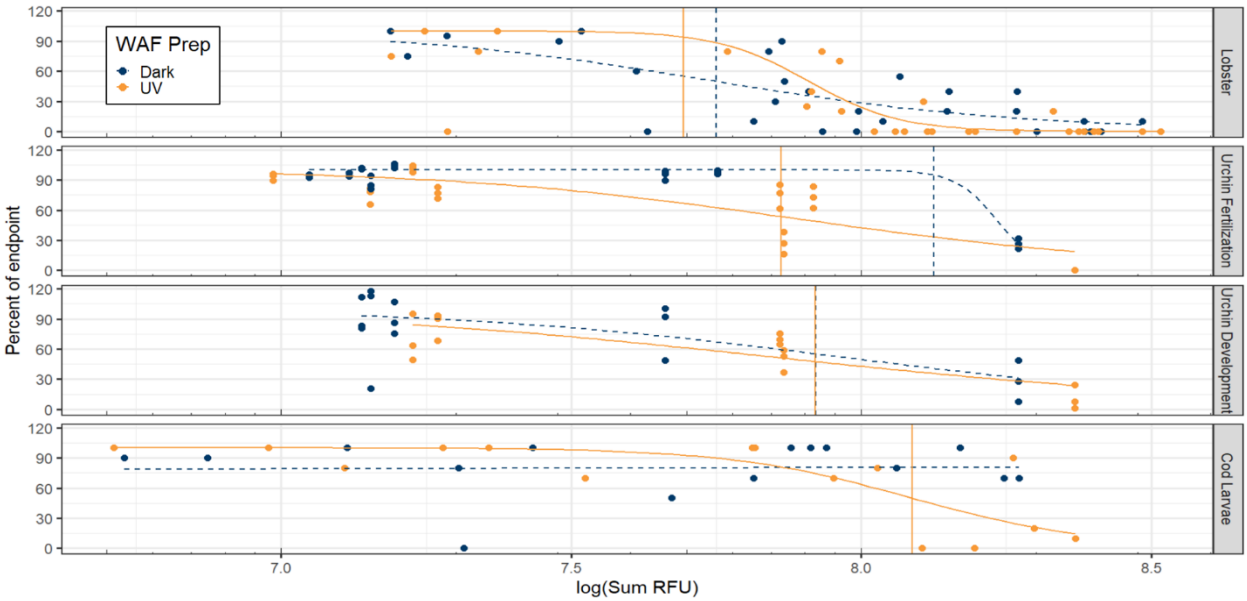


Figure 2: Normalized responses showing lobster immobilization (top), urchin fertilization and development (middle) and cod mortality (bottom) following exposure to irradiated (solid orange circles) and non-irradiated (solid blue circles) WAFs. The species are ordered by sensitivity top to bottom, with the vertical lines showing the EC50 on the basis of fluorometry units

The immobilization response largely followed a concentration gradient with TOC, BE-SPME, and PAC based toxic units. However, using only waterborne PAC concentrations to calculate toxic units, the predicted toxicity was consistently lower than what was observed for many of the VLSFOs products, both with and without UV irradiation. These results suggest that there are other aspects that aren't measured by traditional GC-MS (e.g., oxidized products) which are contributing to the toxicity of the VLSFOs.

The results highlight a wide range of responses across fuel types, with significant differences in sensitivity across species. The impact of UV light on the observed toxicity underscores the importance of addressing and incorporating modifying factors when

determining the toxicity of complex mixtures. The SARA fraction (Saturates, Aromatics, Resins, and Asphaltenes), specifically the aromatics and asphaltenes, have significant correlations with the formation of photoproducts and their associated toxicity.

The data generated within this study will be used to develop and validate models, to predict and assess the toxicity of these new generation fuel oils.

Contents

Figures

Tables

Photomodification **O**f **L**ow-sulfur-fuel-oils **I**nvestigations of **T**oxic **E**ffects (POLITE)

1. Study Overview

The changes in International Maritime Organization (IMO) regulations regarding the lower limits of sulfur content in marine fuel oils (IMO 2020) have initiated the transition to a new generation of low sulfur fuel oils (LSFO) that meet the requirements for lower atmospheric sulfur emissions. These new generation fuels are diverse, and their physical, chemical and toxicological properties are less well understood compared to their traditional counterparts. Only limited data are available about the range of properties and chemical composition LSFOs can exhibit. These properties are subject to change following a spill as environmental processes and weathering begin to take place. The significance of these processes, especially the effect of photooxidation by ultraviolet or visible light is a significant data gap.

Through chemical characterization, toxicity assays, and modeling, we addressed the following THREE GOALS and advanced our understanding of the environmental effects of LSFOs after oil spills:

- **GOAL 1: COMPARE THE TOXICITY AND CHEMICAL COMPOSITION OF VLSFOs RELATIVE TO CONVENTIONAL FUEL OILS**: Low-energy water-accommodated fractions (WAFs) were prepared with a set of U/VLSFO products (at least 4 distinct products). These WAFs are used in toxicity assays with Atlantic cod (*Gadus morhua*) larvae, American lobster (*Homarus americanus*) larvae, and green-sea urchin (*Strongylocentrotus droebachiensis*) embryos, along with appropriate positive and negative controls.
- **GOAL 2: INVESTIGATE HOW PHOTOOXIDATION CHANGES THE TOXICITY AND CHEMICAL COMPOSITION OF VLSFOs**: The selected VLSFOs were subjected to photooxidation by irradiating the oil in a full-spectrum solar simulator for 18h during WAF preparation. The resultant WAFs were used in bioassays and were chemically evaluated.
- **GOAL 3: EVALUATE AND COMPARE METHODS TO PREDICT TOXICITY OF PHOTOOXIDIZED AND NON-PHOTOOXIDIZED LSFOs**: The relationship between chemical composition toxic effects was evaluated based on a set of available models.

The toxicological effects of photooxidized and non-photooxidized oils were determined on early-life stages of marine organisms, while state-of-the-art analytical capabilities were be used to investigate the formation of photoproducts. Collectively these data may be used to validate models which predict toxicity based on the chemical composition of the product and species-specific sensitivity.

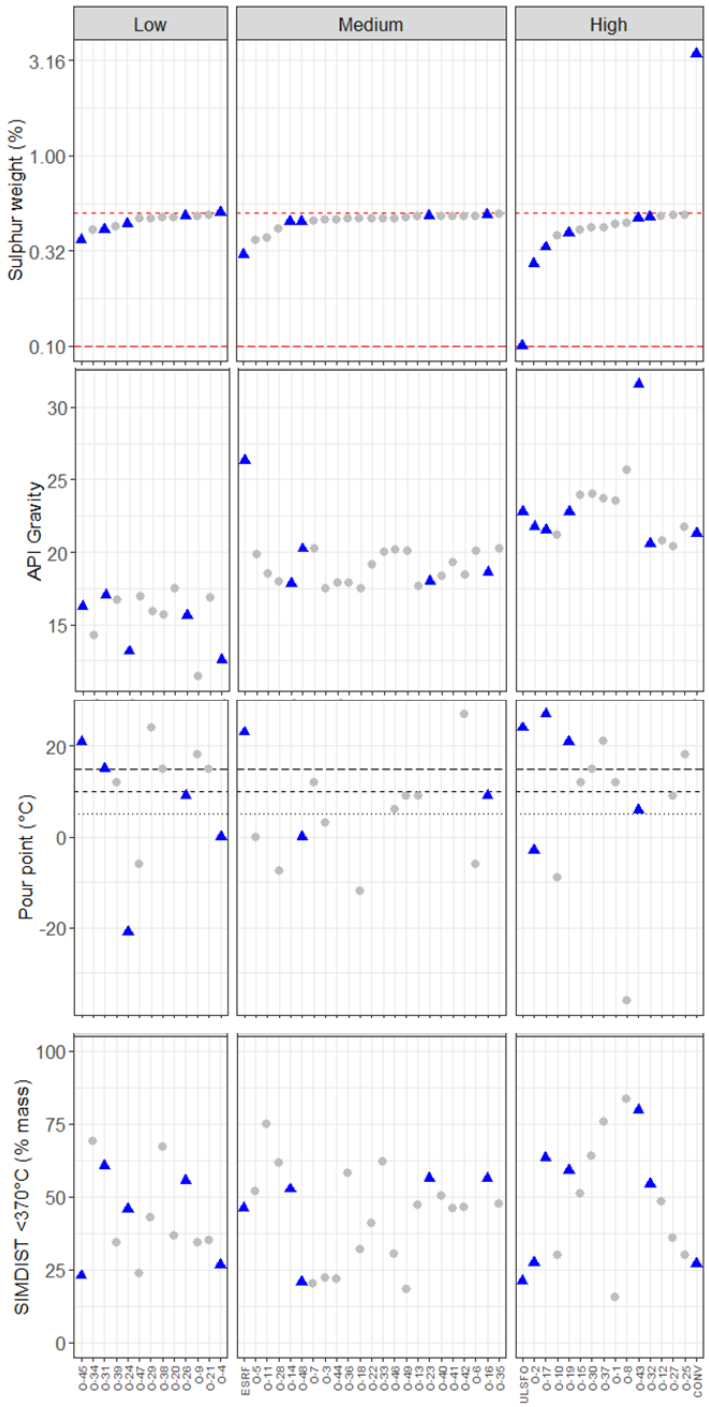
2. Methods

2.1. Test Materials

Materials included an ultra-low sulfur fuel oil (ULSFO Shell 2019, supplied through the 2019 ITOPF R&D Award Winner[1] in collaboration with the Department of Fisheries and Oceans Canada's Multi-Partner Oil Spill Research Initiative [MPRI] program), a conventional heavy crude (CONV, sulfur content ~3.4%, also provided through MPRI), and an artificially weathered (artificially weathered by nitrogen stripping until 10% loss by mass) crude oil sourced from offshore Newfoundland and Labrador (ESRF, sulfur content ~3%). These oils were used to optimize exposure methods and serve as a basis for comparison.

We received an additional 49 VLSFO products from the Australian Maritime Safety Authority (AMSA). The VLSFO products from AMSA were grouped according to their viscosity as low (<48.5 Cst 50 °C), medium (48.5 – 125 Cst 50 °C) and high (>125 Cst 50 °C). Within each grouping, the percent by weight of sulfur was compared and a total of 14 oils were selected for additional toxicological screening based on the distribution of percent sulfur, API gravity, pour point (if water temperatures are below the pour point of the product it is unlikely to flow and will become solid), and the percent of mass that is below 370°C in a boil point distribution (Figure 3).

[1] Sørheim, K.R., Daling, P.S., Cooper, D., Buist, I., Faksness, L., Altin, D., Pettersen, T.A., Bakken, O.M. 2020. Characterization of Low Sulfur Fuel Oils (LSFO) – A new generation of marine fuel oils. https://www.itopf.org/fileadmin/uploads/itopf/data/Documents/RDaward/Final_report_LSFO_Multipartner__3.1_.pdf



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297 *Figure 3: Sulphur content, API gravity, pour point (showing the test temperatures employed in this study,*
298 *6, 10, and 15°C) and %mass less than 370°C from simulated distillation (SIMDIST) of the 49 VLSFOs*
299 *provided by AMSA, the ULSFO, and two conventional crudes (ESRF and CONV) grouped by viscosity, with*
300 *the blue triangles indicating the products (n = 17) selected for toxicity screening.*

2.2. *WAF Preparation*

Low energy water accommodated fractions (WAFs) were prepared following the methods described in Singer et al., (2000). In brief, a known amount of oil was added to a 2L glass aspirator bottle containing 1.6 L of 0.22 µm filtered seawater. The vessel was then set to stir on a stir plate with a magnetic stir bar either under UV light or in the dark on the benchtop, in an environmentally controlled room at test temperature (e.g., 6, 10 or 15 °C). The WAF was stirred for 20 hours, left to settle for 4 hours and then sampled for chemical analyses and use in toxicity testing. All exposures were conducted with 100% concentration WAF solution.

Each WAF was imaged (Figure 4), and an incredible diversity of the products was observed in terms of the physical appearance and behaviour, with some oils spreading and forming a sheen, while others aggregate in waxy clumps.

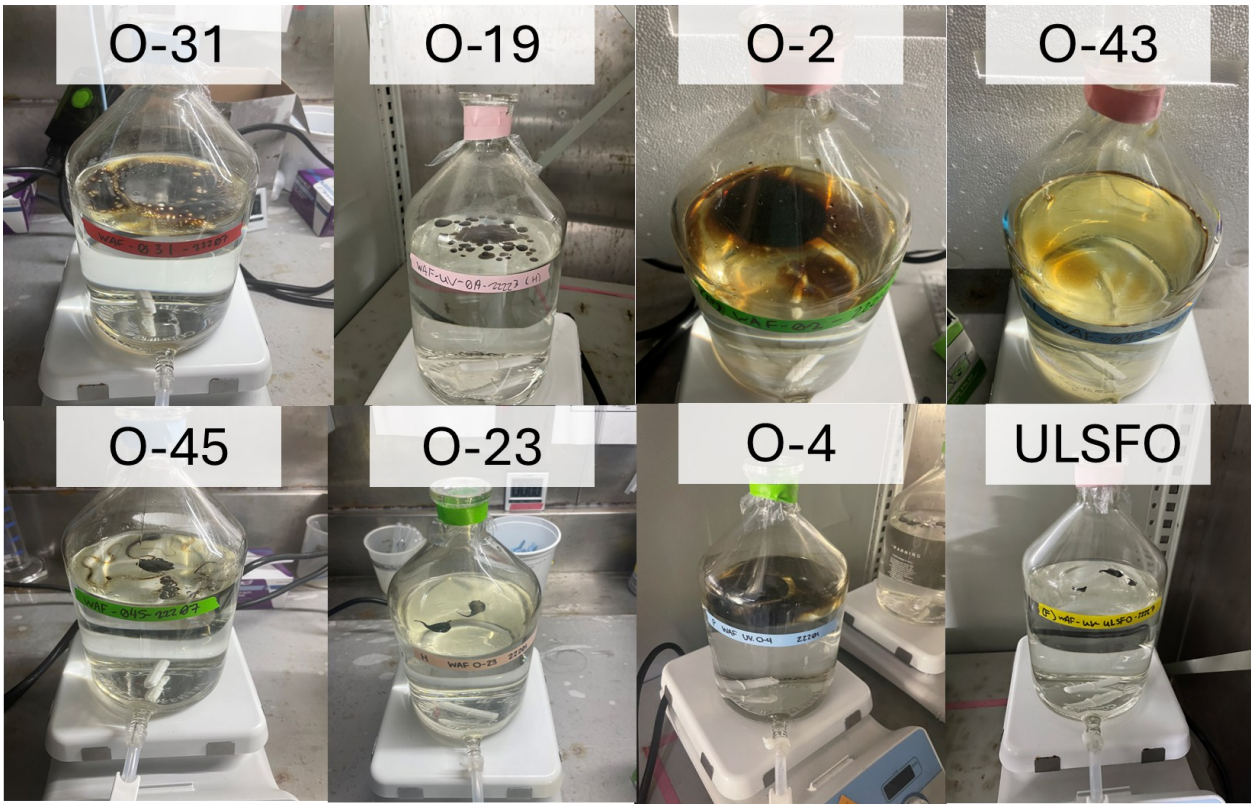


Figure 4: Representative WAFs displaying a range of physical appearance and behaviours upon contact with seawater.

2.3. *UV light exposure*

Full spectrum light exposures (composed of both UV and visible light) were generated using an Atlas Solar Constant MGH 1200 simulator (Atlas Material Testing Technology; Mount Prospect, IL). The spectrum ranges from 280nm to 3000nm and is designed to emulate natural sunlight. Irradiance was measured at the beginning of the UV exposure near the surface of the exposure media using a model UV203-3 Irradian portable radiometer (Irradian Limited, East Lothian, UK). UV doses were also be quantified using a surrogate unit containing a chemical actinometer. The UV light intensity used in the study was selected based on previously published literature of photo-enhanced toxicity at the values recorded on a sunny summer day in St. Andrews, NB using the Irradian radiometer. The radiometer measured the UV dose at the height of the oil being exposed. The average values measured ± the standard deviation were as follows: UV A = 5.43 ± 1.11 W/m², UV B = 0.57 ± 0.13 W/m², and total visible light = 55.56 ± 5.53 W/m². Based on the intensity measured (UV-A + UV-B) the total UV dose for the 18 hours of exposure was 108.0 ± 21.8 W/m^2 x hour.

2.4. *Test organism*

2.4.1. American lobster

Adult commercial size (0.5 - 2.0 kg) ovigerous (“berried”) female American lobsters (*Homarus americanus*) were captured by local fishers in the Bay of Fundy Lobster Fishing Area 36 under authority of a Fisheries and Oceans Canada license. Upon collection, females were transferred to the Huntsman Marine Science Centre (St. Andrews, New Brunswick, Canada) and held in communal tanks in ambient natural seawater (8-14 °C) on a 16:8 light: dark cycle. The berried females were assessed and staged for embryo development weekly to predict release of larvae. Berried females with mature egg clutches were transferred to an individual tank with seawater heated to 18 °C to encourage synchronous larval release. Released larvae were collected daily, with freshly released Stage I larvae (< 72 hours old) used for all bioassays.

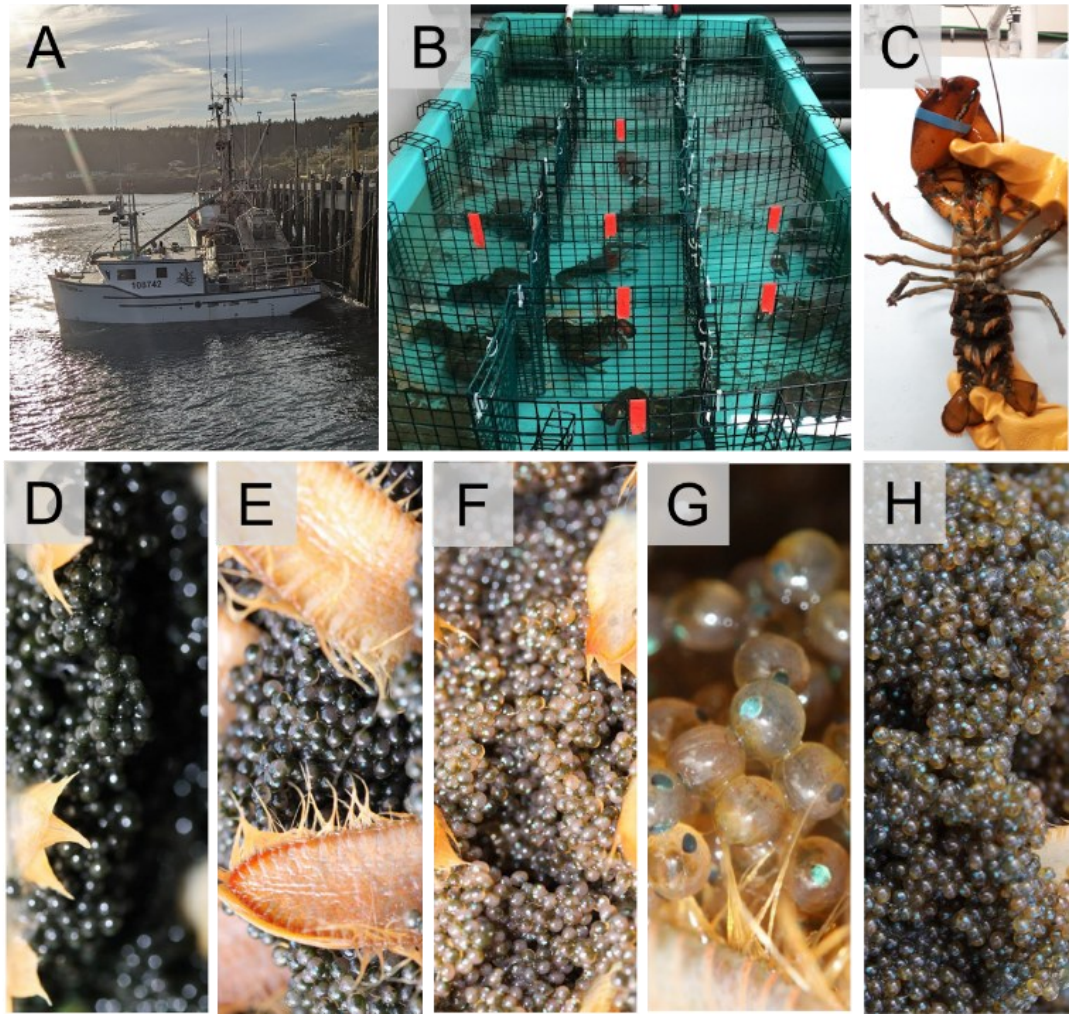


Figure 5: Overview of the process of working with berried lobsters. A) Collection of lobsters from local fishers after receiving required permission to do so, B) holding and feeding of berried female lobsters in individual holding cells, C) staging and measuring the clutch to assess fecundity and development stage in anticipation of larval release, D-H) developmental progression of the embryos.

2.4.2. Atlantic cod

Wild, sexually mature cod were captured August 2020 and were maintained year-round in communal tanks with flow-through natural seawater (4-14°C) and lighting to mimic ambient conditions. To obtain the embryos and larvae needed for toxicity both natural and artificial spawning methods are used. Prior to spawning, female and male fish are assessed to determine the gamete maturity. Once high-quality gametes were observed during the pre-spawning assessments, fish were handled weekly to ensure a regular supply of embryos and larvae throughout the cod spawning season (February – April). In addition to the targeted crosses made in lab, cod also spawned naturally in their holding tank and were captured using egg collectors which were monitored daily. Cod embryos are incubated without feed until hatch, after which they are fed enriched rotifers 3x daily

until they are used for testing. Freshly fertilized embryos (>48hrs post fertilization) and ~200 degree day larvae were used for all studies.

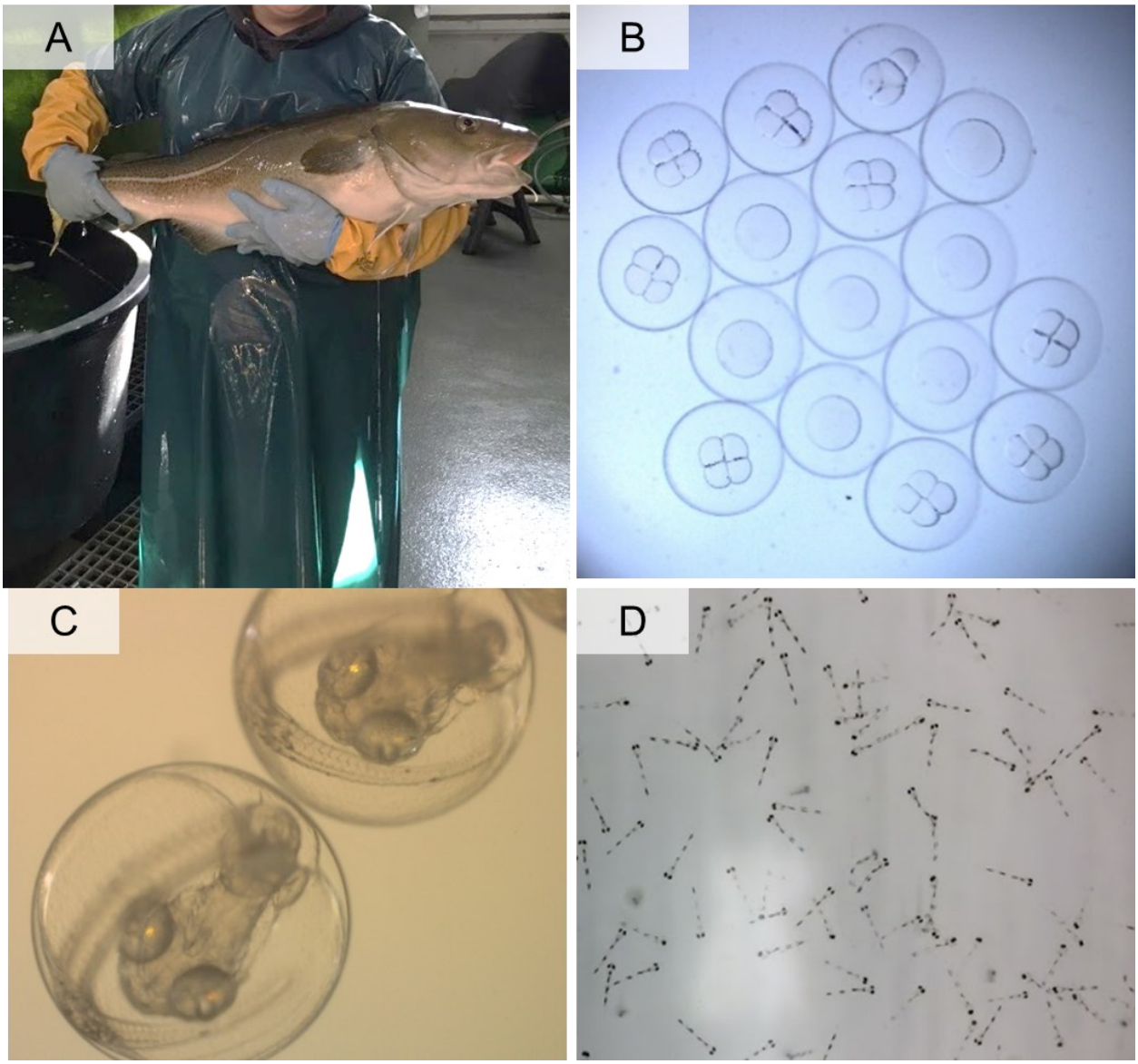


Figure 6: Overview of Atlantic cod embryo and larvae generation for toxicity testing. A) a technician holding a mature cod prior to gamete collection during artificial spawning procedures, B) reference image of cod embryos 24-48hrs post fertilization C) image of cod embryos prior to hatch, D-H) reference image of ~200 degree day cod larvae.

2.4.3. Green sea urchin

Green sea urchin (*Strongylocentrotus droebachiensis*) collected from the Bay of Fundy were maintained at the Huntsman Marine Science Centre in a flowthrough system at 6 ± 2ºC to enhance gonad development and fed *ad libitum* with macroalgae (kelp or rockweed) (Figure 7).

Figure 7: Holding of adult urchins under "perpetual spring" conditions prior to spawning them.

2.5. *Bioassay*

2.5.1. American lobster methodology

Bioassays were conducted in temperature-controlled chambers at 15 °C (+/- 2°C). For each exposure, 20 reference individual organisms were imaged to confirm the developmental stage and ensure larval size did not contribute to the observed differences in toxicity. Mortality and immobilization were assessed after 24-hours of exposure following the methods described in Philibert et al. (2021) and de Jourdan et al. (2022). In all bioassays, water quality (pH, dissolved oxygen content, salinity and temperature) was measured at test start and in pooled samples after 24 hours of exposure. Exposure media was generated using low energy WAFs prepared at a loading of 1 g of oil per L of filtered natural seawater. WAFs were prepared either under UV light (Full spectrum light generated using an Atlas Solar Constant MGH 1200 simulator, Atlas Material Testing

Technology; Mount Prospect, IL) or in the dark on the benchtop. Test solutions were prepared using a gradient dilution design.

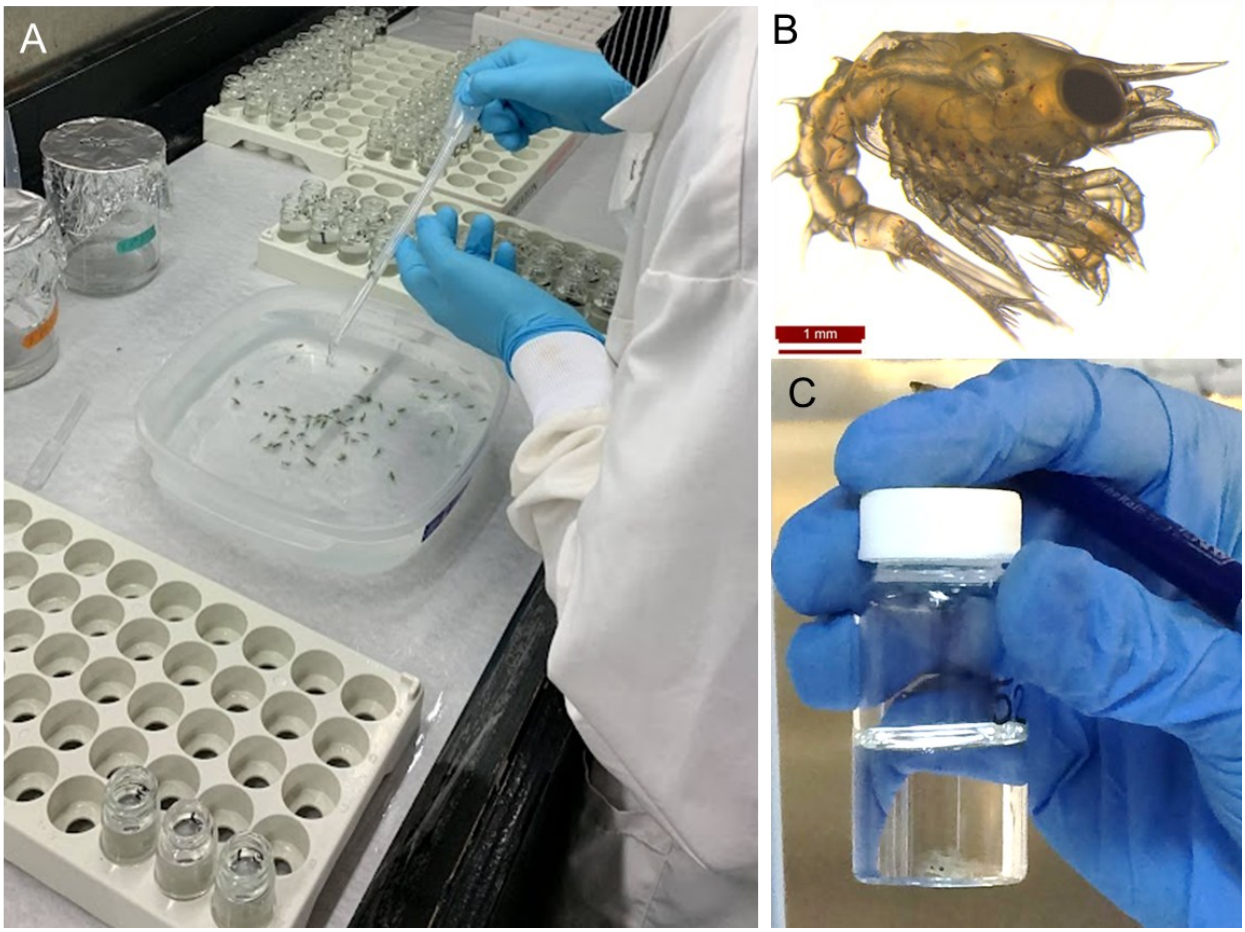


Figure 8: Lobster testing overview A) allocating the larval lobster into exposure vessels. B) Stage I larvae. C) Assessing a lobster larvae in the exposure vessel.

For all trials, exposure solution water quality (pH, dissolved oxygen content, salinity and temperature) was measured at test start and in pooled samples after 24 hours of exposure. The trial was considered valid if the control survival was greater than 80%, dissolved oxygen remained over 60% saturation, and solution temperature change remained within 1.5 °C for the duration of the trial. Trials were performed and data were collected consistent with OECD Principles of Good Laboratory Practice. All assessments were blinded to observational staff and data entry, followed by data quality control, were completed by separate staff independent of the assessments.

2.5.2. Atlantic cod methodology

Bioassays were conducted in temperature-controlled chambers at 6 °C (+/- 2°C). For each exposure, 20 reference individual organisms were imaged to confirm the developmental

stage and ensure embryo and larval size/quality did not contribute to the observed differences in toxicity. The age as degree days (summation of the temperature for each day post hatch) and the size distribution from each trial is shown in Figure 9.

For all trials, exposure solution pH, dissolved oxygen content, salinity and temperature were measured at test start and in pooled samples after 24 hours of exposure. The trial was considered valid if the control survival was met (≥ 60% for embryo and ≥ 80% for larval exposures), dissolved oxygen remained over 60% saturation, and solution temperature change remained within 1.5°C for the duration of the trial. Trials were performed and data were collected consistent with Good Laboratory Practice standards. All assessments were blinded to observational staff and data entry, followed by data quality control, were completed by separate staff independent of the assessments.

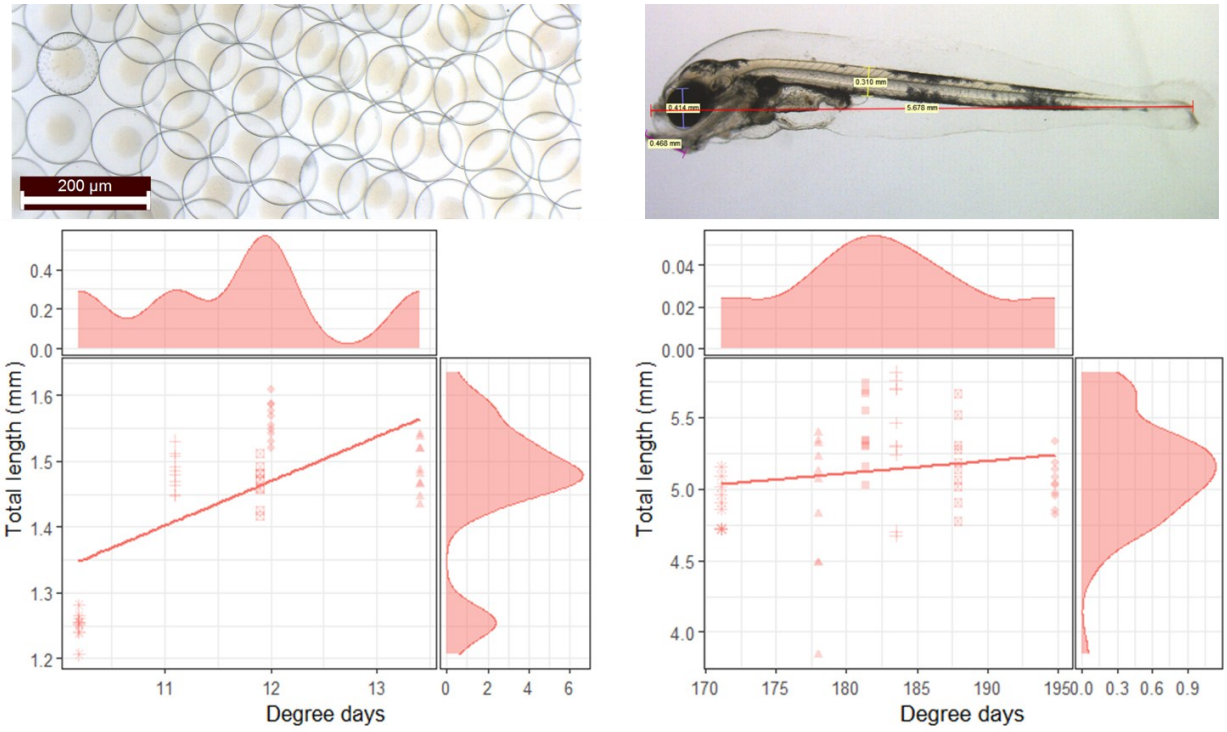


Figure 9: Distribution of the ages and sizes of the reference organisms at for each toxicity trial (different shapes) conducted with embryos (left) and larvae (right)

2.5.3. Green sea urchin methodology

To perform fertilization tests, the method described by Environment Canada (2011) was followed. Briefly, sea urchins were induced to release gametes with a 1 mL injection of 1.5 M KCl. Sperm and eggs were collected and kept separated until needed. Toxicity of the WAFs was tested by exposing gametes to the dilutions (e.g., 100, 10, 1% strength)

plus a negative control (1.0 µm filtered seawater) and a positive control of copper sulfate (0.038 mg/L). Each condition was replicated three times. Exposure was carried out in glass scintillation vials with a final volume of 10 mL, in an environmental chamber (temperature set at 10 ± 2 ºC). Sperm was activated in each test solution (salinity 30 ± 2 PSU), after 10 minutes 2000 ± 200 eggs per vial were added, and after an additional 10 minutes the fertilization assay was stopped by adding 1 mL of glutaraldehyde. Fertilization success was measured by counting the number of fertilized embryos (presence of a fertilization membrane, Figure 10) in 1 mL subsample on a Sedgewick rafter slide under a compound microscope (Olympus model BH2).

The urchin development bioassay was conducted with the same test solutions as the fertilization. Upon confirmation of fertilization, embryos less than 2-hours post fertilization were transferred into the test solution at a loading of 20 embryos/mL (equivalent to 400 embryos per exposure vessel; non fertilized eggs are transferred as well and their presence is accounted for in the final assessment). An additional set of control units were assessed daily to confirm developmental stage, and the trial was terminated when >80% pluteus was observed in the developmental check units. Upon termination of the trial, the contents of the exposure vials were assessed and scored according to developmental stage as either normal pluteus, slightly abnormal pluteus, abnormal pluteus, or affected (e.g., did not develop to pluteus).

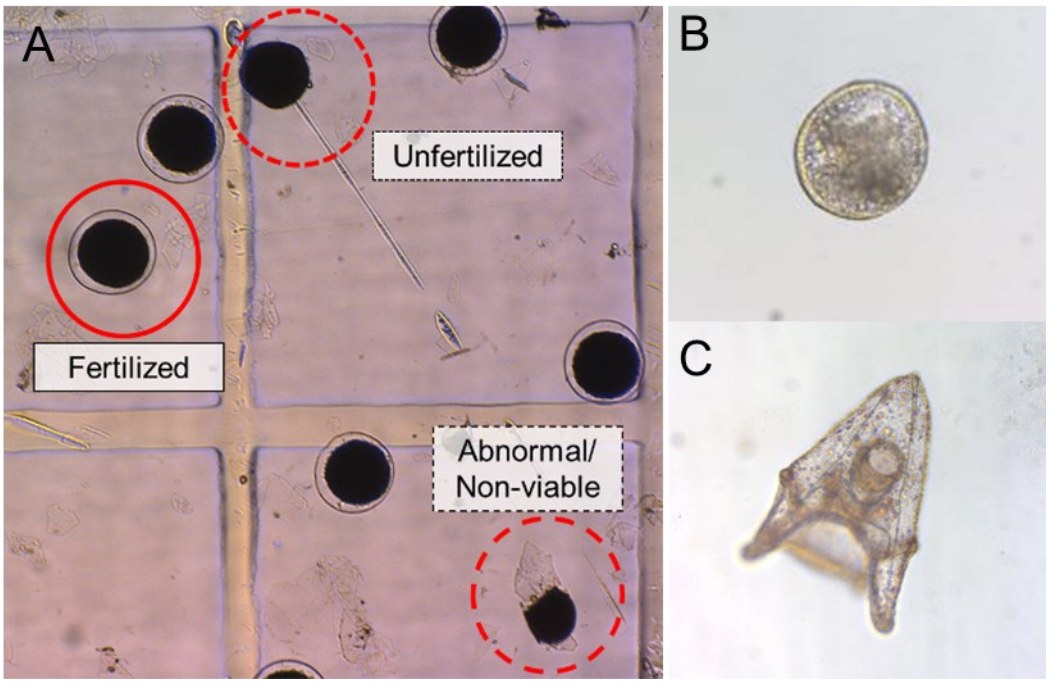


Figure 10: A) Representative eggs (unfertilized) and embryos (fertilized) following a fertilization bioassay. B) 40 hrs post fertilization, C) ~200 hrs post fertilization showing normal development

All trials were performed and data were collected consistent with Good Laboratory Practice standards. All assessments were blinded to observational staff and data entry, followed by data quality control, were completed by separate staff independent of the assessments.

2.6. *Chemical characterization*

2.6.1. Total Organic Carbon

Total organic carbon (TOC), was measured in each WAF by an external laboratory (RPC, Fredericton, NB). TOC was measured using the combustion method following the APHA 5310 B method. The measurement of TOC can track the presence of carbon in the water even as the concentrations of measured analytes have seemingly decreased to minimal levels, supporting research into new types of toxic compounds including photoproducts (Lara-Jacobo et al., 2021; Heshka et al., 2022).

2.6.2. Fluorometry

Fluorometry data (3D excitation and emission matrix) was collected for each of the test solutions both at the start and at the end of the exposure period. Fluorometry, paired with PAH and alkyl-PAH measurements, provide semi-quantitative analysis of the PAC content in each of the different test solutions. For this study, measurements were collected with a Horiba Aqualog under the following conditions: excitation wavelengths ranging from 200-800nm, an integration of 0.5s, accumulations of 1, increments of 3, CCD gain was set to high, and samples were run concurrently with a 0.22 µm filtered seawater blank.

2.6.3. Biomimetic Extraction using Solid Phase Microextraction

A solid phase microextraction coupled to a gas chromatography-flame ionization detection (SPME-GC-FID) instrument was used for biomimetic extraction (BE) measurements following established protocols as described in Katz et al. 2022. Briefly, portions of 20 mL sample were transferred into 20-mL headspace vials, and extracted using a solid phase microextraction (SPME) fiber (30 µm PDMS fiber; Supelco; 0.132 µL PDMS; 23Ga, yellow hub part # 57289-U), installed in an autosampler with automated and temperature-controlled stirring was used (CTC CombiPAL). The extraction time was 100 min at 30°C, using orbital shaking (250 rpm). A GC-FID system (Agilent 7890B) equipped with an Rtx-1 column (Restek; 15m x 0.53mm i.d., 1.5um film) was used. An inlet liner with a narrow diameter was used (1.8 mm, Restek Topaz; 280 °C inlet temperature). The GC oven temperature was 40 °C for 3 min and then ramped to 300 °C at a 45 °C/min rate. The carrier gas (He) flow was 17 mL/min. The FID trace was recorded using a software (ChromaTOF, Leco). External calibration was performed using certified reference material containing toluene, o-xylene, 2-methylnaphthalene, 2,3-dimethylnaphthalene, and 9-methylantracene (Sigma-Aldrich Certified Reference

Material 42127). Serial dilutions of the calibration solution were automatically injected (1 µL injection volume, using a CombiPAL autosampler). The FID signals were exported as machine-readable files (CSV) using the ChromaTOF software and integrated using an R script. The FID signals were baseline-corrected (to subtract the column bleeding) by fitting the column bleed signal from a no-inject temperature ramp run to the BE FID signal. BE calibration was performed using an external calibration using liquid injection of a certified reference material containing DMA (Sigma-Aldrich 42127-2ML, Oil Sand-Affected Water Calibration Standard Kit; 2,3-dimethylnaphthalene concentration 2,000 µg/mL). Using the slope from these calibration curves along with the PDMS volume of the used SPME fiber (0.132 µL), the fiber concentration of each BE sample was calculated as "µmol DMA equivalent per mL PDMS". All BE samples were measured in analytical duplicates.

2.6.4. Gas Chromatography–Mass Spectrometry

Water samples from the WAFs were sent to an external laboratory (RPC, Fredericton, NB) for analytical characterization of polycyclic aromatic compound (PACs) and alkyl PAHs by solvent extraction and gas chromatography–mass spectrometry (GC–MS; using the method previously described in the United States Environmental Protection Agency 3510C/8270C document). Using the results of the water characterization, a toxic unit (TU) approach was employed, where the concentration of the analytes was used to predict the toxicity of the mixture. The toxicity of dissolved oil components was evaluated using an additive TU model:

$$TU_{oil,dis} = \sum_{i}^{N} \frac{C_i}{L(E)C_{50,i}} \qquad \text{(Eq. 1)}$$

where:

$TU_{oil,dis}$ = dissolved phase toxic units
i = individual PAC
N = number of PACs measured in the oil
C_i = dissolved concentration of individual PAC, *i*
$L(E)C_{50,i}$ = dissolved LC_{50} (lethal concentration) or EC_{50} (effect concentration) causing 50% response for each individual PAC *i*

To estimate the EC50 for each measured PAC, the target lipid model (TLM) was applied. The TLM posits that effect endpoints can be explained when the molar concentration at a hypothetical target lipid site within the test organism exceeds a critical threshold value (Parkerton et al., 2023). Based on the TLM, toxicity is described by:

$$\log L(E)C_{50} = -0.94 \log K_{ow} + \log \Delta c_i + \log CTLBB \qquad \text{(Eq. 2)}$$

where:

$L(E)C_{50}$ = lethal or sublethal molar concentration in water causing a 50% effect, ($mmol/L_{water}$)

K_{ow} = octanol-water partition coefficient of hydrocarbon ($L_{water}/L_{octanol}$)

CTLBB = critical target lipid body burden causing a 50% effect ($mmol/L_{octanol}$ = $\mu mol/g_{octanol}$). A CTLBB for lobster of 87.7 µmol/g octanol was applied based on the 24-hour exposure data (Philibert et al. 2021).

Δc_i = class-specific correction. The Δc_i is required for monoaromatics (Δc = -0.025), polyaromatics (Δc = -0.364) and heterocycles such as thiophenes (Δc = -0.412) to account for differences in partitioning between octanol and target lipid (McGrath et al. 2018; McGrath et al. 2021).

The Toxic Units for each compound were calculated by dividing the dissolved concentration of each hydrocarbon by the corresponding predicted effect concentration, and then summing to derive $TU_{TPAH32,dis}$ (the subscript denotes the individual compounds considered in the TU calculation, here the 32 analytes measured by RPC). The $TU_{TPAH32,dis}$ is then multiplied by 50 in order to estimate the percent effect (e.g., a TU of 1 would have 50% mortality).

2.6.5. VLSFO Characterization

The products from AMSA were provided with various physical and chemical characterization (3.1.1). Additional analysis included determining the UV-vis spectrum of each product, and a more comprehensive analysis of the chemical components of the products. GC-MS was also employed at Bigelow to examine the PAC concentration (46 analytes) in the source oil, and following the preparation of the WAF, both in the dark and under UV. And lastly, the VLSFO samples were analyzed using comprehensive two-dimensional gas chromatography coupled to a flame ionization detector (GC×GC-FID) according to Aeppli et al. (2014). Briefly, 1 µL of each sample in dichloromethane (DCM) was injected in a GC×GC-FID system with a dual stage cryogenic modulator (Leco, Saint Joseph, MI), equipped with a Restek Rtx-1 first- dimension column (60 m, 0.25 mm ID, 0.25 µm film thickness) and a SGE BPX-50 second-dimension column (1.5 m, 0.10 mm ID, 0.10 µm film thickness). The inlet temperature was held at 300 °C. The injection mode was splitless, and the carrier gas was H_2 at a constant flow rate of 1.00 mL min^{-1}. The first oven was programed isothermal at 40 °C for 10 min, 40 to 340 °C at 1.25 °C min^{-1} (held for 5 min). The second oven was programmed as follows: isothermal at 45 °C for 10 min, 45 to 355 °C at 1.29 °C min^{-1} (held for 5 min). The modulation period was 15 s. The constituents of the source oils were identified based on their position on GC×GC chromatograms, and FID signals were integrated on the ChromaTOF software (Leco).

3. Results

3.1. *Test Material Characterization*

3.1.1. Physical and chemical data

Physical and chemical data was made available from AMSA regarding the physical and chemical properties of these products, specifically the high temperature simulated distillation (HTSD) curves and the SARA values (SARA = Saturates, Aromatics, Resins, Asphaltenes). This data is presented in Table 1.

Table 1: Additional physical and chemical characterization of the source oils including simulated distillation (Sim. Dist.) and SARA (Saturates, Aromatics, Resins, Asphaltenes) analysis. The products tested in this program are shaded blue.

Sample ID	Country Bunkered	Sulphur (% wt)	Sim. Dist. (% mass)		SARA (**S**aturates, **A**romatics, **R**esins, **A**sphaltenes; % mass)			
			<370°C	>370°C	S	A	R	A
O-1	South Korea	0.4339	15.7	84.3				
O-2	Japan	0.27	27.5	72.5				
O-3	China	0.46	22.2	77.8				
O-4	Germany	0.5	26.5	73.5	38.4	24.5	27.6	9.4
O-5	China	0.36	51.8	48.2				
O-6	New Zealand	0.482	*					
O-7	Singapore	0.452	20.2	79.8				
O-8	Canada	0.44	83.4	16.6				
O-9	China	0.48	34.4	35.6				
O-10	Japan	0.38	30	70				
O-11	China	0.37	75	25				
O-12	Singapore	0.477	48.4	51.6				
O-13	Singapore	0.48	47.1	52.9				
O-14	Turkey	0.45	52.6	47.4	46.1	24.1	22	7.8
O-15	Taiwan	0.406	51.2	48.8	62.5	22.5	10.2	4.8
O-16	Singapore	0.49	56.4	43.6	51.9	20.2	20.2	7.6
O-17	Australia	0.33	63.2	36.8	61.8	28.2	7.6	2.5
O-18	USA	0.47	32.1	67.9	49.1	31.6	17.5	1.9
O-19	Australia	0.39	59.1	40.9	60	32.8	5.7	1.5
O-20	Singapore	0.476	36.6	63.4	51	14.3	27.1	7.6
O-21	Netherlands	0.49	35	65	51.4	17.2	22.3	9
O-22	Singapore	0.47	41	59	59.5	19.7	17	3.8
O-23	Singapore	0.48	56.5	43.5	49.9	19.3	18.2	12.5
O-24	USA	0.44	45.8	54.2	49	27.6	15.9	7.5
O-25	Turkey	0.49	29.8	70.2	61	20	15.5	3.5
O-26	China	0.48	55.6	44.4	49.2	25.9	18.5	6.4
O-27	Singapore	0.487	35.8	64.2				
O-28	Japan	0.41	61.6	38.4				
O-29	China	0.468	42.9	57.1	48.4	25.9	19.7	6

O-30	Taiwan	0.42	64	36				
O-31	USA	0.41	60.5	39.5				
O-32	South Korea	0.473	54.2	45.8	58.9	23.5	13.6	4
O-33	Sweden	0.47	62.2	37.8				
O-34	Vietnam	0.407	69	31				
O-35	Singapore	0.491	47.4	52.6	53.4	20	20.2	6.4
O-36	China	0.465	58.1	41.9				
O-37	Taiwan	0.421	75.8	24.2	61	24.9	9.6	4.6
O-38	China	0.475	67.2	32.8				
O-39	China	0.423	34.4	65.6				
O-40	Singapore	0.48	50.2	49.8				
O-41	Singapore	0.48	46.2	53.8	54.6	24.9	14.5	6
O-42	Singapore	0.482	46.4	53.6	49.8	22.5	20.7	7
O-43	Russia	0.47	79.7	20.3	77.4	15.6	6.8	0.2
O-44	China	0.462	21.6	78.4				
O-45	China	0.36	22.9	77.1				
O-46	Korea	0.47	30.3	59.7				
O-47	China	0.465	23.9	76.1				
O-48	Singapore	0.45	20.6	79.4				
O-49	Singapore	0.475	18.3	81.7				

*According to the AMSA report, sample O-6 was omitted due to the appearance of light gas oil components presumed to be from sample contamination when sampling on the vessel.

This data became available after the first year of toxicity testing was underway so it was not able to inform the initial selection of products for testing, however it was used to identify certain products that had characteristics that had not been covered in our first year of testing and allowed us to include two additional oils. We selected O-19 (high aromatic content) and O-32 (mid-range aromatic and saturates content) to ensure our dataset covered a wide range of physical and chemical characteristics. The HTSD curves (Figure 11) and the report from AMSA also indicated that O-43 is likely a misidentified oil, with AMSA suspecting that it is RMD80 and not appropriate for an RMD380 specification based on its kinematic viscosity of 6.22 cSt (in the bottom panel of Figure 11 the curve for O-43 shows 75% of the cumulative mass has been lost by 343°C). We did include O-43 in our testing initially as it was notably less viscous than our other oil samples. The information from the HTSD curves is used to highlight any trends in the tested products with respect to their likelihood of undergoing photomodification and their general toxicity, following the guidance described in Dettman et al. 2023.

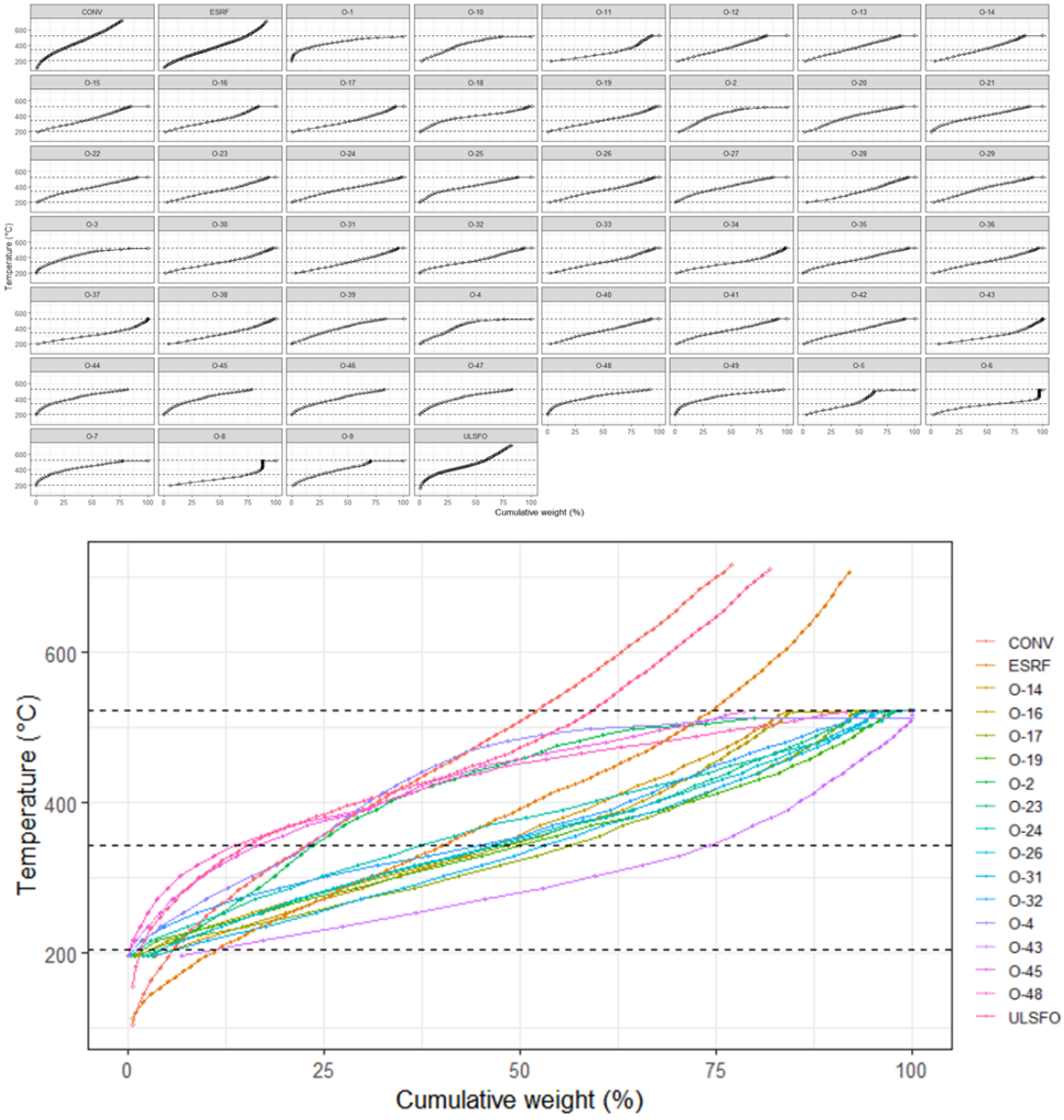


Figure 11: (Top) High temperature simulated distillation curves for the 49 AMSA products and the Huntsman reference oils. The dashed horizontal lines represent the boiling point cutoffs associated with the C11 (204°C), C25 (343°C), and C40 (524°C) alkanes. (Bottom) Overlay of the HTSD curves for the products screened for toxicity with larval lobsters. O-43 (light purple) shows a distinctly different pattern with 75% of the cumulative mass having been lost by 343°C. Note the HTSD data for ULSFO, CONV, and ESRF were generated separately from the other 49 AMSA products.

3.1.1. Gas Chromatography–Mass Spectrometry

Select products were evaluated for alkanes (n-C10 to n-C39 normal aliphatics), and branched alkanes (pristine, phytane), and polycyclic aromatic compounds (PACs) using GC-MS. These analyses were completed on the raw product ("Source"), the product after making a WAF in the dark ("Extract") and the product collected after making the UV WAF ("UV").

For illustrative purposes, alkanes were grouped as C10-C14, C15-C20, C21-C25, C26-C30, and C31-C37 (Figure 12).

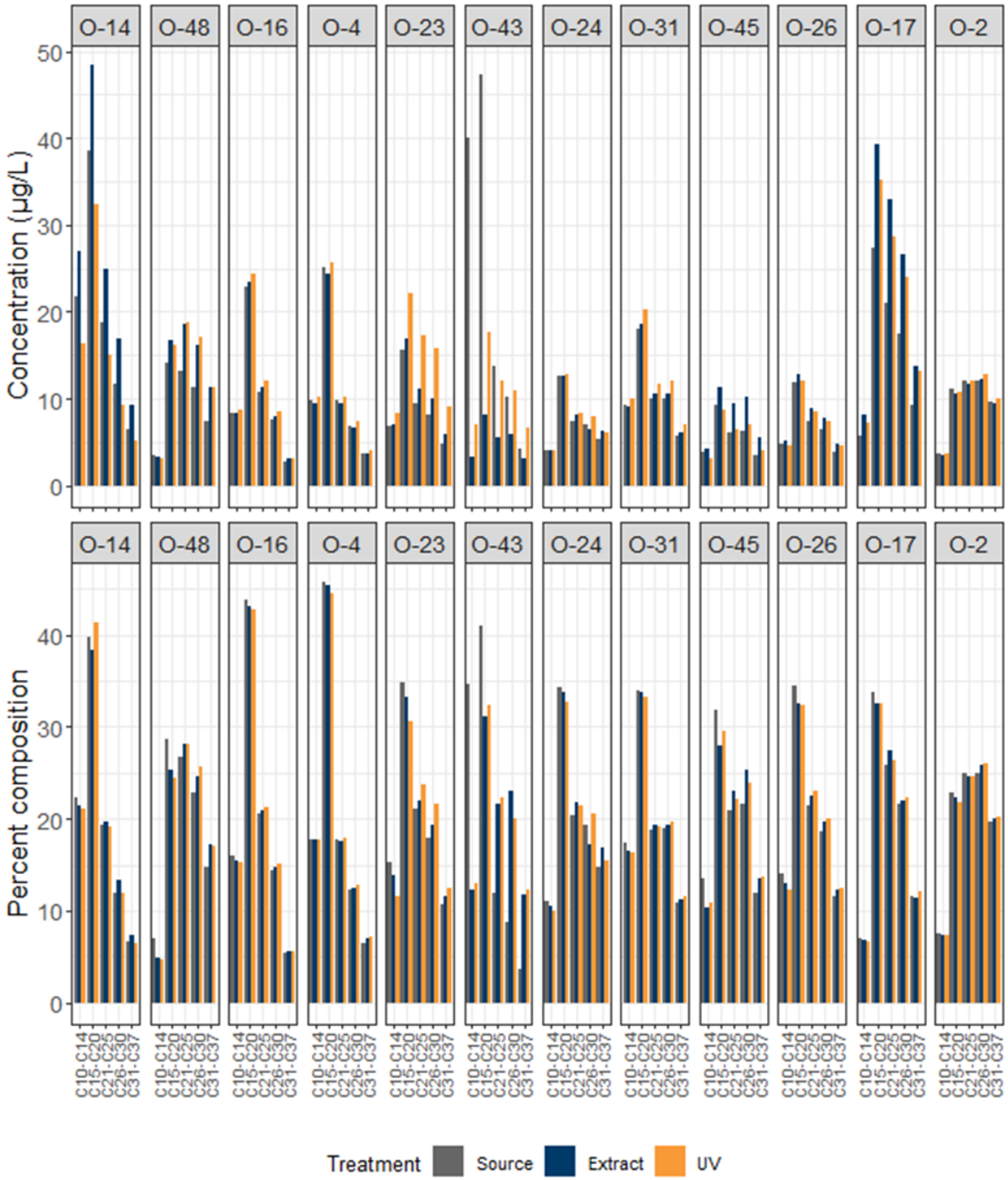


Figure 12: Grouped alkane concentrations for the products, top: concentration, bottom: percent composition.

In most cases the concentration of the alkanes was similar across treatments, with C15-C20 being the most abundant grouping, with some notable exceptions. O-4 and O-17

have slightly greater concentration in the oil that was collected from the dark WAF ("Extract") than the Source or UV treated oil. O-23 shows an increase in concentrations of the higher carbon alkanes (e.g., >C15) following UV treatment. O-43 shows a unique pattern where the Source oil has the highest concentration of the low weight alkanes (<C20), which are considerably reduced in both the light and dark WAF collected oil.

It is generally true that most oils exhibit decreasing concentrations of n-alkanes with increasing carbon number. This trend occurs because higher molecular weight alkanes are less volatile and less soluble in the lighter fractions of crude oil. Additionally, the formation of these long-chain alkanes requires specific geological conditions and longer periods, making them less abundant compared to shorter-chain alkanes. This general trend does not hold for many of the VLSFOs analyzed in this project, where the low molecular weigh alkanes (<C15) are largely depleted in the samples and there is an appreciable amount of long-chain alkanes (e.g., >C30). This pattern is likely due to the blending and catalytic process used to produce these products and achieve the low sulfur requirement while meeting the RMD380 specification.

The products were analyzed for PACs (n = 46), and for visualization purposes the sum PACs are presented in Figure 13.

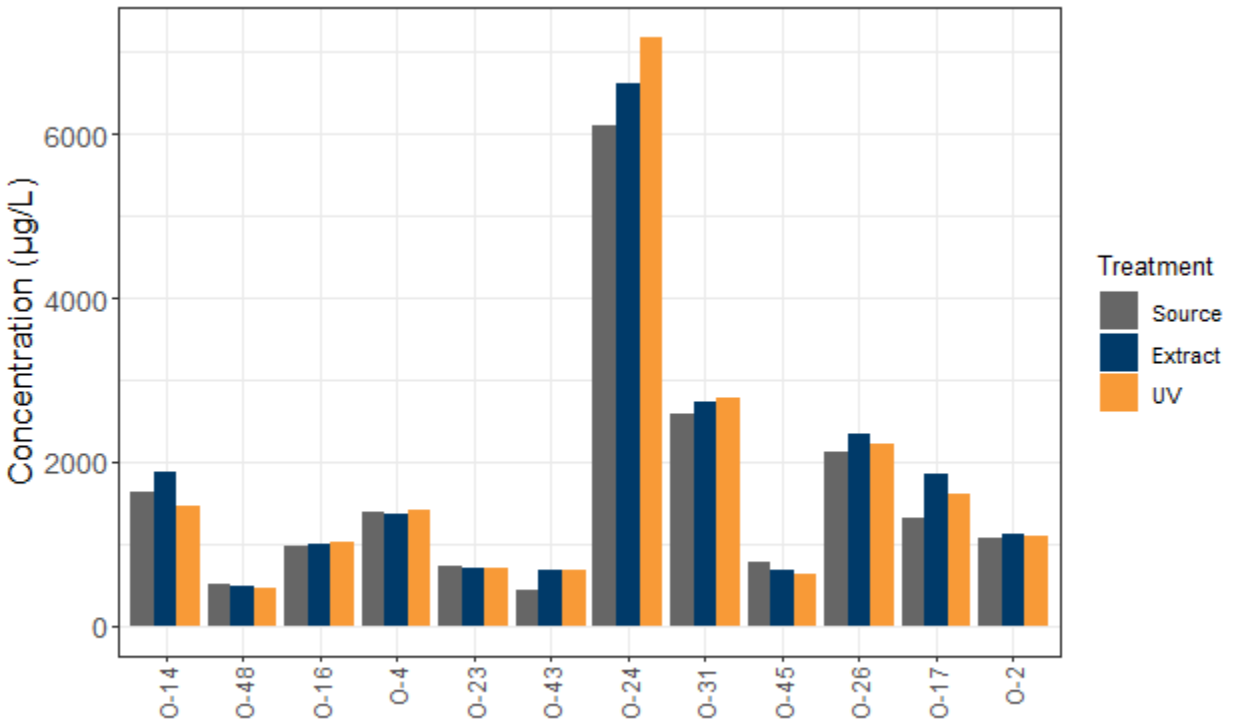


Figure 13: Sum PACs for each of the examined products as the source, extract, and UV treated.

The products in Figure 13 are ordered from left to right as least to most toxic to lobster larvae (see section 3.3.1). The fact the O-14 (least toxic) has a greater sum PAC concentration than O-2 (most toxic) highlights that sum PAC is not a reliable metric as it does not consider the differences in compositional profiles and the varying toxicity of the components (Figure 14).

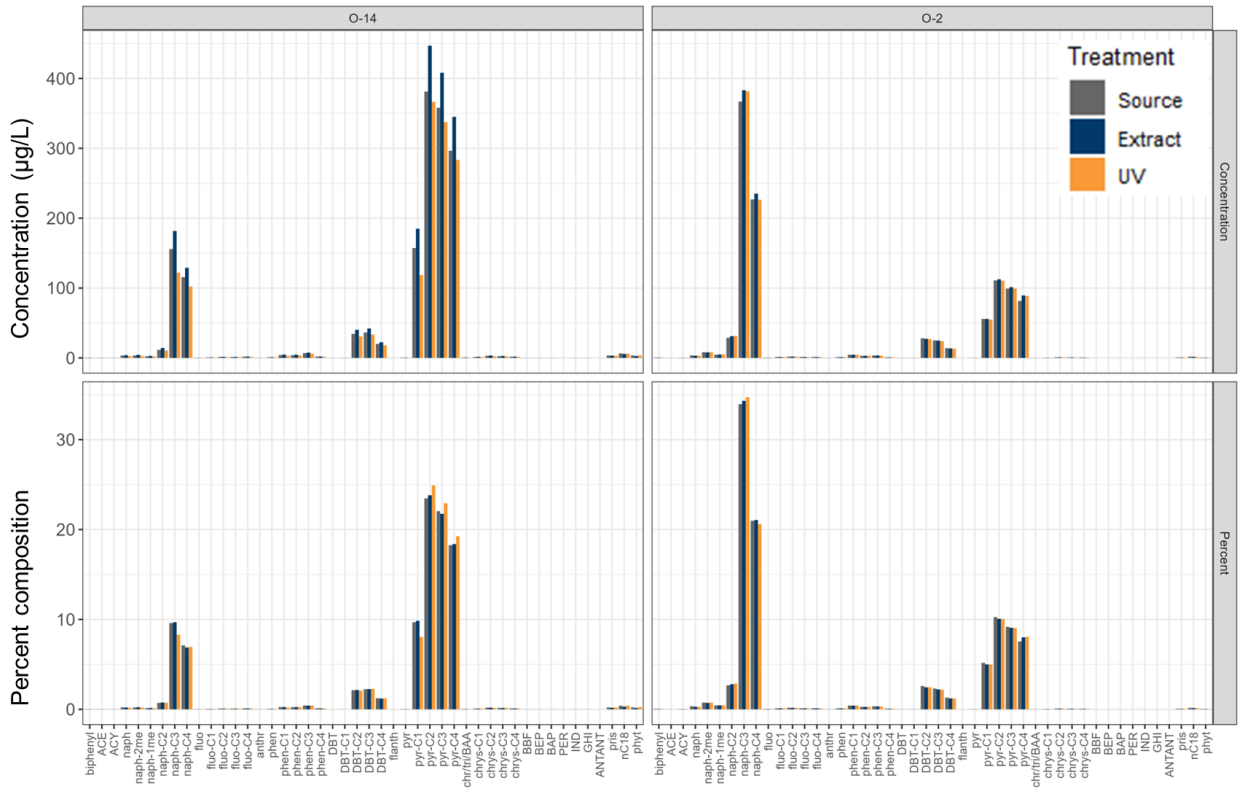


Figure 14: Concentration (top row) and percent composition (bottom row) of the measured PACs for two products, O-14 (least toxic to lobsters) and O-2 (most toxic to lobsters).

The main difference between the two profiles shown in Figure 14, is the relative abundance of napthalenes (C3 and C4-napthalenes) in O-2 compared to O-14. Naphthalene is more acutely toxic than pyrene (the most abundant grouping in O-14), thus the greater contribution of naphthalenes in O-2 is responsible for the greater toxicity observed, despite the lower overall sum PAC concentration than O-14.

The full suite of analytical characterization of the VLSFOs is provided in Appendix.

3.1.1. GC × GC

The samples were analysed by GC × GC – MS, and their profiles revealed differences in abundance and composition of the individual components within the product.

Representative profiles are provided in Figure 15 for products that had varying toxicity towards American lobster larvae.

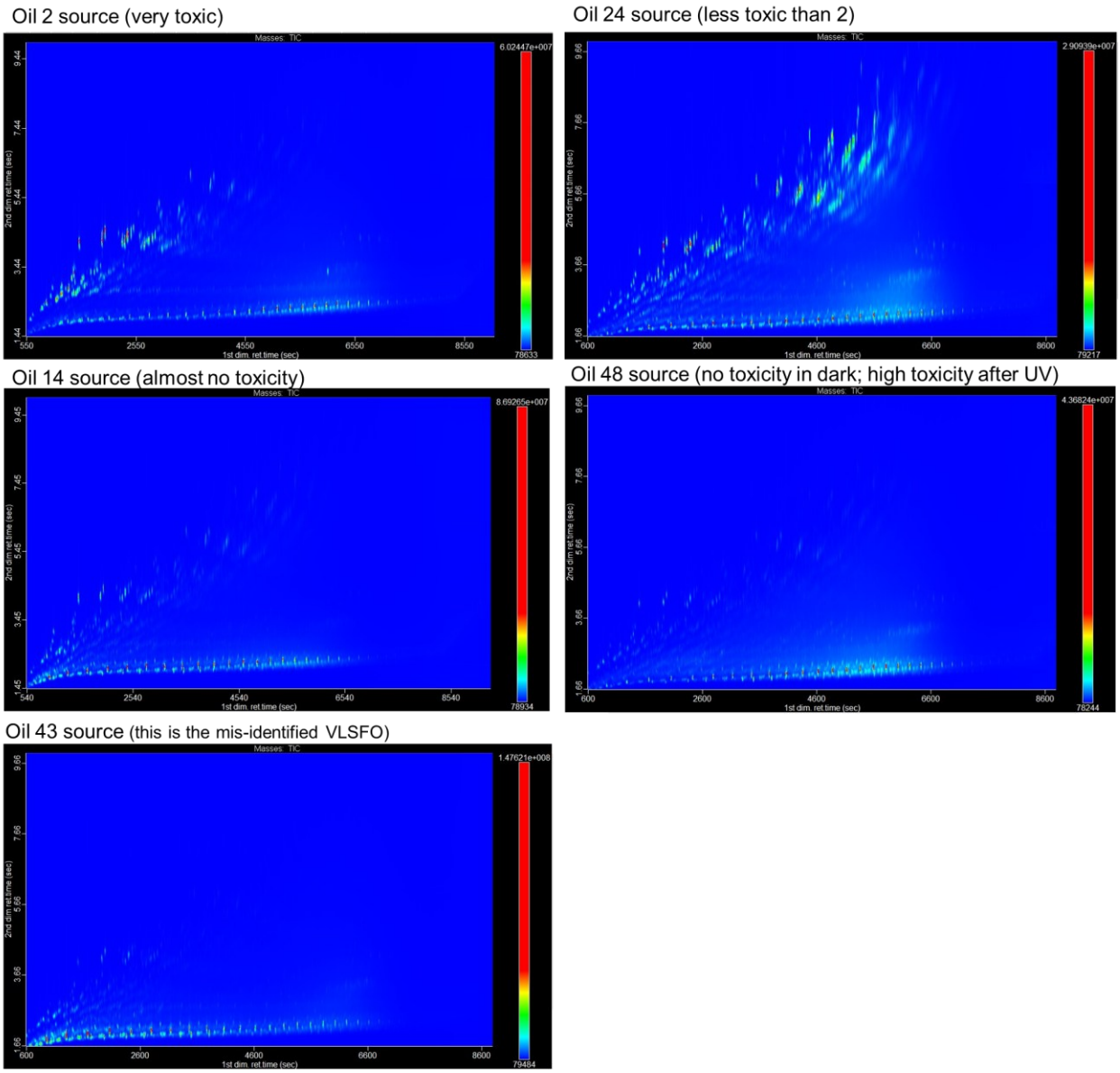


Figure 15: Representative GC × GC plots from select products demonstrating a range of toxicity responses.

Annotation of the GC × GC profiles (Figure 16) will provide inputs for environmental fate and toxicity models which require hydrocarbon blocks or pseudo-components as their input.

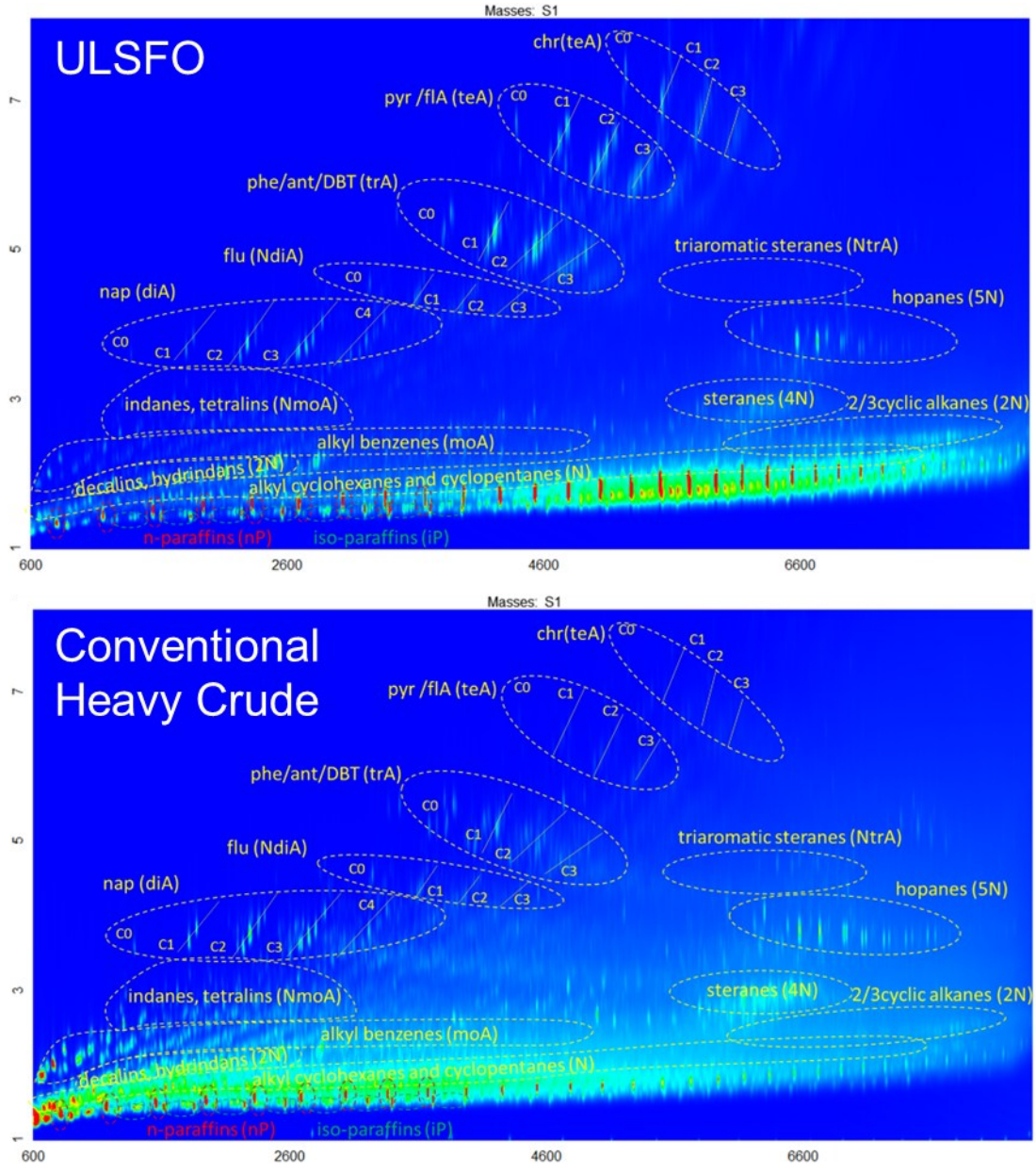


Figure 16: Annotated GC × GC profiles for the ULSFO and CONV products.

3.1.2. UV-Vis

Select VLSFOs had their UV-Vis spectra examine, for both a dark and UV irradiated sample. Each solution was analyzed on a spectrophotometer in the range of 200–800 nm. Evolution of the different absorbance signals (A) in the spectra were plotted with respect to wavelength (λ) to examine changes in the shape and amplitude following UV irradiation (Figure 17).

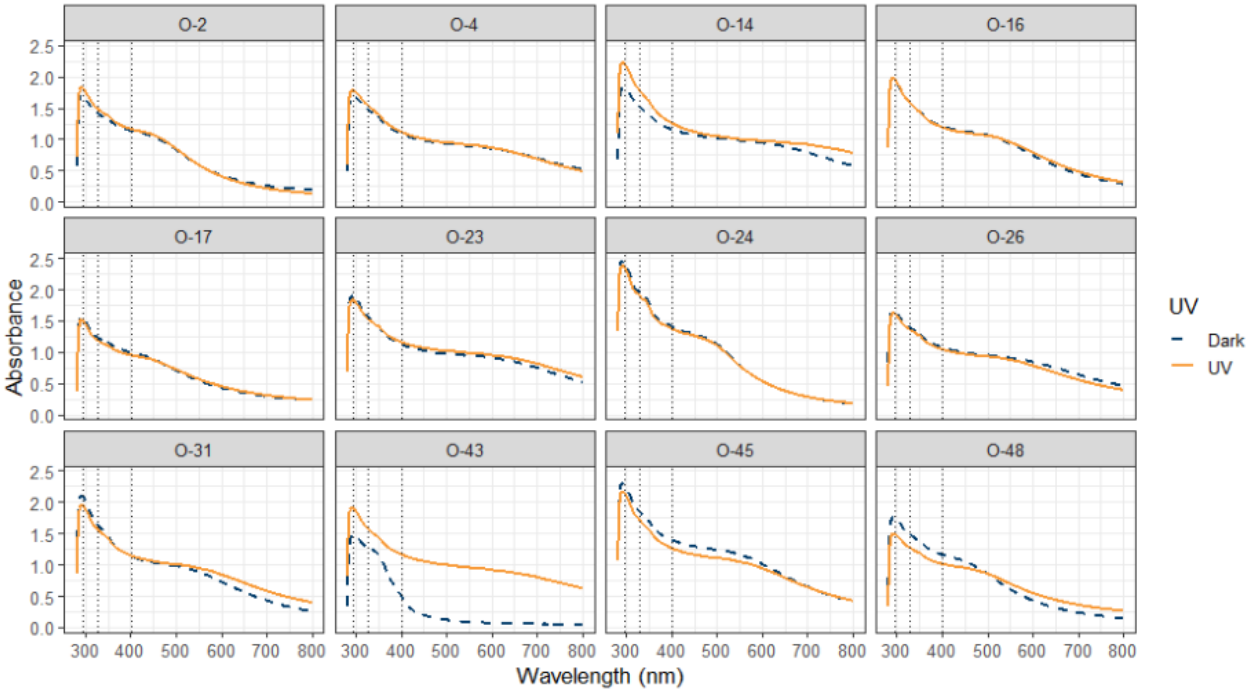


Figure 17: UV spectra profiles for 12 oils. The vertical lines 295 nm for pure phenanthrene, 328 nm for aromatic chromophores with three or four aromatic rings, and 401 nm for the Soret electronic absorption band of vanadyl porphyrins

Most of the products examined showed similar profiles following irradiation, with O-14, O-31 and O-48 showing slight increases in amplitude following irradiation, while O-43 had both a different shape and amplitude following irradiation. These changes in absorbance may be an indicator of the likelihood of whether a product is likely to undergo photomodification.

3.2. *Impact of WAF Preparation Method*

3.2.1. Timing of irradiation

The oil was exposed to light in multiple different steps to compare the effect of photo-modification methods on water chemistry and biological effects. We conducted two trials (one trial using ULSFO, the second trial using CONV) with the following 7 treatments (Figure 18):

- Treatment 1 – Oil was spread to a 1 mm thick layer in a petri dish and exposed to UV light for 18 hours prior to WAF preparation,
- Treatment 2 – Oil was left in a petri dish with no UV light exposure,

- WAF was prepared on a lab bench, collected after 24 hours, and then divided into 2 beakers, one beaker was placed under the UV light for irradiation (Treatment 3) and the other left without UV light exposure (Treatment 4),
- Treatment 5 – WAF was exposed to UV light for 18 hours while mixing,
- Treatment 6 – WAF was prepared normally on a bench without UV exposure, and
- Treatment 7 – was a seawater only control.

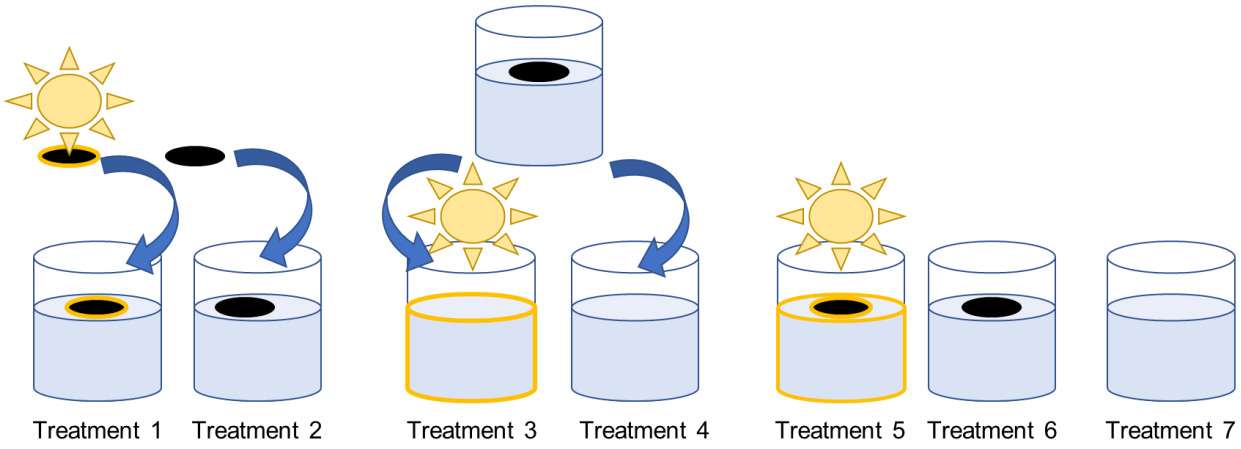


Figure 18: Schematic of the various ways the 18 hour UV dose was applied to the treatments, either directly on the oil (Treatment 1), on the dissolved phase test solution (Treatment 3), or while mixing (Treatment 5). The non-UV treatments (2, 4, and 6) were treated in the same manner as their UV counterparts only in a dark location within the same room.

The media generated from these trials was characterized with fluorometry and had analytical measurements of total organic carbon (TOC), PAH and alkyl-PAH (PAC; with the exception of Treatments 3 and 4 where there was not sufficient volume to complete the analysis), and biomimetic extraction solid-phase microextraction (BE-SPME). The test media was used to expose Stage I larval lobsters for 24 hours to assess their immobilization and mortality response. There was no observed toxicity in these trials and as such the focus is on the changes in concentration in the WAFs following the different UV treatments. There were changes in the measured concentrations depending on the Treatment method, the product, and the measurement method (Figure 19).

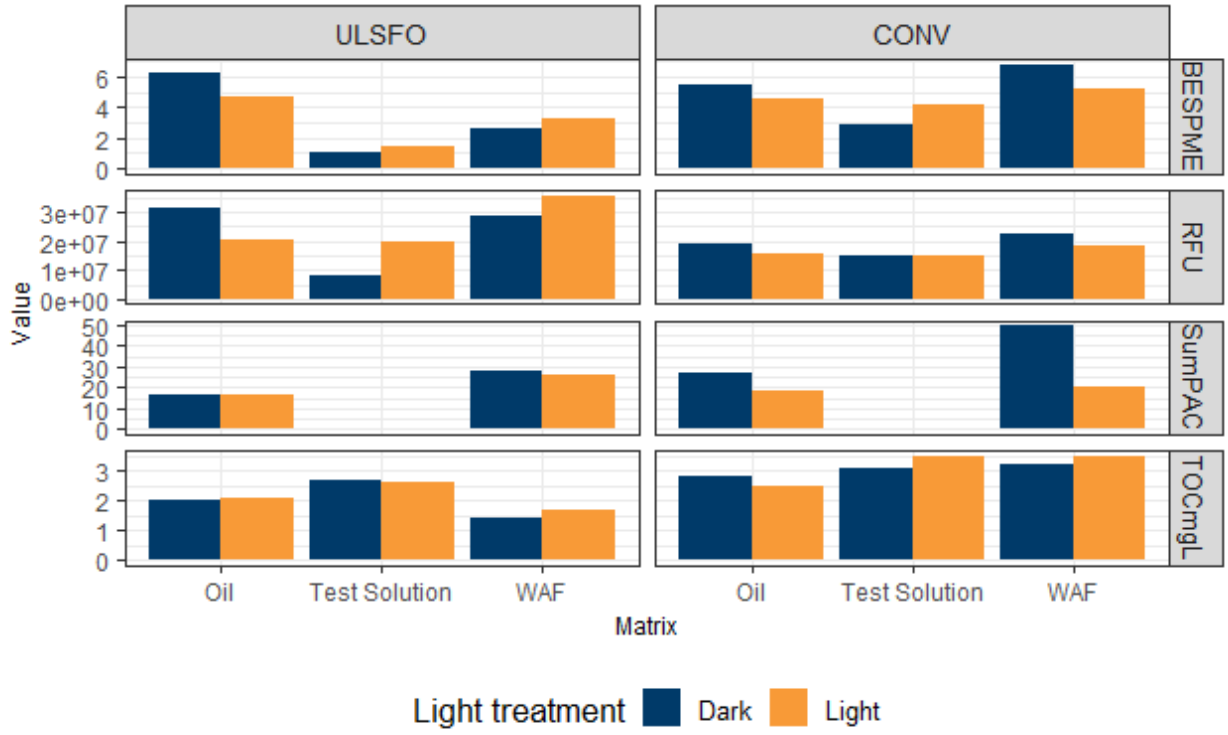


Figure 19: Summary of the measurements of BE (top row), fluorometry (second row), PACs (third row) and TOC (bottom row) following the different Treatment methods and UV irradiation.

For consistency, volume, and relevance purposes, the method of irradiating the WAF was selected for the remainder of the testing program.

3.2.2. Variable loading

To examine the role of slick thickness and photomodification, trials were conducted with variable oil loadings of ULSFO and CONV. Duplicate WAFs were prepared with loading rates of 0.01, 0.1, 1, and 10 g/L oil, and one of each loading rate were either exposed to UV light for 18hrs while mixing or left on the lab bench without any UV light exposure.

There was a concentration dependent increase in BE values with increased loading, and irradiation lead to a slight (ULSFO) and larger (CONV) increase in BE values (Figure 20).

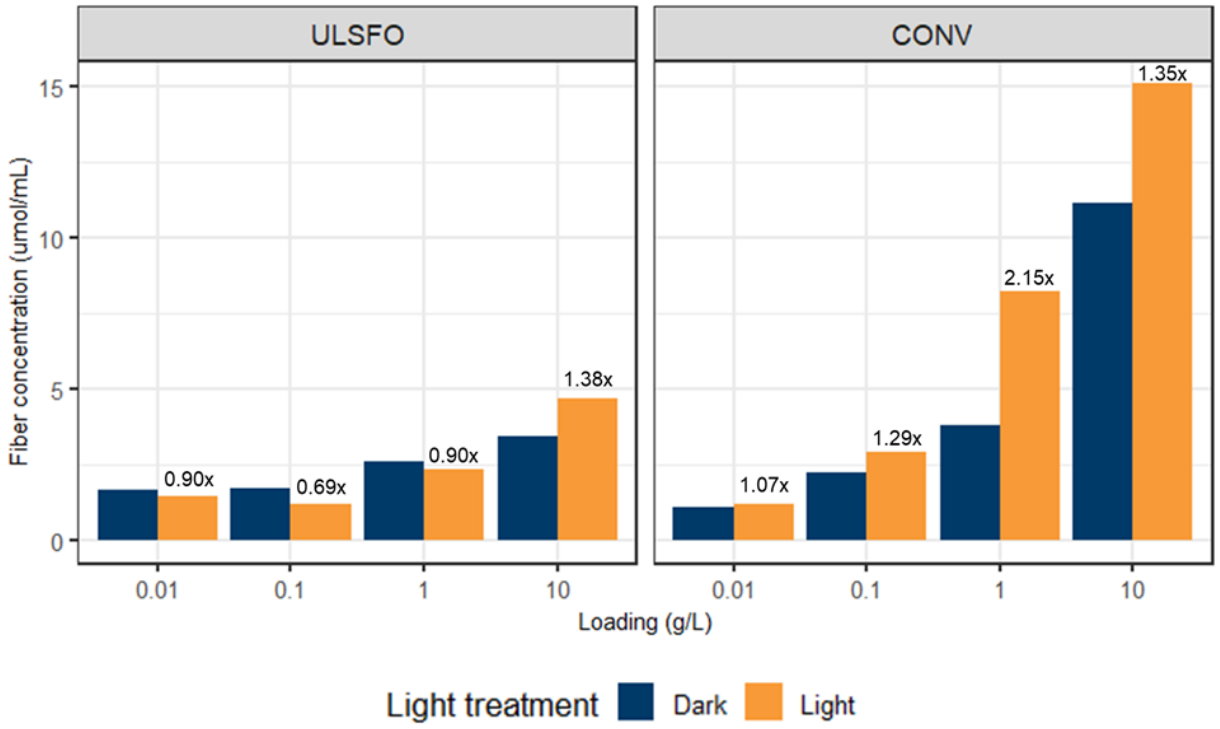


Figure 20: BE values (fiber concentration) for the different loadings of ULSFO (left) and CONV (right) prepared under UV light (orange) and in the dark (blue). The numbers indicate the fold difference between the irradiated and dark samples.

The ULSFO generally had lower BE values than the CONV, suggesting that at equal loadings there is less bioavailable material in the ULSFO preparation than in the CONV. The higher the loading, the higher the BE, and the greater the increase in BE from the irradiated sample, highlighting the importance of slick thickness for the formation of photoproducts.

The increase in BE values mirrored the observed toxicity in a lobster bioassay, where at the 10 g/L loading of CONV there was notable (e.g., >20%) immobilization and mortality, with the irradiated sample having greater effect (Figure 21).

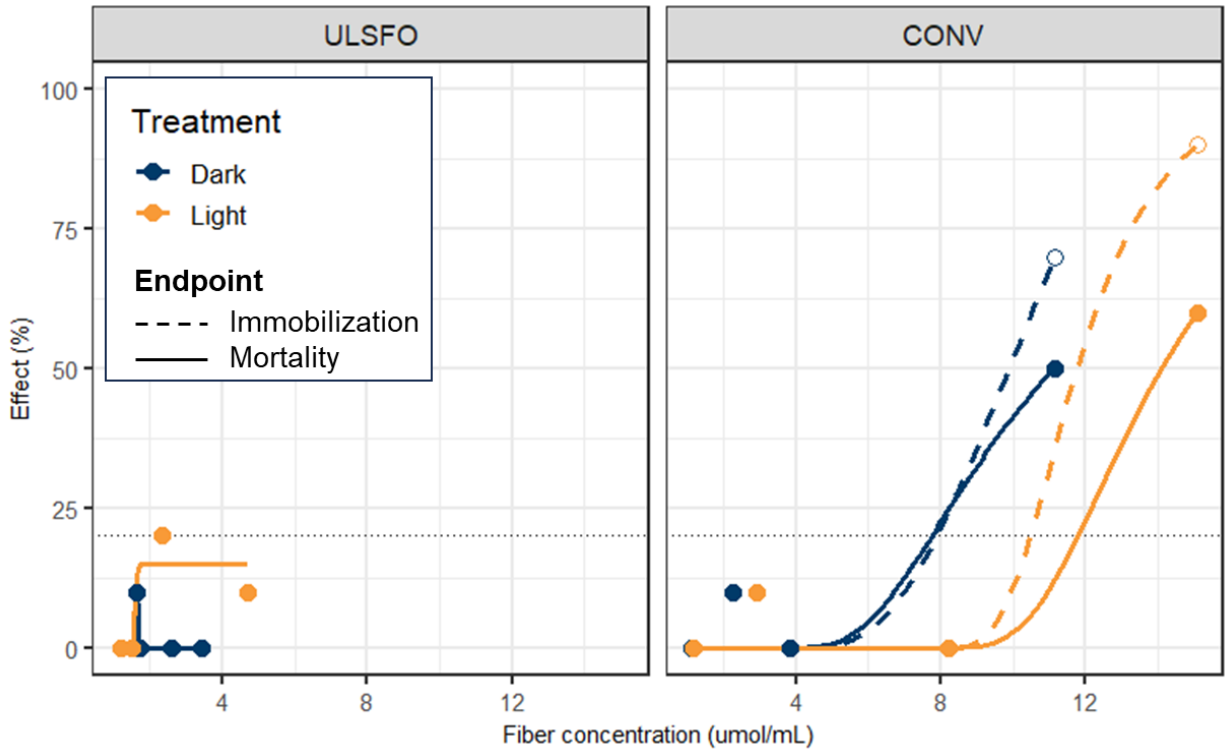


Figure 21: Concentration response relationship for lobsters exposed to variable loadings of ULSFO (left) and CONV (right) that were irradiated (orange) or prepared in the dark (blue). The immobilization response is shown with open circles and dashed line, while mortality is shown with solid circles and lines, both were fit with a 3-parameter Type 1 Weibull model.

Given the small volume of VLSFOs available, the decision was made to proceed with a single loading to maximize the amount of trials that could be conducted with an individual product. All subsequent WAFs for the toxicity testing were made with a 1 g/L loading. WAFs were made with 1.6 L of seawater, and as such the target loading was 1.6 g (+/- 20%) (Figure 22). From the 150 WAFs made a total amount of 278 g of VLSFO product was utilized in this project.

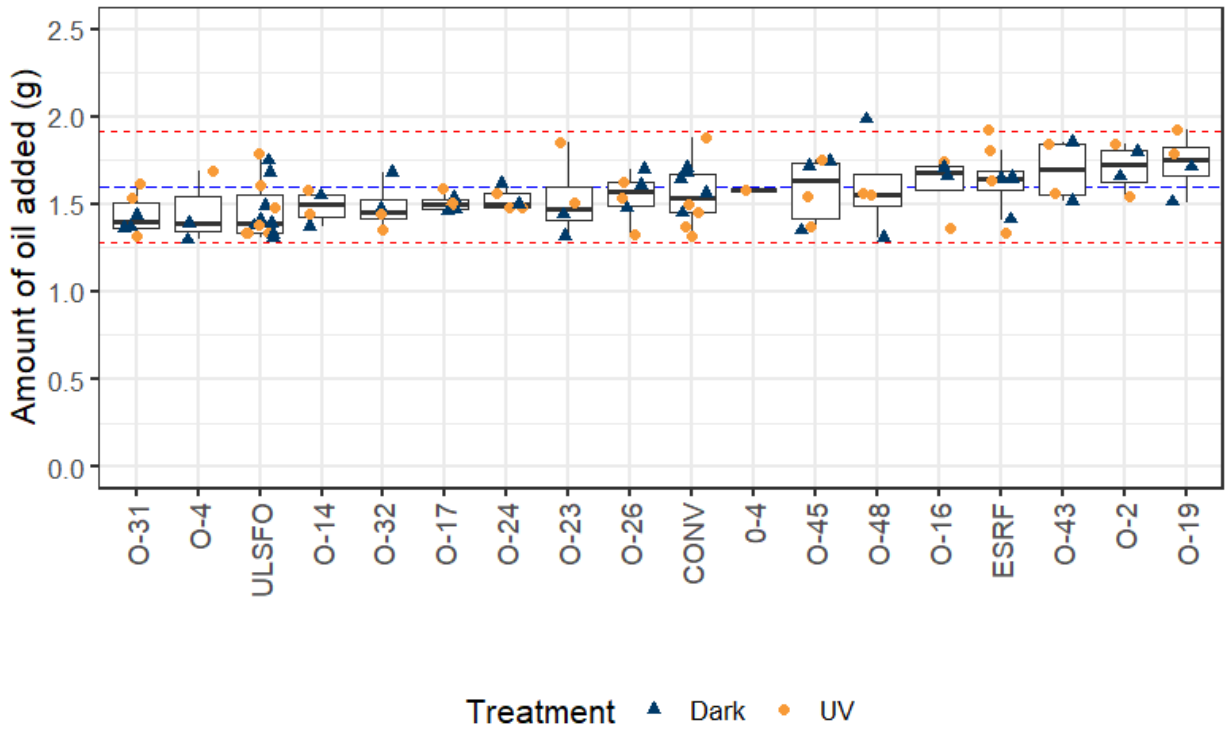


Figure 22: Amount of each product used in making the WAFs. The dashed blue line is the target loading, while the dashed red lines are the acceptable ranges.

3.3. *Toxicity Testing*

3.3.1. American lobster results

WAFs were prepared at a single loading (1 g/L) under UV light and in the dark and were tested using only 100% strength solution of the WAF. The objective of this screening was to determine the relative toxicity of the different products, and to identify any products which showed a significant effect of photomodification (e.g., a change in toxicity relative to the dark preparation) (Table 2).

738 *Table 2: Summary of lobster immobilization (Imm.) results for 2022 and the various exposure metrics. Fold change refers to the Light to Dark.*

Product	UV	Imm. (%)	Fold Change	TOC (mg/L)	Fold Change	Sum RFU	Fold Change	BE SPME	Fold Change	Sum PAC (µg/L)	Fold Change	Predicted Toxic Units	Predicted Imm.	Observed - Predicted Imm.
O-43	Dark	100		2.8		4E+07		12.1		168		0.10	5	95
	Light	100	1.00	8.2	2.93	1E+08	3.09	14.7	1.22	240	1.43	0.35	18	82
O-17	Dark	90		2.6		3E+08		24.5		1053		0.80	40	50
	Light	100	1.11	3.3	1.27	3E+08	0.83	18.9	0.77	1264	1.20	0.86	43	57
O-2	Dark	100		2.5		2E+08		30.7		900		0.78	39	61
	Light	100	1.00	4.7	1.88	3E+08	1.23	30.4	0.99	1045	1.16	0.93	46	54
O-24	Dark	60		2.6		2E+08		16.0		454		2.97	100	-40
	Light	100	1.67	5.4	2.08	2E+08	1.23	28.4	1.77	702	1.55	6.16	100	0
O-16	Dark	60		2.4		1E+08		10.9		222		0.25	12	48
	Light	70	1.17	3	1.25	1E+08	0.90	10.1	0.93	219	0.98	0.23	11	59
O-48	Dark	10		2		3E+07		3.3		65		0.05	3	7
	Light	80	8.00	3.8	1.90	9E+07	3.07	5.9	1.77	133	2.04	0.35	17	63
O-26	Dark	100		5.2		3E+08		19.9		1125		2.25	100	0
	Light	100	1.00	8	1.54	3E+08	1.27	25.8	1.29	1571	1.40	4.33	100	0
O-31	Dark	80		3		1E+08		18.8		454		1.72	86	-6
	Light	100	1.25	4.7	1.57	2E+08	1.12	16.9	0.90	428	0.94	1.26	63	37
O-45	Dark	80		2.2		1E+08		9.9		243		0.22	11	69
	Light	100	1.25	4.7	2.14	2E+08	1.87		0.00	420	1.73	0.79	40	60
O-23	Dark	90		1.9		7E+07		4.0		273		0.18	9	81
	Light	20	0.22	1.9	1.00	6E+07	0.90	6.7	1.68	278	1.02	0.18	9	11
O-14	Dark	10		2		7E+07				326		0.18	9	1
	Light	20	2.00	2.5	1.25	9E+07	1.17	9.5		315	0.97	0.19	9	11
O-4	Dark	60		2.6		8E+07		8.6		153		0.11	6	54
	Light	100	1.67	2.9	1.12	1E+08	1.46	9.6	1.12	229	1.50	0.19	10	90
ULSFO	Dark	0		2		2E+07		2.6		17		0.05	2	-2
	Light	0	-	4.6	2.30	2E+07	1.53	2.3	0.90	33	1.89	0.04	2	-2
CONV	Dark	0		2.1		3E+07		3.8		22		0.03	1	-1
	Light	20	-	2.3	1.10	2E+07	0.66	8.2	2.15	16	0.74	0.13	6	14

739

In nearly all cases there was an increase in toxicity following UV irradiation (Figure 23), which was mirrored by increases in the various exposure metrics (TOC, RFU, BE-SPME and PAC). Using the PAC data the Toxic Units (TU) were calculated, and the predicted toxicity (as immobilization) was compared to the observed response. The TU approach was able to predict the toxicity within +/- 20% for 12 of the 28 products, however it generally was underpredicting the toxicity of the VLSFOs both with and without UV.

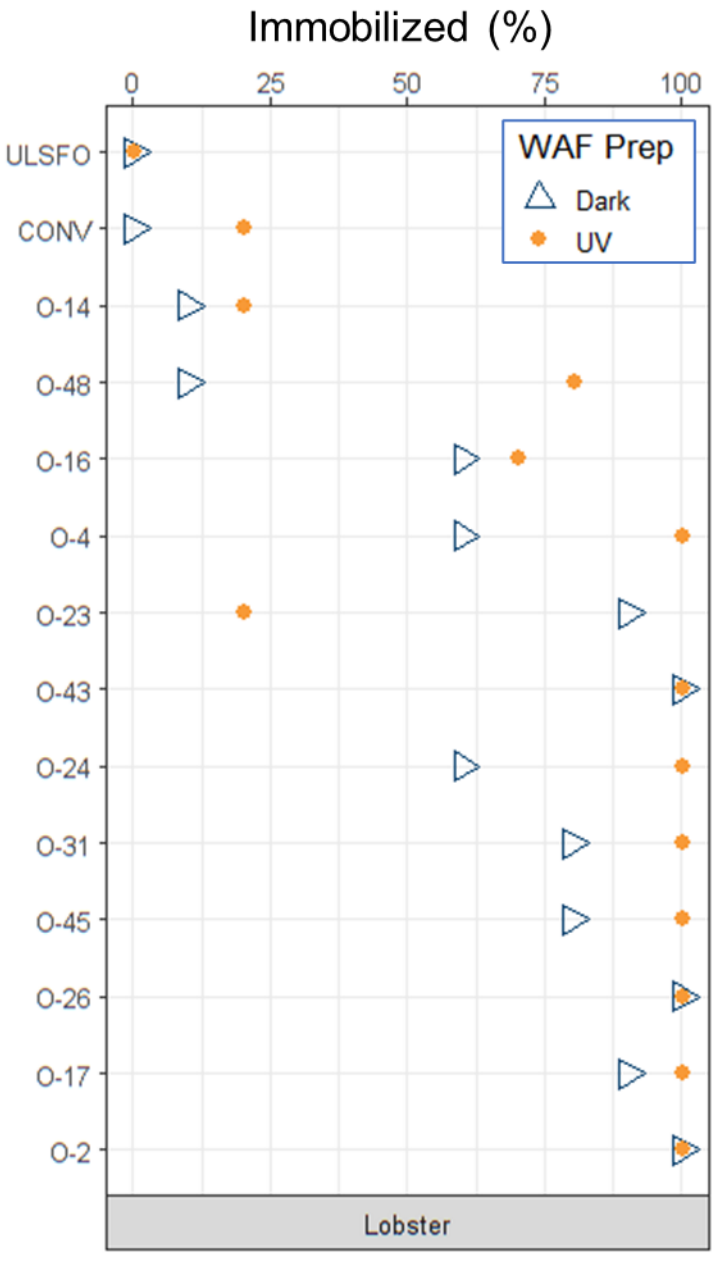


Figure 23: Visual summary of the lobster immobilization response for the 2022 exposures to irradiated (solid orange circles) and non-irradiated (open blue triangles) WAFs.

In 2023, two additional products, O-19 and O-32, were selected for testing based on their physical properties, specifically SARA. The other products that were selected for re-testing included fuels that saw 100% immobilization in the UV treated WAFs including O-2, O-4, O-17, O-24, O-26, O-43 and O-45, and the product O-23 which saw greater toxicity in the dark WAF (90% immobilization) than in the one prepared under UV (20% immobilization). As the products which had 100% immobilization were unable to discern how much toxicity was being "added" due to photo-modification, a dilution series was prepared from each WAF such that the 100, 10 and 1% strength were tested. In nearly each case, the dilution range proved too wide, as at the 10% strength there was minimal toxicity for most products. The notable exception was O-43 which saw sustained toxicity in the UV treated WAF even at 1% strength (90% immobilization, Table 3).

Table 3: Summary of the O-43 testing with larval lobster in 2023

Percent	UV	Immobilized (%)	RFU
1	**Dark**	0	3,206,626
	UV	90	4,336,892
10	**Dark**	0	8,040,701
	UV	90	24,622,922
100	**Dark**	40	40,920,218
	UV	100	220,328,001

The fluorometric signal for the UV treated O-43 WAF was ~5.4 times greater than its dark counterpart. The 10 and 1% strength UV solution had a lower fluorometric signal than the 100% strength Dark WAF yet produced a significantly greater immobilization response. This suggests that the photo-products formed in the during the irradiation of O-43 are not detectable by fluorometer (e.g., addition of an oxygen atom will often decrease fluorescence of aromatic compounds). The 100% strength sample of UV treated O-43 WAF also showed the greatest increase in TOC (4.8 x) and BE-SPME (2.29 x) across all products tested, suggesting that this product is highly photo-reactive, and based on the results of the lobster (and cod, see section 3.3.2.2) bioassay the products formed are very toxic.

The lobster data from 2022 and 2023 were combined to model the relationship between the exposure metrics, TOC (Figure 24), RFU (Figure 25), BE-SPME (Figure 26), PACs (Figure 27), and TUs (Figure 28), and immobilization.

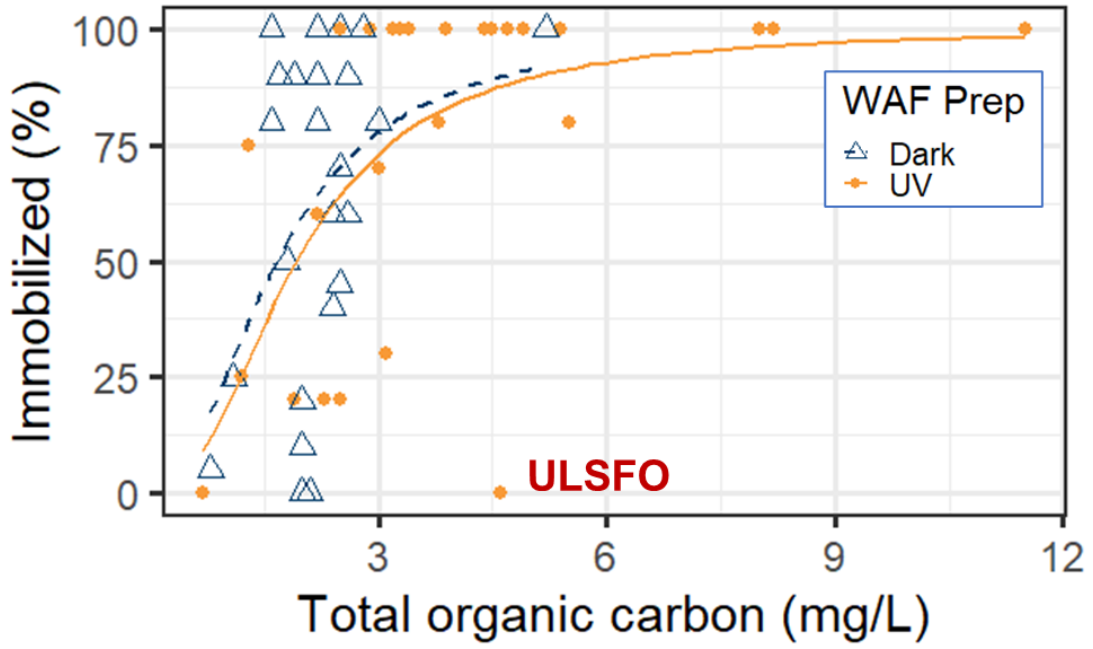


Figure 24: Concentrations of total organic carbon (TOC) and the lobster immobilization response.

TOC concentrations generally had a consistent relationship with immobilization, where increasing concentrations of TOC (especially above 3 mg/L) resulted in nearly complete immobilization. However, the lack of resolution in this measurement and its non-specificity may limit its widespread adoption for predicting toxicity outside of a laboratory setting. The irradiated ULSFO result is unexpected, showing a 2.3-fold increase in TOC relative to the dark preparation, but no change in toxicity (a similar result was observed in the Atlantic cod trials, Table 4). This highlights a challenge in the non-specificity of TOC as it cannot be assumed that all photoproducts that are formed are equally toxic, and in the case of ULSFO where there was a notable increase in TOC, these products did not contribute to toxicity.

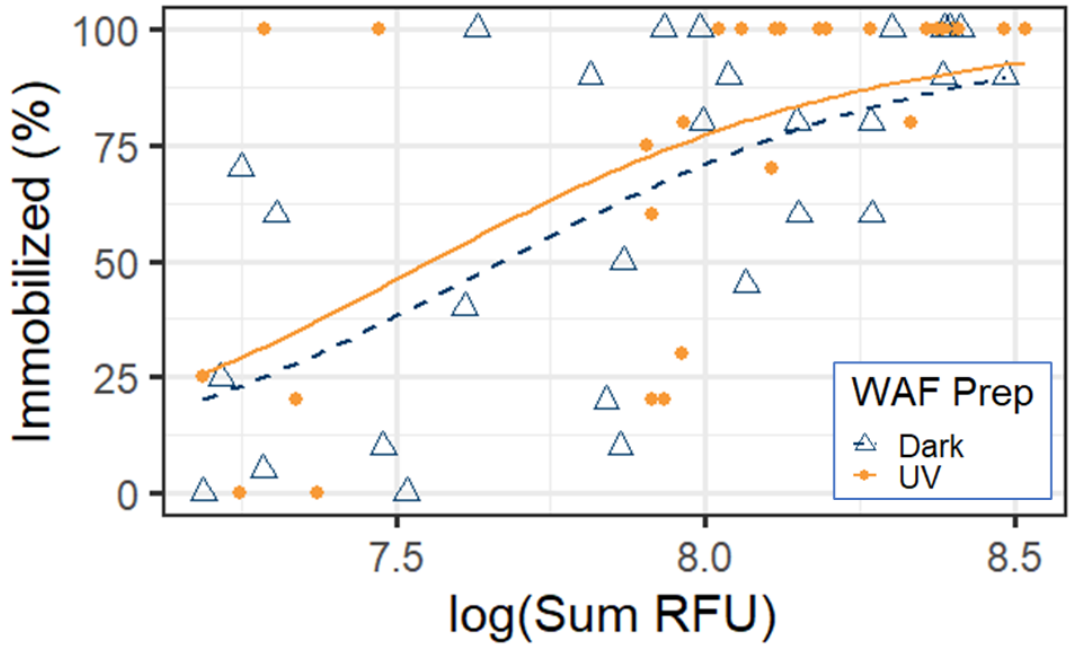
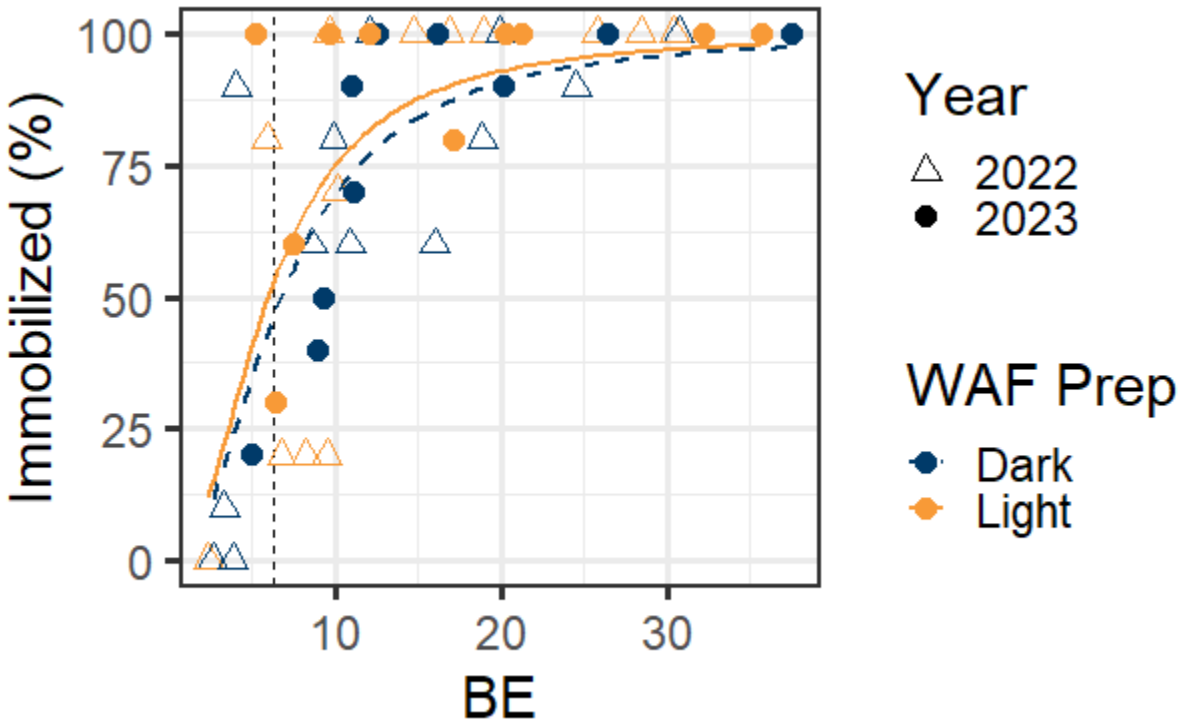


Figure 25: Relative fluorescence units (RFU) and the lobster immobilization response.

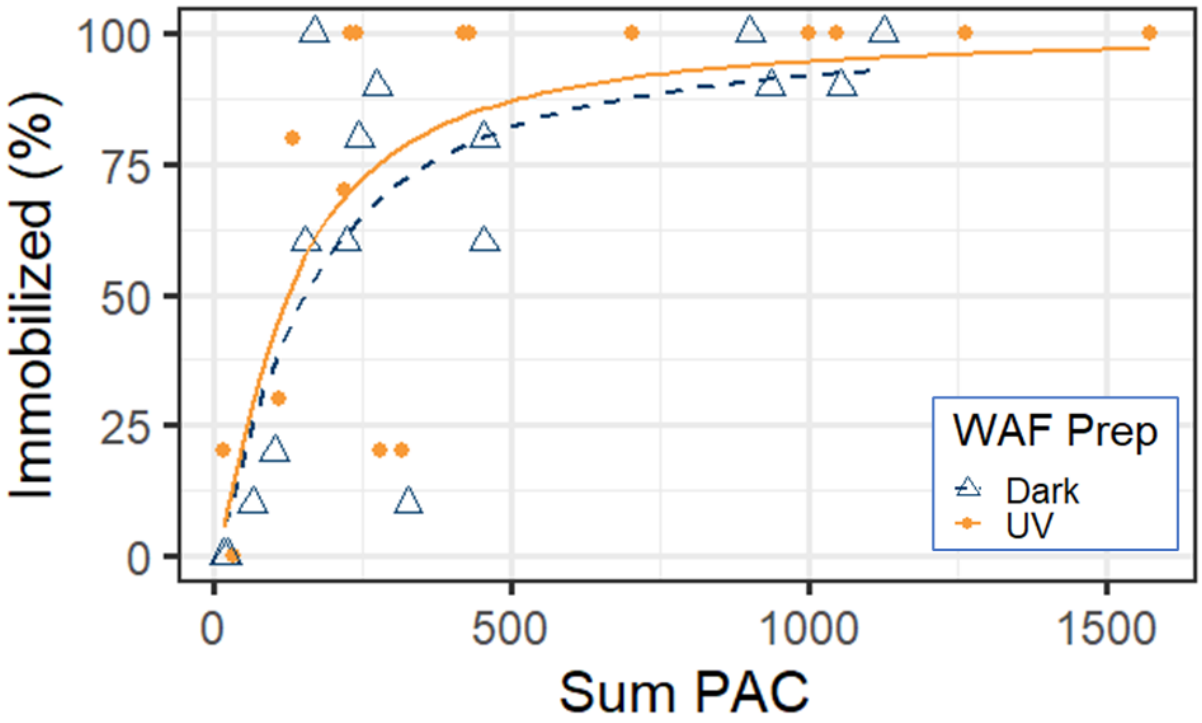
Fluorometry proved to be quite variable in being able to describe the observed toxicity and may not be a reliable predictor of observed toxicity in lobsters. This may be due to the presence of compounds in the VLSFOs (both the dark and irradiated) that are not detectable by fluorometry but contribute to the observed toxicity.



794

795 *Figure 26: Summary of the larval lobster immobilization response data following 24-hour exposure to*
796 *irradiated (orange) and non-irradiated (blue) WAFs* in the 2022 (open triangles) and 2023 (filled circles)
797 *testing season. The dashed vertical line is the calculated BE-critical value of 6.3 µmol per mL PDMS.*

798 When comparing the lobster results from 2022 (circles) to 2023 (triangles), there was very
799 good agreement between BE values and toxicity, which supports the derivation of a BE-
800 critical value (value that corresponds to 50% mortality, analogous to an LC50 value with
801 the exposure metric expressed as a fiber concentration, rather than a water
802 concentration), that can be used to reasonably approximate the toxicity of an unknown
803 WAF, and potentially an environmental sample, to American lobster larvae. The lobster
804 BE-critical value was calculated as a fiber concentration of 6.3 µmol per mL PDMS (0.7
805 standard error) and can be used to compare sensitivity across species.



806

807 *Figure 27: Sum of the polycyclic aromatic compounds (PAC) and the lobster immobilization response.*

808 The immobilization response increases with increasing sum PAC concentrations,
809 however there are a few instances where the immobilization response is less than would
810 be expected for a sample with that sum PAC (µg/L) value. This is largely due to the
811 varying toxicity of the different PACs within that summary metric of sum PAC (or total
812 PAC), where the contribution of each PAC towards toxicity is considered equal. The toxic
813 unit approach accounts for the composition and concentration of the PACs in the mixture
814 and allows for the prediction of toxicity (Figure 28).

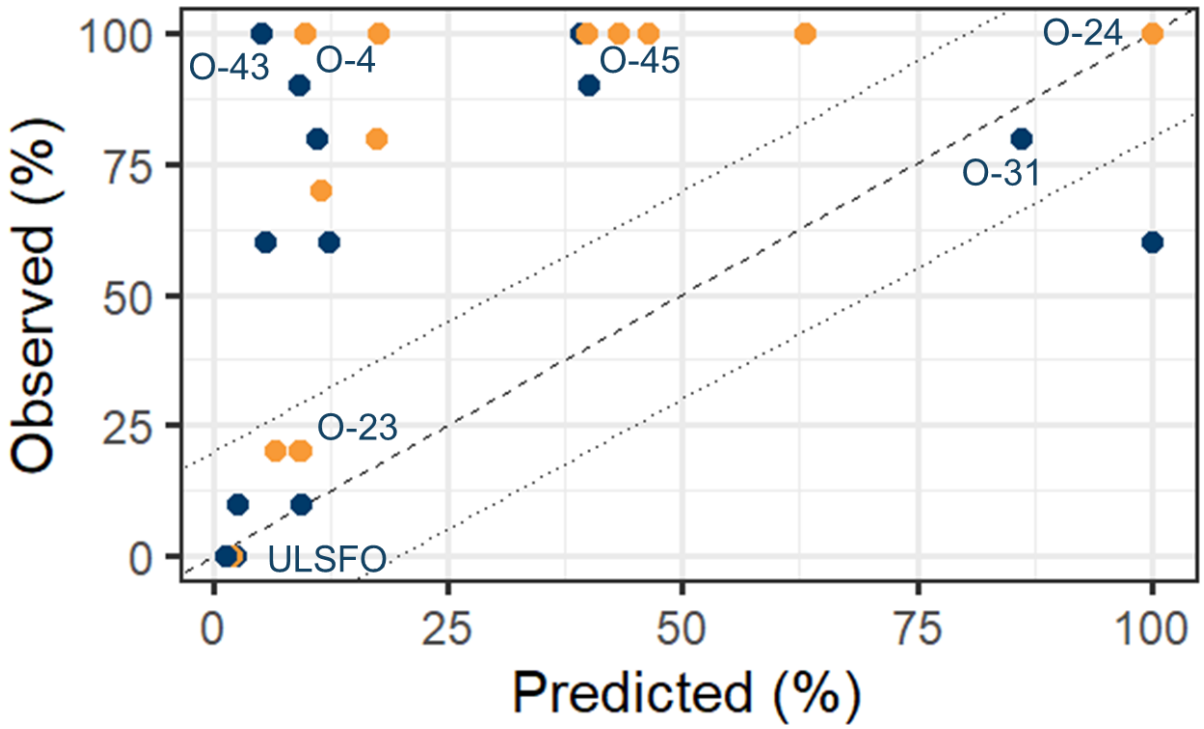


Figure 28: Predicted and observed toxicity. Dashed line is 1:1 with the dotted lines bounding 20%.

Using only waterborne PAC concentration, the toxic unit model consistently underestimates the toxicity of many of the VLSFOs products, both with and without UV. Other aspects that are not measured by traditional GC-MS (e.g., oxidized products) are likely contributing to the toxicity.

The results of two seasons of lobster testing are presented in **Error! Reference source not found.**. Stage I lobster larvae were sensitive to both UV-treated and non-irradiated WAF, and in many cases showed increased toxicity following photomodification. The results between the years were consistent for most products, with the notable exception of O-23 and O-45. With O-23, this product was selected for a repeat exposure based on the observed result of reduced toxicity following photo-modification. In the 2023 exposure, we were not able to repeat that result and on the basis of the exposure metrics (e.g., TOC, fluorometry, and BE-SPME) believe the 2022 result to be anomalous. O-43 was selected for a repeat exposure in 2023 based on the 100% immobilization in both the UV-treated and non-irradiated WAF, and because the AMSA report had suggested that this product had been mislabelled (suspected to be RMD80 specification oil, and not RMD380). This may have also had an impact on the stability of the sample, which would explain the decrease in toxicity in 2023 compared to 2022 for the non-irradiated WAF,

834 however the UV-treated WAF in each year elicited a 100% immobilization response in the
835 larval lobsters.

836

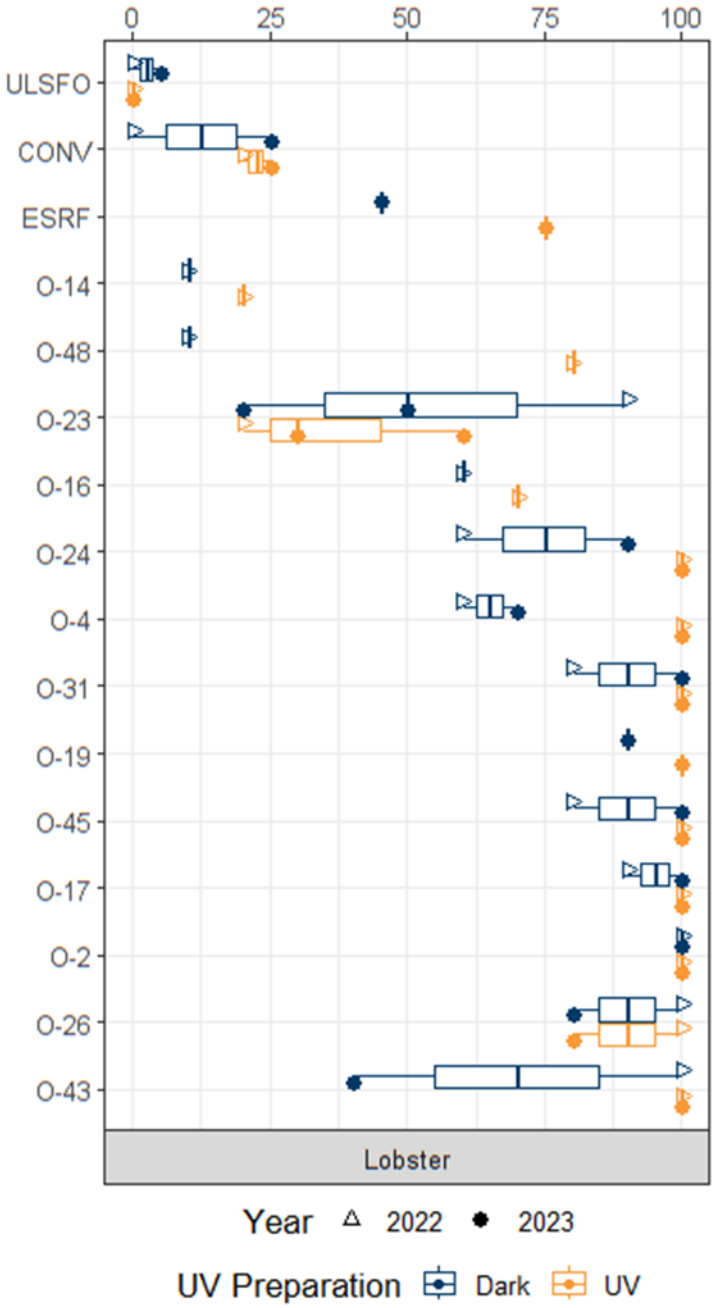


Figure 29: Summary of the lobster immobilization in response to 24-hr exposure to irradiated (orange) and non-irradiated (blue) WAFs in the 2022 (open triangles) and 2023 (filled circles) testing season. Where the product was tested in both years the boxplot illustrates the range and mean response.

3.3.1.1. Photo-modification and Photo-sensitization in lobster

American lobster larvae were exposed to dark-WAF or UV-WAF at 100% strength for 24-hrs. Afterwards the larvae were transferred into clean seawater, and a subset of replicates

from each treatment (dark or UV-WAF) were directed towards a photo-sensitization exposure involving a 3-hour exposure to a single UV dose, while the other units remained in the dark. Each unit was then followed for an additional 24-hours (under dark conditions) of monitoring to assess mortality. The results of which are shown in Figure 30.

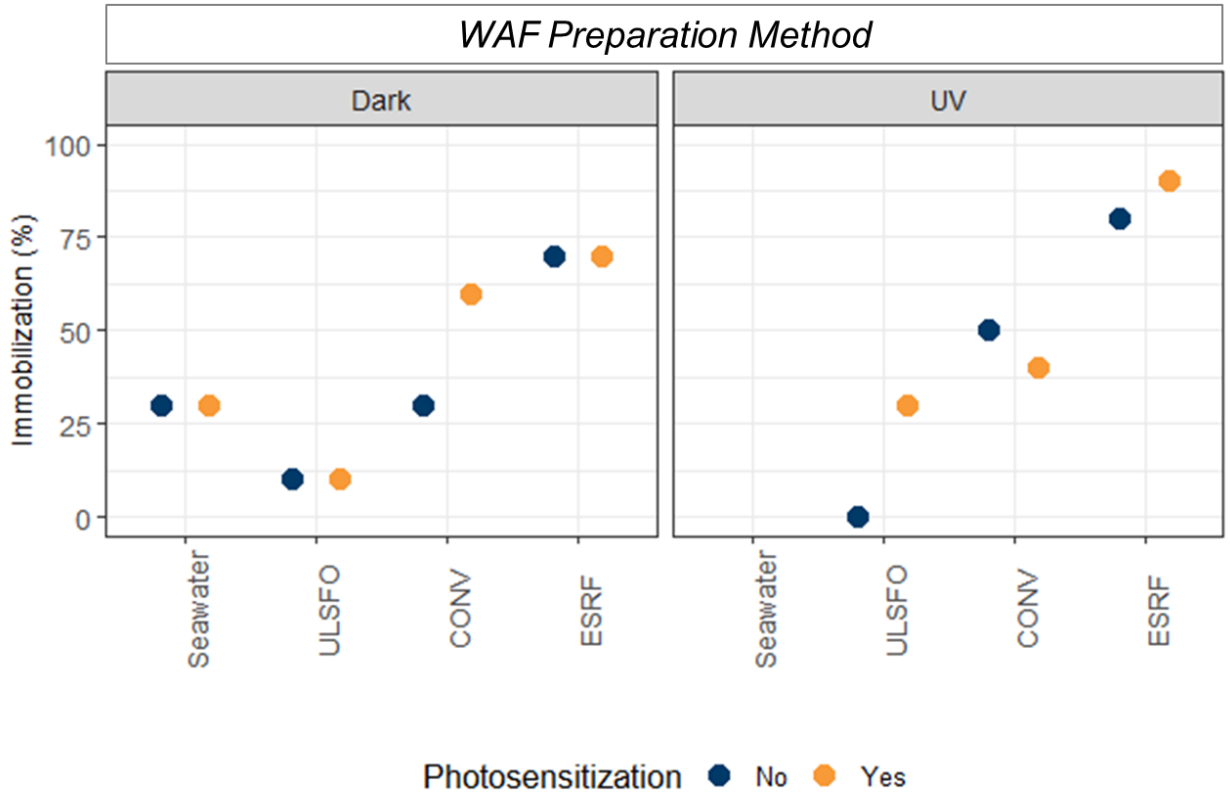


Figure 30: Summary of the photo-modification and photo-sensitization combined trial. The panel on the left represent WAF media that was prepared in the dark (no photo-modification), while the panel on the right is WAF media prepared under UV light (photo-modification). The colours of the points within each panel indicate whether following 24-hrs of exposure to media the replicate (individual point) was placed under UV light for 3-hours (photo-sensitization; orange circles), or left in the dark (blue circles).

Photosensitization (orange points) had minimal effect on larval lobsters exposed to these WAFs under either photomodification regime. While there was an increase in the toxicity observed in the photo-modified ULSFO WAF, the relatively high control responses prevents us from saying with confidence that this is a true effect.

3.3.2. Atlantic cod results

3.3.2.1. Embryo

Six products (5 VLSFOs, and 1 conventional crude) were screened for toxicity with cod embryo exposures. WAFs were prepared at a single loading (1 g/L) under UV light and in the dark and were tested using only 100% strength solution of the WAF, with a 24-hr

exposure, followed by transfer to clean seawater for monitoring for 96 hours. The objective of this screening was to determine the relative toxicity of the different products, and to identify any products which showed a significant effect of photomodification (e.g., a change in toxicity relative to the dark preparation).

The embryo trials showed either no toxicity response (Figure 31) or failed our validity criteria due to poor embryo performance (data not shown).

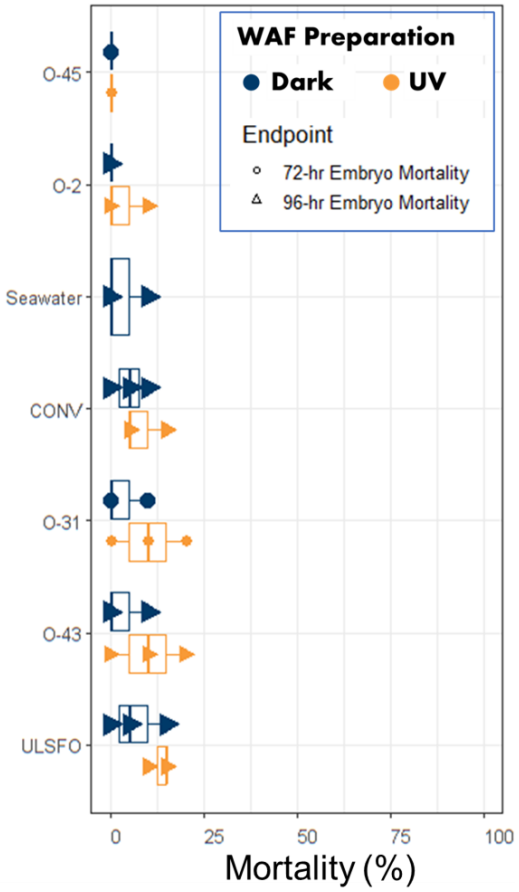


Figure 31: Summary of embryo responses following either 72 (circles) or 96 (triangles) exposure to UV-treated (orange) or dark (blue) WAF.

Due to the embryo quality issues, some of the trials were only valid for 72-hours (as shown by triangles in Figure 31) as compared to the others which ran for 96-hours. In each case there was little mortality response nor indication of significant differences between WAF preparation methods. Given the lack of sensitivity (potentially owing to reduced permeability of the embryo) and the embryo quality issues, the focus of the testing shifted to the larval life stage.

878 *3.3.2.2. Larval*

879 Larval cod were more sensitive to exposure than embryos, so product testing was
880 expanded to include 14 products (12 VLSFOs, and 2 conventional crudes), with the
881 results summarized in Table 4 and Figure 32.

882 *Table 4: Results of larval cod toxicity screening prepared in the dark and under 18 hours of irradiation.*

Oil	UV	Average Mortality (%)	TOC	Sum RFU	BE-SPME	Fold Increase (UV to Dark)			
						Mortality	TOC	Sum RFU	BE-SPME
CONV	Dark	6.7	1.6	20243420	4.50	0.49	1.44	1.13	1.06
	UV	3.3	2.3	22789404	4.77				
ESRF	Dark	20	1.5	65196965	11.25	1.34	1.00	1.37	1.18
	UV	26.7	1.5	89577332	13.29				
ULSFO	Dark	6.7	0.8	13004722	1.30	1.00	3.75	0.99	0.75
	UV	6.7	3.0	12861002	0.98				
O-17	Dark	3.3	1.9	148216323	17.57	4.03	1.37	1.23	0.99
	UV	13.3	2.6	183013329	17.47				
O-26	Dark	23.3	3.3	176393893	17.34	4.00	1.58	1.13	0.92
	UV	93.3	5.2	198524518	15.91				
O-31	Dark	6.7	2.1	81886893	11.90	6.97	1.57	1.30	0.98
	UV	46.7	3.3	106750433	11.72				
O-45	Dark	6.7	1.6	87111250	7.36	0.49	1.25	0.74	0.93
	UV	3.3	2	64872312	6.88				
O-2	Dark	20	1.8	187227317	34.45	4.50	1.61	1.25	0.92
	UV	90	2.9	233979691	31.85				
O-43	Dark	23.3	2.3	47085476	11.83	4.29	3.78	3.33	1.26
	UV	100	8.7	156743506	14.87				
O-24	Dark	33.3	1.6	114884479	13.56	2.40	2.44	1.11	1.12
	UV	80	3.9	127287034	15.13				
O-4	Dark	16.7	1.9	75681677	10.44	0.00	1.16	0.87	0.66
	UV	0	2.2	65543284	6.93				
O-14	Dark	43.3	1.7	20628964	11.37	0.00	1.18	0.92	0.88
	UV	0	2.0	18994446	10.04				
O-16	Dark	3.3	1.9	27143889	11.41	7.06	0.95	1.23	1.05
	UV	23.3	1.8	33425179	12.01				
O-48	Dark	6.7	2.8	7476462	3.64	1.00	2.68	1.27	0.98
	UV	6.7	7.5	9518965	3.55				

883

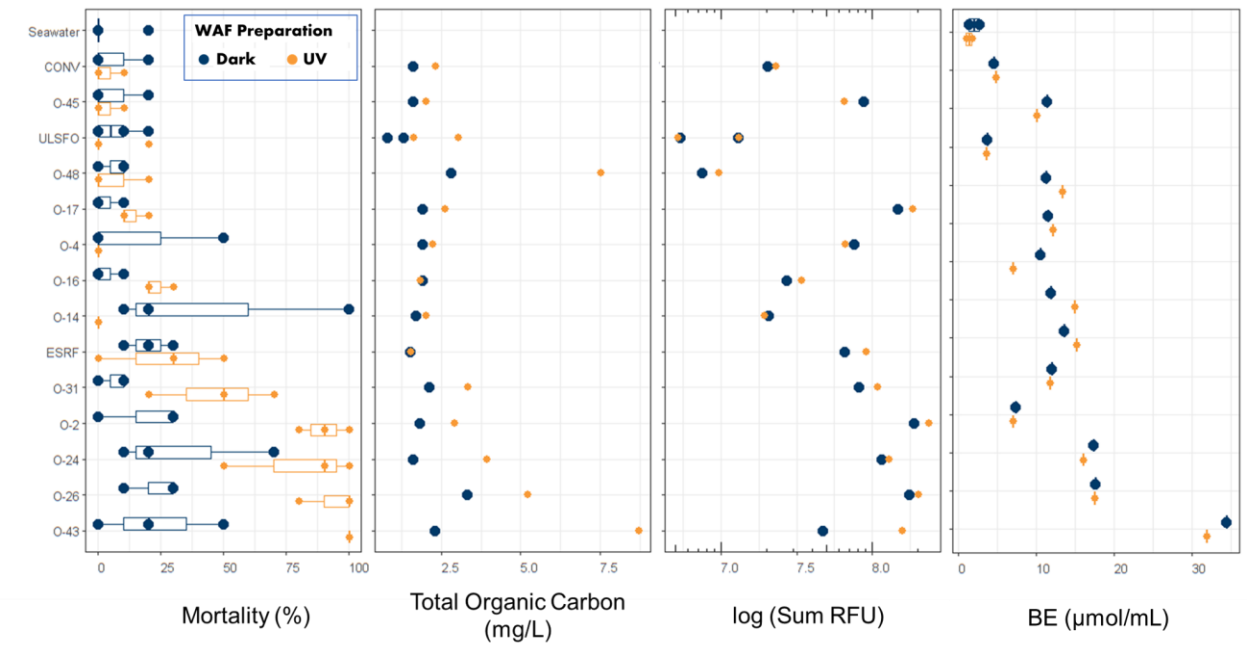


Figure 32: Summary of the larval mortality (left; each point represents a replicate, with the box bounding the interquartile range), total organic carbon measurements (middle-left), the relative fluorometry signal (middle-right), and the BE-SPME (right) for the UV-treated (orange) and dark (blue) WAFs. The products are listed on the y-axis in decreasing order of toxicity based on the UV-treated WAFs (e.g., products on the bottom are more toxic)

In nearly all cases the observed toxicity in the UV treated WAF (orange circles in Figure 32) was equal or greater than the WAF prepared in the dark (blue circles). For all products tested, there was an increase in TOC with UV treatment. While the fluorometer can only measure compounds that fluoresce, in all but one case (O-45) there was an increase in the relative fluorometric signal in the UV treated WAFs compared to their non-irradiated counterpart. It should be noted, however, other compounds may have been formed that are not detectable with this method. There was less difference in the BE values between the irradiated and dark counterparts, however the increase in BE concentration generally followed with increases in toxicity. The increased TOC and RFUs after irradiation suggest that there were additional photoproducts being formed and that the amount formed varied between oil samples. The VLSFO WAFs showed a full range of mortality response from 0 to 100% when irradiated, however there was seldom more than 50% mortality for any VLSFO WAF prepared in the dark.

The mortality data was modelled using the TOC, RFU, and BE-SPME as exposure metrics (Figure 33).

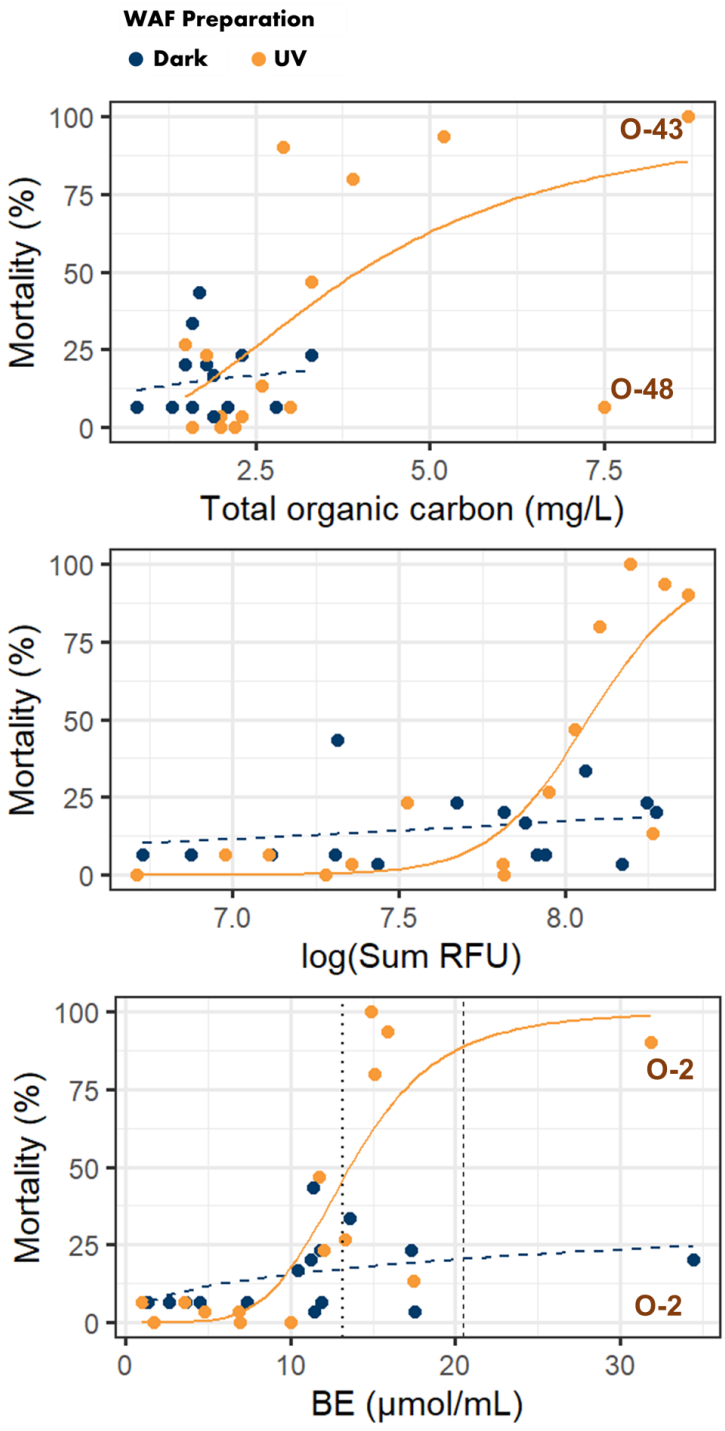


Figure 33: Fitting the larval cod mortality data to the exposure metrics of total organic carbon (top), relative fluorometry signal (middle), and BE (bottom) for the UV-treated (orange) and dark (blue) WAFs.

As TOC increased greater than 2.5 mg/L there was more pronounced mortality, with the notable exception of O-48, which had the second highest TOC concentration (7.5 mg/L),

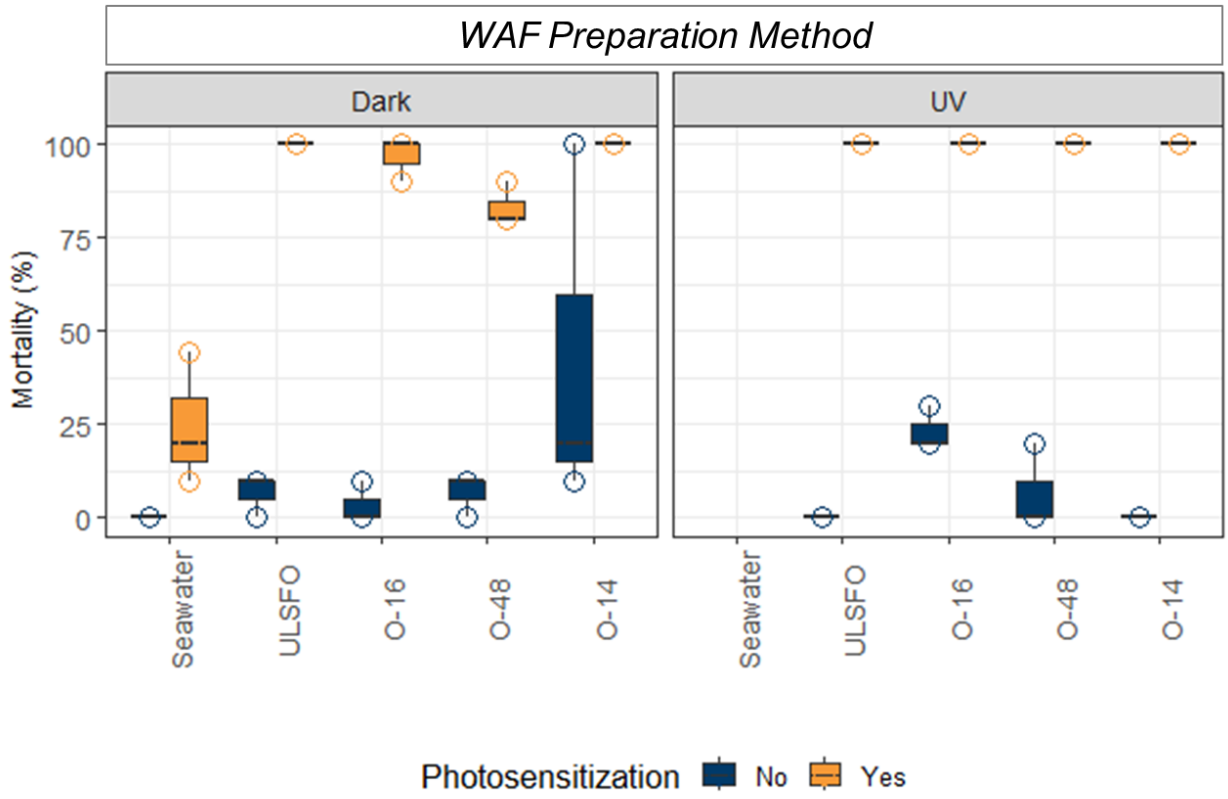
but very little mortality (mean 6.7% mortality). The greatest TOC measurements were from WAFs that had been irradiated, highlighting the formation of photoproducts. There was a stronger relationship with the fluorometry signal strength (RFU) and the observed mortality when looking at the WAFs prepared under UV light. However, the dark WAFs did not have as strong of a relationship, partially owing to the limited mortality seen in the dark preparations. If the formed photoproducts are not detectable by fluorescence, this may explain why at similar RFUs there is increased mortality observed in the UV WAFs than those in the dark.

The BE exposure metric (µmol/L) generally increased with increasing mortality. There were three results from the same trial where the BE-values were high (e.g., 17.3 – 17.5 µmol/L) but showed less mortality than expected. These values came from O-17 (UV and dark) and O-26 (dark) and are consistent with the magnitude observed in the lobster studies for those products (18.9 – 24.5 µmol/L). For lobster, these two products were among the most toxic, however in cod they presented a much lower response. This could be due to an overall reduced sensitivity of cod as compared to lobster or may reflect that the exposure and monitoring time in the colder temperature cod, was not sufficient to observe the toxicological effects (e.g., may have required >24-hrs to observe the effects). The other result of interest for the BE relationship is O-2, which exhibited the greatest BE concentrations (31.9 and 34.5 µmol/L for UV and dark preparation respectively; again in line with the measured values from the lobster studies), and also showed extreme differences in mortality, 20% for the dark preparation and 90% for the UV preparation. The lower than expected mortality in the dark preparation of O-2 may be due to the colder temperatures and requiring more time to observe mortality, or could be due to the higher degree of variability observed within Atlantic cod and their responses to petroleum products (Scovil et al. 2022); however as the fish were from the same batch, the latter explanation is less likely in this case. A concentration response model was applied to the BE and mortality results, both with all the data and excluding the 4 data points where the mortality was lower than expected, yielding a BE-critical value of 20.5 µmol/L (5.9 standard error) and 13.2 µmol/L (0.5 standard error) respectively (indicated as the dashed and dotted vertical lines in the bottom panel of Figure 33). The BE-critical value is greater than the value for lobster (6.3 µmol/L), confirming the greater acute sensitivity of lobster as compared to cod larvae.

3.3.2.3. Photo-modification and Photo-sensitization in cod

Larval cod were exposed to dark-WAF or UV-WAF at 100% strength for 24-hrs. Afterwards the cod were transferred into clean seawater, and a subset of replicates from each treatment (dark and UV-WAF) were directed towards a photo-sensitization exposure involving a 3-hour exposure to a single UV dose, while the other units remained in the

948 dark. Each unit was then followed for an additional 24-hours (under dark conditions) of
949 monitoring to assess mortality. The results of which are shown in Figure 34.



950

951 *Figure 34: Summary of the photo-modification and photo-sensitization combined trial. The panel on the left*
952 *represent WAF media that was prepared in the dark (no photo-modification), while the panel on the right is*
953 *WAF media prepared under UV light (photo-modification). The colours of the boxes within each panel*
954 *indicate whether following 24-hrs of exposure to media the replicate (individual point) was placed under UV*
955 *light for 3-hours (photo-sensitization; orange boxes), or left in the dark (blue boxes).*

956 Photo-sensitization (orange boxes) had a slightly greater effect on organisms pre-
957 exposed to a photo-modified WAF (right panel) than the dark-WAF (left panel). This result
958 could indicate that photo-products generated during irradiation were bioavailable to the
959 organism, were at least mildly persistent within the organism, and were photo-reactive.
960 However, because the responses in the photo-sensitization exposure units were so high
961 (nearly showing 100% mortality) it is difficult to tease out the magnitude of the difference
962 between the photo-sensitization potential of photo-modified products compared to their
963 non-modified counterparts. It is also worth noting that in the seawater alone control
964 treatment, a 3-hour UV exposure did result in mortality that was not observed in the no
965 photo-sensitization group. This highlights that photo-sensitization exposures are
966 fundamentally a co-exposure of two stressors (photo-reactive compounds and UV
967 irradiation) and their potential synergistic effect (Alloy et al. 2023). The impact of photo-

sensitization was greater in cod larvae than what was observed in lobster. This difference may be attributable to the different biology of the organisms, where the larval lobster has a semi-transparent carapace made of chitin which may limit the transmission of UV light into the body, whereas the larval cod are more transparent and thus allow more UV light into the body where it may react with the accumulated PACs, which otherwise may have been at levels where toxicity would not be expected to occur.

3.3.3. Green sea urchin results

Five products (3 VLSFOs, and 2 conventional crudes) were screened for toxicity with green sea-urchin bioassays. WAFs were prepared at a single loading (1 g/L) under UV light and in the dark and were diluted to generate exposure solutions of 100, 10, and 1% strength. The urchin results are presented in Figure 35 for fertilization and development.

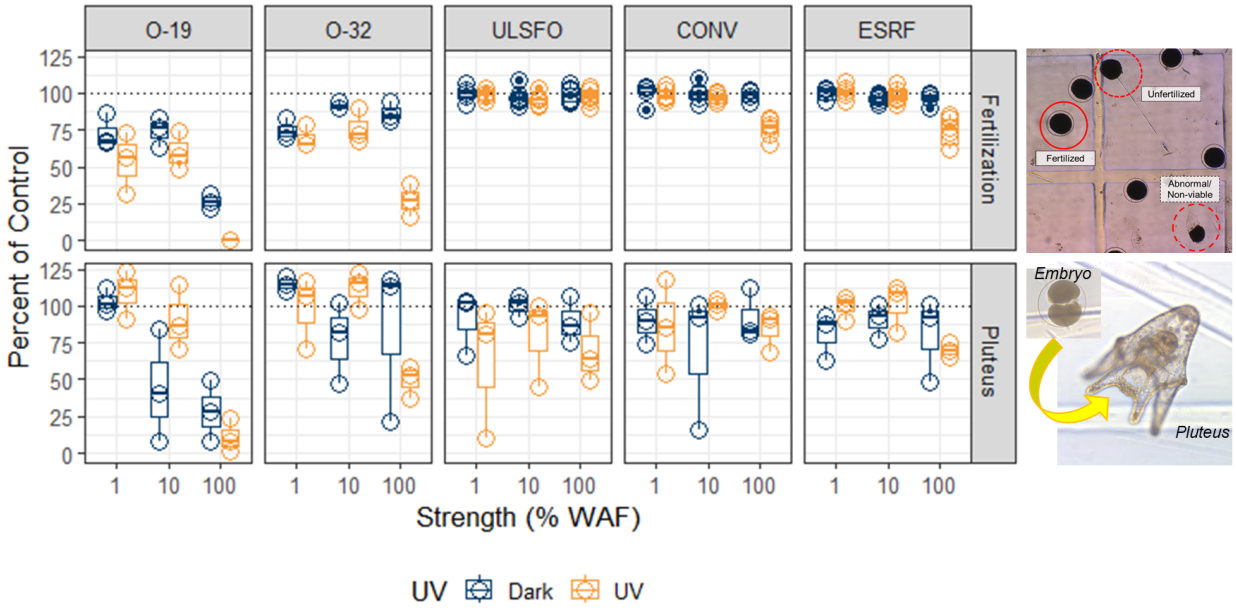
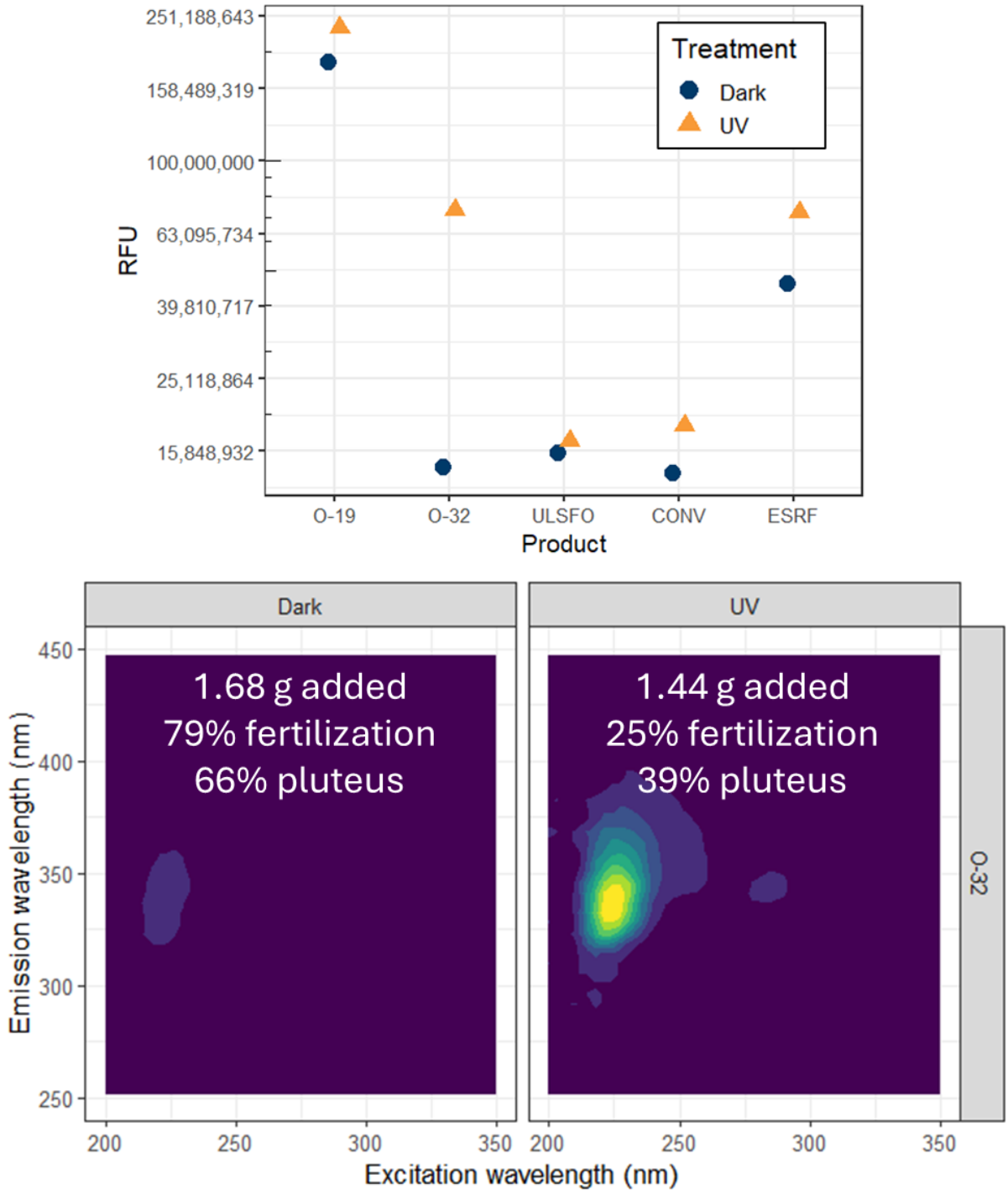


Figure 35: Urchin fertilization (top) and development (bottom) results for the UV-treated (orange) and dark (blue) WAFs.

Fertilization and development are sensitive toxicological endpoints, and at the highest tested concentrations, both VLSFO products, O-19 and O-32, exhibited a significant decrease relative to the control performance, while there was minimal impact on these endpoints for the ULSFO, Conventional Heavy Crude (CONV) or the offshore Newfoundland crude (ESRF). For the VLSFOs, O-19 and O-32, the UV treated WAFs did exhibit greater toxicity for both endpoints than did the WAFs prepared in the dark, and demonstrated an increase in fluorometric signal following irradiation (Figure 36).



989

990 *Figure 36: (top) The sum of the fluorometric signals from the urchin exposures for the WAFs prepared in*
991 *the dark (blue circles) and under UV (orange triangles). (bottom) Summary of the fluorometry excitation*
992 *and emission spectra (colour represents signal intensity with yellow being the most intense and purple the*
993 *least) and toxicological responses for O-32.*

994

At the time of reporting, only the fluorometry data was available for the urchin exposures, and it was used to fit the fertilization and development responses (Figure 37).

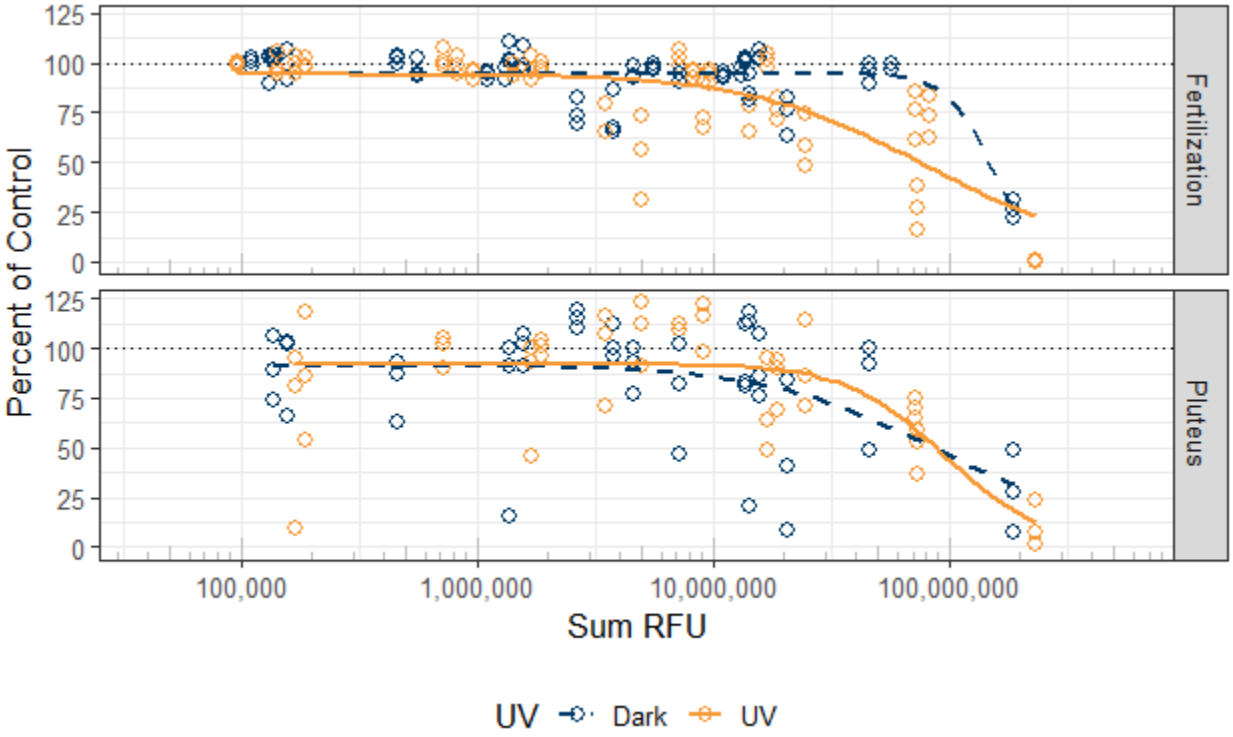


Figure 37: Concentration response relationship for the UV-treated (orange) and dark (blue) WAFs.

The response for both endpoints generally followed that with increasing fluorometric signal there was reduction in the percent fertilized and reduced development to the pluteus stage. When modelling the response data with both preparations combined, the EC50 for fertilization was 99,981,951 (sum RFUs) and the EC50 for development to pluteus was 82,994,263 (sum RFUs). This highlights that the development endpoint is slightly more sensitive than the fertilization. However, as the fertilization bioassay can be run in a single day (as compared to the 4-5 days for the development endpoint), it offers a better opportunity to rapidly screen for toxicity.

3.4. *Modelling*

Using the measured PAC and alkane concentrations in the products (3.1.1), collected after making WAF in both the dark and under UV, the expected concentrations in the WAF were estimated, and those were used to calculate toxic units to the lobster and predict the immobilization response. To estimate the concentrations, the mass of the analyte in the sample was multiplied by partitioning transfer rate (estimated based on log KOW), and the equilibrium partitioning between water and oil. The estimated values were

compared to the subcooled solubility to ensure the modelled estimates did not exceed solubility. From the estimated water concentrations, the toxic unit approach was applied to predict the toxicity. The predicted and observed toxicity to American lobster larvae for the products tested in presented in Figure 38.

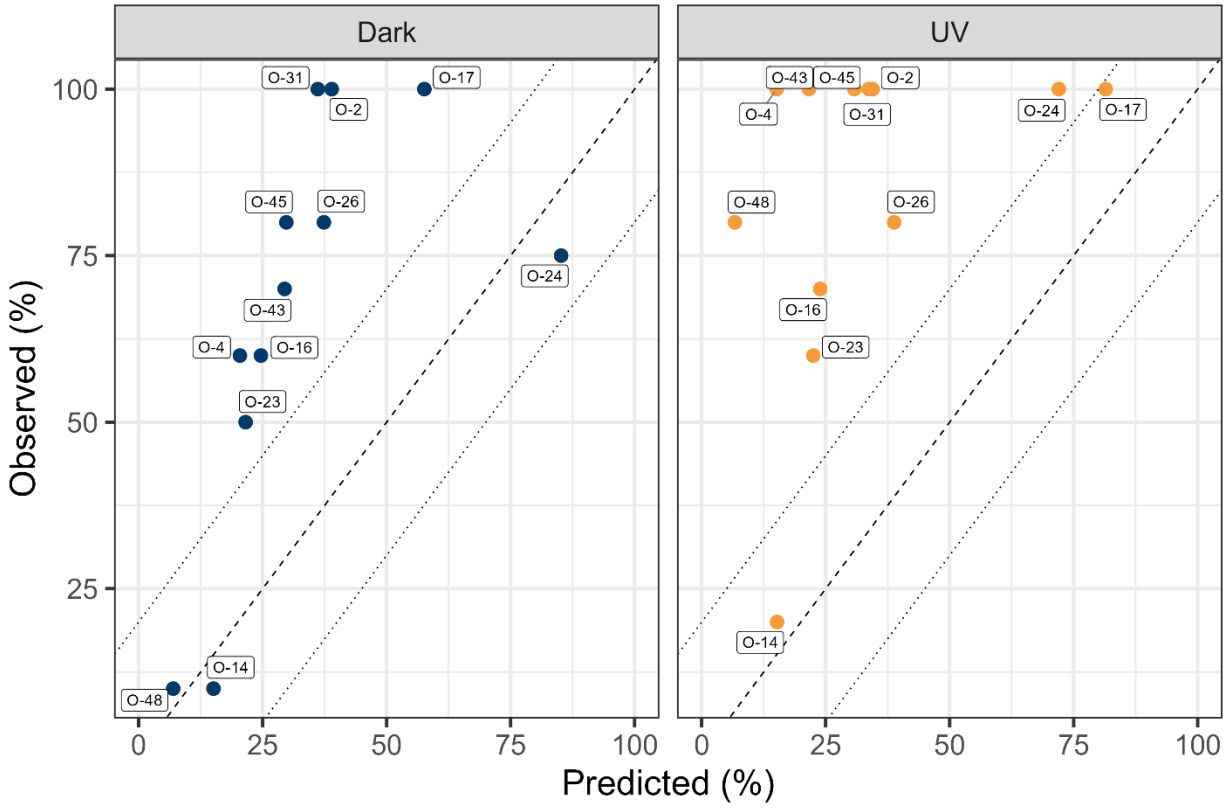


Figure 38: Predicted toxicity (as immobilization) to American lobster larvae based on the measured concentrations in the source oil following the preparation of a WAF in the dark (left) or under UV light (right). The dashed line is showing 1:1 and the dotted lines bound a +/- 20%.

In the case of the dark and irradiated VLSFO, the predicted toxicity was consistently less than the observed toxicity, with only 3 products (O-14, O-24, O-48) in the dark preparation and 2 products (O-14 and O-71) in the UV preparation having predictions within 20% of the observed response. The dark preparations have a slightly better predictive ability (e.g., more points closer to equality), than the UV preparations, again suggesting the formation of compounds that are not accounted for in traditional chemical analysis but are contributing to toxicity. These results highlight that to have more accurate predictions of toxicity more comprehensive and additional characterization of test media (e.g., fractionation and liquid chromatography high resolution mass spectrometry (LC-HRMS), gas chromatography-quadrupole time-of-flight mass spectrometry (GC-QTOF)) is required.

3.4.1. Relationships in water chemistry

When comparing the WAF preparations for lobster and cod, there was generally consistent relationship between the measurements of the exposure metrics (Figure 39).

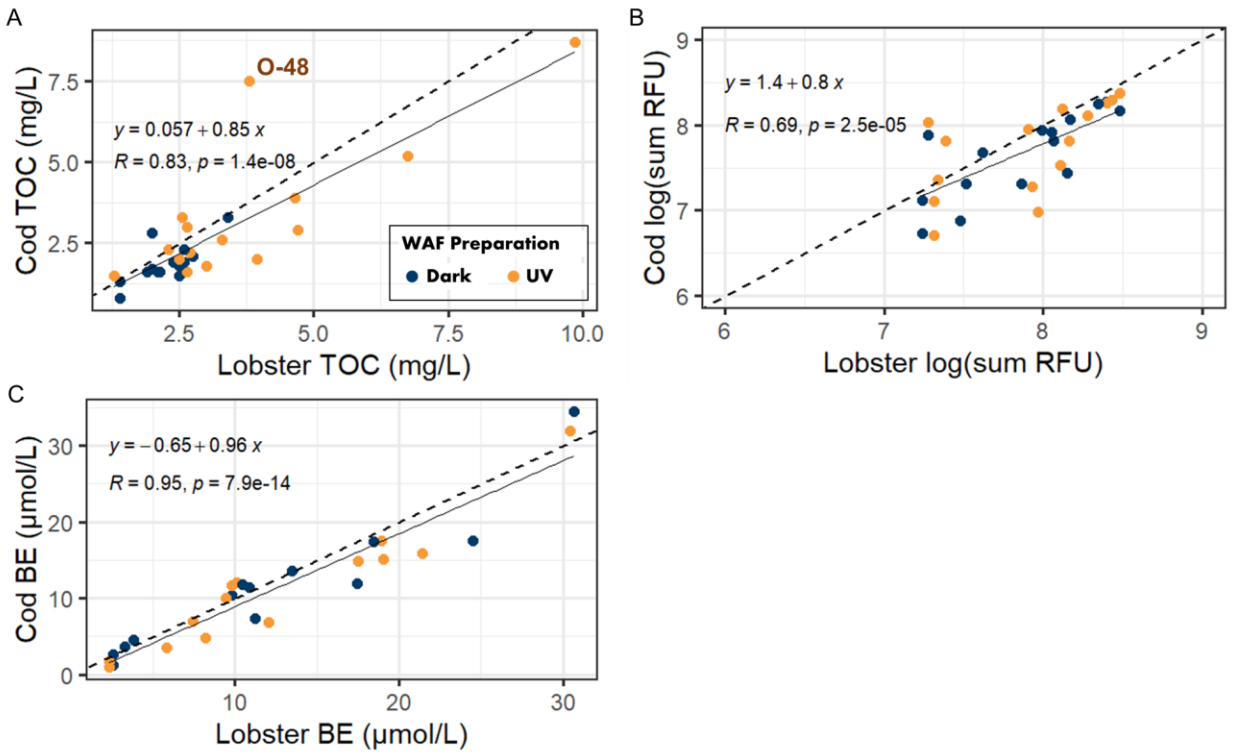


Figure 39: Comparison between the measured exposure metrics TOC (A), RFU (B), and BE-SPME (C) between lobster (x-axis) and cod (y-axis) for the same products (n = 15) prepared in the dark (blue circles) and under UV light (orange circles).The dashed diagonal line is 1:1 and the solid line is the linear regression relationship with the equation in the insert.

In nearly all cases, the measured values in the cod preparations are slightly lower than the same product prepared in the lobster testing (e.g., points tend to fall below the dashed diagonal line at 1:1 in Figure 39). This difference may be attributable to two things. The first being temperature, with the WAFs used in lobster testing having been prepared at 15°C, while the WAFs for cod testing were prepared at 6°C, the warmer temperature may have impacted the dissolution rates, resulting in greater concentration in the lobster preparation. The second option could reflect weathering state/stability of the products, as the lobster testing was performed first, followed by the cod testing approximately 4 months later. However, the closeness of the data, and the significant relationship between the preparations for each species highlights that a consistent WAF preparation and analytical characterization was preformed for all trials.

In comparing between exposure metrics, there is a significant relationship the fluorometric signal and the BE values (Figure 40), while the other relationships are less strong or consistent.

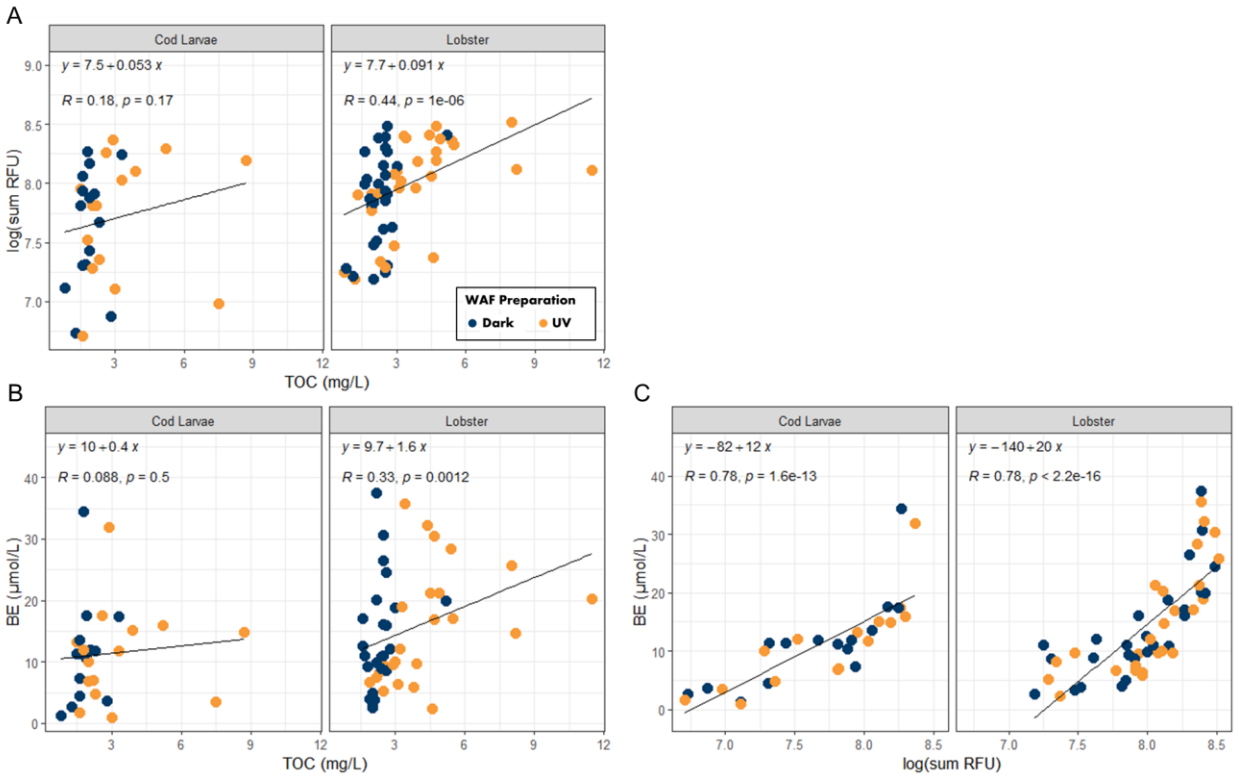


Figure 40: Relationship between the exposure metrics TOC and RFU (A), TOC and BE (B), and RFU and BE (C) prepared in the dark (blue circles) and under UV light (orange circles).

The relationship between the different exposure metrics highlights the importance of fully characterizing exposure solutions, as each measurement method can only detect certain compounds (e.g., fluorometry can only detect compounds which fluoresce) and each of those compounds may be contributing towards toxicity in a different manner. The BE metric, which measures the non-descript bioavailable fraction, has a good relationship with observed toxicity in both lobster and cod, and can account for compounds which may not by typically analyzed by other methods. This metric should be further explored as a rapid analytical tool that can provide information regarding the potential impact of a water sample.

3.4.2. Relationships between properties of the VLSFOs and bioassay results

The physical and chemical properties of the tested products (only considering the VLSFOs and ULSFOs; excluding O-43 and the conventional products) were examined against the measured changes in exposure metrics (e.g., TOC, PAC, RFU, and BE) and

the toxicological response (lobster immobilization in dark, with UV, and fold difference), to identify significant relationships (Figure 41).

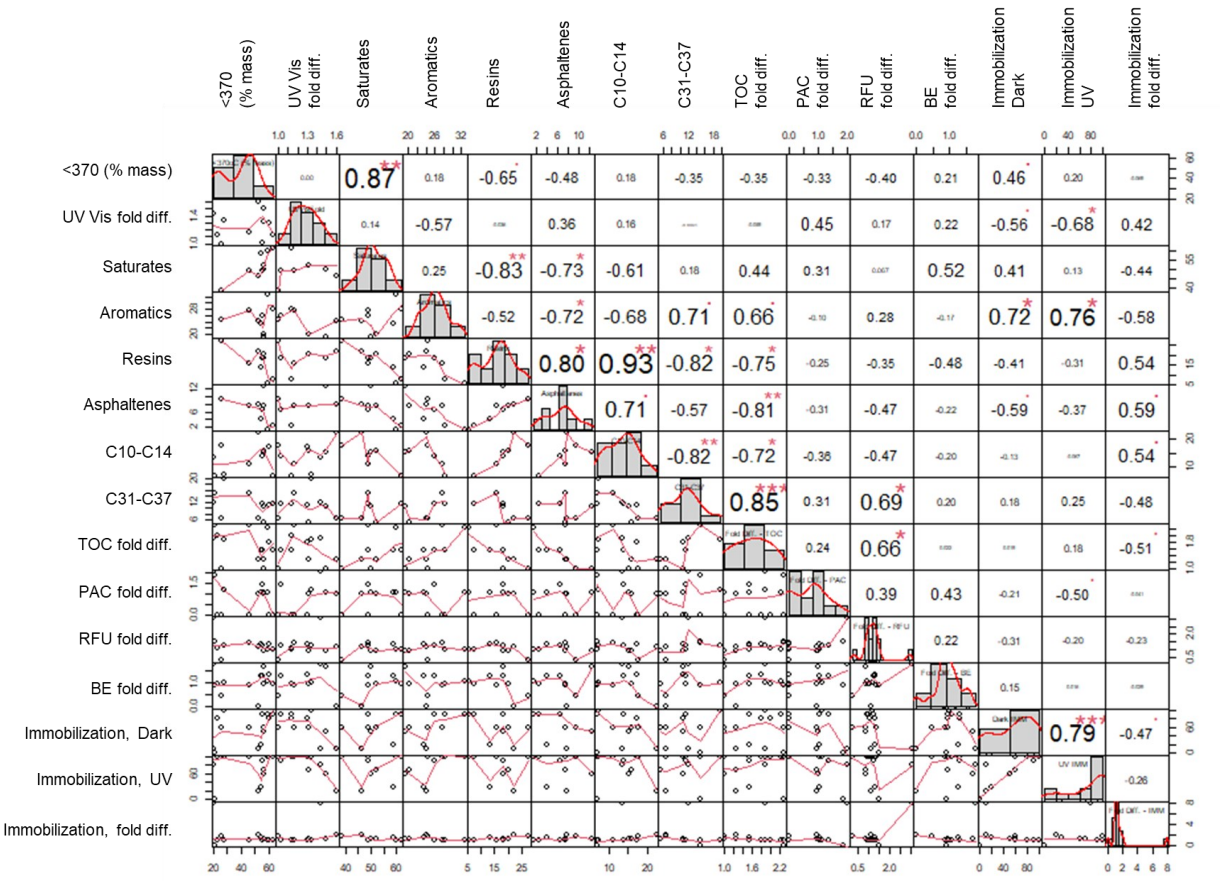


Figure 41: Correlation matrix with scatterplots (lower half), histograms (diagonal), and significance levels (upper half) for a suite of measures.

The diagonal shows histograms representing the distribution of each measure. The lower triangle displays scatterplots illustrating pairwise relationships between the measures, while the upper triangle contains the Spearman correlation coefficients (r) for each pair. Asterisks (*, **, ***) in the upper triangle indicate the significance of the correlations: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$). Cells without asterisks represent correlations that are not statistically significant ($p > 0.05$).

The physical-chemical properties of the VLSFOs are related to each other, as can be seen by the significant correlations between the SARA components. When looking at the changes in exposure metric concentration from UV to dark, there were few of these physical-chemical properties that had significant relationships. With fold increase in TOC concentrations, there was a positive and significant relationship with aromatics and the

C31-C37 fractions. This suggests that for a product with more aromatics and more in the C31-C37 fraction, there is likely to be more of an increase in TOC, as photoproducts, following UV irradiation. Similarly, there was a negative and significant relationship with resins, asphaltenes, and the C10-C14 fraction, suggesting that as those measures increase there is less of an increase in TOC following irradiation. There was also a positive and significant relationship between fold increase in RFUs and the C31-C37 fraction of the VLSFOs. There were no significant relationships for fold increase in PAC or BE with any of the physical-chemical measures.

The toxicological responses for lobster, immobilization in the dark WAF exposure, immobilization in the UV WAF exposure, and fold difference in immobilization following UV exposure, did have significant correlations with some of the physical-chemical measures. Notably the immobilization in the dark and UV were both positively and significantly correlated to the percent aromatic (e.g., greater the percent aromatic, the greater the immobilization); however the percent aromatic was not statistically correlated to the fold difference in immobilization response. The fold difference in immobilization was weakly correlated with the percent asphaltenes and the C10-C14 fraction (increasing amounts of each being correlated with a greater fold difference in immobilization), and the fold difference in TOC (increasing difference in TOC correlated to reduced difference in immobilization); however, the Spearman correlation coefficient for these relationships were all less than 0.6 and should be interpreted with caution.

The SARA components, specifically the aromatics and asphaltenes, proved to have significant relationships with whether photomodification is likely to occur, and whether there is likely to be toxicity. The percent aromatics were significantly correlated with an increase in TOC (suggesting more formation of photoproducts), as well as the observed toxicity in the dark and UV WAFs. The percent asphaltenes was significantly correlated with increases in TOC following UV irradiation, and whether there would be an increase in immobilization following UV irradiation (albeit a weaker relationship). These relationships may provide a first pass screening value to determine the likelihood of photomodification and the significance of the photoproducts towards increasing the toxicity of the product.

4. Conclusions

There was an incredible diversity in the physical appearance, behaviour, and toxicity of the WAFs made with the VLSFOs, both alone and in the presence of UV light.

The results highlight a wide range of responses across fuel types, with significant differences in sensitivity across species. Some of the VLSFOs and the ULSFO showed very little acute toxicity in these bioassays, while others resulted in significant changes

(e.g., immobilization, mortality, delayed development). Lobster immobilization was the most sensitive endpoint, however the Atlantic cod saw significant increases in toxicity following a photo-sensitization exposure. The differences between these sensitivity to photo-modification and photo-sensitization highlight the importance of testing multiple species with different life histories and biology to ensure that the potential impacts of a contaminant are fully understood.

The impact of UV light on the observed toxicity underscores the importance of addressing and incorporating modifying factors when determining the toxicity of complex mixtures in the laboratory. The likelihood of a product undergoing photomodification seems to be reasonably predicted by the SARA fraction, however this relationship should be further explored with more detailed characterization of the photoproducts. Photomodification can significantly increase the toxicity of some VLSFOs and it should be considered when assessing the potential damage these products can cause in a fuel spill scenario.

The toxicity of many of the tested VLSFOs did not follow that of traditional crude oils, where PACs alone may adequately describe toxicity. The SARA components, particularly the percent aromatic, provided a general estimate for observing toxicity in larval lobster. The percent asphaltenes had a weakly significant relationship with the increase in toxicity observed following irradiation, and may provide an indicator for the potential toxicity of the formed photoproducts. Additional work needs to be done to understand what components within these VLSFOs are contributing towards toxicity, and the data generated in this study will help to develop and validate models to predict and assess the toxicity of these new generation fuel oils.

5. Dissemination

During this project the results of this study were presented at numerous scientific conferences:

SETAC-Europe Dublin, IRE: April 30th – May 4th 2023
AMOP Technical Seminar, Edmonton, AB, CAN: June 6 – 8th 2023
Canadian Ecotoxicology Workshop Ottawa, ON, CAN: October 2 – 5th 2023
SETAC-North America Louisville, KY, USA: Nov 12 – 16th 2023
SETAC-North Atlantic Chapter, Woods Hole, MA, USA: April 11 – 12th, 2024
ACCESS/BoFEP Joint Conference, St. Andrews, NB: June 4 – 7th, 2024
Canadian Ecotoxicology Workshop Kitchener, ON, CAN: October 6 – 9th 2024

Additionally, aspects of the research were presented to 19 high school and University groups from New Brunswick, Quebec, and Ontario, Canada. The findings presented in this study are being prepared for publication in the peer reviewed literature.

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7. Appendix

7.1. *Alkanes and PAC Data from VLSFOs*

Instrument II	071124co_15.D	071124co_16.D	071124co_17.D	071124co_18.D	071124co_19.D	071124co_20.D	071124co_21.D	071124co_22.D	071124co_23.D	071124co_24.D	071124co_27.D	071124co_28.D	071124co_29.D	071124co_30.D	071124co_31.D	071124co_32.D
Sample->	Source O-2	Source O-4	Source O-14	Source O-16	Source O-17	Source O-23	Source O-24	Source O-26	Source O-31	Source O-43	Source O-45	Source O-48	Extract O-2	Extract O-4	Extract O-16	Extract O-31
	7/12/2024 4:05 PM	7/12/2024 5:07 PM	7/12/2024 6:09 PM	7/12/2024 7:11 PM	7/12/2024 8:13 PM	7/12/2024 9:14 PM	7/12/2024 10:16 PM	7/12/2024 11:18 PM	7/13/2024 12:20 AM	7/13/2024 1:22 AM	7/13/2024 4:28 AM	7/13/2024 5:30 AM	7/13/2024 6:31 AM	7/13/2024 7:33 AM	7/13/2024 8:35 AM	7/13/2024 9:37 AM
biphenyl	6.005	1.456	2.693	1.01	8.617	2.37	1.574	10.131	1.351	1.58	1.126	0.506	7.024	1.5	0.968	1.254
ACE	1.069	0.327	1.783	0.582	2.582	0.449	0.585	2.909	0.524	0.212	0.85	0.131	1.344	0.317	0.536	0.534
ACY	3.668	1.887	1.541	2.509	8.632	1.595	2.066	4.762	1.884	1.093	3.362	0.454	4.488	2.004	2.515	1.796
naph	40.307	11.824	24.832	9.199	63.142	24.247	7.689	68.104	12.897	8.746	14.429	5.421	43.79	10.851	7.995	10.407
naph-2me	91.404	20.462	24.369	27.123	186.215	35.975	32.652	46.57	35.525	17.16	35.932	7.644	107.247	22.217	28.237	34.332
naph-1me	52.338	13.539	15.971	17.746	111.833	20.994	19.277	32.64	20.991	14.484	23.106	4.874	65.98	14.737	18.212	20.15
naph-C2	322.15	88.323	81.01	130.085	772.316	123.764	153.435	101.189	137.906	76.319	166.202	27.656	419.594	97.801	137.26	137.323
naph-C3	4096.08	1261.541	1087.134	1815.572	8478.686	1509.028	2217.449	1044.202	1823.054	993.871	2361.32	395.61	5166.814	1349.282	1846.659	1789.674
naph-C4	2531.944	1055.301	806.764	1419.602	6404.362	997.239	1583.269	702.678	1296.107	918.719	1733.6	353.451	3170.652	1126.643	1427.471	1264.136
fluo	4.938	2.014	2.158	2.255	11.001	2.53	2.449	5.795	1.964	1.68	2.664	0.69	5.947	2.1	2.179	1.86
fluo-C1	14.081	5.208	4.709	8.272	33.482	6.955	9.59	7.345	7.455	3.256	9.641	2.204	16.932	5.477	8.296	7.089
fluo-C2	21.626	11.055	9.423	19.226	62.802	13.938	22.861	12.289	16.985	6.904	21.401	6.337	25.187	11.339	19.03	15.867
fluo-C3	14.71	9.79	8.61	18.865	46.613	13.137	25.667	15.408	16.835	6.357	21.332	7.485	16.977	9.846	18.642	15.302
fluo-C4	14.668	14.233	12.061	24.597	35.827	16.087	37.588	25.739	22.137	10.595	25.354	14.841	17.138	13.93	24.824	21.942
anthr	1.681	0.966	1.322	1.363	3.306	1.041	2.175	13.383	1.01	0.25	1.883	0.422	2.078	0.932	0.918	1.764
phen	12.644	6.658	5.751	11.13	34.444	8.647	12.648	12.259	9.029	3.182	13.975	2.661	15.616	6.987	11.355	9.145
phen-C1	50.611	25.35	28.147	51.895	143.977	45.349	81.081	46.002	54.842	9.948	69.786	15.334	60.801	25.321	52.193	55.238
phen-C2	33.261	24.375	27.444	52.104	103.944	45.046	122.144	77.058	65.176	9.767	71.085	16.817	40.775	24.643	52.824	66.556
phen-C3	39.813	44.05	47.55	85.681	103.623	70.663	289.961	174.216	122.641	17.806	113.378	30.711	47.981	43.822	85.165	123.569
phen-C4	8.956	14.157	13.056	21.824	20.73	17.496	75.192	49.382	30.193	6.644	26.326	10.733	10.582	13.738	21.03	29.654
DBT	1.436	1.903	0.559	1.569	7.38	1.195	0.781	0.883	0.81	1.241	2.02	0.388	1.689	1.968	1.623	0.812
DBT-C1	2.11	3.148	1.301	3.139	12.654	2.885	1.899	2.475	1.705	1.591	4.263	0.989	2.509	3.209	3.198	1.71
DBT-C2	313.395	471.454	240.15	448.496	1564.362	503.367	390.007	847.099	306.375	246.92	602.937	176.11	368.231	464.506	445.595	305.408
DBT-C3	279.692	447.421	254.441	356.805	960.602	470.415	556.053	1381.483	366.691	221.239	527.149	204.871	333.238	440.652	371.418	363.037
DBT-C4	156.769	242.765	139.9	182.951	354.262	226.325	338.718	844.377	202.185	133.739	250.036	136.999	183.202	236.103	182.951	202.579
flanth	0.312	0.98	0.424	0.498	1.165	0.627	1.634	1.853	0.743	0.092	0.787	0.113	0.361	0.85	0.47	0.671
pyr	2.274	3.375	2.94	4.694	5.007	4.076	18.563	9.809	7.91	0.382	7.137	1.433	2.777	3.565	4.83	8.166
pyr-C1	623.936	1149.858	1097.635	1463.576	1369.915	1643.803	7502.554	3840.208	2952.412	256.67	2203.027	509.555	752.507	1202.365	1471.429	2980.605
pyr-C2	1238.127	2997.446	2660.076	3209.555	2037.275	3355.535	24673.888	8667.55	7711.637	381.835	4746.452	1136.708	1516.84	3180.795	3215.043	7801.135
pyr-C3	1107.758	2731.693	2498.013	3448.25	1753.169	2983.917	20516.083	8204.636	6580.322	466.509	4760.833	1310.123	1366.036	2893.377	3399.622	6556.197
pyr-C4	910.974	2548.439	2068.874	3399.622	1469.631	2290.918	15919.678	6329.518	5117.408	711.637	4488.269	1340.114	1205.393	2748.25	3456.481	5476.064
chr/tri/BAA	1.908	4.563	4.033	7.357	1.946	5.394	12.992	10.491	8.17	0.756	9.336	1.899	2.255	4.713	7.23	8.003
chrys-C1	4.207	11.265	9.09	15.85	4.164	11.078	50.35	27.01	19.879	1.352	20.447	3.817	5.073	11.603	15.777	20.285
chrys-C2	10.335	30.62	21.068	37.363	11.017	25.55	135.68	71.359	52.001	3.918	50.268	10.884	12.713	32.875	36.528	51.073
chrys-C3	8.211	26.985	17.688	31.012	9.691	19.777	120.12	57.939	42.47	3.637	40.31	9.609	10.123	28.615	29.852	41.174
chrys-C4	6.638	21.547	11.766	20.288	7.035	12.027	66.54	35.443	26.248	3.836	24.896	7.954	8.103	22.953	19.147	26.189
BBF	0.433	1.528	0.759	1.396	0.33	1.073	5.222	2.47	1.937	0.117	2.228	0.233	0.529	1.706	1.403	1.907
BEP	0.493	1.985	0.89	1.58	0.397	1.109	5.4	3.019	2.485	0.207	2.272	0.592	0.614	2.146	1.57	2.536
BAP	0.317	2.262	0.643	0.961	0.77	0.9	5.821	1.957	1.714	0.294	1.332	0.298	0.402	2.398	0.836	1.674
PER	0.126	0.899	0.255	0.382	0.306	0.358	2.313	0.778	0.681	0.117	0.529	0.118	0.16	0.953	0.332	0.665
IND	0.373	4.104	1.062	0.709	0.196	0.763	3.81	1.625	1.148	0.11	0.921	0.303	0.49	4.119	0.632	1.083
GHI	0.339	3.736	0.966	0.646	0.178	0.695	3.468	1.479	1.045	0.1	0.839	0.276	0.446	3.749	0.575	0.986
ANTANT	0.132	0.652	0.261	0.4	0.107	0.35	1.568	0.615	0.494	0.018	0.48	0.078	0.167	0.702	0.352	0.402
nC10	2.935	7.083	27.68	17.771	7.298	20.774	3.826	9.396	14.491	74.997	17.326	4.786	3.578	7.962	16.661	11.967
nC11	8.964	16.121	31.19	23.125	11.863	26.686	6.789	13.231	20.699	102.878	20.76	6.653	9.554	16.616	22.601	19.02
nC12	8.875	20.94	30.023	26.366	22.604	28.186	10.682	15.242	20.059	89.535	22.705	7.384	10.385	21.753	26.113	19.295
nC13	8.895	23.587	30.222	32.494	31.378	30.314	12.814	17.175	19.403	86.537	24.942	8.743	10.425	24.63	32.811	19.272
nC14	10.695	26.884	32.381	40.196	40.331	33.954	15.773	19.231	22.088	81.808	28.959	11.284	12.657	28.131	40.313	22.074
nC15	11.075	26.837	31.269	42.153	43.752	33.403	16.72	20.38	21.961	78.322	31.758	12.733	12.926	30.098	43.021	22.481
nC16	13.667	31.143	32.151	44.666	54.592	37.997	19.052	21.758	23.67	70.679	32.858	16.1	15.815	31.14	45.393	24.588
nC17	17.796	46.39	45.367	71.47	114.792	60.479	26.486	31.054	27.987	106.262	46.677	20.831	19.105	45.367	64.058	25.272
pris	8.469	16.901	24.583	46.691	57.057	24.69	10.152	14.314	18.929	56.563	18.22	10.436	8.884	17.363	43.493	16.678
nC18	20.007	46.315	45.026	67.981	133.64	62.012	27.598	32.277	27.819	81.172	45.984	25.718	21.776	45.91	60.575	24.724
phyt	5.965	18.436	24.943	41.209	20.062	18.436	10.845	10.845	15.182	75.912	11.929	12.471	6.507	17.351	35.787	13.556
nC19	19.767	28.723	33.889	43.751	78.964	41.904	18.401	24.421	22.571	41.066	34.956	24.364	22.908	30.249	47.258	22.625
nC20	19.19	21.094	29.499	38.347	72.637	36.893	17.492	22.33	19.371	36.353	32.219	25.061	22.678	22.489	39.526	20.514
nC21	23.344	20.861	31.104	39.324	81.311	39.937	18.723	24.066	20.96	32.743	34.055	28.091	27.024	21.554	40.321	21.039
nC22	26.553	19.626	29.223	39.707	84.796	39.989	18.967	23.052	21.365	30.29	35.926	29.081	29.988	20.43	41.266	22.311
nC23	28.452	19.045	28.053	39.033	86.434	42.425	19.007	24.717	21.573	32.184	38.253	32.329	32.608	19.673	40.381	22.533
nC24	28.338	17.82	23.41	32.973	82.042	35.053	18.023	21.786	20.906	28.04	34.208	30.264	34.097	18.712	34.247	20.952
nC25	28.448	17.004	19.533	30.06	81.803	34.861	16.35	20.908	19.829	26.254	36.008	28.104	33.415	17.734	31.468	20.448
nC26	29.681	16.985	18.178	29.43	75.958	33.113	17.454	20.934	21.062	24.493	36.844	27.106	34.673	17.564	30.113	21.41
nC27	29.711	15.314	17.875	29.236	81.104	36.432	17.676	21.95	22.74	23.633	41.264	26.596	34.228	16.054	30.392	23.024
nC28	29.288	13.175	16.395	26.539	74.227	33.66	17.566	19.757	22.554	22.741	38.594	26.186	33.948	13.713	28.069	22.584
nC29	26.701	10.979	15.296	22.795	62.923	32.204	17.53	19.193	21.371	22.097	35.529	25.067	31.676	11.759	23.968	21.814
nC30	20.116	9.288	13.163	18.294	54.102	28.267	16.636	17.154	18.109	17.124	31.88	21.591	31.08	10.085	19.487	18.106
nC31	23.705	8.467	10.979	14.538	52.212	25.206	15.562	15.918	16.333	14.141	27.501	19.848	28.425	9.236	15.763	16.492
nC32	18.442	5.847	8.536	9.708	38.63	18.066	10.802	11.068	10.586	8.962	18.749	14.261	21.941	6.456	9.892	10.922
nC33	16.732	4.927	7.507	6.972	31.039	15.198	11.9	9.428	9.461	7.677	17.823	12.648	19.948	5.81	7.591	9.749
nC34	13.646	4.33	5.142	5.057	20.738	11.681	8.31	7.577	7.382	5.944	12.136	10.639	16.348	4.915	5.597	7.611
nC35	12.516	3.837	4.635	4.134	17.953	10.657	7.61	6.507	6.652	4.123	10.254	9.265	15.088	4.299	4.551	6.908
nC36	11.512	3.74	4.059	3.563	13.183	9.116	6.54	5.769	5.129	2.969	8.384	8.173	13.913	4.077	3.803	5.466
nC37	10.171	3.536	3.607	2.798	11.476	7.92	5.774	4.864	4.875	1.624	6.915	7.138	12.462	3.831	3.054	4.969
nC17pris	19.906	45.065	49.742	78.429	94.608	54.898	28.039	32.65	39.504	93.656	47.858	28.067	23.386	47	79.823	37.43
nC18phyt	22.036	44.061	47.538	66.173	84.414	52.237	27.941	30.844	34.305	89.05	44.707	31.344	25.815	47.308	66.797	34.785
oTP	0.011	0	0	0.01	0.049	0	0	0	0.011	0	0	0	0.012	0	0	0.012
C30hop	11.162	9.665	6.978	16.839	19.909	20.294	12.306	15.545	10.488	10.88	29.191	11.2	13.485	10.405	16.516	10.08

Instrument Id	071124co_33.D	071124co_34.D	071124co_35.D	071124co_36.D	071124co_39.D	071124co_40.D	071124co_41.D	071124co_42.D	071124co_43.D	071124co_44.D	071124co_45.D	071124co_46.D	071124co_47.D	071124co_48.D	071124co_51.D	071124co_52.D
Sample->	Extract O-2 UV	Extract O-4 UV	Extract O-16 UV	Extract O-31 UV	Extract O-14	Extract O-17	Extract O-24	Extract O-26	Extract O-14 UV	Extract O-17 UV	Extract O-24 UV	Extract O-26 UV	Extract O- 23	Extract O- 43	Extract O-48	Extract O-43 UV
	7/13/2024 10:39 AM	7/13/2024 11:40 AM	7/13/2024 12:42 PM	7/13/2024 1:44 PM	7/13/2024 4:49 PM	7/13/2024 5:51 PM	7/13/2024 6:53 PM	7/13/2024 7:55 PM	7/13/2024 8:57 PM	7/13/2024 9:59 PM	7/13/2024 11:00 PM	7/14/2024 12:02 AM	7/14/2024 1:04 AM	7/14/2024 2:06 AM	7/14/2024 5:11 AM	7/14/2024 6:13 AM
biphenyl	6.106	1.128	0.902	1.122	2.356	6.109	1.454	9.543	2.524	8.542	1.191	10.023	2.054	0.978	0.466	2.114
ACE	1.194	0.227	0.519	0.477	1.656	2.141	0.582	2.84	1.68	2.936	0.483	2.973	0.433	0.704	0.127	0.446
ACY	3.93	1.498	2.436	1.639	1.415	6.782	1.982	4.484	1.452	9.514	1.636	4.711	1.455	3.034	0.428	1.503
naph	37.19	8.309	7.084	8.406	21.69	43.836	6.429	65.822	19.245	66.215	5.304	67.735	20.168	11.719	4.639	21.227
naph-2me	98.69	17.234	26.638	31.127	24.479	159.112	34.605	49.994	21.837	220.705	30.233	50.907	37.221	32.948	7.226	38.697
naph-1me	59.841	11.263	17.433	18.548	15.765	96.344	20.436	34.237	15.253	134.678	17.585	36.097	21.036	21.981	4.536	21.434
naph-C2	381.713	74.382	132.928	128.319	78.258	671.091	160.131	99.231	81.111	940.198	135.748	104.822	125.02	161.86	26.224	126.904
naph-C3	4622.916	1008.31	1784.014	1653.12	1005.025	7077.084	2193.153	971.425	930.895	9699.144	1811.679	991.773	1390.53	1996.659	371.977	1405.522
naph-C4	2741.717	814.798	1363.998	1148.316	713.501	4995.141	1499.586	642.38	779.79	7223.716	1200.773	659.829	900.055	1601.298	332.109	910.022
fluo	5.436	1.631	2.152	1.722	2.02	9.207	2.423	5.62	2.129	13.383	2.022	5.89	2.337	2.489	0.647	2.393
fluo-C1	14.942	4.143	8.098	6.543	4.315	27.364	9.515	6.989	4.181	29.801	7.868	7.269	6.483	8.082	2.181	6.525
fluo-C2	21.763	8.107	18.336	14.427	8.4	49.308	22.029	11.526	9.186	72.622	17.868	11.766	12.798	19.768	5.954	12.725
fluo-C3	14.539	6.962	17.761	14.138	7.706	34.93	25.038	14.305	8.458	51.495	20.207	14.87	12.375	19.61	6.837	12.4
fluo-C4	15.023	10.014	23.643	20.459	10.512	26.689	37.81	25.351	11.377	39.43	30.602	26.251	14.585	23.654	13.581	14.45
anthr	1.698	0.93	1.357	1.153	1.225	2.593	1.752	1.712	0.968	3.768	1.253	1.847	1.019	1.852	0.429	1.01
phen	14.239	5.401	11.463	8.661	5.56	30.155	13.249	12.466	5.474	42.977	11.013	13.147	8.768	13.329	2.661	8.664
phen-C1	54.421	19.154	51.48	51.94	26.388	123.457	85.897	44.975	21.94	153.714	68.725	46.374	43.609	56.255	14.905	43.243
phen-C2	35.778	18.18	51.51	61.395	25.172	85.232	131.412	77.562	26.983	127.185	104.604	81.755	43.926	69.754	16.181	43.722
phen-C3	41.258	31.774	82.066	112.373	42.264	77.809	313.013	175.454	46.39	116.934	247.222	185.764	65.994	109.54	29.086	65.491
phen-C4	9.115	9.926	20.058	26.916	11.096	14.711	77.456	47.804	12.023	22.147	61.151	50.451	15.43	23.983	10.024	15.233
DBT	1.456	1.453	1.583	0.756	0.523	5.902	0.716	0.793	0.518	8.636	0.584	0.821	1.113	1.827	0.389	1.109
DBT-C1	2.212	2.387	3.13	1.588	1.208	9.745	1.832	2.312	1.294	14.497	1.487	2.378	2.759	3.925	0.948	2.729
DBT-C2	326.827	345.451	434.527	284.241	222.994	1186.605	378.403	803.725	234.599	1760.351	319.878	823.854	475.287	559.742	169.305	470.415
DBT-C3	290.616	324.928	353.259	334.885	232.987	693.696	561.605	1337.213	255.337	1044.592	465.115	1390.079	440.437	490.723	190.079	438.216
DBT-C4	160.888	175.466	177.722	186.927	124.499	249.391	350.251	816.297	137.034	372.6	297.457	852.042	205.05	231.447	131.375	204.799
flanth	0.332	0.691	0.461	0.76	0.397	0.937	1.464	1.704	0.546	0.488	1.272	1.853	0.58	0.665	0.138	0.574
pyr	2.357	2.691	4.73	7.817	2.855	4.06	20.243	10.557	2.778	5.819	17.232	11.013	4.188	6.273	1.458	4.133
pyr-C1	664.144	898.959	1438.884	2829.801	1022.422	1071.523	7041.154	3892.337	904.163	1582.498	5838.221	3934.059	1587.701	1604.068	508.136	1577.578
pyr-C2	1337.181	2347.588	3140.776	7279.47	2471.712	1577.105	27464.806	8986.187	2795.932	2378.997	23710.312	9633.302	3304.73	4841.911	1151.939	3309.555
pyr-C3	1203.311	2121.665	3300.284	6034.532	2257.427	1346.452	22334.721	8378.24	2573.888	2068.212	19240.303	9013.623	2854.588	4752.318	1322.99	2880.322
pyr-C4	1073.415	2008.325	3355.724	5115.799	1908.042	1177.2	17536.613	6707.001	2161.022	1798.959	14453.926	7107.096	2255.535	4535.099	1450.142	2249.669
chr/tri/BAA	2	3.558	7.128	7.532	3.867	1.525	11.572	8.946	2.079	1.872	299.874	7.764	4.793	4.302	1.885	4.836
chrys-C1	4.534	8.72	15.203	18.713	8.571	3.215	49.039	25.445	7.735	4.408	1313.987	26.168	9.88	17.585	3.752	9.924
chrys-C2	11.244	24.334	35.369	47.322	19.636	8.412	126.663	64.42	18.757	12.279	3280.528	64.675	22.891	40.073	10.751	23.141
chrys-C3	8.815	21.04	28.922	37.339	15.572	7.71	113.384	52.614	16.939	11.422	2807.852	53.776	17.438	38.409	9.583	17.642
chrys-C4	7.069	16.478	18.161	23.522	9.95	5.5	64.559	31.16	10.965	8.04	1584.049	31.021	10.737	24.82	7.604	10.847
BBF	0.463	1.256	1.226	1.745	0.637	0.276	6.573	1.783	0.953	0.397	116.33	1.662	0.948	1.441	0.326	0.939
BEP	0.548	1.644	1.534	2.372	0.848	0.323	0.581	2.868	0.784	0.465	141.534	2.89	1.052	1.896	0.596	1.056
BAP	0.356	1.768	0.881	1.521	0.617	0.715	13.345	1.405	1.605	0.509	109.218	1.244	0.727	0.984	0.271	0.725
PER	0.141	0.702	0.35	0.604	0.245	0.284	5.303	0.636	0.198	0.202	41.426	0.604	0.276	0.342	0.109	0.287
IND	0.375	3.043	0.613	0.963	0.94	0.135	0.537	1.321	0.071	0.178	11.709	1.288	0.547	0.131	0.265	0.543
GHI	0.341	2.77	0.558	0.877	0.855	0.123	0.488	1.202	0.668	0.162	57.203	1.172	0.498	0.511	0.241	0.495
ANTANT	0.133	0.465	0.323	0.351	0.213	0.072	0.241	0.411	0.184	0.097	27.404	0.411	0.245	0.071	0.061	0.237
nC10	2.812	5.399	14.35	11.467	24.587	4.644	2.381	7.842	17.306	6.459	2.357	7.205	16.621	12.593	4.017	16.609
nC11	9.306	12.718	21.764	19.43	30.589	8.645	5.945	12.46	25.247	12.514	5.119	11.996	25.323	17.31	6.157	24.8
nC12	9.572	16.816	25.607	19.528	29.931	17.645	10.162	15.053	26.342	25.46	8.718	15.094	28.938	20.232	7.068	28.452
nC13	9.844	19.26	32.993	19.509	30.417	26.949	13.321	17.806	27.577	39.419	12.065	18.57	32.974	24.27	9.024	33.615
nC14	11.776	21.931	41.41	22.089	33.562	33.901	16.417	20.193	28.905	49.048	14.563	20.895	37.408	27.393	12.293	38.932
nC15	12.162	22.901	41.236	22.436	32.526	35.973	16.28	21.298	29.842	57.656	14.964	21.32	38.337	28.084	15.753	40.502
nC16	14.809	25.695	45.997	23.878	34.465	47.23	19.849	22.036	28.98	69.105	17.742	23.666	42.749	33.548	19.183	45.148
nC17	14.728	30.607	57.476	22.141	31.981	69.712	19.425	23.067	45.591	114.824	15.655	23.61	45.048	39.297	14.281	43.546
pris	7.229	13.149	36.972	15.792	18.439	36.664	8.427	10.287	23.264	58.157	7.169	10.543	18.952	15.554	8.786	18.3
nC18	17.06	30.251	55.048	21.113	31.172	80.582	20.597	23.729	45.099	131.982	16.47	24.245	45.873	39.167	17.76	44.584
phyt	4.88	10.845	30.365	10.302	15.182	11.929	7.049	7.591	27.654	22.774	5.422	7.049	12.471	10.845	8.133	11.929
nC19	21.223	22.455	47.687	22.556	35.483	70.161	21.852	26.184	29.514	97.458	20.143	27.925	48.031	34.582	35.849	51.661
nC20	20.806	17.417	41.417	20.244	30.691	63.876	18.593	23.694	25.652	93.298	17.171	25.538	42.663	31.149	36.161	46.196
nC21	25.101	16.456	40.59	20.63	32.399	72.427	20.067	25.885	26.426	101.752	18.246	28.077	45.509	33.259	39.856	49.629
nC22	27.958	15.579	41.955	22.458	29.969	76.058	20.234	26.071	25.808	108.958	18.687	27.272	46.481	35.679	45.058	50.645
nC23	30.724	15.601	41.567	22.681	30.191	78.469	21.408	27.062	25.306	112.558	19.368	29.712	49.283	38.145	48.36	54.135
nC24	32.133	14.556	33.99	21.444	23.509	73.134	20.132	24.032	18.986	105.147	18.251	26.587	40.635	35.692	46.598	45.004
nC25	30.832	14.11	31.843	20.571	21.473	72.183	18.324	23.512	17.589	105.555	16.439	25.534	41.407	35.069	42.952	45.81
nC26	32.217	13.751	31.225	22.235	20.155	64.686	19.618	22.544	16.06	95.183	17.588	22.868	40.301	35.359	41.618	44.109
nC27	31.824	12.679	30.986	23.298	19.592	69.779	0.257	24.562	16.105	104.905	17.257	26.581	43.506	42.226	42.653	48.73
nC28	31.937	10.675	28.607	23.457	18.117	62.968	19.906	22.426	13.478	95.858	18.314	24.493	40.495	40.737	41.601	45.604
nC29	29.724	9.272	24.428	22.468	17.032	56.34	20.651	22.197	13.344	81.65	16.931	24.25	39.306	37.854	40.97	44.069
nC30	29.324	8.053	19.661	19.044	18.277	45.886	18.766	19.24	12.249	72.006	17.138	21.483	34.188	33.77	27.379	39.076
nC31	26.164	7.179	15.894	17.054	12.48	45.134	17.881	17.165	10.287	70.374	15.578	19.059	30.764	28.622	32.919	34.369
nC32	20.68	5.077	10.473	11.331	9.518	32.607	13.093	13.234	6.93	50.92	12.322	14.12	22.663	19.916	26.772	25.52
nC33	18.693	4.292	6.405	10.052	7.289	25.775	14.181	10.486	6.258	40.501	10.255	11.597	16.642	16.793	21.029	21.343
nC34	15.664	3.943	5.459	7.98	6.457	17.261	9.32	8.707	4.037	27.63	7.617	7.792	14.769	11.453	16.965	16.826
nC35	14.45	3.362	4.783	7.14	5.703	14.667	8.423	7.256	4.299	22.805	7.311	7.952	13.031	11.111	14.982	14.688
nC36	13.098	3.342	3.979	5.755	4.881	10.849	7.515	6.535	3.872	17.224	6.531	7.319	11.089	9.051	12.916	12.143
nC37	11.758	3.15	3.225	5.111	4.606	9.01	6.852	5.657	3.378	15.022	6.024	6.197	9.349	0.578	10.852	10.297
nC17pris	21.629	34.84	80.754	40.081	52.212	83.957	29.478	34.255	45.284	120.97	26.635	36.301	62.293	46.831	36.289	66.221
nC18phyt	23.921	36.806	67.708	35.009	49.947	71.325	29.453	32.701	42.373	104.064	26.907	34.98	59.738	43.641	42.29	64.303
oTP	0.01	0	0	0.011	0	0.038	0	0.026	0	0.066	0	0.032	0.016	0.012	0	0.016
C30hop	12.111	7.433	15.716	9.213	5.531	11.274	12.218	14.338	7.628	18.681	10.853	16.019	19.948	31.739	11.973	20.318

Instrument II	071124co_53.D	071124co_54.D	071124co_63.D	071124co_64.D
Sample->	Extract O-45 UV	Extract O-48 UV	Extract O-23 UV	Extract O-45
	7/14/2024 7:15 AM	7/14/2024 8:17 AM	7/15/2024 1:25 PM	7/15/2024 2:27 PM
biphenyl	0.952	0.45	2.066	0.879
ACE	0.776	0.132	0.468	0.661
ACY	2.962	0.402	1.549	2.834
naph	9.969	4.325	23.711	12.203
naph-2me	33.825	6.96	41.783	34.919
naph-1me	21.316	4.35	22	21.304
naph-C2	160.126	25.112	130.55	150.848
naph-C3	2069.023	346.853	1523.247	2043.705
naph-C4	1540.447	301.242	975.207	1431.971
fluo	2.577	0.667	2.394	2.493
fluo-C1	8.805	2.135	6.737	8.794
fluo-C2	19.106	5.874	13.1	19.162
fluo-C3	18.865	6.777	12.701	19.188
fluo-C4	23.293	13.476	14.995	22.441
anthr	1.682	0.372	1.027	1.843
phen	13.835	2.634	8.853	13.377
phen-C1	64.469	14.145	46.14	66.929
phen-C2	68.526	15.635	44.094	65.531
phen-C3	105.098	27.937	66.14	100.733
phen-C4	23.367	9.579	15.582	22.49
DBT	1.775	0.365	1.134	1.793
DBT-C1	3.863	0.913	2.752	3.762
DBT-C2	548.854	165.688	479.513	537.894
DBT-C3	488.789	189.685	437.643	461.497
DBT-C4	226.397	130.91	208.56	216.798
flanth	0.672	0.132	0.596	0.695
pyr	6.618	1.371	4.3	6.531
pyr-C1	2071.334	503.784	1634.059	2068.212
pyr-C2	4655.345	1129.328	3272.564	4373.888
pyr-C3	4643.898	1313.245	2782.308	4291.012
pyr-C4	4519.678	1437.465	2094.229	4087.512
chr/tri/BAA	5.734	1.81	5.391	8.873
chrys-C1	18.091	3.565	10.915	19.311
chrys-C2	42.293	10.216	24.213	46.001
chrys-C3	37.72	8.968	17.918	36.199
chrys-C4	21.838	7.107	10.692	22.058
BBF	1.381	0.311	0.981	1.919
BEP	1.96	0.588	1.122	2.204
BAP	0.753	0.225	0.88	1.214
PER	0.381	0.095	0.35	0.483
IND	0.739	0.249	0.544	0.692
GHI	0.672	0.227	0.495	0.63
ANTANT	0.227	0.054	0.223	0.31
nC10	11.854	3.826	19.185	15.574
nC11	17.072	6.034	29.418	20.841
nC12	21.049	7.147	33.386	24.019
nC13	26.36	9.107	39.008	28.931
nC14	31.484	12.381	45.307	33.557
nC15	34.498	14.04	47.381	39.012
nC16	37.842	18.991	54.953	43.204
nC17	36.677	14.345	42.748	32.077
pris	15.326	8.12	19.936	13.69
nC18	36.514	17.797	41.82	30.803
phyt	9.76	7.591	11.387	7.049
nC19	41.965	34.975	68.683	49.761
nC20	37.094	36.794	61.948	46.293
nC21	41.571	41.786	69.484	51.865
nC22	43.688	44.559	70.573	54.739
nC23	46.676	50.865	75.13	58.765
nC24	43.98	47.254	63.709	53.923
nC25	44.799	44.306	64.636	57.017
nC26	46.502	42.673	61.902	59.576
nC27	52.516	43.654	69.025	66.178
nC28	50.236	42.736	64.869	63.396
nC29	47.392	42.05	62.06	60.369
nC30	42.359	36.591	55.186	53.299
nC31	36.076	34.156	49.801	45.821
nC32	25.785	24.804	35.633	32.701
nC33	25.338	21.836	28.898	27.471
nC34	15.555	17.533	21.975	19.701
nC35	13.897	15.73	17.618	15.435
nC36	11.278	13.563	14.391	11.867
nC37	9.314	11.177	12.271	9.504
nC17pris	55	36.13	84.321	63.606
nC18phyt	52.421	42.264	81.873	60.614
oTP	0	0.011	0.01	0.01
C30hop	34.098	12.188	19.914	29.499