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Investigation of the effects of East Coast Canada water accommodated fraction and chemically enhanced water accommodated fraction on early life stages of commercially harvested marine species

Enquête sur les effets de la fraction adaptée à l'eau et de la fraction chimiquement dynamisée sur les premiers stages de vie des espèces marines exploitées commercialement sur la côte Est du Canada

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Investigation of effects of east coast Canada WAF and CEWAF on early life stages of commercially harvested marine species

Executive Summary

- This ESRF funded research program sought to address a critical data gap regarding the use of the dispersant Corexit 9500A by performing toxicity tests with both treated and untreated offshore Newfoundland & Labrador weathered crude oil on the less commonly studied, vulnerable, early life stages of commercially important species.
- The exposure media used in the toxicity testing was generated to be reflective of chemical concentrations and physical droplet size and distributions that may occur during a spill offshore Newfoundland (Chapter 3).
    - Huntsman contracted SL Ross Environmental Research Ltd. (Ottawa, ON) to perform wave tank tests with our weathered test material to determine the physical and chemical characteristics of dispersed (physically and chemically with Corexit 9500A) and dissolved oil under breaking wave conditions. The preliminary dispersant effectiveness assessments showed that Corexit 9500A produced a good dispersion of the crude at 13^{o}C, with slightly reduced effectiveness at 4^{o}C.
    - A series of trials were performed at Huntsman to identify the laboratory conditions (e.g., mixing speed, mixing duration, settling duration) that would produce comparable results in terms of droplet profile, mean droplet size, and concentration (as total petroleum hydrocarbon concentration, TPH) as observed in the SL Ross wave tank study.
    - The mixing speed of 150 rpm, with a mixing and settling duration of 60 minutes, with an oil loading of 1 g oil per L of filtered natural seawater to generate a water accommodated fraction (WAF), and a dispersant to oil ratio of 1:20 to generate a chemically enhanced water accommodated fraction (CEWAF) was employed for all toxicology trials.
- Toxicology trials were conducted with species of considerable ecological and economic importance for Canada. Each of the chosen commercially harvested

species (Atlantic cod, Atlantic herring, American lobster, and Snow crab) represent a different spawning strategy and have different seasonal distributions, which present a unique scenario for exposure to crude oil.

- Atlantic Cod (*Gadus morhua*)
    - A series of trials were undertaken to examine the effects of exposure to physically and chemically dispersed crude oil around the fertilization window in order to understand the vulnerability of Atlantic cod during this earliest life stage (Chapter 4). We did not observe a significant effect of exposure to gametes at the concentrations (up to 30.7 mg/L TPH and 2.7 mg/L BTEX) and exposure durations (20 minutes) tested. When exposure (7 hours) to petroleum hydrocarbons began during fertilization, we observed adverse latent effects on Atlantic cod embryos, with hatching and post-hatch larval survival each showing a significant reduction.
    - Outside of the fertilization window, the sensitivity of the developing Atlantic cod embryos to exposure to petroleum hydrocarbons was characterized (Chapter 5). The results showed susceptibility to contaminants early in development, which then diminished over time, with effects on hatching only observed in the earliest exposure window (~approximately 6 days post fertilization) following a 24-hr exposure to concentrations of 30 mg/L TPH from a CEWAF exposure.
    - Sublethal effects in Atlantic cod embryos and larvae exposed to dilutions of WAF and CEWAF and dispersant alone were examined (Chapter 6). A 24-hour exposure during the hatching window resulted in the presentation of morphological abnormalities (e.g., curved spine) with an EC50 of 3.59 mg/L TPH (WAF exposure) and 6.7 mg/L TPH (CEWAF exposure). With a 24-hour exposure of larval cod followed by 6 days in clean water, we observed a significant effect on growth at concentrations of 2.9 mg/L TPH (WAF exposure) and 1.1 mg/L TPH (CEWAF exposure).
    - The importance of familial variability in the exposure response for the Atlantic cod larvae exposed to physically and chemically dispersed crude oil was assessed (Chapter 7). The results of our study show that there was

significant variability in the lethal response of Atlantic cod to petroleum hydrocarbons with LC50s ranging from 0.02 to 31.3 mg/L TPH (CEWAF exposures), and that a specific male Atlantic cod parent (sire), specific female Atlantic cod parent (dam), or the combination of specific male and female parents (crosses or families) may affect the resulting survival of siblings within a family. This tolerance/susceptibility to the effects of an oil spill is a heritable trait within Atlantic cod

- Atlantic herring (*Clupea harengus*)
    - We used a modification of the herring “sticky-egg” bioassay that gained prominence following the Exxon Valdez spill to assess the toxicity of physically and chemically dispersed oil on Atlantic herring at several early life stages and across relatively short, realistic exposure durations (Chapter 8). The results demonstrated that a 1-hour, static exposure to sufficient concentration (>2 mg/L TPH), at a critical developmental window (fertilization to 2 days post fertilization) is sufficient to observe significant latent effects, such as reduced hatch and increases in abnormal hatching. The difficulty in working with wild caught organisms was made apparent with the difficulty in maintaining good control survival. Where there was good survival there was significant variability depending on the pedigree of the population.
- American lobster (*Homarus americanus*)
    - We investigated the sensitivity of American lobster larvae exposed to physically and chemically dispersed crude oil and whether the response was consistent amongst different batches released from the same lobster and amongst different lobsters American lobster variability (Chapter 9). The 24-hour EC50 values ranged 2.15 - 12.8 mg/L TPH. These exposure conditions were static non-renewal and thus the derived toxicity values may overestimate the real-world exposure scenario of oil/dispersed oil to lobster larvae, which is expected to be shorter and more dynamic.
    - The effects of chemically and physically dispersed oil to American lobster larvae were characterized at different exposure durations (Chapter 10).

The three planktonic larval stages of the American lobster did not show a significant difference in response to exposure to petroleum hydrocarbons, and there was rapid and significant onset of immobilization within 6 hours of exposure.

- Northern shrimp (*Pandalus borealis*) and Snow crab (*Chionoecetes opilio*)
    - Northern shrimp (*Pandalus borealis*) toxicology trials proved difficult over the course of the project given difficulties to live capture, transport and hold berried female individuals for later hatching of larvae to support exposures, and as such we instead worked with larvae from snow crab (*Chionoecetes opilio*) (Chapter 11). The LC50s calculated after a 24-hour exposure indicated that exposure to concentrations of TPH between 1 to 2 mg/L were sufficient to cause latent mortality effects that were not observed within the first 48-hours post exposure. The 48-hour LC50 was estimated to be 1.0 mg/L TPH (WAF exposure) and 2.9 mg/L TPH (CEWAF exposure), which is comparable to the values for Northern Shrimp derived from the literature (1.7 - 13 mg/L).
- Select results are summarized in Table 1, additional data and context are provided in the specific sections of the text.

Table 1: Summary of select toxicity results from each species

Species	Exposure	Endpoint	EC50 mg TPH/L (standard error)	
			WAF	CEWAF
Atlantic cod	7-hr exposure during fertilization	Hatch of fertilized (Figure 32)	1.35 (2.2)	7.27 (2.6)
	24-hr exposure, hatching window	Blue sac disease presentation (Figure 44)	3.5 (0.4)	6.7 (0.8)
	24-hr exposure, 200dd larvae	Survival (Figure 52)	15.6 (18.3)	9.76 (0.4)
Atlantic herring	1-hr exposure during fertilization	Overall survival (Figure 62)	16.5 (2200)	6.13 (10)
	24-hr exposure, 48 hpf embryos	Abnormal hatch (Figure 65)	4.8 (na)	2.5 (1.5)
	24-hr exposure, 48 hpf embryos	Overall survival (Figure 66)	21.6 (48.2)	28.5 (26.6)
American lobster	24-hr exposure, Stage I larvae	Immobilization (Figure 78)	3.5 (0.1)	5.8 (0.2)
Snow crab	24-hr exposure, Z1 larvae	Latent Mortality at d7 (Figure 87)	1.1 (0.3)	1.9 (2.4)

- It is prudent to consider dispersant application as a possible spill response option considering the history of, and potential for future, oil spills and that response times with conventional methods are likely to be slow for some high-risk areas offshore Newfoundland and Labrador (Chapter 12)
    - The observation of toxic effects following exposure to petroleum hydrocarbons, whether they are derived from physically or chemically dispersed means, is dependent on the life stage, exposure concentration and duration. Our results show significant variability within the life history of

a species, with specific developmental points (e.g., hatching) and early life stages being more vulnerable. We observed significant lethal effects with exposure durations of 1- (herring; Chapter 8, Figure 61), 6- (lobster; Chapter 10, Table 25), 7- (cod; Chapter 4, Figure 30) and 24-hours (snow crab; Chapter 11, Figure 86), and sublethal effect concentrations following 24-hours of exposure were observed at 1.1 mg/L TPH (reduced growth in larval cod, Chapter 6, Figure 47). These cold water commercially important species are among the most sensitive species to hydrocarbon exposure.

- The toxicity of the dispersant Corexit 9500A was examined in cod and lobsters. We found that cod embryos exposed to Corexit 9500A for 24-hours during the vulnerable hatching window had significant sublethal effects (increase in blue-sac disease presentation) occurring at 405 mg/L, but there was no significant effect on hatching. In larval lobsters, the 24 and 48-hour LC50s for exposure to Corexit 9500A were 38 and 36 mg/L, respectively. The highest dispersant-only concentrations in field applications are expected to range between 3 and 10 mg/L in the first minute to several hours following successful application based on operational dispersant application rates at the surface. Our results support the low inherent toxicity of the dispersant alone.
- The decision to use dispersants as a spill response option is likely to differ throughout the year as environmental parameters and biological assemblages vary along with efficacy and potential impact of dispersant application.

- The results of this research program directly addressed a data gap related to the toxicity of dispersed and non-dispersed crude oil on vulnerable early life stages of commercially harvested fish and invertebrate species, and will greatly improve the ability of researchers to predict effects and for responders to employ the best available and most appropriate response strategies based on the particular scenario.

Enquête sur les effets de la WAF et de la CEWAF de la côte est du Canada sur les premiers stades de vie des espèces marines exploitées commercialement

Résumé

- Ce programme de recherche financé par le Fonds pour l'étude de l'environnement (FEE) a cherché à combler une lacune critique dans les données sur l'utilisation du produit dispersant Corexit 9500A en effectuant des essais de toxicité avec du pétrole brut altéré, traité et non traité, en provenance de sources au large de Terre-Neuve-et-Labrador, sur les premiers stades de vie plus vulnérables d'espèces commerciales qui sont plus rarement étudiées.
- Le milieu d'exposition utilisé lors des essais de toxicité a été généré de manière à tenir compte des concentrations chimiques ainsi que de la taille et de la distribution physique des gouttelettes susceptibles se produire lors d'un déversement d'hydrocarbure au large de Terre-Neuve-et-Labrador (Chapter 3).
    - Huntsman a fait appel à SL Ross Environmental Research Ltd. (Ottawa, Ontario) pour effectuer des essais en cuve à houle avec notre matériel d'essai altéré afin de déterminer les caractéristiques physiques et chimiques du pétrole dispersé (sur les plans physiques et chimiques avec le Corexit 9500A) et dissous dans des conditions de vagues déferlantes. Les évaluations préliminaires de l'efficacité du produit dispersant ont montré que le Corexit 9500A produisait une bonne dispersion du pétrole brut à 13^{o}C, et que son efficacité se voyait légèrement réduite à 4^{o}C.
    - Une série d'essais a été réalisée à Huntsman pour déterminer les conditions en laboratoire (la vitesse de mélange, la durée de mélange, la durée de décantation) qui permettraient d'obtenir des résultats comparables en ce qui concerne le profil de gouttelettes, leur taille moyenne et leur concentration (en tant que concentration totale d'hydrocarbures pétroliers, TPH) telles qu'elles ont été observées dans l'étude en cuve à houle de SL Ross.

- Les paramètres ci-après ont été employés pour tous les essais toxicologiques : une vitesse de mélange de 150 tr/min, une durée de mélange et de décantation de 60 minutes, une charge de pétrole de 1 g de pétrole par litre d'eau de mer naturelle filtrée pour générer une fraction adaptée à l'eau (WAF), et un rapport dispersant-pétrole de 1:20 pour générer une fraction adaptée à l'eau améliorée chimiquement (CEWAF).

- Les essais toxicologiques ont été menés avec des espèces qui revêtent une importance écologique et économique considérable pour le Canada. Chacune des espèces choisies exploitées à des fins commerciales (morue, hareng de l'Atlantique, crabe des neiges et homard) représente une stratégie de frai différente et présente une répartition saisonnière différente, ce qui présente un scénario unique d'exposition au pétrole brut.
- Morue (*Gadus morhua*)
  - Une série d'essais a été entreprise pour examiner les effets de l'exposition au pétrole brut physiquement et chimiquement dispersé pendant la période correspondant à la fenêtre de fécondation de la morue afin d'en comprendre la vulnérabilité à cette étape précoce de son cycle de vie (Chapter 4). Nous n'avons observé aucun effet considérable de l'exposition des gamètes pour les concentrations (jusqu'à 30,7 mg/L de TPH et 2,7 mg/L de BTEX) et les durées d'exposition (20 minutes) des essais. Lorsque l'exposition (7 heures) aux hydrocarbures pétroliers commençait pendant la fécondation, nous avons observé des effets latents néfastes sur les embryons de morue, l'éclosion et la survie des larves après l'éclosion, présentant chacune une réduction.
  - En dehors de la fenêtre de fécondation, la sensibilité des embryons de morue en développement à l'exposition aux hydrocarbures de pétrole a été caractérisée (Chapter 5). Les résultats indiquent une sensibilité aux contaminants au début du développement, et cette sensibilité diminue ensuite avec le temps, les effets sur l'éclosion n'étant observés que dans la première fenêtre d'exposition (environ six jours après la fécondation) après

une exposition de 24 heures à des concentrations de 30 mg/L de TPH provenant d'une exposition à la CEWAF.

- Les effets sublétaux chez les embryons et les larves de morue exposés à des dilutions de WAF et de CEWAF et au dispersant seul ont été examinés (Chapter 6). Une exposition de 24 heures pendant la fenêtre d'éclosion a entraîné la présentation d'anomalies morphologiques (colonne vertébrale courbée, par exemple) et une concentration efficace médiane (CE50) de 3,59 mg/L de TPH (exposition à la WAF) et de 6,7 mg/L de TPH (exposition à la CEWAF). Après une exposition de 24 heures des larves de morue suivie de 6 jours dans de l'eau propre, nous avons observé un effet significatif sur la croissance à des concentrations de 2,9 mg/L de TPH (exposition à la WAF) et 1,1 mg/L de TPH (exposition à la CEWAF).
- Nous avons évalué l'importance de la variabilité familiale dans la réponse à l'exposition pour les larves de morue exposées à du pétrole brut physiquement et chimiquement dispersé (Chapter 7). Les résultats de notre étude indiquent que la réponse létale de la morue aux hydrocarbures pétroliers avec des CL50 allant de 0,02 à 31,3 mg/L de TPH (expositions à la CEWAF) présente une variabilité considérable, et qu'un individu parent mâle de la morue (père), un individu parent femelle de la morue (mère), ou la combinaison d'individus parents mâles et femelles (croisements ou familles) peut avoir une incidence sur les résultats liés à la survie des frères et sœurs au sein d'une famille. Cette tolérance ou sensibilité aux effets d'un déversement d'hydrocarbures est un trait héréditaire chez la morue.

- Hareng de l'Atlantique (*Clupea harengus*)
  - Nous avons utilisé une modification de la technique d'essaies biologiques sur les œufs collants du hareng, qui a pris de l'importance à la suite du déversement de l'Exxon Valdez, pour évaluer la toxicité du pétrole dispersé physiquement et chimiquement sur le hareng de l'Atlantique à plusieurs étapes précoces de son cycle de vie et pendant des durées d'exposition relativement courtes et réalistes (Chapter 8). Les résultats ont indiqué qu'une exposition statique d'une heure à une concentration suffisante

(>2 mg/L de TPH), à un moment critique du développement (de la fécondation à 2 jours après la fécondation), était suffisante pour observer des effets latents significatifs, tels qu'une réduction des éclosions et une augmentation des éclosions anormales. La difficulté de travailler avec des organismes capturés à l'état sauvage est devenue manifeste en raison de la difficulté à maintenir un bon taux de survie des organismes témoins. Dans les cas où les taux de survie étaient bons, il y avait une variabilité considérable en fonction du pedigree de la population.

- Homard (*Homarus americanus*)
    - Nous avons étudié la sensibilité des larves de homard exposées à du pétrole brut physiquement et chimiquement dispersé et déterminé si la réponse était uniforme parmi les différents lots relâchés provenant du même homard et chez différentes variabilités du homard (Chapter 9). Les valeurs de la CE50 sur 24 heures étaient comprises entre 2,15 mg/L et 12,8 mg/L de TPH. Ces conditions d'exposition étant statiques et non renouvelables, les valeurs de toxicité dérivées peuvent donner lieu à la surestimation du scénario d'exposition réelle au pétrole ou au pétrole dispersé des larves de homard, qui devrait être plus court et plus dynamique.
    - Les effets du pétrole dispersé chimiquement et physiquement sur les larves de homard ont été caractérisés à différentes durées d'exposition (Chapter 10). Les trois stades larvaires planctoniques du homard n'ont pas présenté de différence considérable en réponse à l'exposition aux hydrocarbures pétroliers, et les premiers symptômes d'immobilisation sont apparus rapidement et étaient importants dans les six heures suivant l'exposition.
- La crevette nordique (*Pandalus borealis*) et le crabe des neiges (*Chionoecetes opilio*)
    - Les essais toxicologiques sur la crevette nordique (*Pandalus borealis*) se sont avérés ardus tout au long du projet en raison des difficultés à capturer, à transporter et à conserver des individus femelles œuvés vivants aux fins d'éclosion ultérieure de larves qui feraient l'objet d'expositions. Nous avons

donc plutôt opté pour des larves de crabe des neiges (*Chionoecetes opilio*) (Chapter 11). Les concentrations létales (CL50) calculées après une exposition de 24 heures ont indiqué que l'exposition à des concentrations de TPH de 1 mg/L à 2 mg/L était suffisante pour provoquer des effets de mortalité latents qui n'ont pas été observés dans les 48 heures suivant l'exposition. La CL50 sur 48 heures a été estimée à 1,0 mg/L de TPH (exposition à la WAF) et à 2,9 mg/L de TPH (exposition à la CEWAF), ce qui est comparable aux valeurs pour la crevette nordique tirées de la documentation (1,7 mg/L à 13 mg/L).

- Des résultats sélectionnés sont résumés dans le Tableau 2 et les données supplémentaires et le contexte sont fournis dans les sections précises du texte.

Tableau 2 : Résumé de résultats de toxicité sélectionnés pour chaque espèce

Espèces	Exposition	Résultat final	CE50 mg TPH/L (erreur type)	
			WAF	CEWAF
Morue	Exposition de 7 heures pendant la fécondation	Éclosion de l'œuf fécondé (Figure 32)	1,35 (2,2)	7,27 (2,6)
	Exposition de 24 heures, fenêtre d'éclosion	Manifestation de la maladie du sac bleu (Figure 44)	3,5 (0,4)	6,7 (0,8)
	Exposition de 24 heures, larves à 200 DJ	Survie (Figure 52)	15,6 (18,3)	9,76 (0,4)
Hareng de l'Atlantique	Exposition de 1 heure pendant la fécondation	Survie générale (Figure 62)	16,5 (2 200)	6,13 (10)
	Exposition de 24 heures, embryons à 48 HPF	Éclosion anormale (Figure 65)	4,8 (S.O.)	2,5 (1,5)
	Exposition de 24 heures, embryons à 48 HPF	Survie générale (Figure 66)	21,6 (48,2)	28,5 (26,6)
Homard	Exposition de 24 heures, larves au stade I	Immobilisation (Figure 78)	3,5 (0,1)	5,8 (0,2)
Crabe des neiges	Exposition de 24 heures, larves Z1	Mortalité latente au 7^{e} jour (Figure 87)	1,1 (0,3)	1,9 (2,4)

- Il est prudent d'envisager l'application de dispersants comme option possible d'intervention lors de déversements compte tenu de l'historique et du potentiel de déversements futurs de pétrole et du fait que les délais d'intervention quant aux méthodes traditionnelles seront probablement lents pour certaines zones à haut risque au large de Terre-Neuve-et-Labrador (Chapter 12).
    - L'observation des effets toxiques après une exposition aux hydrocarbures pétroliers, qu'ils soient produits par dispersion physique ou chimique, dépend de l'étape du cycle de vie, de la concentration et de la durée de l'exposition. Nos résultats indiquent une grande variabilité dans le cycle de vie d'une espèce, les stades de développement précis (l'éclosion, par exemple) et les premières étapes du cycle de vie étant plus vulnérables. Nous avons observé des effets létaux considérables lors des durées d'exposition de 1 (hareng; chapitre 8, Figure 61), 6 (homard; chapitre 10, Table 25), 7 (morue; chapitre 4, Figure 30) et 24 heures (crabe des neiges; chapitre 11, Figure 86), et des concentrations présentant des effets sublétaux après 24 heures d'exposition ont été observées à 1,1 mg/L de TPH (croissance réduite chez les larves de morue, chapitre 6, Figure 47). Ces espèces vivant en eau froide sont importantes sur le plan commercial et sont parmi les espèces les plus sensibles à l'exposition aux hydrocarbures.
    - La toxicité du dispersant Corexit 9500A a été examinée chez la morue et le homard. Nous avons constaté que les embryons de morue exposés au Corexit 9500A pendant 24 heures au cours de la fenêtre d'éclosion vulnérable présentaient des effets sublétaux considérables (augmentation de la manifestation de la maladie du sac bleu) se produisant à 405 mg/L, mais il n'y avait aucun effet important sur l'éclosion. Chez les larves de homard, les CL50 sur 24 et 48 heures d'exposition à Corexit 9500A étaient respectivement de 38 mg/L et 36 mg/L. Les concentrations les plus élevées de dispersant employé seul dans les applications sur le terrain devraient se situer entre 3 mg/L et 10 mg/L de la première minute à plusieurs heures après une application réussie, selon les taux d'application de dispersant

opérationnel à la surface. Nos résultats appuient la faible toxicité inhérente du dispersant employé seul.

- La décision d'utiliser des dispersants comme option d'intervention en cas de déversement est susceptible de varier tout au long de l'année, car les paramètres environnementaux et les assemblages biologiques varient, tout comme l'efficacité et l'incidence potentielle de l'application de dispersants.

- Les résultats de ce programme de recherche ont directement comblé une lacune dans les données relatives à la toxicité du pétrole brut dispersé et non dispersé sur les premières étapes vulnérables du cycle de vie des espèces de poissons et d'invertébrés exploitées à des fins commerciales, et amélioreront considérablement la capacité des chercheurs à prévoir les effets et la capacité des intervenants à employer les meilleures stratégies d'intervention disponibles qui sont les plus appropriées en fonction du scénario particulier.

Introduction

Chapter 1 Background

Definitions

- **Asphaltene content:** This material is defined by its solubility: the components that dissolve in toluene and precipitate in n-alkane solvents are the asphaltenes, essentially the dissolved solids component of crude oil. As asphaltene content increases from 0 to 40%, viscosity and density increase, and color changes from clear to dark brown
- **Barrel:** 42-US gallons = 159-L = 0.159 m^3
- **Biodegradation:** process by which organic substances are decomposed by micro-organisms into simpler substances such as carbon dioxide, water and ammonia**.**
- **Dispersant:** Mixtures of solvents, surfactants, and other additives that are applied to oil slicks to reduce the oil-water interfacial tension, and , with the input of mechanical energy from wave action, break down oil into small droplets
- **Emulsify:** the process whereby water droplets become incorporated in the oil to form a water-in-oil emulsion, thereby increasing the volume. The rate at which an oil emulsifies determines the window of opportunity for spill response options.
- **Entrainment:** the transport of oil from a surface slick into the water column by wind and waves
- **FPSO:** floating production, storage, and offloading vessel
- **NEBA:** Net Environmental Benefit Analysis
- **OLS:** Offshore loading system
- **Pour point:** Temperature at which a liquid becomes semisolid and loses its flowing characteristics
- **SIMA:** Spill Impact Mitigation Assessment
- **Specific gravity/API°:** American Petroleum Institute (API) measure of specific gravity of crude oil or condensate in degrees. An arbitrary scale expressing the gravity or density of liquid petroleum products.
- **Viscosity:** Having a resistance to flow (typically reported as centristrokes, cSt, equivalent to mm^2/second)
- **Volatility:** Refers to how quickly oil evaporates into the air
- **Weathering:** Action of UV light, wind, waves, and water that leads to disintegration or deterioration of the substance

Oil and gas exploration and production activities have existed for decades within the productive environment offshore of Newfoundland & Labrador and Nova Scotia. These activities are expected to continue with the potential for further development for resources

extraction expanding to new areas of the Canadian offshore. These same areas overlap with historically highly productive oceanic feeding and spawning habitat for complex marine species assemblages, including numerous species that are the target of commercial fisheries – including Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*), American lobster (*Homarus americanus*) and Northern shrimp (*Pandalus borealis*). Both of these sectors – offshore oil production and offshore fisheries – are very important to the economic and social well-being of Atlantic Canadian provinces. The physical overlap of these sectors creates the potential for negative impacts to the fisheries, and the environment, in the event of a significant spill or release of crude oil. The number of spills offshore Newfoundland have generally been decreasing, however, single spill events have the capacity to introduce significant amounts of hydrocarbons to the environment (Figure 1).

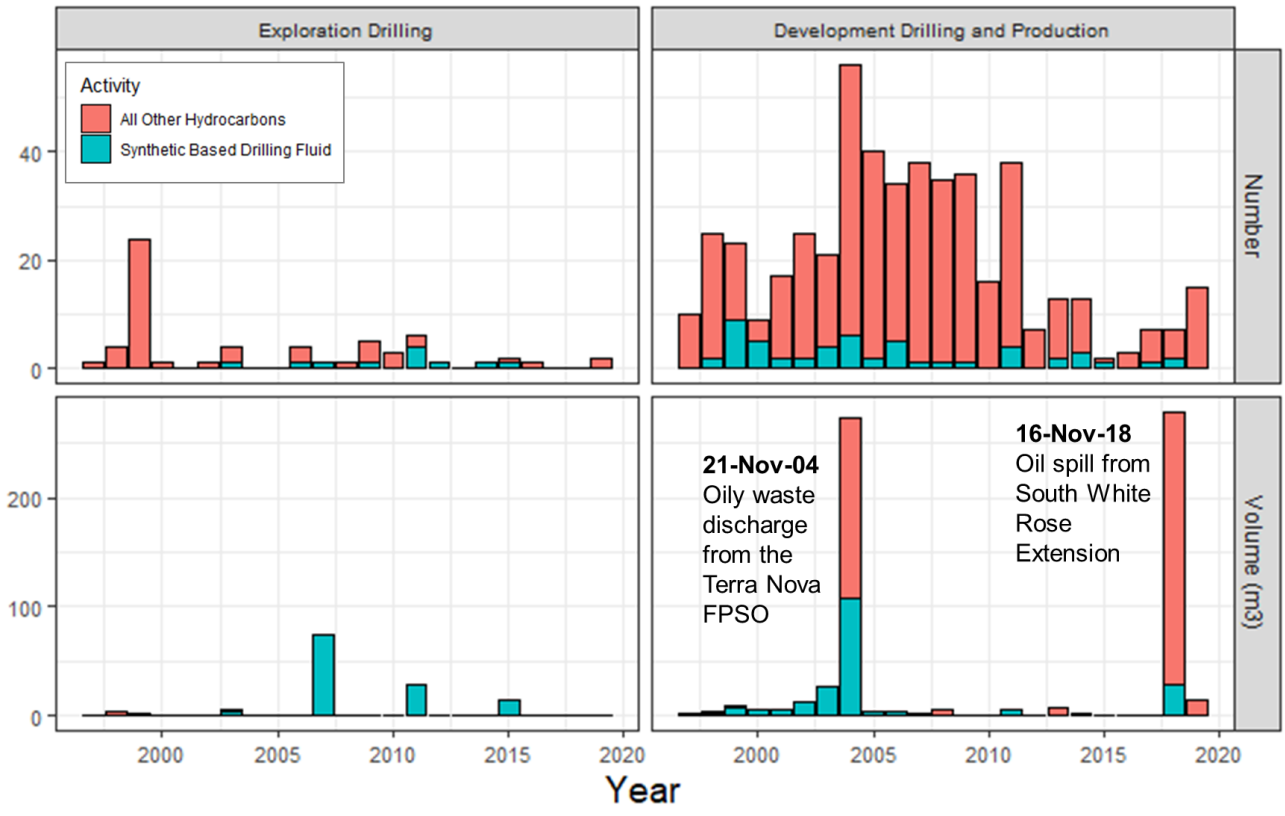


Figure 1: Summary of the number of spills (top row) and volume spilled (bottom row) of hydrocarbons (red) and synthetic based drilling fluid (blue) during the exploration (left column) and production (right column) phases of petroleum resource extraction. Source: https://www.cnlopb.ca/wp-content/uploads/spill/sumtab.pdf (Accessed March 2020; the data is not inclusive of an additional three significant spills that occurred in 2019) FPSO = floating production, storage, and offloading vessels

The potential environmental impact of these spills or future spills is of great concern and data are required to assess the best options to mitigate the amount of damage should a catastrophic spill occur. This research program aimed to address data gaps surrounding the toxicity of crude oil on commercially important Atlantic species and the impacts following the use of a chemical dispersant on the toxicological responses.

1.1 Previous Spills and Toxicity Studies

Decades of laboratory testing and field research has generated a wealth of information of potential use in oil spill response decision making processes. A query of the Europe PMC repository of life science literature from 1980 to 2019 using the key word 'oil spill', normalized to the total number of publications each year, reveals a steady increase in publication output (Figure 2).

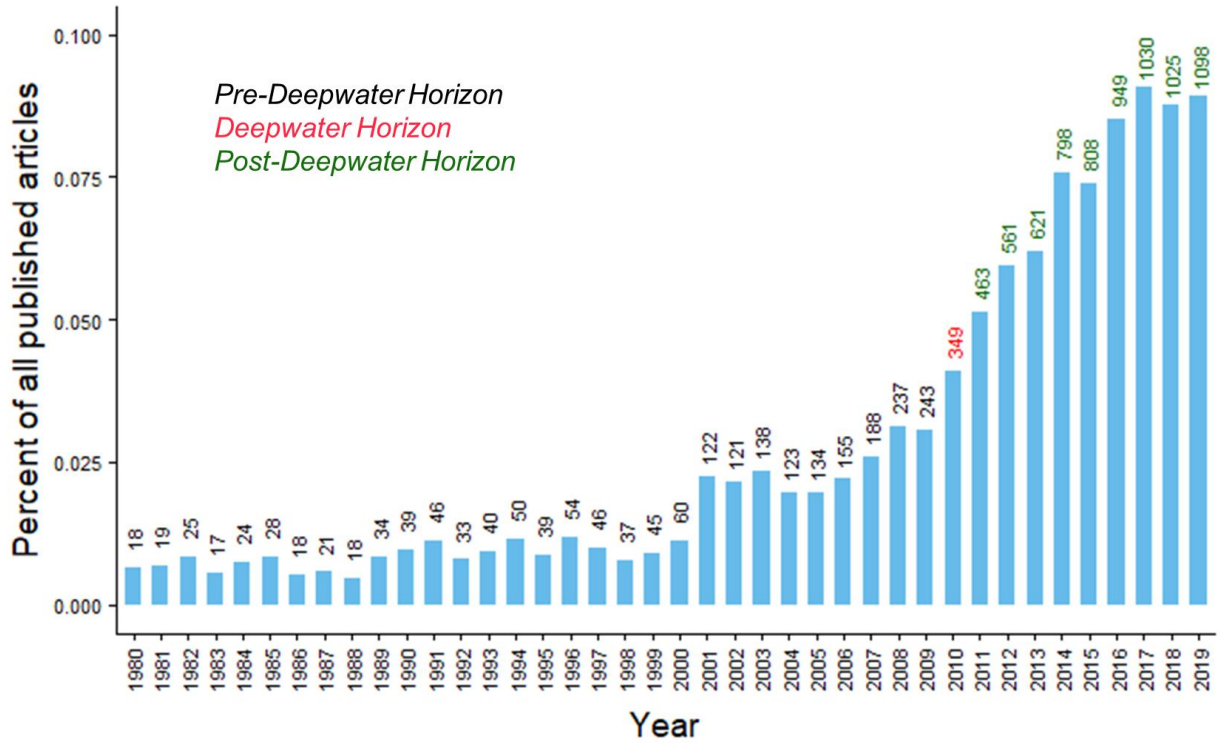


Figure 2: Normalized number of 'oil spill' publications from 1980 to 2019, with the absolute number of 'oil spill' publications for each year given above the bar, as queried from the Europe PMC database.

There was a marked increase in publication output following the 2010 Deepwater Horizon accident. Indeed, our understanding of the effects of crude oil on biota has been greatly

advanced following large scale environmental disasters, such as the Exxon Valdez (1989), Cosco Busan (2007), Hebei Spirit (2007), and Deepwater Horizon (2010). The research generated from these spills is often very case or site specific and has little value for planning and environmental risk assessments in other areas and with different petroleum products and environmental conditions, if not properly conducted or reported. For example, after the Exxon Valdez oil spill there were numerous studies on Alaska North Slope crude oil and its effects on fish development, particularly the Pacific herring (*Clupea pallasil*), where effects were widespread, significant, and persistent (Carls et al. 2002; Marty et al. 1997; Barron et al. 2003) However, the Braer oil spill (1993) off the Shetland Islands spilled more than twice as much oil as the Exxon Valdez (84,500 tonnes compared to an estimated 38,500 tonnes), yet due to three major factors the environmental impacts were surprisingly limited. These key factors that combined to minimize the impact were: i) the oil composition, Gullfaks crude oil is light and more easily biodegradable, ii) the winds and waves were exceptionally strong and persistent during and after the spill, and iii) the particular area of Shetland lacks vulnerable landforms such as low-energy beaches and saltmarshes but has an exposed coastline, which produced strong reflection and turbulence effects (Ritchie 1993).

The dynamic nature of field conditions makes the realistic design and execution of laboratory or field-based toxicological tests challenging (Bejarano et al. 2014). However, their real world utility lies in the ability to compare concentrations of oil that cause impacts in laboratory settings with measured concentrations of oil and dispersants in the water column following dispersant use during actual spills (Coelho et al. 2013). The conduct of a ‘realistic’ oil toxicity test seeks to reproduce one or more conditions at a spill site (or expected spill site) to better reflect the environmental concentrations and conditions. The implicit assumption is that the closer the test conditions are to the site-specific conditions then the more useful and reliable the data collected will be for that particular scenario and environment. For these reasons, our research program sought to create conditions that were reflective of offshore Newfoundland so that the data generated would be useful for spill scenarios in that specific environment.

1.2 Factors affecting the severity of environmental impact

1.2.1 Geographical location and Environmental Factors

The Island of Newfoundland has a coastline of ~9,655 km consisting of numerous bays, coves, and inlets. Lying to the southeast of Newfoundland are the Grand Banks with a diverse ecosystem that provides a significant marine environment for seabird colonies, marine mammals, and significant contributor towards a $1.4 billion seafood industry (Seafood Industry Year in Review, 2016). The Grand Banks provides an ideal habitat for diverse and economically important fish species, migratory and resident seabirds, and marine mammal assemblages.

The main offshore oil and gas industry in Newfoundland is currently also concentrated on the Grand Banks. Beginning in 1966, there has been extensive exploration of the petroleum reserves located beneath the Grand Banks, with significant finds in 1979 (Hibernia), 1981 (Hebron), and 1984 (Whiterose). The Grand Banks are considered one of the harshest oceanic environments in which oil and gas operations take place owing to three major determinants: 1) located at the convergence of three major storm tracks in North America; 2) at the relatively unsheltered convergence point of two major ocean currents; and 3) in the path of sea ice and icebergs. The presence of sea ice and icebergs, in combination with the high winds, waves, cold temperatures, and reduced visibility (Table 3) present a challenging environment for all aspects of offshore oil and gas operations: exploration, production, shipping, and response.

Table 3: Offshore Newfoundland weather conditions

Parameter	**Value**
Fog	90 days per year
Sunshine	~40 hours per month
Mean surface winds	8.5 - 10.5 m/s (February) 6.5 - 7.75 m/s (August)
Mean sea surface temperature	0 - 5^{o}C (February) 11 - 17^{o}C (August)

Source: Review of Offshore oil spill Prevention and Remediation Requirements and Practices in Newfoundland and Labrador, https://www.gov.nl.ca/nr/files/publications-energy-nloffshore-oil-review-appendix-package.pdf

1.2.2 Oil type and quantity

Crude oils from offshore Newfoundland and Labrador are generally characterized as medium density and viscosity. When crude oil is spilled in the marine environment, its physical and chemical properties will change through weathering processes, such as evaporation, emulsification, dispersion, and spreading. These changes alter the fate and behaviour, and thus biological effects, of the product and will dictate the specific response options/countermeasures most appropriate to deploy under the given conditions. With Newfoundland crudes, the pour point tends to be higher than ambient temperatures in winter and after some weathering summer temperatures as well. Oils with a pour point close to, or higher than the sea surface temperature, are not likely to be dispersible, as high viscosity oils don't allow penetration of the dispersant into the oil film. This also means that if these oils cool 8 - 10^{o}C below their pour point, they could gel and may be difficult to skim, pump, or disperse, but not burn. These oils also tend to emulsify after some weathering, but generally at a slower rate thereby allowing for skimming, burning, and/or dispersant usage (Turner, 2010). Lab studies have shown that Hibernia crude (which has a low density, high pour point and high wax content) forms a stable emulsion with a water content of up to 80% within a few hours of the spill, particularly in rough seas (Hurlbut et al. 1991). This can lead to a five - ten-fold increase in relative slick volume and alters the rate at which it disperses and evaporates.

Applied Science Associates Inc. (2011) performed stochastic modelling based on several spill scenarios from the Hebron platform to determine the probability that oil on the water surface would exceed a 10 ppb (10 µg/L) threshold and that shoreline oiling would exceed a thickness threshold of 0.01 mm (10 µm; selected based on seabird sensitivity). The 95th percentile values were determined by ranking 100 spills in each scenario according to the area of sea surface exposed to oil, the length of shoreline exposed, and the volume of entrained (naturally dispersed) oil above the selected thresholds (Table 4).

Table 4: Simulation results of stochastic modelling of spill scenarios (Galagan et al. 2011)

Oil Release Scenario	Duration	Total Spill volume	Season	95th percentile Surface area oiled at >0.01 mm (km^2)	95th percentile Shoreline oiled at >0.01 mm (km)	95th percentile Entrained oil volume after 30 days (m^3)
Platform Blowout (D-94 crude; 70 m above the sea surface)	30 days	1,050,000 bbl (166,936.6 m^3)	Summer	388,892	0	31,200
			Winter	586,907	0	71,852
			Winter (ice)	646,973	0	55,122
	60 days	1,050,000 bbl (166,936.6 m^3)	Summer	1,383,424	0	501
			Winter	1,901,033	47.7	573
			Winter (ice)	1,791,808	42.4	550
	100 days	3,500,000 bbl (556,455.4 m^3)	Summer	2,306,565	10.6	159,925
	120 days	4,200,000 bbl (667,746.5 m^3)	Winter	4,225,306	636.2	263,493
			Winter (ice)	4,841,586	742.2	463,493
Seafloor Blowout (Ben Nevis crude; ~98 m depth)	100 days	2,000,000 bbl (317,975 m^3)	Summer	2,416,717	5.3	3,706,057
	120 days	2,400,000 bbl (381,569 m^3)	Winter	4,415,367	466.5	4,309,446
			Winter (ice)	4,615,041	471.8	4,182,299
Batch Transfer (marine diesel)	Instantaneous	5,031 bbl (800 m^3)	Summer	251,672	0	496
			Winter	447,298	0	548
			Winter (ice)	461,520	0	547
Batch OLS Transfer (crude)	24 hours	31,449 bbl (5000 m^3)	Summer	250,351	0	25
			Winter	433,132	0	28
			Winter (ice)	454,550	0	32

Both of the crude oils used in their blowout simulations were persistent and did not readily disperse into the water column from natural processes. They found that when this characteristic was combined with long duration releases then a large fraction of the oil remained on the sea surface and shoreline oiling of up to 700 km was a possible outcome. The batch transfer spill scenarios (marine diesel and crude from the offshore loading system (OLS)) demonstrated that the marine diesel is more easily dispersed into the

water column than the crude (as seen by the volume of oil entrained in Table 4) and that neither spill scenario is predicted to reach the Newfoundland shoreline.

The above scenarios provide insight into potential worse case scenarios and demonstrate that under certain conditions, with no additional intervention or response, the amount of oil spilled could cause serious adverse environmental effects.

1.2.3 Oil Spill Response Options

There are several options available to mitigate the impacts of an oil spill. The decision and appropriateness of each response option is dependent on numerous factors, such as oil properties (e.g., specific gravity/API^{o}, viscosity, pour point, volatility, asphaltene content), available resources, location of spill, and time elapsed. The available response techniques (which vary by jurisdiction) are weighed based on the technique that will remove the most oil, is likely to be the most effective under the prevailing conditions, and is feasible with the available resources. This process is termed a Net Environmental Benefit Analysis (NEBA), or more recently Spill Impact Mitigation Assessment (SIMA), and follow a structured approach to respond to a spill as follows:

- Compile and evaluate data: identify an exposure scenario and potential response options to understand the potential impacts of that spill scenario;
- Predict outcomes: for the given scenario to determine which techniques are effective and feasible;
- Balance trade-offs: weigh a range of ecological benefits and drawbacks resulting from each feasible response option. Note that SIMA also includes socio-economic benefits and costs resulting from each feasible response option; and,
- Select best-available response options: for the given scenario based on which tools and techniques will minimize impacts.

The preferred response method is to collect the oil mechanically in booms and remove it with skimmers. However, weather can quickly overwhelm or prevent booming and skimming efforts (Prince et al. 2017). The application of dispersants is a response option that gained significant attention after their unprecedented usage during the Deepwater

Horizon oil spill response. Chemical dispersants are a mixture of components (e.g., non-ionic surfactants, anionic surfactants, and solvents) designed to break up an oil slick. A floating oil slick will break up into smaller oil droplets (10-100 µm) following effective dispersant application and sufficient mixing energy (usually in the form of wave action) for rapid dilution and dispersion into the water column with subsequent biodegradation by naturally occurring microbes (Bejarano 2018; Echols et al. 2019; George-Ares and Clark 2000a; Lee et al. 2013; NRC 2005). One of the main objectives for dispersant application is to reduce the amount, and potential impact, of floating oil to marine wildlife, coastal shorelines, and sensitive habitats. The decision to use dispersants as part of an operational response must be made with full consideration of the benefits and drawbacks of its application, some of which are described in Table 5.

Table 5: Potential benefits and drawbacks of dispersant use

Benefits	**Drawbacks**
Reaches and treats more oil than other response options	Window of opportunity for use may be limited, ranging from hours to days depending on the oil type and ambient conditions
Speeds up oil removal from the water by enhancing natural biodegradation	Does not collect the oil from the environment
Prevents oil in a subsea spill from surfacing, thus mitigating harm to sea birds, wildlife, and responders	Potential effects of dispersed oil on organisms living in the water column

The environmental toxicity of dispersants, specifically Corexit 9500A, have been extensively studied with rigorous reviews compiled by George-Ares and Clark (2000b); NRC (2005); Barron et al. (2013); Hansen et al. (2014); Echols et al. (2016); Bejarano (2018) and NASEAM (2020). The relatively low toxicity of the current formulations of dispersants, combined with their rapid dilution and dissipation in the environment, and the reduction in air borne exposure of VOCs to responders and wildlife, lends to their popularity as a viable response option. However, one concern with dispersant use is whether dispersed oil is more toxic than untreated oil (NASEM 2019). There is insufficient knowledge of the consequences of oil spills for key northern species (Keitel-gröner et al.

2020), as well as of specific oil spill response options to mitigate large impacts (Wilkinson et al. 2017)(Wenning et al. 2018). This is particularly true for the less commonly studied but commercially important species and especially during the vulnerable early life stages. This ESRF funded research program sought to address this critical data gap regarding the use of the dispersant Corexit 9500A by performing toxicity tests with both dispersant treated and untreated offshore Newfoundland & Labrador weathered crude oil on the less commonly studied, vulnerable, early life stages of commercially important species.

1.3 Objective

The objective of this research program was to provide ecotoxicity data (e.g., LC50, EC50, LOEC, NOEC) for an offshore crude oil that has been physically and chemically dispersed to the early life stages of commercially and environmentally important species. These data will help inform risk assessments, NEBA/SIMA processes, and contribute towards the ability of the industry and regulators to appropriately respond in the event of an oil spill in the region.

Project Methods

Chapter 2 Project Setup

Definitions

- **CEWAF:** chemically enhanced water accommodated fraction (WAF), as a result of dispersant application
- **Definitive Trial:** a *Trial* (see below) which met internal validity criteria related to performance
- **ESRF:** Environmental Studies Research Fund
- **Exposure Solution**: The media surrounding the organism. Once the organism has been introduced to the Test Solution, it is termed the Exposure Solution.
- **Gradient dilution**: Method of preparing Test Solutions by using the same Stock Solution for the preparation of each solution in the series. Contrast to serial dilution, where a Test Solution is used to make the next Test Solution in the series through dilution.
- **HMSC:** Huntsman Marine Science Centre (Huntsman)
- **LC50**: lethal concentration 50%, median concentration to kill 50% of sample, used in assessment of acute toxicity
- ***LO(A)EC***: The lowest-observed (adverse) effect concentration. Determined by the chosen concentrations and defined as the first concentration for which there is a significant difference from the control for the effect under consideration.
- **LT50**: lethal time 50%, median time point when 50% of sample dies at a specific dosage, used in assessment of chronic toxicity
- ***NO(A)EC:*** The no-observed (adverse) effect concentration. Determined by the chosen concentrations and defined as the last concentration for which there is no significant difference from the control for the effect under consideration.
- **Stock Solution**: A concentrated version of the test substance, to be used in the preparation of Test Solutions
- **Test Solution**: Media to which the organism will be exposed.
- **WAF:** water accommodated fraction
- **Trial:** an individual, discrete study. May be related to method development, or conduct of a toxicity test

2.1 Test Facility

The Huntsman Marine Science Centre (Huntsman) is a private not-for-profit research and education institution located on the shores of the Bay of Fundy (1 Lower Campus Rd, St. Andrews, NB, Canada, E5B 2L7). Huntsman maintains a surveillance program to monitor for pesticides and heavy metals within its natural, UV treated, seawater supply. Water

samples were collected quarterly throughout the research program and sent to Maxxam Analytics (currently, Bureau Veritas; Bedford, Nova Scotia) for analysis for total mercury, total metals in water and organophosphorus pesticides in water. All inorganics were within expected values for seawater and none of the 28 organophosphorus pesticides analysed were detected.

All toxicity trials described in this report took place within a controlled environment room in the Christofor Research Laboratory. All glassware was pre-cleaned (solvent rinsed with DCM, methanol, acetone, hexane), dried, and then equilibrated to the same temperature as the environment room prior to use.

Animal Use Protocols that meet the criteria of the Canadian Council for Animal Care were approved for all fish broodstock holding and handling (N.B., such protocols are not required for invertebrate or fish early life stages prior to exogenous feeding). All of the required live animal holding facilities throughout the Huntsman Lower Campus (i.e., tanks and life support systems) required to hold broodstock, early life stages and live feed production for the species and life stages of interest were monitored daily and held within optimal conditions for the species. All toxicology trials were conducted in temperature controlled environmental rooms in the Christofor Research Laboratory.

2.2 Acquisition of Animals and Spawning

All species examined in this research program are of considerable ecological and economic importance for Canada. Each of the chosen commercially harvested species represents a different spawning strategy (e.g., broadcast spawning, external or internal fertilization) and have different seasonal distributions, which presented a unique scenario for exposure to crude oil. Vulnerable early life stages of each species were targeted for toxicity testing. A captive spawning and husbandry approach, which relied on partnerships with local fishers, was required to ensure high quality test organisms representing these early life stages. The general acquisition, husbandry, and spawning conditions for each test species are described in the following sections.

2.3 Toxicity Trials

Transition to performing toxicity trials was dependent on successful acquisition and spawning on a species-by-species basis. The toxicity tests were designed to represent environmentally realistic exposures of oil to an organism at several important early life stages. All trials were conducted in full (natural) seawater and using a range of environmentally relevant exposure durations. Each trial was given a unique identifier based on species being tested (e.g., GM = *Gadus morhua* for Atlantic cod studies) and sequential numbering. Wherever feasible, trials were conducted blinded and masked to the technicians performing the biological assessments. All data collection and study conduct was completed in the spirit of Good Laboratory Practices (GLP). The trials generated effects data (e.g., LC50, NOEC, LOEC) from exposures to water accommodated fractions (WAF) of an East Coast Atlantic oil and chemically enhanced water accommodated fractions (CEWAF) using the dispersant Corexit 9500A. Each WAF and CEWAF preparation was physically characterized using a LISST-100X instrument (Laser in Situ Scattering and Transmissometry, Sequoia Scientific Inc., Seattle, WA). Select samples were periodically sent to external laboratories (e.g., Maxxam Analytics, RPC, EMBSI) for analytical confirmation and characterization. The number of replicates, concentrations, and test organisms exposed varied with each trial based on the specific objectives (e.g., method development vs. definitive trial) and endpoints (e.g., fertilization, mortality, sublethal). Water quality parameters (e.g., salinity, temperature, dissolved oxygen, pH, ammonia) were regularly sampled in test and exposure media and if these parameters drifted outside of acceptable ranges then the trial would not be considered valid. Other validity criteria included control response (e.g., <20% mortality), logical concentration response relationship (e.g., increasing response with increasing concentration), and consistency between replicates (e.g., coefficient of variation <30%). A visual summary of all trials completed is presented in Figure 3.

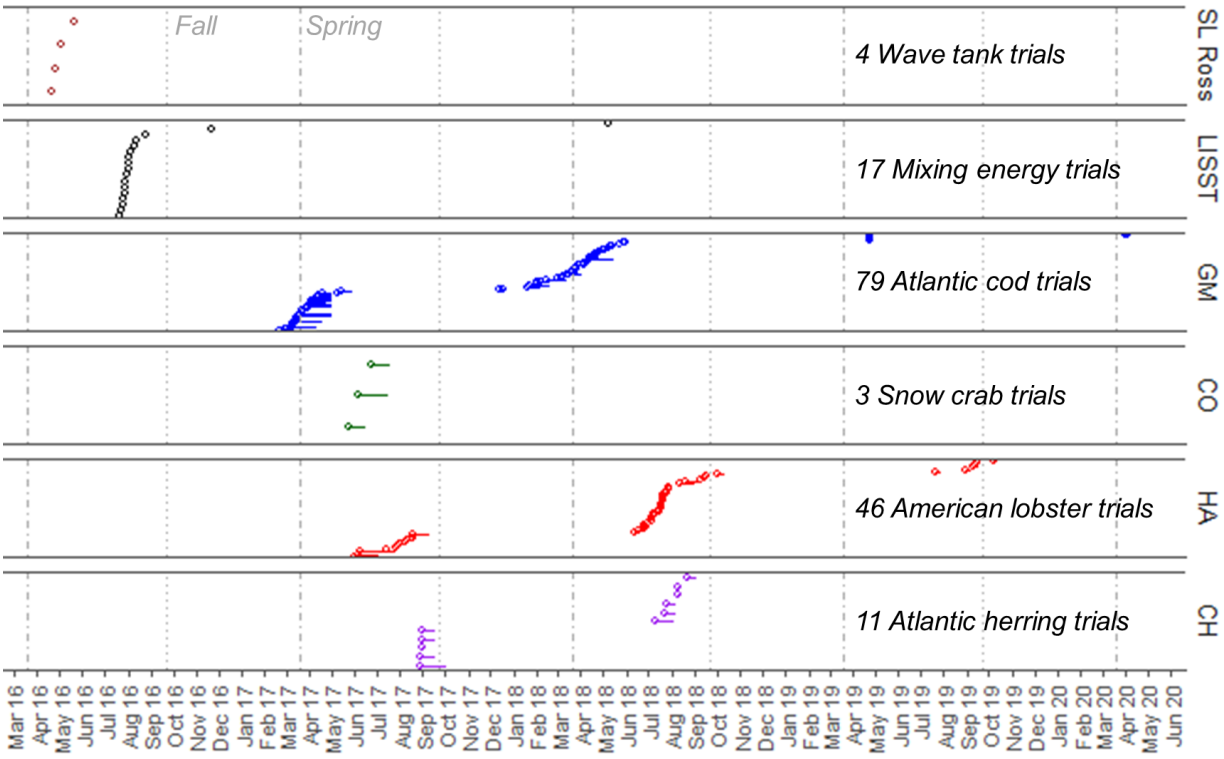


Figure 3: Timeline for all trials conducted during this research program.

2.4 Analysis, Reporting and Knowledge Transfer

All data collected during the trials were quality checked prior to transfer to electronic format. Data analyses were all conducted within the open source, freely available R (R Core Team 2012) with a significance level of 0.05. The specific statistical tests required varied with each trial. Generally, all data were tested for outliers using Grubbs' test (which checks the value which shows the largest absolute deviation from the mean of a normally distributed data as being an outlier). Summary statistics including arithmetic mean, minimum, maximum, standard deviation and median were calculated for all environmental parameters. Analysis of the test endpoints (e.g., mortality and growth) followed the data handling methods prescribed in the USEPA Method 1007.0, including evaluation of the NOEC and LOEC endpoints using ANOVAs and Dunnett's procedure following assessment of normality (Shapiro-Wilk's Test) and homogeneity of variance (Bartlett's Test). The data were transformed (e.g., arcsin) or analysed using a step-down test (Jonckhee trend test) when assumptions of normality were not met. Concentration-

response modelling (*drc* package in R, Ritz et al. 2016; Ritz and Streibig 2005; Ritz 2016) was used to fit the data and estimate the effect concentrations (and 95% confidence interval) associated with various levels of response (e.g.,10 and 50% decrease in survival from the control). Results were reported on the basis of percent strength of the WAF and CEWAF for the purposes of identifying if and where significant differences existed from the controls. All effect concentration data were calculated and reported on total petroleum hydrocarbons (TPH) with other measures (e.g., PAH, and µmol/mL PDMS) reported where available.

Compilation of the results presented in this report is underway for publication as a series of peer-reviewed publications. Many of the results have been shared at conferences (e.g., North American Chapter of Society of Environmental Toxicology and Chemistry Annual General Meeting, Canadian Ecotoxicology Workshop), with the ESRF Management Board, response organisations, government and industry partners.

Chapter 3 Exposure Media Characterization

Definitions

- **BE:** Biomimetic extraction, process by which analytes in a samples are allowed to selectively partition into a surrogate lipid phase, and thereby act to simulate the bioconcentration process
- **BTEX:** monocyclic aromatic hydrocarbons, primarily benzene, toluene, ethylbenzene, and xylene
- **Dissolved concentrations:** the concentration of oil components in only the aqueous phase. This has also been referred to as “truly” or “freely” dissolved components.
- **DOR:** Dispersant to oil ratio
- **GC-FID:** gas chromatography & flame ionization detector, used to identify different organic molecules within a gas mixture
- **GC-MS:** gas chromatography & mass spectrometry, used to identify different molecules within a mixture by measuring mass to charge ratio
- **OWR:** Oil to water ratio
- **SPME:** Solid phase microextraction
- **Total Concentrations:** the sum of the concentration of oil components in the aqueous phase and the oil phase. It is the mass of oil contained in the aqueous phase and in the microdroplets per unit bulk volume of solution.
- **TPAH:** total polycyclic aromatic hydrocabons
- **TPH:** total petroleum hydrocarbons

3.1 Introduction

Oil and its refined products are among the most complex and variable mixtures to evaluate from a toxicology perspective. Crude oil is a mixture of thousands of chemical compounds, each with varying properties, proportions, and toxicities. The monocyclic aromatic hydrocarbons, primarily benzene, toluene, ethylbenzene, and xylene (BTEX), are assumed to account for most of the acute toxicity to fish through a narcosis mechanism (Short et al. 2003). While BTEX may be among the most abundant aromatic hydrocarbons in many oils (mainly lighter, and fresh oils), they are also the least persistent because of their relatively high vapour pressure, leading to quick volatilization and loss to the atmosphere within a short time (hours to days) following the release of oil into the environment. The polycyclic aromatic hydrocarbons (PAHs) is an abundant class of hundreds of compounds that are more persistent than BTEX.

Standard methods for preparing petroleum-water mixtures for laboratory toxicity testing were first developed by Anderson et al. (1974) and have since been modified by several investigators to create more consistent petroleum-water and petroleum-dispersant-water mixtures (Aurand and Coelho 2005; Barron et al. 2003; George-Ares et al. 1999; Olsvik et al. 2010; Singer et al. 2000). The standard for preparing exposure media and performing oil toxicity testing is the result of the Chemical Response to Oil Spills Ecological Effects Research Forum (CROSERF) working group. CROSERF sought to standardize and improve quality and usefulness of laboratory research on the aquatic effects of oil spill treating agents with the primary objective to standardize test methods and reduce inter-laboratory variability. CROSERF identified several key factors for the consistent preparation of water accommodated fractions (WAFs), including pre-treatment of dilution water (filtration, sterilization etc.), mixing energy and duration (mixing energy is a key determinant in dispersion formation), vessel size, geometry, headspace, and surface area-to-volume ratio.

For oil toxicity tests, it is essential to measure the composition and concentration of hydrocarbons in the source oil and in test solutions. Measured concentrations are needed to compare toxicity of WAF and CEWAF following use of dispersants (Clark et al. 2001). CROSERF recommended that a single concentration value, referred to as total hydrocarbon content (THC; in mg/L) and defined as the sum of C10-C36 total petroleum hydrocarbon (TPH) and <C10 volatile hydrocarbons, be used in reporting estimates of toxicity test endpoints. This approach ensures a common baseline metric for reporting to ensure comparability of data. In practice, effects concentrations are often reported as a percentage of the original test media (nominal concentrations). Toxicity reported as nominal concentrations represent a percentage of a particular loading and are most closely tied to the laboratory method. Results based on nominal concentrations are difficult to relate to field data or to use in models, unless coupled with measured concentrations of hydrocarbons. Measured concentrations can include several analytical methods for expressing petroleum hydrocarbons. TPH are the most commonly reported measure for both laboratory and field studies. As such, they allow for comparisons between lab and field exposures. Total petroleum aromatic hydrocarbons (PAHs) are

believed to be more specific to the toxic fractions and the composition of PAHs modulates the toxicity, while non-PAH constituents may also contribute to observed toxicity.

For all the strengths of the CROSERF method, particularly with respect to comparative assessment of oil toxicity, it has been criticised for its limited representation of real world environmental scenarios (Bejarano et al. 2014; Coelho et al. 2013), perhaps no more so than in terms of the role and contribution of microdroplets towards observed toxicity. Micron and submicron-sized oil droplets/colloids (undissolved-phase hydrocarbons) are formed and entrained in open ocean and coastal areas after chemical (dispersant) and/or natural physical (wave action) dispersion of oil in water (Li et al. 2007). With laboratory testing in a closed system, these droplets can serve as a 'passive dosing' source for maintaining dissolved oil exposures and may exhibit physical effects, that in turn enhance the observed toxicity. This result is an artefact of the test system and not reflective of the reality of an open field system, where droplets experience rapid and extensive dilution. The extensive use of dispersants during the Deepwater Horizon accident highlighted the need for knowledge related to the formation, persistence, and impacts of oil microdroplets (Figure 4).

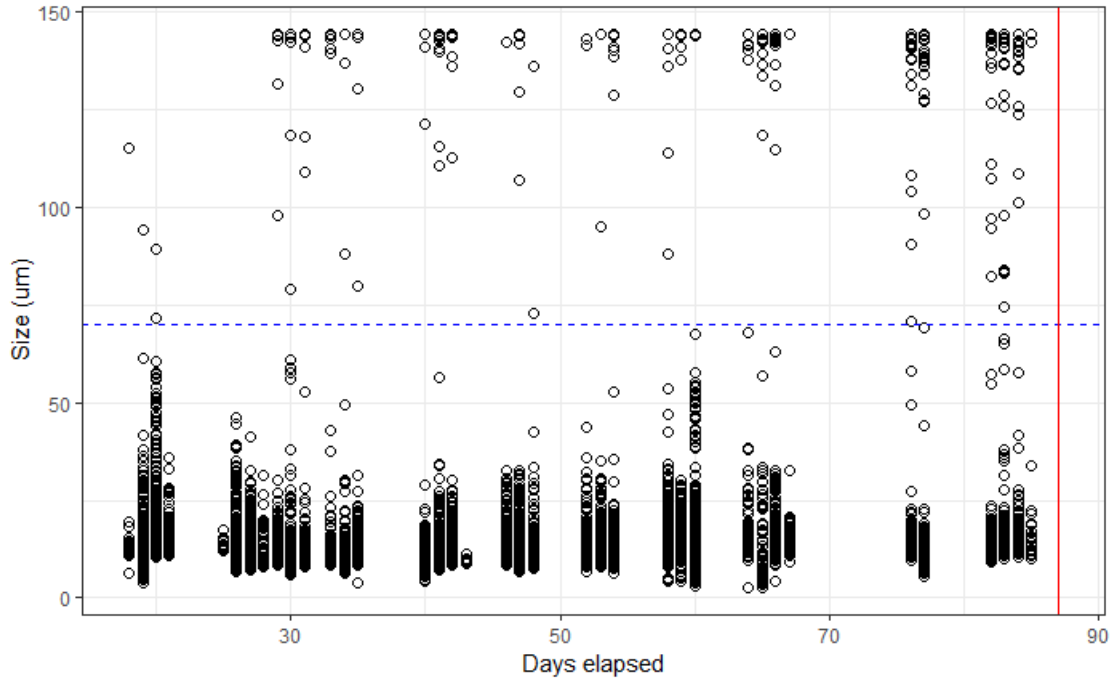


Figure 4: Mean oil droplet size measured by Department of Fisheries and Oceans scientists during the Deepwater Horizon accident response operation, from numerous stations, depths, and time points since the accident occurred. The vertical red line is at 87 days when the well was declared sealed, and the horizontal blue line is at 70 µm diameter, which is considered to be permanently dispersed or neutrally buoyant.

We sought to develop a laboratory method that prepared a consistent exposure media for use in toxicity studies of physically and chemically dispersed crude oil that also reflected realistic and specific real world environmental scenarios to offshore Newfoundland & Labrador. We calibrated our method to results obtained from wave tank trials conducted under conditions meant to mimic offshore Newfoundland & Labrador to ensure the relevance of our preparations.

3.2 Test Material

Huntsman received 13.5-L of fresh offshore Newfoundland & Labrador crude oil (specific production field confidential) on 31-Jan-16. The crude oil was artificially evaporated to 10% loss by mass, by stripping with nitrogen (Figure 5).

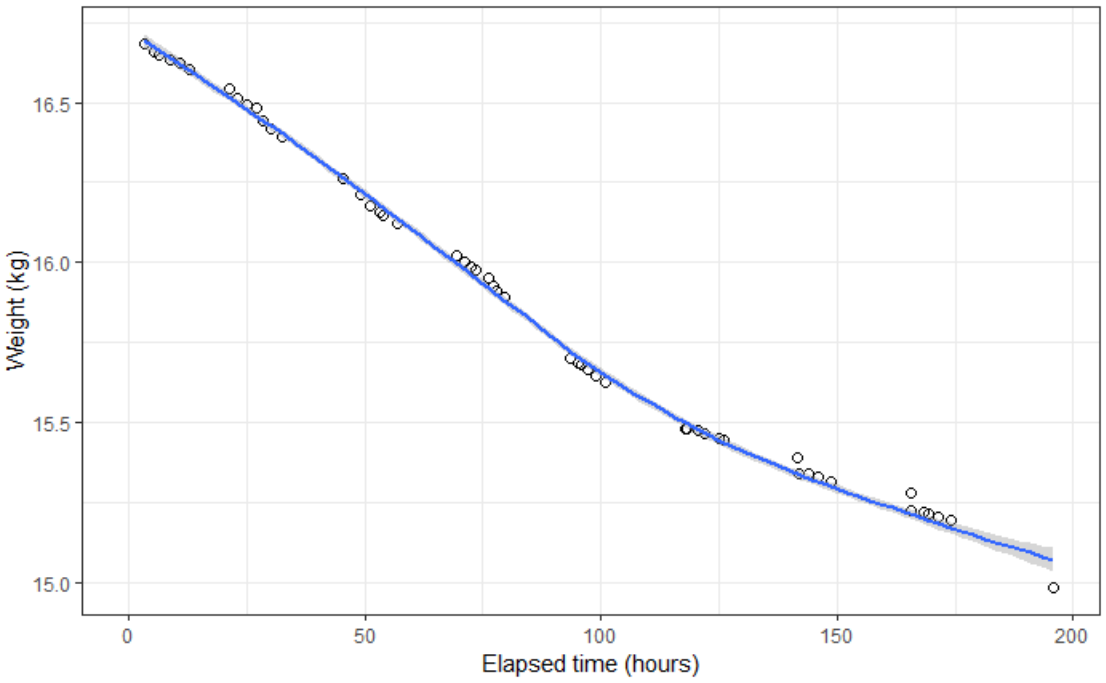


Figure 5: Mass profile as the crude oil was artificially weathered by nitrogen sparging

The use of 'fresh' oil in toxicity testing may overstate the hydrocarbon exposure for most all scenarios where dispersants could be used. Typically, it would take a minimum of 6 hours for a spill response effort to employ dispersant application, so by weathering the oil prior to testing we create a more realistic exposure media that is more reflective of the state of the oil at the time of dispersant application. After the evaporative weathering, the crude oil was split into 25 individually labelled stainless steel bottles (18 x 16 oz, and 7 x 32 oz, on 18-Mar-16) then sealed and stored until use. Only one bottle was opened and used at a time and each bottle was used for a maximum of three months before opening a new bottle to help ensure consistency of the test material.

The physical properties of the specific crude weathered by 9% at three different temperatures were reported by Environment Canada's Environmental Science and Technology Centre (http://www.etc-cte.ec.gc.ca/) and reported in Table 6.

Table 6: Physical properties of 9% evaporated (by weight) crude oil

Temperature (°C)	Density (g/mL)	Dynamic Viscosity (mPa*s)	Chemical Dispersibility (volume %, with Corexit 9500A)
0	**0.9059**	**615**	21
15	**0.8926**	**87**	20
25	**0.8846**	**39**	16

Source: http://www.etc-cte.ec.gc.ca/databases/

While these values are considered to be representative of the specific crude, these physical properties were not confirmed in our test material. The test material was chemically characterized for TPH and PAH content.

3.3 Methods

3.3.1 Wave tank

Huntsman contracted SL Ross Environmental Research Ltd. (Ottawa, ON) to perform wave tank tests with our weathered test material to determine the physical and chemical characteristics of dispersed (physically and chemically with Corexit 9500A) and dissolved oil under breaking wave conditions. Their report is included in Appendix 1. A preliminary dispersant effectiveness assessment was conducted prior to wave tank testing to assess the performance of Corexit 9500A on dispersing the crude at two temperatures: 4 and 13°C. The method involved addition of 1.5 mL oil into two 100-mL glass cylinders filled with 80 mL artificial seawater with one of the cylinders also having 60 µL of dispersant added (1:25 dispersant to oil ratio, DOR). The cylinders were gently inverted for one minute, then observed to visually characterize the dispersion efficacy as either:

- **good** – formation of brown dispersion of small oil droplets that slowly rise to the surface,
- **reduced** – formation of dark/black large oil droplets that quickly rise to the surface, or
- **poor** – little or no difference from the untreated oil cylinder with fast rising, large droplets.

Following the efficacy testing, larger-scale tank tests were completed in the 11 m long x 12 m wide x 1.2 m deep indoor wave tank. The wave tank was programmed to generate

a train of waves that combined to form a breaking wave comparable to offshore Newfoundland sea state conditions (total height 18 cm at a frequency of 2 waves per minute) in the middle of the containment barrier where the crude oil was initially added for a total run time of 30 minutes. During that time, water quality in the tank was monitored every 1.5 seconds using a LISST laser particle size analyser (Sequoia Scientific Inc. Model 100x Type C) that was suspended 40 cm below the water surface to characterize the droplet size distribution and concentration in the dispersed oil. Grab water samples were collected beneath the oil slick at a depth of 40 cm immediately prior to introducing the oil and at 2, 8, 15, and 30 minutes following the first wave impact. The samples were analysed for petroleum hydrocarbons (F1 through F4 fractions) and benzene, toluene, ethylbenzene, and xylenes (BTEX) by Maxxam Analytics Inc. Oil remaining in the containment area after the 30 minutes run was collected and weighed to calculate the dispersant effectiveness based on comparison to the known mass of oil added to the water surface. Four runs were completed with oil alone and oil + dispersant (DOR 1:20) tested at 4 and 13^{o}C thereby providing seasonally relevant seawater temperatures for our species and life stages of interest.

3.3.2 Mixing Exposure Media

Our goal was to replicate the results observed in the SL Ross wave tank trials, which served as a proxy for offshore Newfoundland conditions, within a laboratory setup and cognizant of the logistical demands of performing a toxicity test. Ultimately, we employed a benchtop mixing method that was a modification of the baffled flask method for dispersant effectiveness used by Environment Canada and the United States Environmental Protection Agency. Briefly, 0.22 µm filtered seawater was poured into a clean baffled flask (size of the flask depended on the volume required for testing while maintaining a 20% headspace but generally was 2-L). An appropriate aliquot of oil (1 g of oil per L of seawater) was drawn up into a gastight syringe and dispensed onto the central surface of the water in the flask. In the case of a CEWAF preparation, dispersant was also added to the centre of the surface oil slick at a dispersant to oil ratio of 1:20. The flask was then sealed with DuraSeal, secured on an orbital shaker, shaken at the prescribed energy level for a specific duration that was determined to mimic the wave

tank proxy results (e.g., 150 rpm for 60 minutes in a 2-L flask with 1.6 L of water), and allowed to settle for 1-hour prior to use as exposure media (Figure 6).

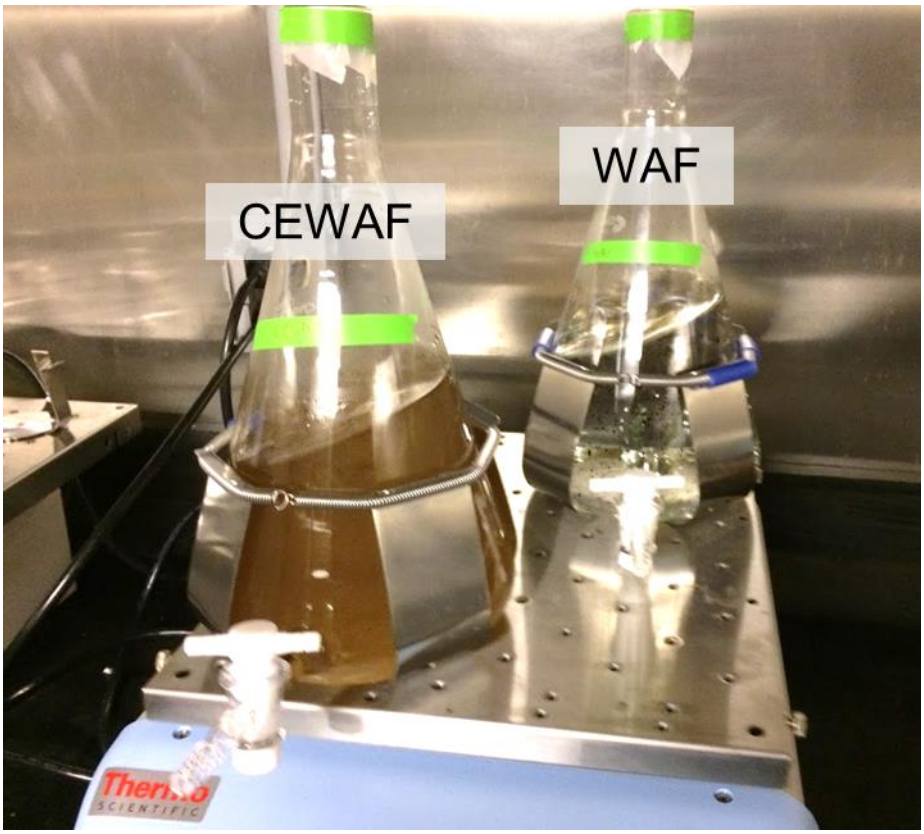


Figure 6: WAF and CEWAF preparation

Required volume of prepared WAF or CEWAF was poured out through the baffled flask spigot, with the first 100 mL being discarded and the last 100 mL not used for exposures. Exposure media for use in toxicity testing was prepared using gradient dilutions of the aliquot from the WAF or CEWAF preparation. All preparations were characterized for microdroplet size profile distribution using a LISST-100X instrument (LISST-100X, Sequoia Scientific Inc., Seattle, WA). Samples were periodically taken to complete detailed chemical analysis (e.g., PAH and Alkylated PAH, BTEX, TPH).

3.3.3 Physical Characterization

The LISST-100X particle size analyzer (Type C) is an optical device that measures the size (range from 2.5 to 500 µm) and volume of particles in a given sample based on the physical properties of light as it is scattered off a particle. The LISST operates by emitting a laser beam across the sample chamber and any of this light that interacts with particles present within the sample (e.g., microdroplets of crude oil) is scattered (diffracted) and focused by a specialized lens onto a series of 32 detector rings. Light intensity readings on each ring are processed based on an inversion algorithm to automatically calculate the volume concentrations (in µL/L) for the 32, logarithmically spaced (the upper size in each bin being 1.18 times that of the lower), particle size bins. For each sample preparation, the mean concentration value per bin class, the particle size distribution (defined as the average number of particles within a given size class), and the mean droplet size were calculated from 60 independent measurements with data collected and recorded once per second.

3.3.4 Chemical Characterization

Chemical analyses were performed by commercial analytical laboratories. Maxxam Analytics (currently Bureau Veritas, Bedford, NS) completed chemical analysis following the Atlantic RBCA Tier I and Tier II Petroleum Hydrocarbon methods on the 2016 and 2017 samples. The Tier I method provides the concentration of benzene, toluene, ethylbenzene and xylene(s) (BTEX) and total petroleum hydrocarbon (TPH) concentration minus BTEX. This procedure reports the volatile petroleum hydrocarbons (C6-C10), including BTEX (VPH analysis), the extractable hydrocarbons (>C10-C32) (EPH analysis) as well as the modified TPH (sum of C6-C32 less BTEX). In the Tier II analysis, both the VPH and EPH ranges are subdivided into aromatic and aliphatic fractions and narrower carbon ranges are reported. VPH is measured by direct purge and trap gas chromatography/mass spectrometry (GC/MS) and the EPH is partitioned into hexane and the extracts are measured by GC-FID (flame ionization detector).

In 2018, the analytical analyses were performed by RPC (Fredericton, NB). RPC performed the same hydrocarbon analysis as Maxxam (Tier I), as well as a detailed

analysis of PAH and alkyl PAH concentrations (31 analytes, GC/MS based on USEPA 3150C/8270C) in the crude oil as well as the test solutions. The analytical reports are included in the Appendix 2.

In addition to the traditional metrics for measuring exposure concentrations (e.g., BTEX, TPH, PAH), samples were collected and sent to ExxonMobil Biomedical Sciences (Annandale, NJ, USA) to perform a biomimetic extraction (BE). This method uses solid phase microextraction (SPME) fibers as passive samplers to characterize the dissolved hydrocarbon concentration. Following the methods of Letinski et al. (2014), the absorbed concentration on the fibers was quantified by thermal desorption using GC/FID. The concentration on the fibers is well correlated to the concentration that partitions into target lipid and has been proposed as an improved exposure metric for toxicity predictions (Redman et al. 2018).

3.4 Results

3.4.1 Preliminary Dispersant Effectiveness and Wave Tank Tests

The preliminary dispersant effectiveness assessments showed that Corexit 9500A produced a good dispersion of the crude at 13^{o}C, with slightly reduced effectiveness at 4^{o}C (Figure 7).

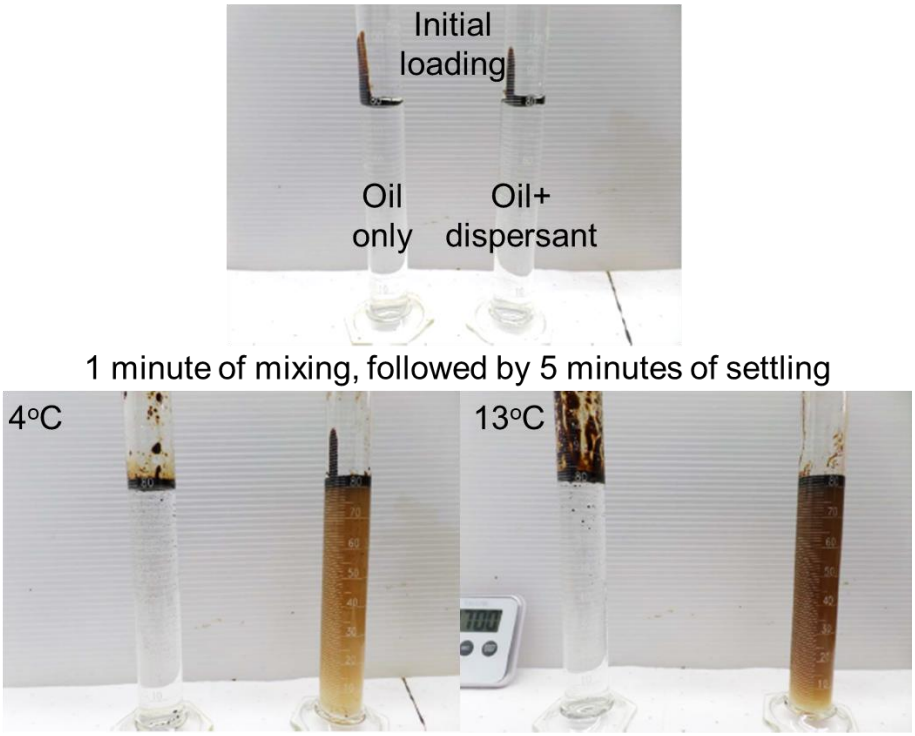


Figure 7: Visual assessment of dispersant efficacy at 4 and 13°C. Modified from SL Ross report (Appendix 1)

The droplet size distribution results from the wave tank tests are shown on a concentration basis (µL droplet/L water) in Figure 8.

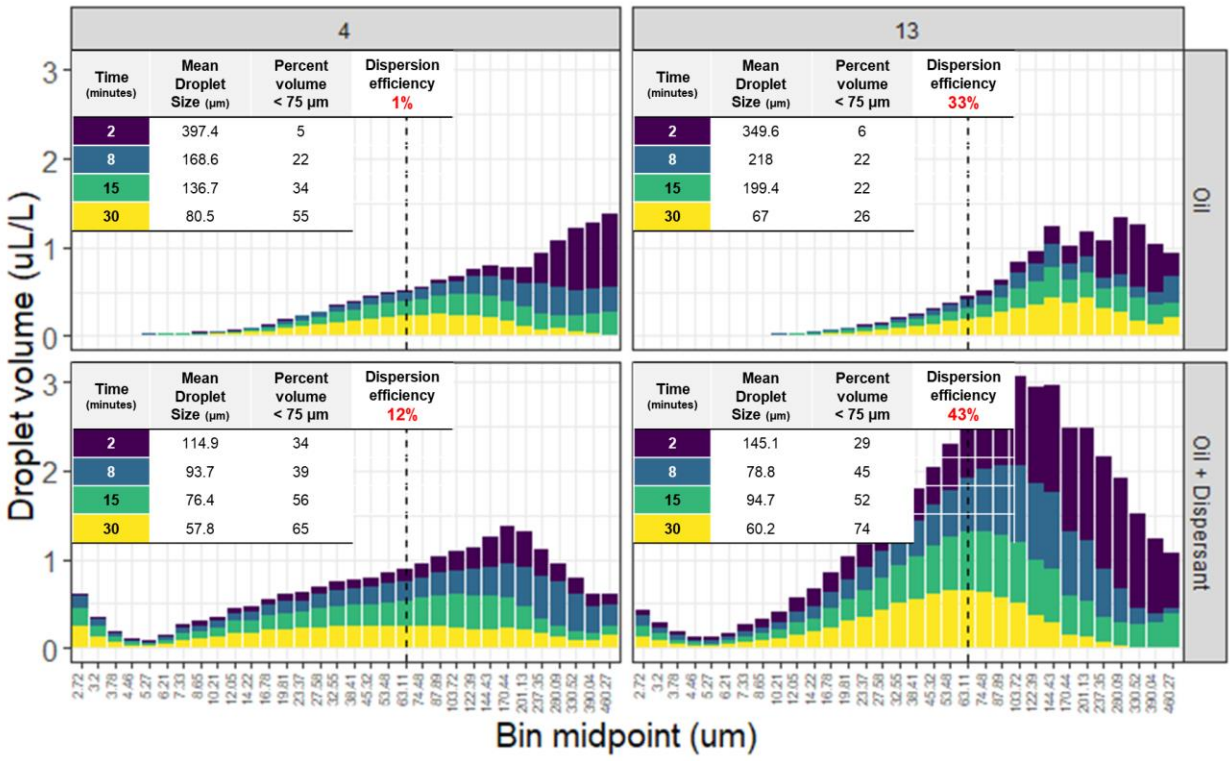


Figure 8: Droplet size distribution profiles for the oil alone (top row) and the oil plus dispersant (bottom row) at 2, 8, 15, and 30 minutes (purple, blue, green, and yellow bars, respectively) after the first wave broke, for the 4 (left column) and 13°C (right column) trials. The dashed vertical line is at ~75 µm, below which droplets are unlikely to resurface.

The droplet profiles for each trial type (oil or oil + dispersant) were similar in distribution, however differed in amplitude, with the greater droplet concentrations in the warmer temperature. The profiles between the two trial types differed, with the addition of dispersant resulting in a greater shift towards smaller droplets over the course of the run. The dispersion efficacy ranged from 1 (4°C oil only) to 43% (13°C oil + dispersant), with greater efficiency in the warmer temperature.

The chemistry concentrations for each run type are presented in Figure 9.

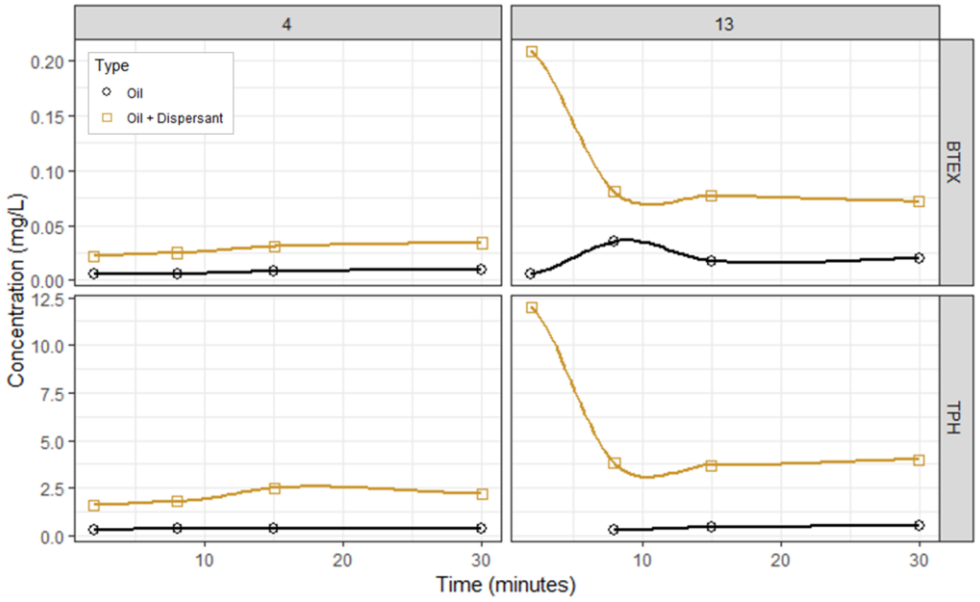


Figure 9: Changes in BTEX and TPH concentration over time for runs at 4 (left column) and 13°C (right column), for oil alone (black circles) and oil plus dispersant (brown squares). Note there was no TPH sample for the 2-minute time point in the 13°C oil alone trial.

For each temperature, the addition of dispersant caused an increase in chemical concentrations (BTEX and TPH) relative to the oil alone treatment. Greater concentrations were observed in the warmer temperature, with peak concentrations occurring within 2 minutes of the first wave breaking onto the slick, then levelling out (~3-fold reduction) for the duration of the run.

3.4.2 Physical Characterization

A series of baffled-flask dispersant-effectiveness trials were performed from 26-Jul to 22-Nov-16 at Huntsman to identify the conditions (e.g., mixing speed, mixing duration, settling duration) that would produce comparable results in terms of droplet profile and mean droplet size as observed in the SL Ross wave tank study.

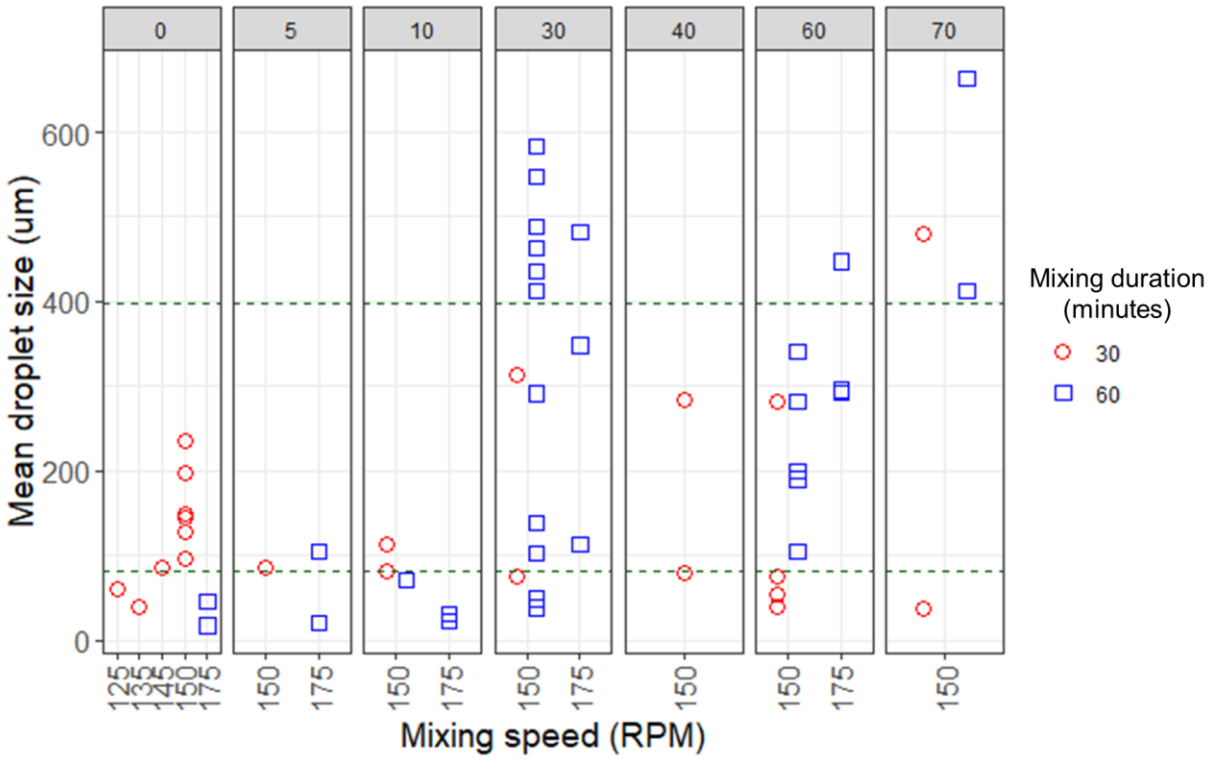


Figure 10: Mean droplet size (µm) from different mixing energies (x-axis) and durations (30 minutes, red circles; 60 minutes, blue squares) and settling times. The dashed horizontal lines are the maximum and minimum mean droplet sizes observed in the SL Ross wave tank trials at 4°C with oil only. Each panel represents a different settling duration (minutes).

The mixing speed of 150 rpm, with a mixing and settling duration of 60 minutes, was selected for all toxicology trials as these parameters resulted in droplet sizes that were consistent and within the target droplet size window to reflect offshore Newfoundland field conditions.

With each toxicology trial, the test solutions had their particle size distributions analysed and mean droplet size calculated. The LISST measurements provided insight into the repeatability and consistency of the WAF and CEWAF preparations. The volume concentration (VC) in each bin increased linearly with increasing strength of test solution. An example of the VC relationship is shown in Figure 11 for a CEWAF series.

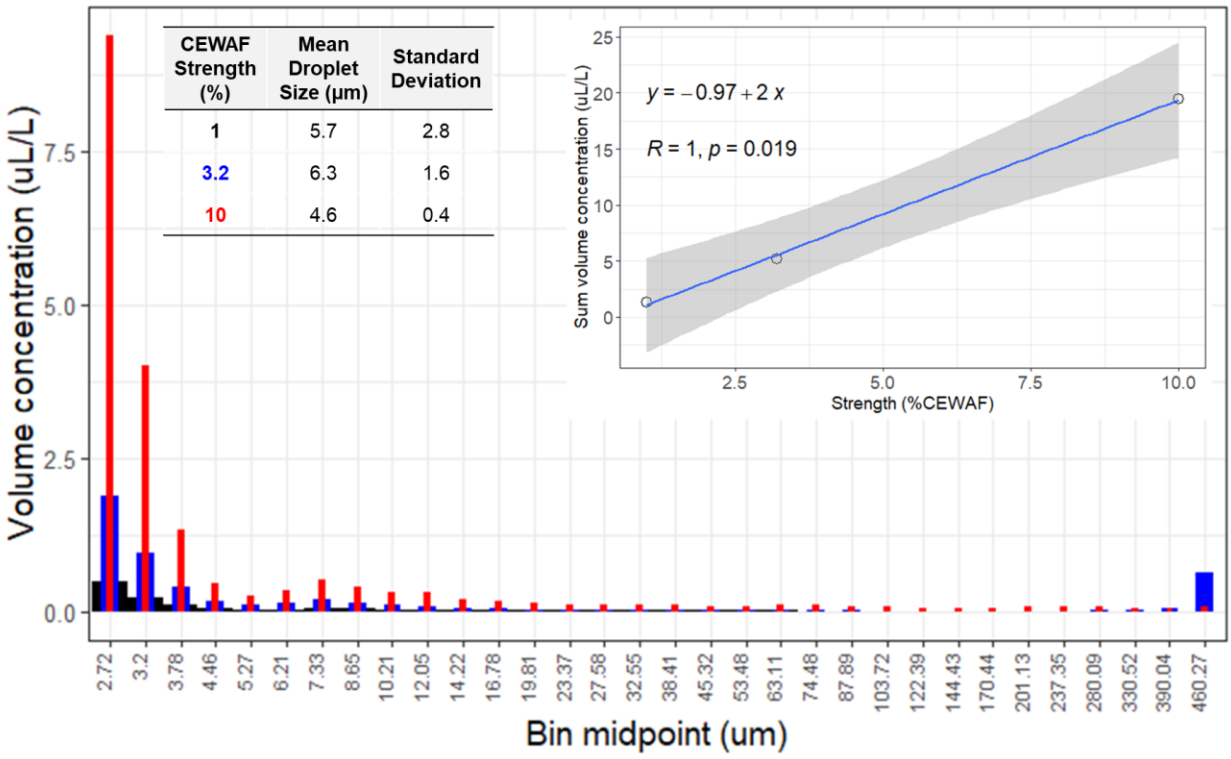


Figure 11: Volume concentration profile for each 1 (black bars), 3.2 (blue bars), and 10% (red bars) dilution of CEWAF prepared at 15°C, with the sum concentration regressed against nominal strength in the insert.

The mean droplet size in in the 1, 3.2 and 10% CEWAF solutions shown in Figure 11 were 5.7, 6.3, and 4.6 µm respectively, demonstrating that the mean droplet size was conserved and not impacted by dilutions. At higher CEWAF concentrations (>18%) the instrument was saturated and transmissivity was too low to allow for a reliable reading, resulting in a plateau in VC concentrations, and an instrument warning that the sample was “too turbid”. Similarly, in the lower WAF concentrations (<56%), the transmissivity was too great (>85%) resulting in an unreliable reading and an instrument warning that the sample was “too clear”. We encountered a problem (likely an alignment issue) with rings 24 and 26 (corresponding bin midpoints of 122.39 and 170.44 µm) of the LISST. Unfortunately, after consultation with Sequoia Instruments we were unable to rectify this issue. As this was consistent across all preparations, any potential impact on droplet calculations (e.g., potentially underestimating droplet size) is expected to be equally distributed across all treatments, and the data collected still considered reliable for comparisons between treatments.

The cumulative distribution frequency (CDF) of the size of droplets and the volume concentration in each bin are shown for the 18% CEWAF test solution from 10 separate trials in Figure 12.

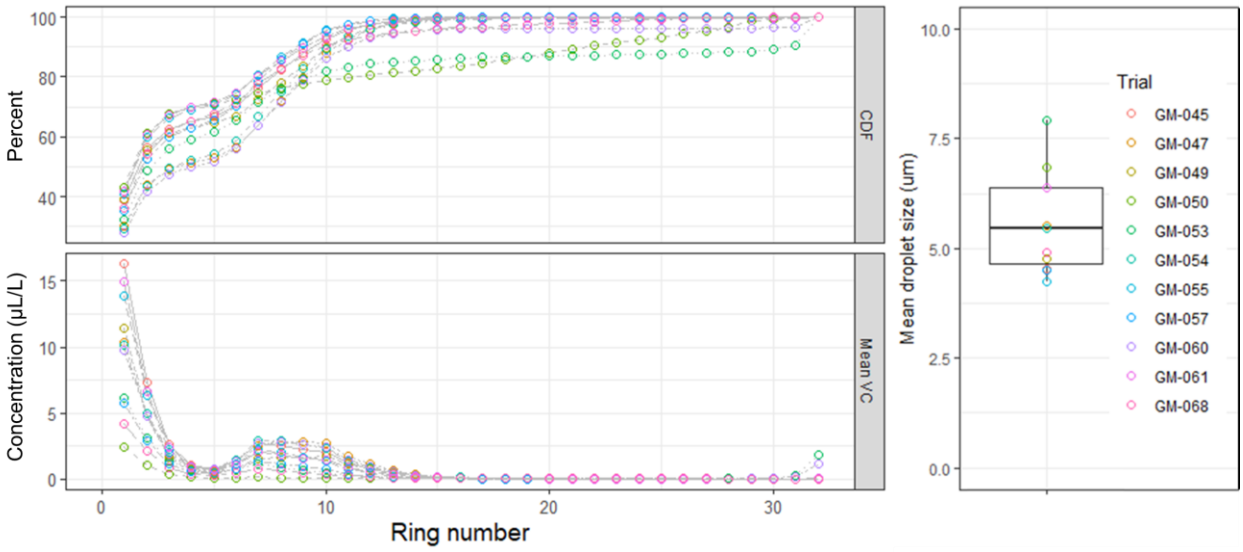


Figure 12: Cumulative distribution frequency of droplet size (CDF, top), mean volume concentration (VC, bottom), and mean droplet size (right, boxplot) calculated from 60 runs of each 18% CEWAF solution from 11 different trials (colours) conducted between March and May 2018 at 5°C.

There was very good consistency between preparations in terms of droplet profile and concentrations. The mean droplet size from the 11 different trials ranged from 4.2 to 7.9 µm with a mean value of 5.6 (standard deviation = 1.16).

We noted a difference in dispersion efficacy (evidenced by changes in droplet VC) with temperature, as was observed with the SL Ross wave tank results, with warmer temperatures having greater volume concentrations (Figure 13).

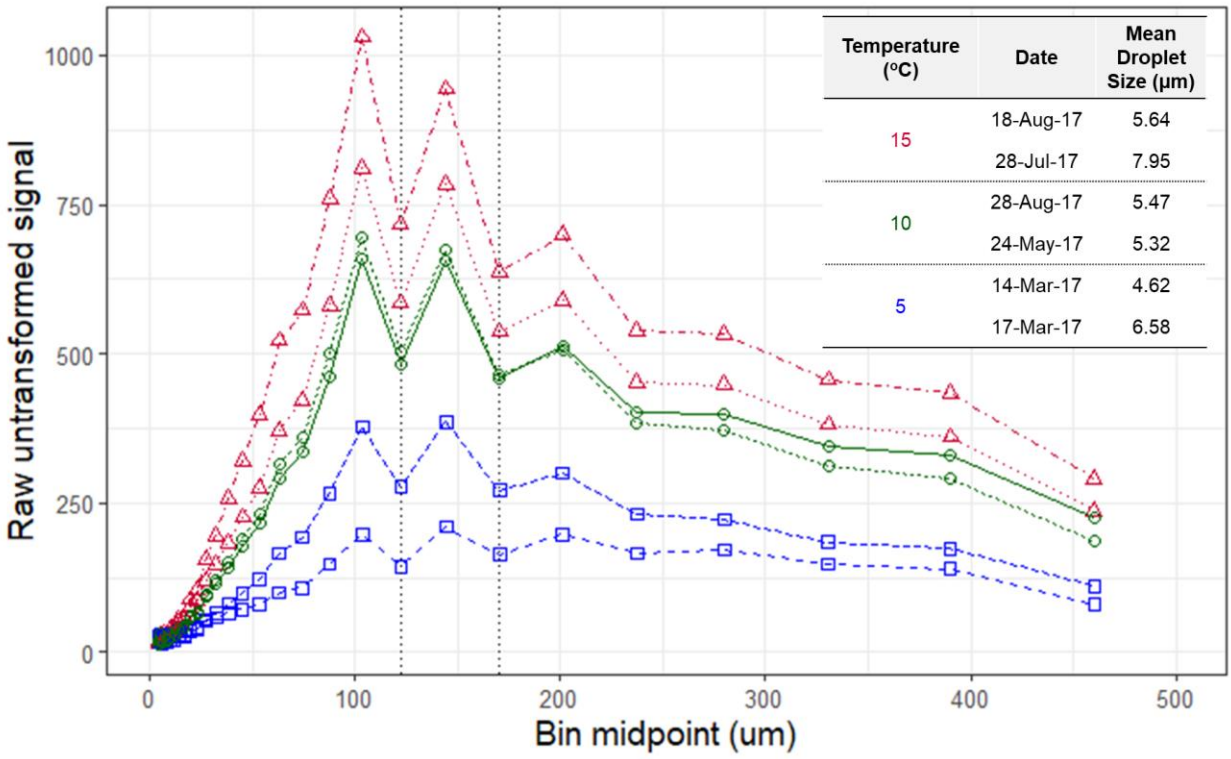


Figure 13: Untransformed signal on each ring of a 3.2% strength CEWAF solution at 5 (blue squares), 10 (green circles), and 15°C (red triangles) from trials conducted in 2017 between 14-Mar to 28-Aug.

The droplet distribution and mean sizes were relatively consistent across temperatures, ranging from 4.62 to 7.95 µm, despite the differences in efficacy. The dashed vertical lines in Figure 13 correspond to the likely alignment issue with rings 24 and 26 (corresponding bin midpoints of 122.39 and 170.44 µm) of the LISST.

3.4.3 Chemical results

Full chemistry reports from the analytical laboratories are provided in Appendix 2.

The weathered crude oil test material was characterized by RPC Laboratories (Table 7).

Table 7: Chemical composition of the test material

Analytes	Reporting Limit (mg/kg)	Concentration (mg/kg)
Benzene	0.005	51
Toluene	0.05	2500
Ethylbenzene	0.01	1200
Xylenes	0.05	6100
VPH C6-C10 (Less BTEX)	2.5	69000
EPH >C10-C16	12	180000
EPH >C16-C21	12	130000
EPH >C21-C32	12	180000
EPH (>C16-C32)	12	310000
Modified TPH Tier 1	*21*	*560000*
Analytes	**Reporting Limit (µg/g)**	**Concentration (µg/g)**
Naphthalene	0.1	730
Acenaphthylene	0.1	13
Acenaphthene	0.1	15
Fluorene	0.1	130
Phenanthrene	0.1	240
Anthracene	0.1	< 2.0
Fluoranthene	0.1	5.4
Pyrene	0.1	12
Bz(a)anthracene	0.1	< 2.0
Chrysene/Triphenylene	0.1	< 2.0
Bz(b)fluoranthene	0.1	3.7
Bz(k)fluoranthene	0.1	2.0
Bz(e)pyrene	0.1	13
Bz(a)pyrene	0.1	< 2.0
Indenopyrene	0.1	< 2.0
Bz(g,h,i)perylene	0.1	2.5
Dibz(a,h)anthracene	0.1	< 2.0
C1-Naphthalenes	0.1	1300
C2-Naphthalenes	0.1	1500
C3-Naphthalenes	0.1	750
C1-Phenanthrenes	0.1	280
C2-Phenanthrenes	0.1	300
C3-Phenanthrenes	0.1	130
Dibenzothiophene	0.1	< 2.0
C1-Dibenzothiophenes	0.1	50
C2-Dibenzothiophenes	0.1	32
C3-Dibenzothiophenes	0.1	< 2.0
1-methylnaphthalene	0.1	1200
2-methylnaphthalene	0.1	1000
Perylene	0.1	< 2.0
Biphenyl	0.1	230

In 2016, a series of variable loading experiments were performed with a 30-minute mixing time at 150 rpm with no settling. The results for BTEX showed good linearity, however for TPH the results did not follow an expected increase in concentration with increase in loading (Figure 14).

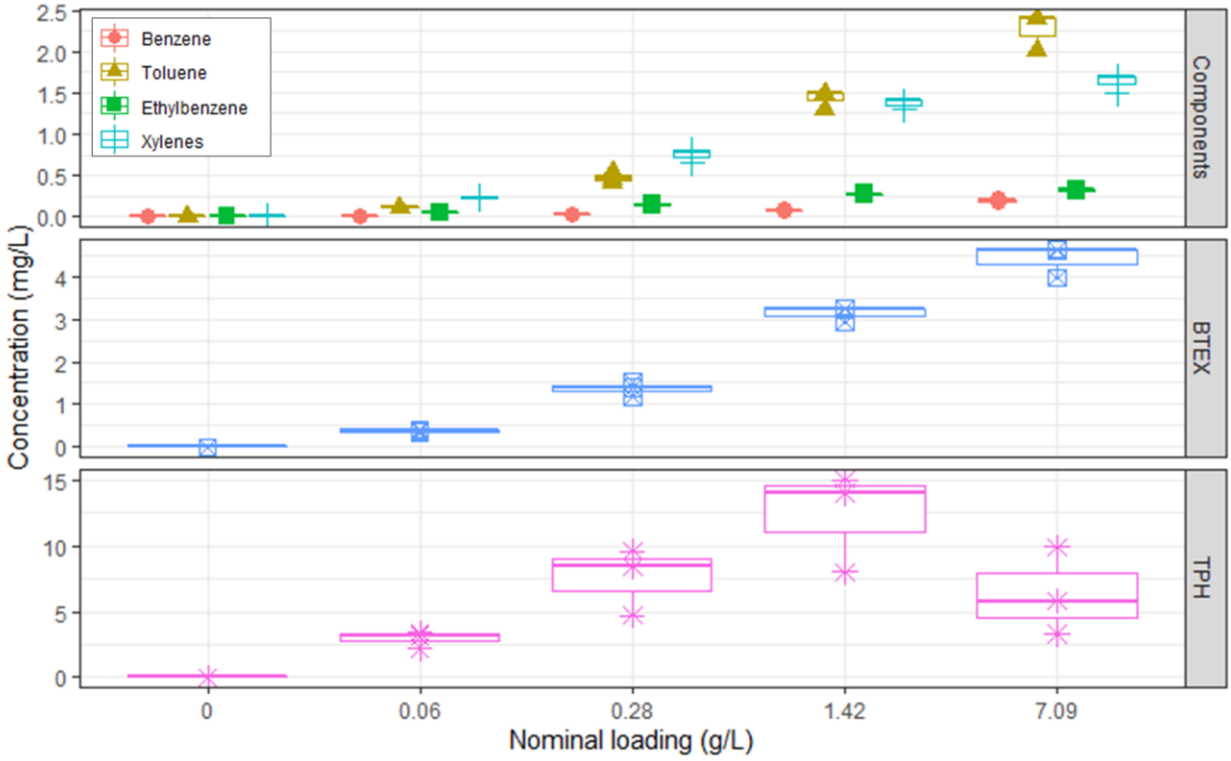


Figure 14: Concentrations of BTEX components (top), sum BTEX (middle) and TPH (bottom) from four different WAF loadings in three separate trials.

The results in Figure 14 demonstrate that 30-minutes mixing and no settling time was not sufficient to reach equilibrium when loadings are greater than 1.42 g/L. A nominal loading of 1 g of oil/L seawater (oil to water ratio of 1:1000) was selected for all subsequent trials based on these results. This loading is consistent with established practices (Adams et al. 2017) and not so large that the dissolution and equilibrium kinetics are limited and are able to occur within relatively short time frames (e.g., 1 hour).

Toxicological studies began in 2017 after the appropriate mixing conditions were determined that mimicked the SL Ross wave tank results as a proxy to offshore Newfoundland field conditions. A visual summary of the concentrations in the 2017 exposure solutions is presented in Figure 15.

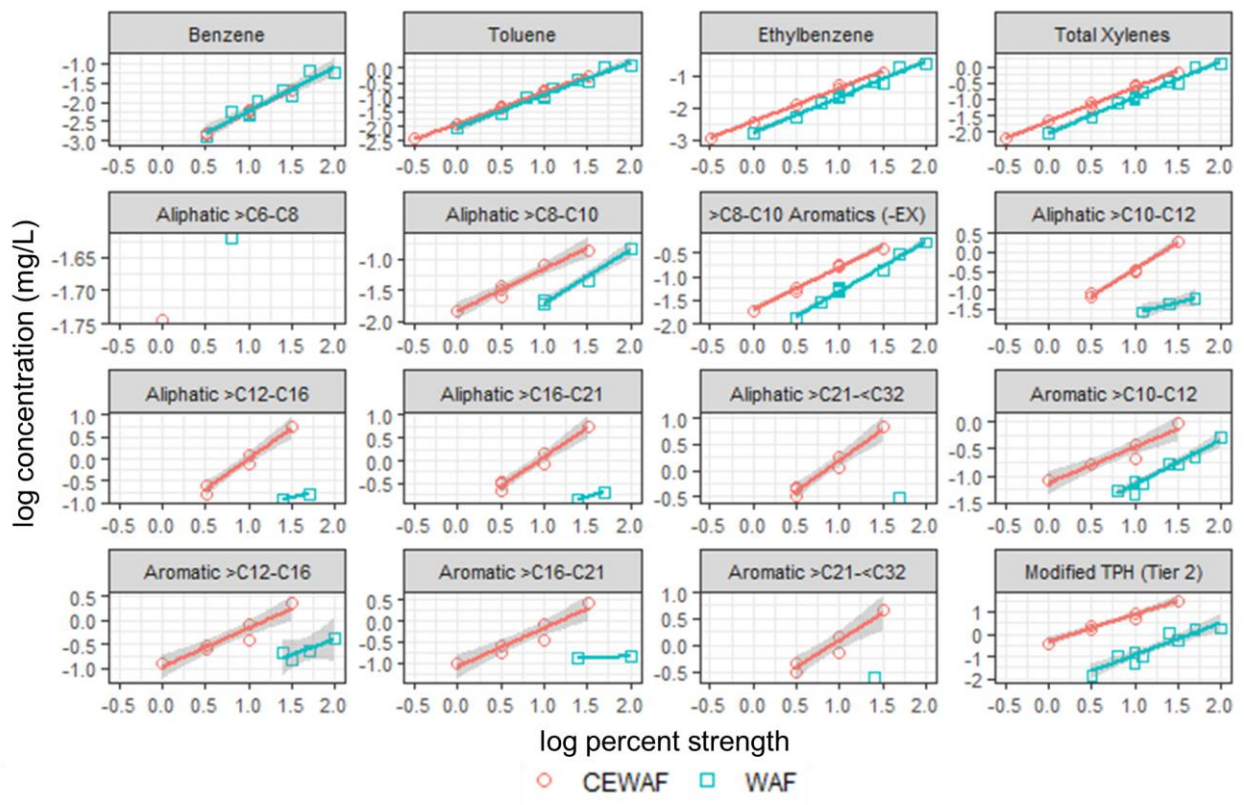


Figure 15: Measured concentration of analytes by nominal strength for CEWAF (red circles) and WAF (blue squares)

There was very good linearity between dilution strength and the measured concentrations for each analyte and preparation type. There was little difference between WAF and CEWAF preparations in terms of BTEX concentrations, while for every other measure at equal strength dilution, the CEWAF had greater measured concentration. These results highlight the importance of reporting effects based on measured concentrations and not the nominal percent strength or dilution when comparing toxicity between WAF and CEWAF.

Three separate WAF and CEWAF preparations were prepared on three different days to assess repeatability, with the 10% WAF and 3.2% CEWAF test solutions sent for analytical characterization (Maxxam Analytics, Bedford NS). The coefficient of variation for each analyte from three separate preparations is reported in Table 8.

Table 8: Coefficient of variation for three WAF and CEWAF preparations

Analyte	Coefficient of variation (CV%)	
	10% WAF	**3.2% CEWAF**
Benzene	3.61	6.67
Toluene	0.58	6.15
Ethylbenzene	5.59	4.56
Total Xylenes	9.09	9.76
BTEX	*4.53*	*7.90*
Aliphatic >C8-C10	8.66	18.15
>C8-C10 Aromatics (-EX)	7.19	11.27
Aliphatic >C10-C12	-	11.49
Aliphatic >C12-C16	-	22.77
Aliphatic >C16-C21	-	21.31
Aliphatic >C21-<C32	-	19.93
Aromatic >C10-C12	0.74	0.00
Aromatic >C12-C16	-	9.56
Aromatic >C16-C21	-	18.95
Aromatic >C21-<C32	-	21.26
Modified TPH (Tier 2)	*3.44*	*15.92*
Average CV	**4.82**	**12.85**

The preparations showed very good consistency, with all analytes in the WAF preparation having a CV less than 10%. The CEWAF results were more variable, but still demonstrated a consistent response, with an average CV of <15%. These results, combined with the consistency in the droplet profiles (Figure 12), support the use of a regression relationship for predicting TPH concentrations for each preparation in the absence of full analytical characterization.

In 2018, RPC (Fredericton, NB) provided more detailed and speciated chemical analysis results (PAH and alkylated PAHs) for the test solutions (Table 9).

Table 9: Chemical analysis of the exposure solutions from RPC (2018). Full report is attached in Appendix 2.

Analytes	RL (mg/L)	WAF (% strength)				CEWAF (% strength)			
		0	32	56	100	0*	18	32	56
Benzene	0.001	<0.001	0.017	0.03	0.057	<0.001	0.01	0.018	0.032
Toluene	0.001	<0.001	0.27	0.46	1.1	<0.001	0.2	0.47	0.76
Ethylbenzene	0.001	<0.001	0.052	0.089	0.21	<0.001	0.046	0.11	0.16
Xylenes	0.001	<0.001	0.27	0.47	1.1	<0.001	0.25	0.54	0.86
VPH C6-C10, less BTEX	0.01	<0.01	0.44	0.74	1.7	0.02	0.58	1.3	2.1
EPH >C10 - C16	0.05	<0.05	0.27	0.46	0.85	<0.05	2	3.3	6.3
EPH >C16 - C21	0.05	<0.05	0.06	0.09	0.18	0.1	1.3	2.2	4.2
EPH >C21-C32	0.1	<0.1	<0.1	<0.1	0.2	0.5	1.9	3.1	6
Modified TPH Tier 1	*0.1*	*<0.1*	*0.8*	*1.3*	*2.9*	*0.6*	*5.8*	*9.9*	*19*
Analytes	**RL (µg/L)**	**0**	**32**	**56**	**100**	**0***	**18**	**32**	**56**
Naphthalene	0.05	<0.05	46	80	150	<0.05	39	67	120
Acenaphthylene	0.01	<0.01	<0.1	<0.2	<0.5	<0.01	0.37	0.6	1.1
Acenaphthene	0.01	<0.01	<0.1	<0.2	<0.5	<0.01	0.45	0.7	1.3
Fluorene	0.01	<0.01	1.0	1.6	2.9	<0.01	4.1	6.8	12
Phenanthrene	0.01	<0.01	1.0	1.7	2.8	<0.01	8.2	14	24
Anthracene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	<0.05	<0.1	<0.1
Fluoranthene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.25	0.4	0.7
Pyrene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.38	0.7	1.2
Bz(a)anthracene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	<0.05	<0.1	<0.1
Chrysene/Triphenylene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	<0.05	<0.1	<0.1
Bz(b)fluoranthene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.10	0.2	0.3
Bz(k)fluoranthene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.10	0.2	0.3
Bz(e)pyrene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.60	1.0	1.8
Bz(a)pyrene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.06	0.1	0.1
Indenopyrene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	<0.05	<0.1	<0.1
Bz(g,h,i)perylene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.08	0.2	0.3
Dibz(a,h)anthracene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	0.05	<0.1	0.2
C1-Naphthalenes	0.1	<0.10	25	42	78	<0.10	47	90	160
C2-Naphthalenes	0.1	<0.10	8.0	13	25	<0.10	47	88	160
C3-Naphthalenes	0.1	<0.10	1.5	2.2	4.0	<0.10	23	41	79
C1-Phenanthrenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	9.5	18	32
C2-Phenanthrenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	10	19	32
C3-Phenanthrenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	4.3	7.9	13
Dibenzothiophene	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	<0.5	<0.1	<1.0
C1-Dibenzothiophenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	1.7	3.1	5.8
C2-Dibenzothiophenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	1.1	2.0	3.6
C3-Dibenzothiophenes	0.1	<0.10	<1.0	<2.0	<4.0	<0.10	<0.5	<1.0	<1.0
1-methylnaphthalene	0.05	<0.05	22	37	66	<0.05	39	67	120
2-methylnaphthalene	0.05	<0.05	19	33	60	<0.05	33	57	100
Perylene	0.01	<0.01	<0.1	<0.2	<0.4	<0.01	<0.05	<0.1	<0.1
Biphenyl	0.05	<0.05	3.1	5.1	9.3	<0.05	7.9	15	27

*Corexit control applied at same concentration as highest strength CEWAF solution tested, 56%

As with the 2017 data, there was good linearity between the nominal strength dilutions and the measured concentrations. The CEWAF preparations generally had higher measured concentrations than the paired WAF dilutions with the exception of the BTEX compounds. In the WAF preparations, the majority of the PAHs were at or below the detection limits, whereas they were more frequently detected in the CEWAF preparations.

The chemistry results of the 2017 (Maxxam) and 2018 (RPC) sampling events were compared to assess any changes over time with respect to TPH (Figure 16).

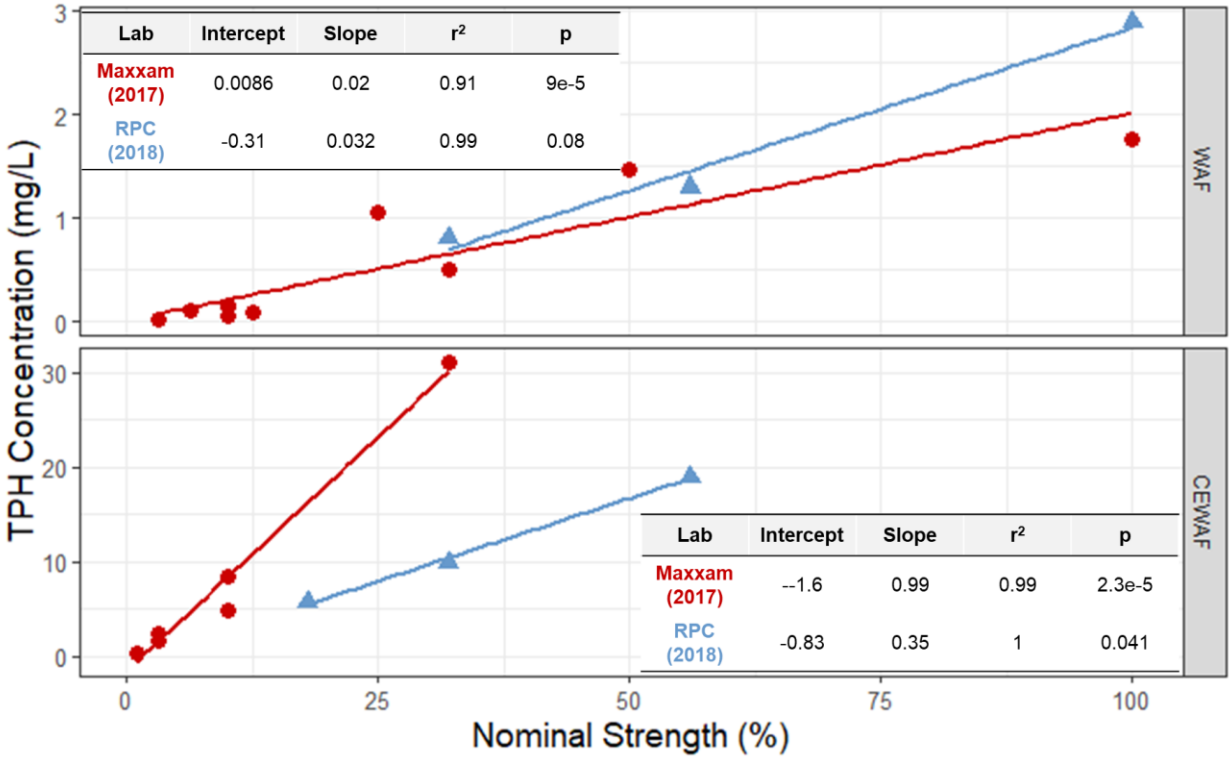


Figure 16: Nominal dilution strength of WAF (top) and CEWAF (bottom) and the measured concentrations from Maxxam (red circles, 2017) and RPC (blue triangles, 2018)

There were differences between the magnitude of concentrations between the two laboratories (and years) while each year demonstrated good linearity with TPH. RPC (2018) had lower concentrations in the CEWAF as compared to the same dilutions of CEWAF measured by Maxxam in 2017. This may speak towards the reduced efficacy of the dispersant over time from a single opened bottle, as the differences between the WAF were less pronounced. These regression curves were assumed to be representative and were used for estimating TPH concentrations for exposures in each year.

The preliminary work with the biomimetic extractions (BE) showed excellent linearity for the WAF and CEWAF dilutions (Figure 17).

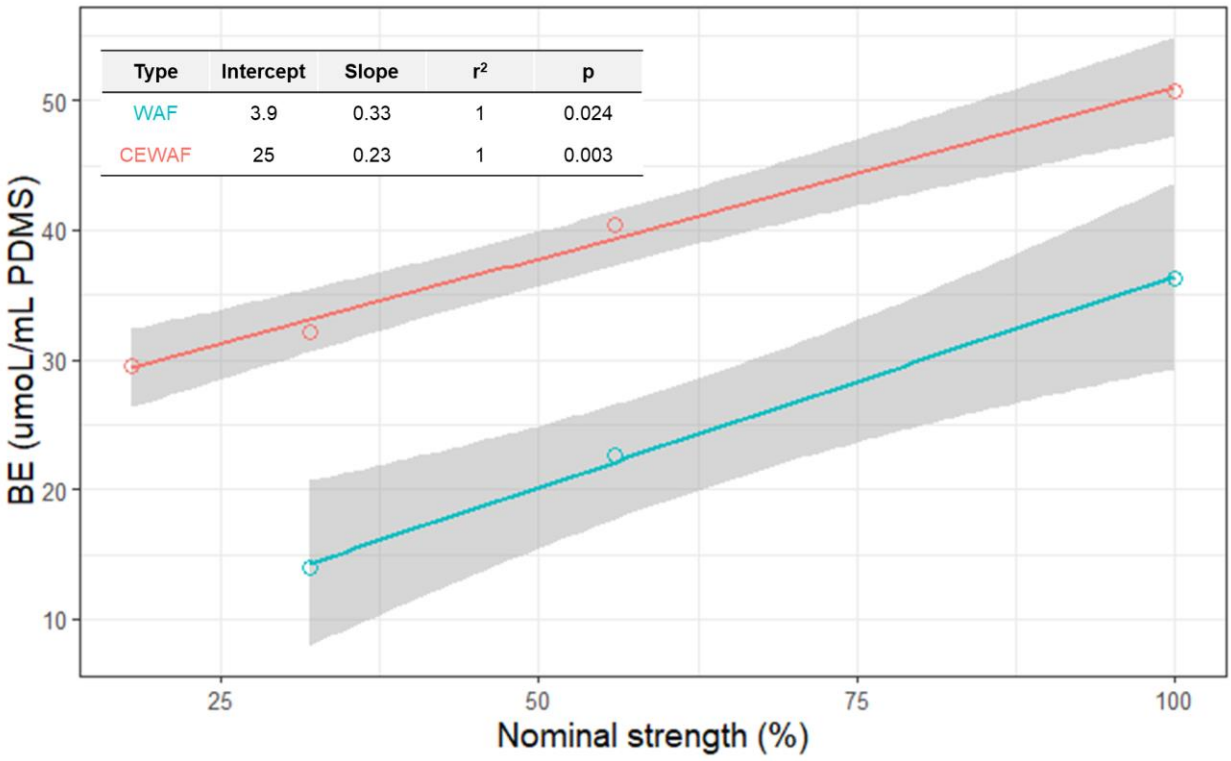


Figure 17: SPME fiber concentrations by the nominal strength of WAF (blue circles) and CEWAF (red circles)

The fiber concentrations in Figure 17 demonstrate that the CEWAF concentration is ~2 times greater at the same dilution strength due to the increased availability of hydrocarbons from the action of the dispersant.

3.4.4 Toxicity results

Our method of 1-hour mixing on an orbital shaker with a 1-hour settling time is a departure from the traditional mixing method of slow stirring for 20-hours with 4-hours settling time. We performed a 24-hour acute bioassay with larval cod to compare the results of a WAF and CEWAF that had been mixed for 1- or 24-hours (Figure 18).

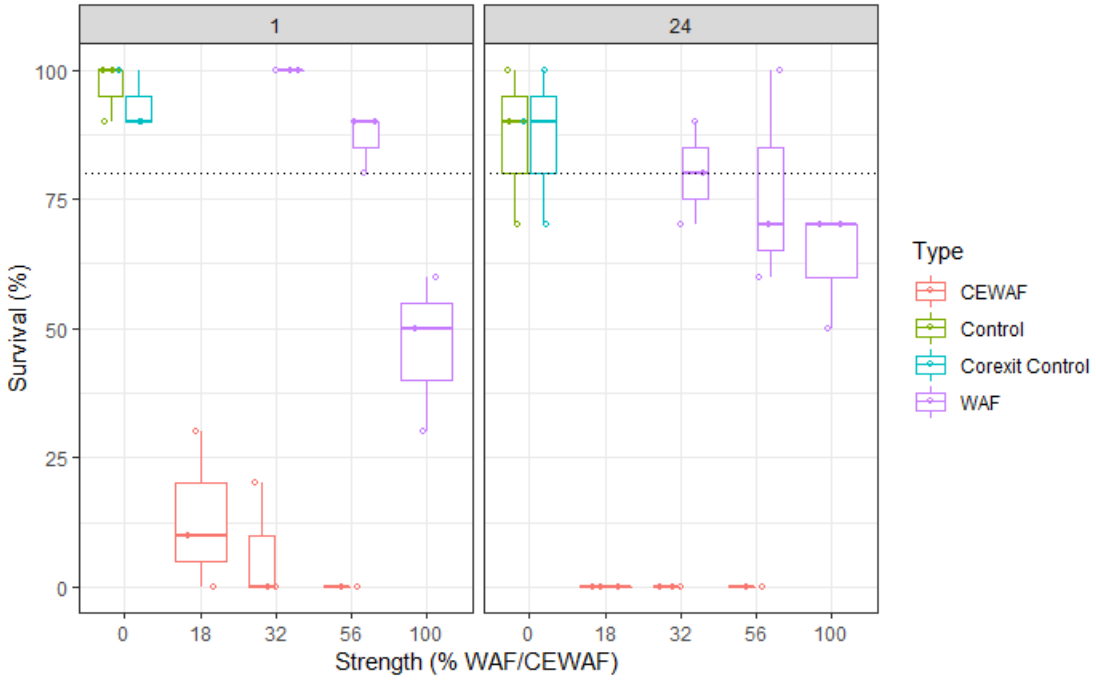


Figure 18: Survival results from larval cod exposed for 24-hours to dilutions of WAF and CEWAF that had been mixed for 1 (left) or 24 hours (right).

The NOEC and LOECs for each the WAF and CEWAF were calculated for the 1 and 24 hour mixes. The mixes had the same NOEC and LOEC for regardless of mix duration for both the WAF (NOEC = 100%, LOEC >100%) and CEWAF (NOEC < 18%, LOEC = 18%).

The nominal strength results were converted to TPH (mg/L) and the concentration response relationship was modelled (responses in WAF and CEWAF combined) and the LC50 was calculated for each mixing duration (Figure 19).

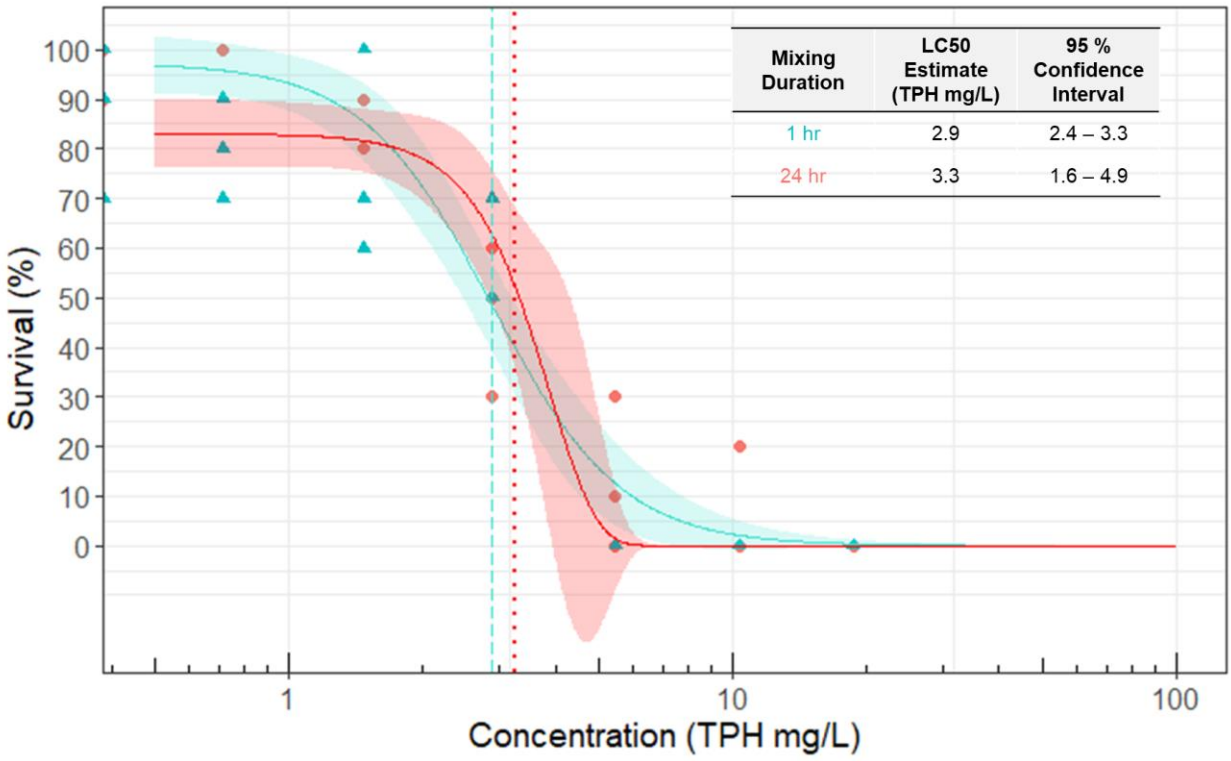


Figure 19: Concentration response relationship for larval cod exposed to dilutions of WAF and CEWAF that were mixed for 1 (blue) or 24 hours (red). The vertical dashed lines are the estimated LC50 values

There was no significant difference (p = 0.618) between the LC50 estimates from each mixing duration. These results support the use of the shorter mixing time for use in acute and short-term exposure toxicology studies involving crude oil WAF and CEWAF exposure media.

3.5 Discussion

During the Deepwater Horizon spill response effort, research scientists and technicians from the Centre for Offshore Oil Gas and Energy Research (COOGER), Fisheries and Oceans Canada (DFO) were requested by the U.S. government and BP to conduct at-sea monitoring. From 8-May to 24-August-2010, the DFO COOGER team maintained a continuous monitoring program that recovered a total of 3,197 unique water samples from 404 stations at depths down to 2000 m for analysis of dispersant effectiveness by characterizing oil droplet size using a LISST (Li et al. 2011). Their results, along with the mean droplet sizes from the SL Ross wave tank experiments, and a collection of our laboratory prepared WAF and CEWAF solutions are shown in Figure 20.

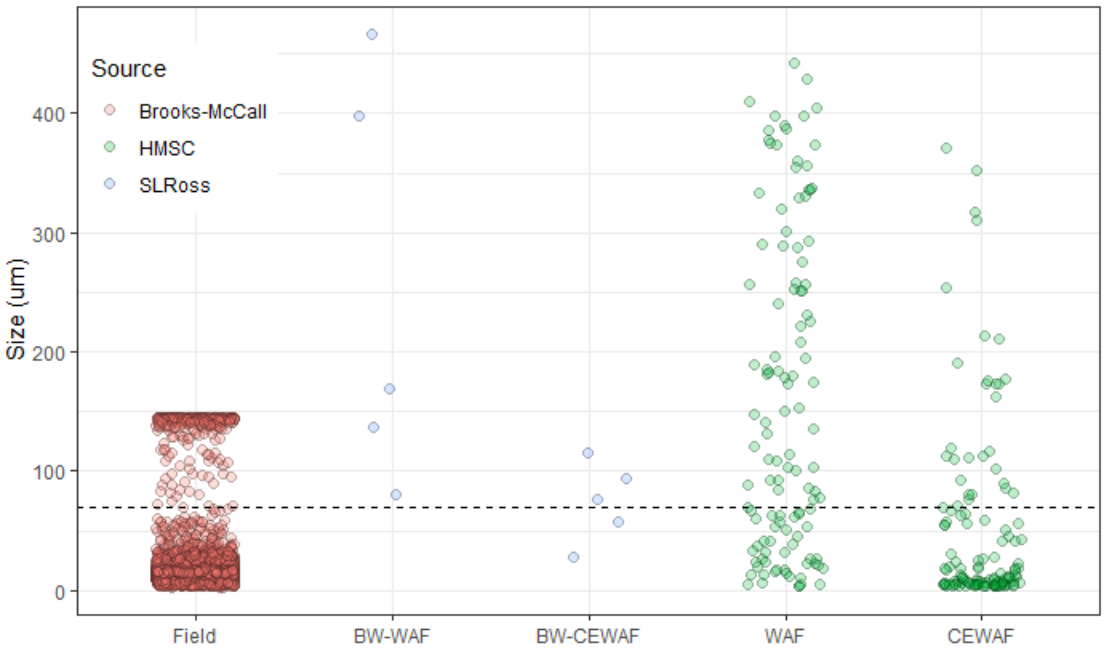


Figure 20: Summary of mean droplet sizes collected from field studies (Brooks-McCall, red), wave tanks (SL Ross, blue), and laboratory preparations (Huntsman, green). The horizontal dashed line is at 70 µm, where droplets are considered to be permanently dispersed.

Due to a misalignment issue with the COOGER LISST, the larger particle size bin data was deemed unreliable and was rejected, which is why the values are cut-off at 150 µm. The breaking-wave data (BW-WAF and BW-CEWAF) from SL Ross are ordered from top to bottom by time post-first wave contact and indicate a progression towards the neutrally buoyant, permanently dispersed droplet size of 70 µm. The droplet sizes generated under our laboratory mixing conditions are reflective of what may be expected to be observed in real-world scenarios, however every situation will be different and result in differing profiles and sizes.

Characterization of exposure media is crucially important to ensure the reliability and comparability of results generated from toxicology studies. Our results demonstrate that crude oil may remain stable and provide consistent analytical results when prepared as a WAF when proper storage and handling is taken. The stability and shelf-life of our dispersant likely waned during this research program (possibly due to having only a single bottle of dispersant that was opened multiple times throughout the program, which is not what would be experienced for properly stored stockpiles of dispersant) as we saw

decreased concentrations (both in droplets and measured analytes) year over year. However, within each specific year we saw consistent results for WAF and CEWAF prepared at the same temperature over a period of two years.

Based on the studies described above we proceeded with a consistent exposure media preparation method that is summarized in mixing method Table 10.

Table 10: Summary of mixing conditions used to generate exposure media

Parameter	**Condition**
Dilution water	Natural seawater, filtered to 0.22 µm
Oil loading	1 g oil per 1 L water
Dispersant to oil ratio	1:20
Mixing vessel	2-L baffled flask
Headspace	20%
Mixing energy	150 rpm
Mixing Duration	1 hour
Settling Duration	1 hour
Generation of test solutions	Discard first 100 mL, refrain from using last 100 mL. Gradient dilution

Atlantic cod (*Gadus morhua*)

Definitions

- **BSD:** blue-sac disease; phenotypic presentation of an assemblage of developmental abnormalities characterized by vertebral abnormalities, edemas, hemorrhaging, reduced growth, and survival
- **CTLBB:** Critical target lipid body burden
- **CYP1A:** Cytochrome P450 1A subfamily
- **Dam**: the female parent of an animal
- **Degree days/Accumulated thermal units**: unit of measurement used to describe the cumulative effect of temperature over time. A thermal unit is one degree for a 24-hr period. For example, if a cod embryo is exposed to 5oC water temperature for 24-hr period, they have gained 5 thermal units. The accumulation of thermal units over time are referred to as degree days
- **Edema**: swelling caused by the buildup of fluid
- **Eggs**: fully ripe, unfertilized internal egg masses in the ovaries, or the released external egg masses of fish and certain marine animals
- **Embryo**: the fertilized, and developing egg
- **Family/Cross**: offspring that carry part of the genetic material from the same two individuals
- **Full-sibling**: individuals sharing the same dam and sire
- **Half-sibling**: individuals sharing one of either the same dam or sire
- **Heritability**: fraction of the total variation observed in a trait that can be accounted for by genetics
- **Milt**: the semen of a male fish
- **Sire**: the male parent of an animal

Background

Atlantic cod has been a commercially important fish species in the North Atlantic for centuries, in capture fisheries and recently as an aquaculture species (Puvanendran et al. 2008). The Huntsman has extensive experience in cod aquaculture and was able to translate those procedures and practices to maintain adult cod and acquire high quality gametes for use in toxicity studies. Atlantic cod (n = 30; 20 females, 10 males) were captured using longline from offshore Nova Scotia, Canada in October 2016 then held within Huntsman wetlab facilities. Holding and husbandry of the cod broodstock received animal care approval from the Regional Animal Care Committee and followed Huntsman Standard Operating Procedures (SOPs) for Animal Use and Care Ethics. The broodstock

individuals were held within two 7.5 m^3 tanks with a single pass through of natural seawater at ambient salinity (28 - 32 psu) and temperature (Figure 21).

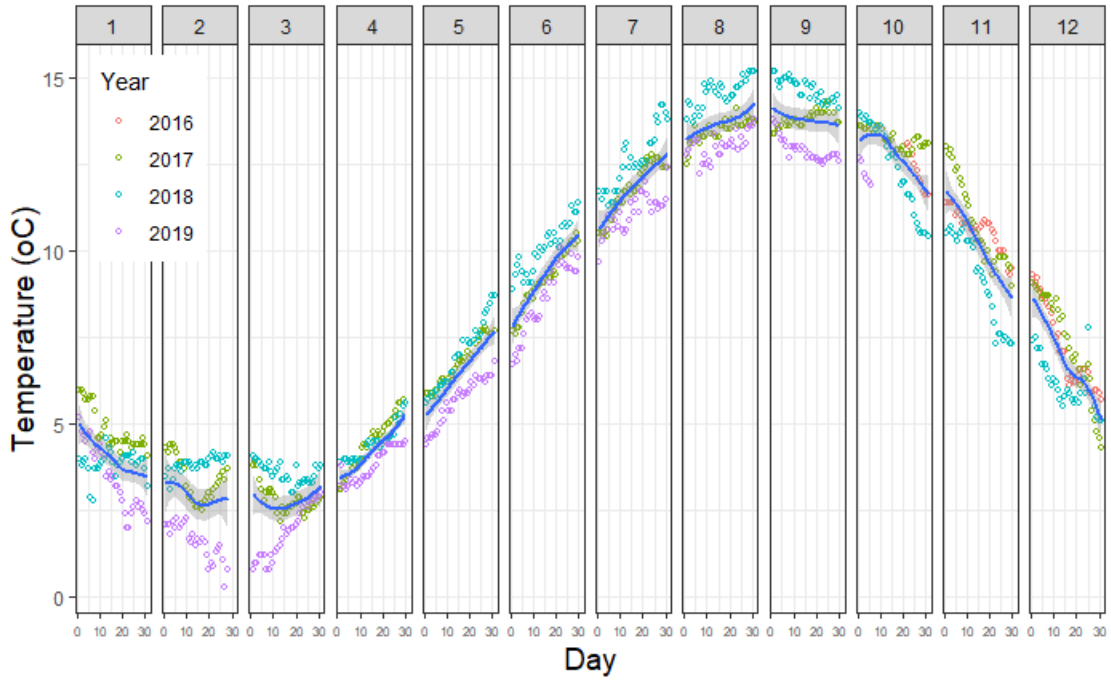


Figure 21: Ambient temperature in the Atlantic cod broodstock tanks by year (colour) and month (panels).

Daily husbandry routines (7 days a week and all holidays) included: water quality measurement (water flow, temperature, dissolved oxygen concentration), feed administered by hand (wild food diet consisting primarily of squid, shrimp, and herring) and daily visual observations with variations from normal behavior recorded daily.

After acclimatization to culture conditions, individual cod were tagged with Passive Integrated Transponders (PIT) in the left side of the dorsal region to allow individual fish tracking and recording of morphometric data. Fin clips were also taken and sent for genotyping to estimate the relatedness based on six microsatellite markers (GMO19, GMO37, GM08, PGMO38, TCH11, and TCH5). The Fortran program, **MER** (Moment Estimate of Relatedness), based on the moment methods developed by Wang et al (2002), was used to estimate the 2- and 4-gene relatedness coefficients between 2 individuals from codominant genetic markers. Bootstrapping over loci was used to estimate standard deviations of the estimates. The relatedness values between each fish are summarized in Figure 22, with those pairings having relatedness values between -

0.2 and 0.2 preferred when spawning to minimize the likelihood for inbreeding that might affect survival results.

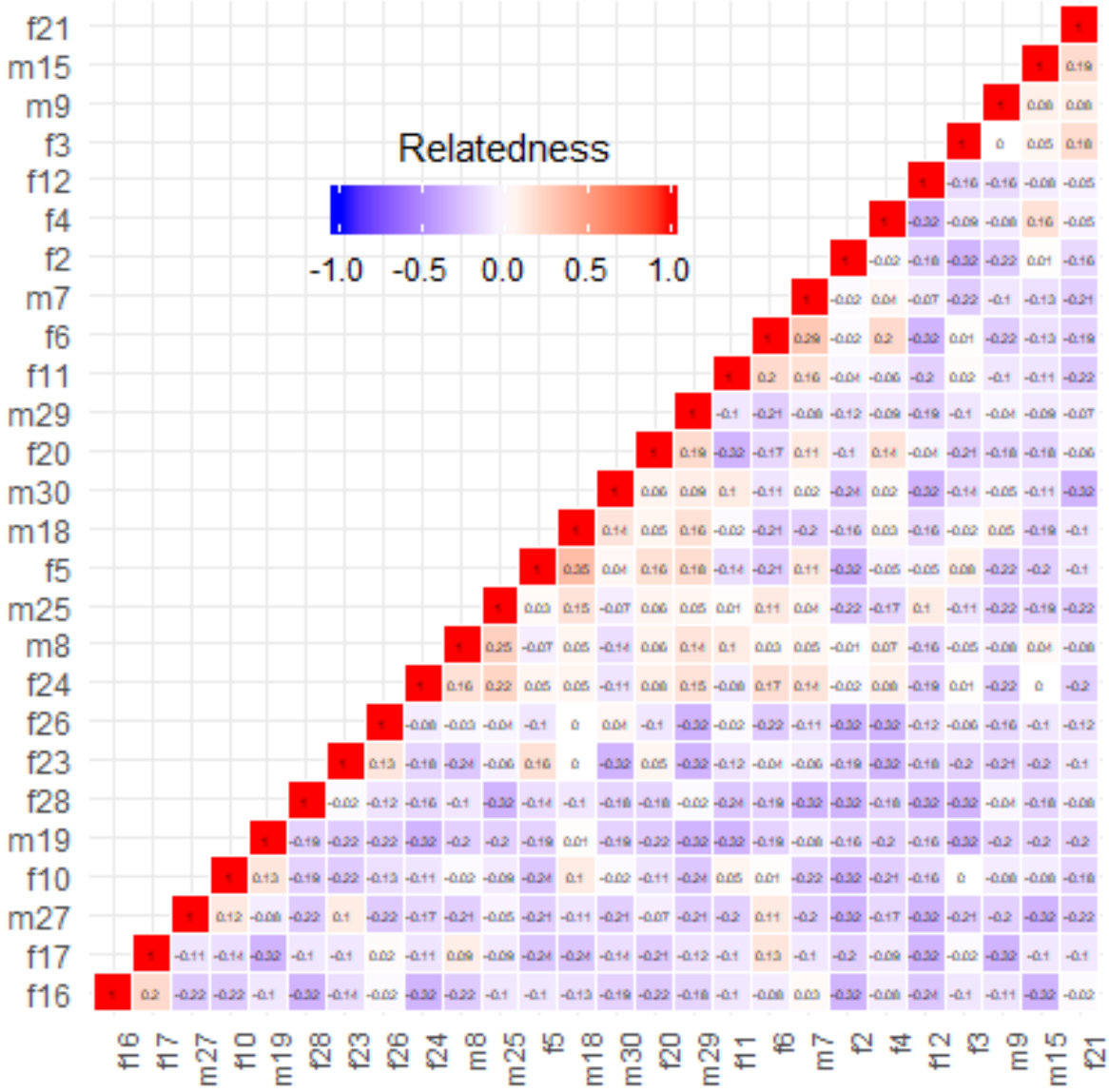


Figure 22: Relatedness values between each cod with values between -0.2 and 0.2 preferred for spawning.

The maturity status of each fish was tracked through visual examination and in the case of females through biopsy of the oocytes. For biopsy and spawning, fish were anesthetized with TMS (tricaine methanesulfonate, 100 ppm) in a separate seawater holding tank with Vidalife stresscoat added to the seawater to protect the mucous layer during handling. The biopsied oocyte diameter was determined and the days to ovulation calculated using the equation:

$y = 4.41*10^{6}\ x^{-1.817}$, from Kjesbu 1994

Where y is the time to ovulation in days and x is the mean egg diameter (mm).

Fish were spawned according to Huntsman SOPs (Figure 23A) and the collected gametes screened for suitability for crossing. Collected eggs were first screened tank-side using a float test to estimate viability by measuring the buoyant floating fraction of eggs (Figure 23B).

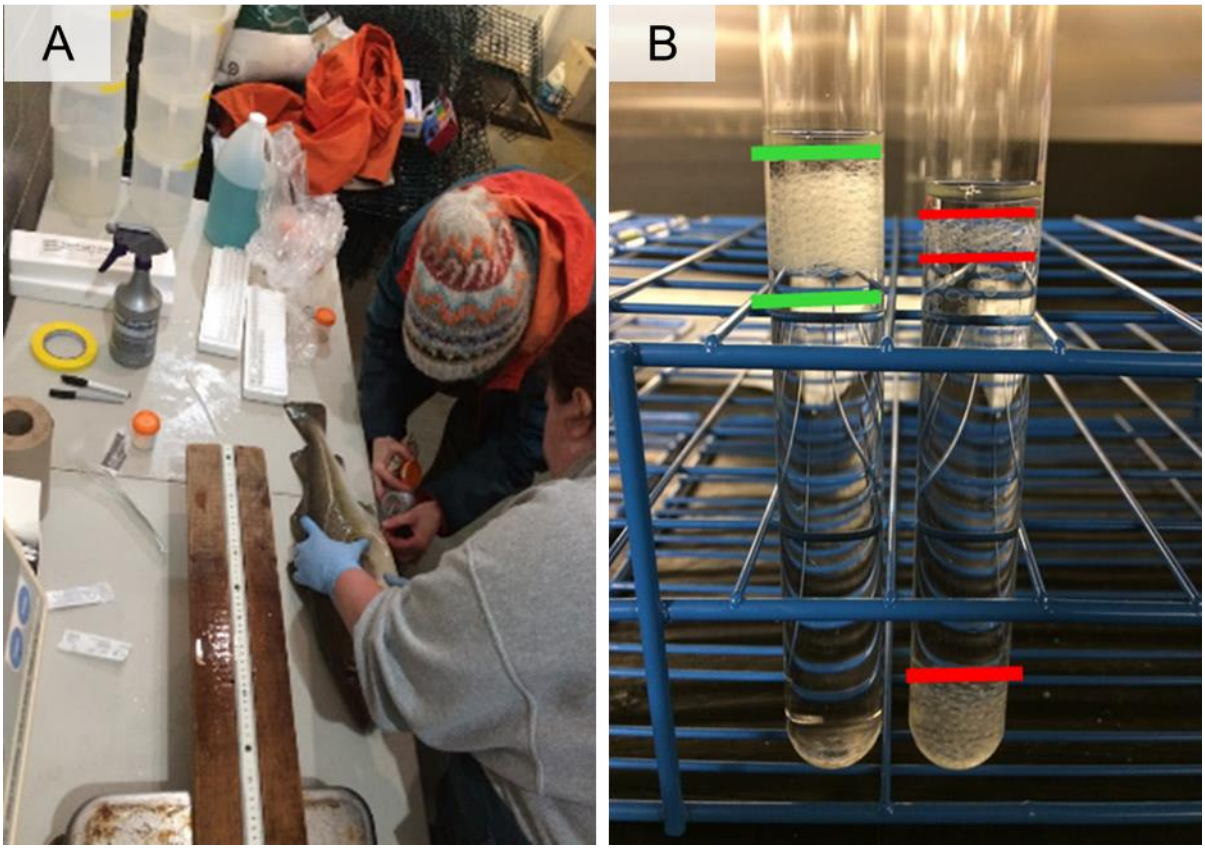


Figure 23: A) Collection of Atlantic cod gametes. B) tank side float test showing a batch of viable (green bands) and non-viable (red bands) eggs.

A subsample from the viable eggs (at least 100 eggs) was collected from the buoyant fraction then imaged at 5.8X magnification using the Leica Application System Software (version 4.8) mounted on the Leica Wild M420 microscope to measure egg diameter. The number of eggs per mL was determined and used when performing the test crosses in order to ensure an optimal sperm to egg ratio.

Milt was collected separately from individual males in clean, dry 120 mL specimen cups using disposable catheters to avoid urine and fecal contamination. Each milt sample had motility visually confirmed then the additional metrics of spermatocrit (Kimble Chase plain capillary tubes I.D. 1.1-1.2 mm sealed with Leica critoseal, spun for 10 minutes on an IEC micro-MB centrifuge and read with an IEC microcapillary reader) and sperm density (Minitube SDM6 milt photometer) were collected.

Fertilizations were performed dry (e.g., addition of sperm directly to eggs prior to the activation in seawater) with the target of 1,000,000:1 sperm to egg ratio. Toxicology exposures were performed on various early life stages of the Atlantic cod (e.g., individual gametes, during fertilization, embryogenesis, hatching, and/or larval fry) and husbandry conditions facilitated access to all of these life stages when required.

The toxicology trials involving Atlantic cod are described in Chapters 4-7.

Chapter 4 Effects of physically and chemically dispersed crude oil on the fertilization of Atlantic cod (*Gadus morhua*)

4.1 Introduction

Atlantic cod (*Gadus morhua*) have planktonic gametes, which are released into the environment from females and males in relatively close proximity of each other to maximise the opportunity of the gametes mixing and achieving fertilization. Fertilization success is determined by the intrinsic quality and quantity of gametes and the environmental conditions upon which the gametes are released. Gametes may be particularly vulnerable to contaminant exposure as sperm and egg cells have undeveloped metabolic systems and lack the ability to avoid exposures through swimming.

Male fitness is assessed by fertilization success, i.e. the male's contribution to the next generation. The concentration of spermatozoa in milt indicates the quantity, and to some extent the quality, of the milt from a particular male and may be linked to fertilization success. Concentration, or density, is easily measured through counting, spectrophotometry, or determining spermatocrit values. Most fertilizations occur within seconds of gamete release in nature and sperm swimming speed is therefore expected to be linked to fitness (Purchase et al. 2010), with fast-swimming sperm being more likely to encounter the micropyle of an egg for fertilization, than slow-swimming sperm (Trippel and Neilson 1992). Sperm characteristics, such as swimming velocity, are measureable using microscopy and computer software (e.g., computer-assisted sperm analysis, CASA), and can provide insight into alterations in swimming behaviour with contaminant exposure. The ecological significance of contaminant exposure to spermatozoa can range from alterations in swimming behaviour and reduced motility to a complete loss of fertilizing ability (Hatef et al. 2013).

The quality of the fish egg can be defined as the ability of the egg to be fertilized and subsequently develop into a normal embryo (Bonnet et al. 2007). The potential of an egg to produce a viable and normal embryo can be impacted by many environmental (e.g., diet, exposure to environmental contaminants) and biological (e.g., maternal hormone)

factors. Non-viable eggs are often discoloured, dimpled, over-ripe, or negatively-buoyant and are not able to be fertilized (McEvoy (Barton) 1984). The presence of ovarian fluid surrounding released eggs can affect sperm swimming (Beirão et al. 2014). It also serves to maintain close contact between gametes, reducing dispersion and maintaining an ionic concentration optimal for fertilization. Disruption of the ovarian fluid microenvironment by exposure to contaminants may lead to reduced fertilization success.

Fertilization success is the ultimate measure of male and female fitness. In Atlantic cod, the act of fertilization is the end result of a complex lekking mating system, whereby the males perform elaborate courtship displays (e.g., circling, producing sounds, fin displays) before a ventral mount results in the broadcast release of the sperm and eggs (Zemeckis et al. 2014). The nucleus of the egg cell is located close to the periphery, often beneath the micropyle, and following sperm entry the sperm nucleus condenses inside the egg and the two nuclei fuse to form the zygote nucleus (Hall et al. 2004). Upon fertilization, the embryonic life stage is still vulnerable to contaminant exposure as it remains free-floating in the water column and is undergoing key developmental processes. Abnormal blastomere cleavage (e.g., asymmetry, poor cellular adhesion, poor differentiation of margins) has been linked with mortality during embryogenesis, low hatching success, and larval abnormalities in serial-spawning marine fishes, including Atlantic cod (Avery et al. 2009; Kjorsvik and Lonning 1983).

A series of trials were undertaken to examine the effects of exposure to physically and chemically dispersed crude oil around the fertilization window in order to understand the vulnerability of Atlantic cod during this earliest life stage. The objectives of these trials were to determine the impact that exposure had on gamete performance (expressed as ability to fertilize or be fertilized), fertilization rates (including presence of abnormalities), and hatching and larval success.

4.2 Methods

All fertilization activities took place within a controlled environment room (4.5^{o}C +/- 1^{o}C) in the Christofor Research Laboratory at the Huntsman Marine Science Centre (St. Andrews, New Brunswick). All glassware was pre-cleaned (solvent rinsed with DCM,

methanol, acetone, hexane), dried, and then equilibrated to the same temperature as the environment room prior to use.

4.2.1 Gamete Exposures

Atlantic cod gametes (milt and eggs) were separately exposed and their ability to successfully fertilize was assessed using unexposed gametes. The gametes were collected from anesthetized (with 100 mg/L tricaine methanesulfonate) broodstock according to Huntsman Standard Operating Procedures. Each milt sample was characterized for density using a spermatocrit apparatus (Kimble Chase plain capillary tubes I.D. 1.1-1.2 mm sealed with Leica critoseal, spun for 10 minutes on an IEC micro-MB centrifuge and read with an IEC microcapillary reader) and a photometer (minitube SDM6 milt photometer) before motility was verified under a microscope. Milt swim performance videos were captured using a microscope (Olympus BH2) fitted with a camera (MC-190HD) set at 40x objective following exposure to test solutions. Test solutions were dilutions of WAF (water accommodated fraction of 1, 3.2, 10, 32, 100%), CEWAF (chemically enhanced water accommodated fraction of 0.32, 1.0, 3.2, 10, 32%), seawater control (bookended), and dispersant only control (applied at equal ratio as to the highest tested concentration of CEWAF; nominally 15 mg/L), each replicated three times. Swim performance exposures used 70 µL of a milt-seminal fluid suspension (70 µL of milt diluted in 2 mL of seminal fluid) transferred into 1 mL of exposure solution in a 25-mL glass scintillation vial and gently mixed by inversion for 5 seconds. From the milt exposure solution, 5 µL was added to a microscope slide and at 1-minute post-activation the sperm activity was recorded for 30 seconds. The sperm performance data was analysed using ImageJ (https://imagej.nih.gov/ij/index.html) with the plugins CASA (https://imagej.nih.gov/ij/plugins/casa.html) (Wilson-Leedy and Ingermann 2007) and CASA automated (http://www.ucs.mun.ca/~cfpurchase/publications.html) (Purchase and Earle 2012) as previously described by Beirão et al. (2018). The video files were imported into ImageJ and processed (e.g., converted to 8-bit, adjusted contrast, threshold adjustment) to facilitate running the CASA automated plugin with the settings listed in Table 11.

Table 11: CASA settings

Parameter	Input Value	Parameter	Input Value
Minimum sperm size (pixels)	2	Maximum percentage of path with low VAP	25
Maximum sperm size (pixels)	10	Low VAP speed 2 (um/sec)	10
Minimum track length (frames)	50	Low VCL speed (um/sec)	15
Maximum sperm velocity between frames (pixels)	10	High WOB (percent VAP/VCL)	80
Minimum VSL for motile (um/sec)	4	High LIN (percent VSL/VAP)	80
Minimum VAP for motile (um/sec)	20	High WOB 2 (percent VAP/VCL)	50
Minimum VCL for motile (um/sec)	25	High LIN 2 (percent VSL/VAP)	60
Low VAP speed (um/s)	2	Frame Rate (frames per second)	17
Maximum percentage of path with zero VAP	1	Microns per 1000 pixels	4400

Output from the CASA included the following measures: percent motility, velocity curvilinear (VCL), velocity average path (VAP), velocity straight line (VSL), linearity (LIN), wobble (WOB, calculated as VAP/VCL), progression (PROG), beat cross frequency (BCF), and number of sperm tracked.

A 0.1 mL aliquot of milt was added to 0.5 mL of exposure media in a 25-mL glass scintillation vial to determine the ability of exposed milt to fertilize unexposed eggs. Exposure media included dilutions of WAF (1, 10, 100%), CEWAF (0.32, 3.2, 32%), seawater control (bookended), and dispersant only control (applied at the rate equal to the highest tested concentration of CEWAF), for a total of nine treatments completed in triplicate. The addition of milt into each exposure solution was staggered by 5 min with a seawater control solution bookended at the beginning and end of the order to ensure effect not erroneously attributed to the highest concentration but accounting for possible poor sperm quality over time. A 0.5 mL volume of un-activated eggs (from a single female cod) was added to each replicate (15-mL Falcon tube), which would achieve a target sperm to egg ratio of 1,000,000:1. After 20 minutes (+/- 1 minute) of exposure, 360 µL of

the milt exposure media solution was added to the eggs then 14 mL of 0.22 µm filtered seawater was added to the Falcon tube. The effect of exposure on the ability of eggs to be fertilized followed similar methods, with the eggs receiving a 20-minute exposure, then being gently rinsed on an 800 µm mesh screen prior to the addition of unexposed milt in a 15-mL Falcon tube with 14 mL of filtered seawater. After 7 hours, the units were imaged to assess for fertilization and in the case of the egg exposures the units were transferred to ongoing monitoring until hatch.

4.2.2 Fertilization Exposures

Eggs and milt were collected from individual cod and individual crosses were identified based on buoyancy screen of the eggs, density readings of the milt, and genetic relatedness information (Figure 22). The fertilization procedure involved adding 1 to 5 mL of eggs to 125-mL flask. The flasks were loaded on the orbital shaker (MaxQ 2000, Thermo Scientific). A volume of milt required to achieve a nominal 1,000,000:1 sperm to egg ratio was added to the flask, immediately followed by the addition of exposure media (Table 12) at a volume equal to 80% of the total volume (e.g., 100 mL in 250-mL flask) to each flask. Addition of eggs, milt, and exposure media to each flask was completed within approximately 30 seconds. The flasks were loaded on anorbital shaker and mixed for 7 hours at 120 rpm. After the fertilization and mixing period, the embryos were collected on an 800 µm mesh screen and rinsed with seawater while keeping the embryos on the mesh submerged. The embryos were then transferred to pre-labelled 15-mL Falcon Tubes filled with seawater (filtered to 0.22 µm). The embryos were allowed to settle for 30 minutes before collecting an aliquot to assess fertilization (minimum 100 embryos/eggs required).

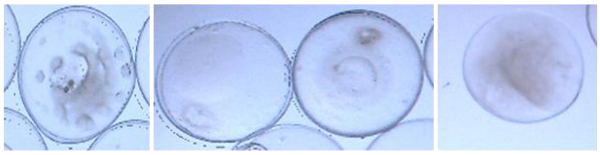
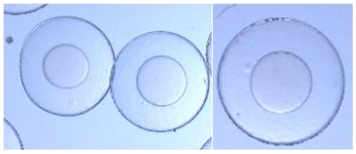
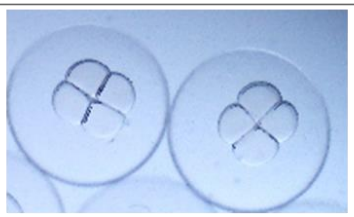
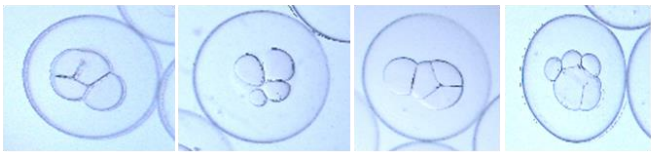
Table 12: Summary of trials to explore effects of exposure around the fertilization window

Cross (♀ x ♂) Trial	Relatedness	Sperm:Egg Ratio	Exposure Type and Loading	Duration	Treatments	Endpoints
5 x 27 GM-016	-0.21	1000000	Milt; 900 eggs per unit	20 minutes	WAF (100, 10.0, 1.00%) CEWAF (32, 3.2, 0.32%) Seawater (bookending exposure series) Corexit control (15 mg/L)	Fertilization, Abnormal fertilization
11 x 18 GM-023	-0.0156	555544	Eggs; 375 eggs per unit	20 minutes	WAF (100, 10.0, 1.00%) CEWAF (32, 3.2, 0.32%) Seawater (bookending exposure series) Corexit control (15 mg/L)	Fertilization, hatch
20 x 8 GM-004	0.059	1000000	Fertilization; 400 eggs per unit	7 hours	WAF (100, 32, 10.0, 3.2, 1.00%) CEWAF (32, 10.0, 3.3, 1.00, 0.32%) Seawater Corexit Control (15 mg/L)	Milt performance, Fertilization, Abnormal fertilization, Hatch
12 x 15 GM-009	-0.079	1000000	Fertilization; 1425 eggs per unit	7 hours	WAF (100, 32, 10.0, 3.2, 1.00%) CEWAF (32, 10.0, 3.3, 1.00, 0.32%) Seawater Corexit Control (15 mg/L)	Fertilization, Abnormal fertilization, Hatch
12 x 7 GM-013	-0.07	1000000	Fertilization; 500 eggs per unit	7 hours	CEWAF (31.6, 10.01, 3.17, 1.00, 0.32%), Seawater (bookending exposure series), Corexit Control (15 mg/L)	Fertilization, Abnormal fertilization, Hatch
12 x 27 GM-015	-0.32	1000000	Fertilization; 500 eggs per unit	7 hours	WAF (100, 31.6, 10.01, 3.17, 1.00%), Seawater (bookending exposure series)	Fertilization, Abnormal fertilization, Hatch

4.2.3 Assessments

Fertilization assessments were made using images taken from the microscope (Leica Wild M420) using 5.8x objective magnification. The eggs/embryos were counted and categorized by class listed according to Table 13.

Table 13: Fertilization Assessment Criteria

Class	Description	Example
1	Non viable: opaque, discolored, oversized, blown-out	
2	Unfertilized: no cleavage, well defined nucleus	
3	Normal fertilized: 4 blastomeres of equal size and symmetric	
4	Abnormal fertilization: signficany asymmetry and/or uneven size	

The percent viable eggs were determined by:

$$\frac{\Sigma(Class\ 2 + Class\ 3 + Class\ 4)}{\Sigma(Class\ 1 + Class\ 2 + Class\ 3 + Class\ 4)} * 100$$

The non-viable eggs usually account for 5% of the eggs (Trippel et al. 2005). Percent fertilized embryos was determined by:

$$\frac{\Sigma(Class\ 3 + Class\ 4)}{\Sigma(Class\ 2 + Class\ 3 + Class\ 4)} * 100$$

The percent of fertilized embryos that displayed abnormal phenotypes was determined by:

$$\frac{Class\ 4}{\Sigma(Class\ 3 + Class\ 4)} * 100$$

At 24-hrs post fertilization, the top buoyant fraction of each Falcon Tube was photographed, for later enumeration, using a Canon EOS 20D tripod setup then transferred to a 600-mL beaker that was brought up to a volume of 500 mL with filtered seawater. The dropped out fraction remaining in the Falcon Tubes was preserved with 10% buffered formalin and enumerated using automated particle analysis in ImageJ and manual methods. Each beaker with the transferred embryos was kept in the environmental room for the duration of the trial, with daily removal of dead embryos (evidenced by cloudy appearance and confirmed under microscope) and ~80% seawater renewal at least every 4 days. Environmental conditions and water quality parameters (e.g., dissolved oxygen, pH, temperature, salinity) were collected from pre- and post renewal solutions.

Upon hatch (~95 degree days), live fish were either moved to a new beaker and monitored until completion of yolk sac absorption or removed. Fish that were dead (evidenced by lack of movement upon gentle prodding) or died while hatching were counted and removed. Hatching success was defined as the percentage of fertilized eggs that hatched to larvae (live or dead). Endpoints included embryo survival, percent hatch, median time to hatch, percent larval survival, and overall survival.

4.3 Results

4.3.1 CASA Results

The sperm swim performance results are visually summarized in Figure 24.

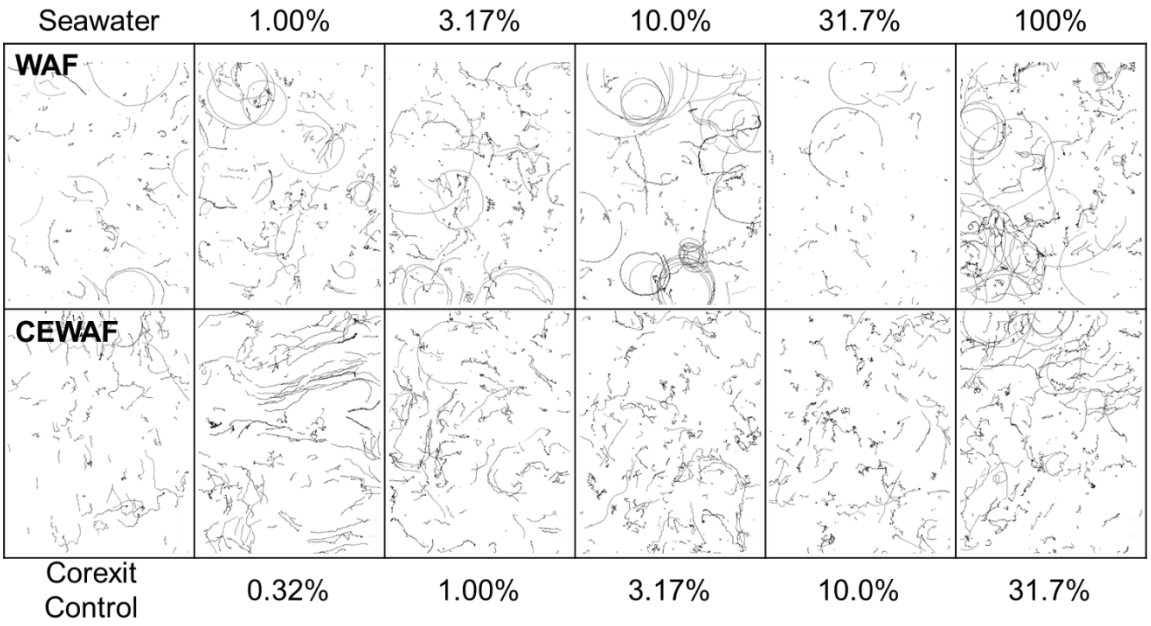


Figure 24: Representative sperm paths from Atlantic cod milt exposed to dilutions of WAF (top row) and CEWAF (bottom row).

The change in swim performance measures (beat cross frequency (BCF), linearity (LIN), percent motility (MOT), progression (PROG), velocity average path (VAP), velocity curvilinear (VCL), velocity straight line (VSL), wobble (WOB, calculated as VAP/VCL)) was assessed against the concentration of the exposure media (TPH and BTEX) for each the WAF and CEWAF treatments alone and combined on a concentration basis in Figure 25.

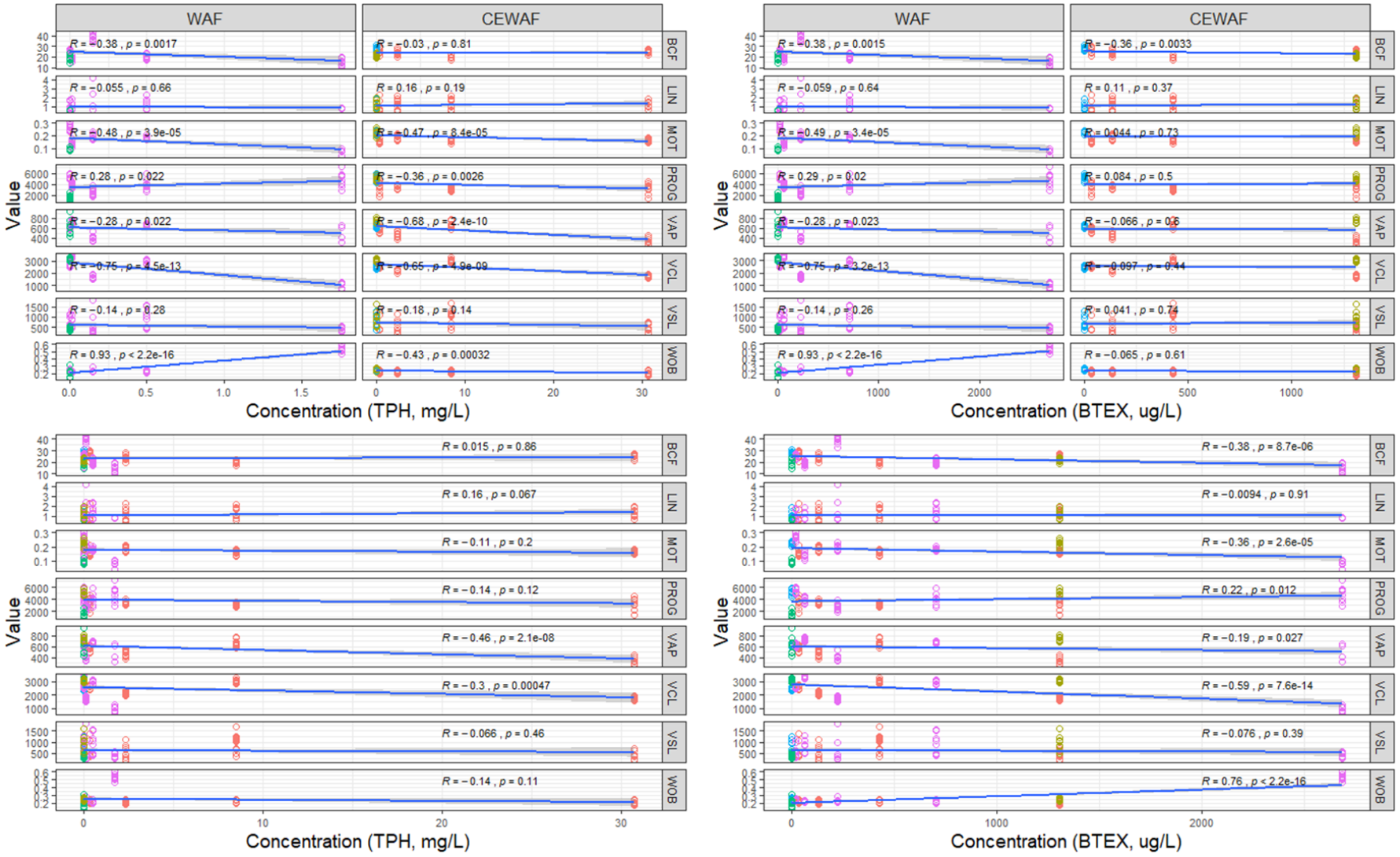


Figure 25: CASA results TPH (left) and BTEX (right) by exposure media type (top row) and combined on a concentration basis (bottom row).

Several parameters showed significant relationships ($p < 0.05$) with changes in concentration of TPH and/or BTEX when analysed separately by WAF and CEWAF and combined. Sperm responded more to changes in BTEX concentrations than TPH for more of the parameters examined. BTEX concentrations in the 100% WAF treatment (2.7 mg/L) were greater than 32% CEWAF treatment (1.3 mg/L) and this corresponded to reduced motility and other parameters in the highest WAF treatment that were not seen in the highest CEWAF treatment despite it having a greater TPH concentration (30.7 mg/L; WAF = 1.77 mg/L).

There was no significant difference in fertilization rate across treatments ($p = 0.06$). The changes in milt performance had a minimal effect on fertilization success with decreases in velocity (both VAP and VCL) and increases in wobble associated with decreases in fertilization. However, this relationship was only significant when WAF and CEWAF were combined whereas alone only WAF showed a significant relationship with fertilization outcomes. VAP, VCL, and WOB are each highly correlated with WOB showing the inverse relationship of VAP and VCL, whereby an increase in WOB was associated with a decrease in percent fertilization. The relationship with VCL is shown in Figure 26.

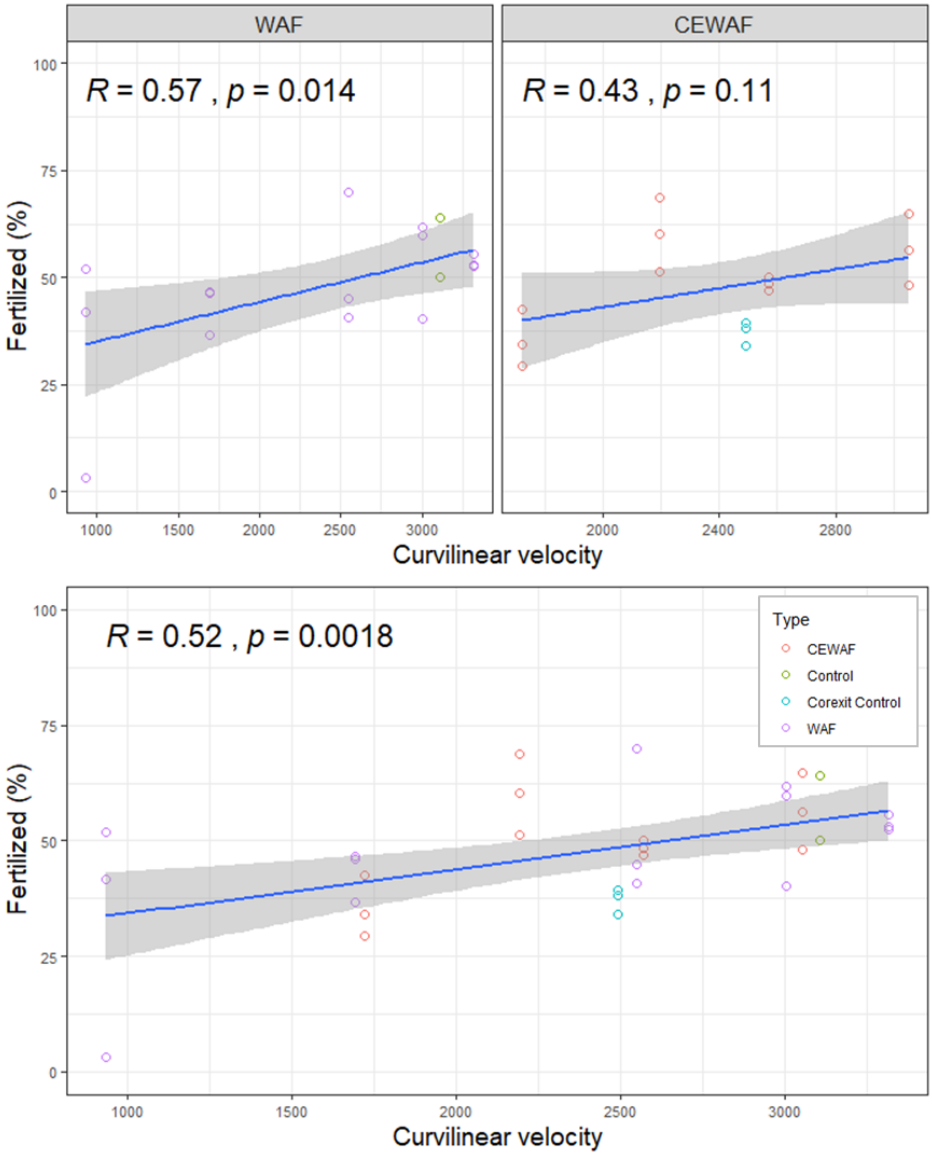


Figure 26: Curvilinear velocity (VCL) and fertilization outcomes

4.3.2 Gamete exposed

The fertilization results from the gamete exposed trials are shown in Table 14.

Table 14: Summary of gamete exposed fertilization trials

Treatment	Number of Cells Assessed		Percent Fertilization	
	Milt Exposure	Egg Exposure	Milt Exposure	Egg Exposure
Seawater Control	172	179	74.17	89.60
	179	120	59.62	87.07
	197	83	67.22	83.95
Mean (st. dev.)	182.7 (12.9)	127.3 (48.4)	67 (7.3)	86.9 (2.8)
1.0% WAF	197	106	67.44	85.29
	282	149	59.57	73.97
	228	93	81.07	79.78
Mean (st. dev.)	235.7 (43)	116 (29.3)	69.4 (10.9)	79.7 (5.7)
10.0% WAF	240	128	64.22	81.25
	229	152	81.05	82.55
	258	131	79.84	90.08
Mean (st. dev.)	242.3 (14.6)	137 (13.1)	75 (9.4)	84.6 (4.8)
100.0% WAF	317	136	72.20	88.15
	273	169	79.01	79.88
	282	98	85.34	83.33
Mean (st. dev.)	290.7 (23.2)	134.3 (35.5)	78.9 (6.6)	83.8 (4.2)
Corexit Control (15 mg/L)	188	132	64.10	86.92
	233	93	59.81	83.70
	286	158	68.02	82.05
Mean (st. dev.)	235.7 (49.1)	127.7 (32.7)	64 (4.1)	84.2 (2.5)
0.32% CEWAF	255	177	74.04	82.66
	306	137	61.95	91.18
	220	190	69.01	87.17
Mean (st. dev.)	260.3 (43.2)	168 (27.6)	68.3 (6.1)	87 (4.3)
3.2% CEWAF	259	166	52.42	87.65
	263	193	70.87	80.42
	358	176	53.85	78.36
Mean (st. dev.)	293.3 (56.0)	178.3 (13.7)	59 (10.3)	82.1 (4.9)
31.65% CEWAF	243	111	57.97	86.36
	228	215	66.33	85.10
	218	126	67.20	87.90
Mean (st. dev.)	229.7 (12.6)	150.7 (56.2)	63.8 (5.1)	86.5 (1.4)
Seawater and Time Control	201	129	79.44	78.51
	185	126	70.55	80.17
	218	164	67.25	78.34
Mean (st. dev.)	16.5 (8.2)	21.1 (15.1)	6.3 (8.7)	1 (1.3)

There were no significant differences in percent fertilization between treatments for either the milt ($p = 0.09$) or egg exposed ($p = 0.73$) trials. Abnormal fertilization (Figure 27) was assessed in only the milt exposed trial.

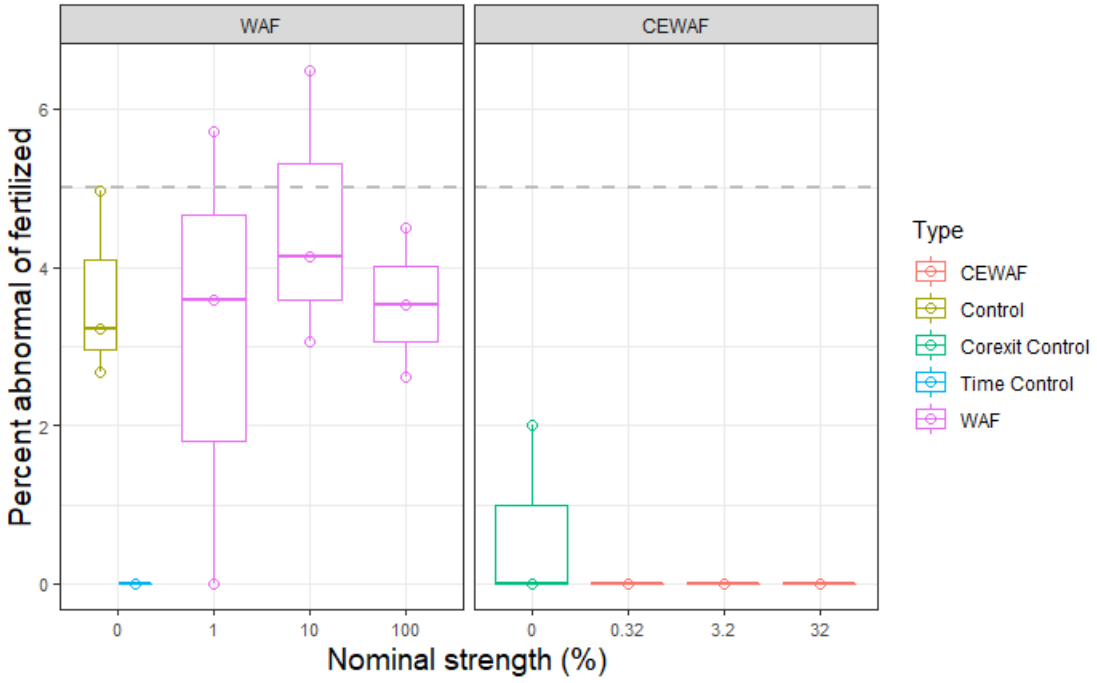


Figure 27: Abnormal fertilization in the WAF (left panel) and CEWAF (right panel) treatments. The dashed horizontal line is at 5%

There was a significant effect of treatment on abnormal fertilization in the milt exposed trial ($p = 0.01$); however, the control had an equal or greater number of abnormal fertilizations compared with all other treatments. Only two replicates had greater than 5% abnormal fertilization (5.7% in 1% WAF treatment and 6.5% in 10% WAF treatment).

The embryos from the egg exposed trial were monitored until hatch (Figure 28).

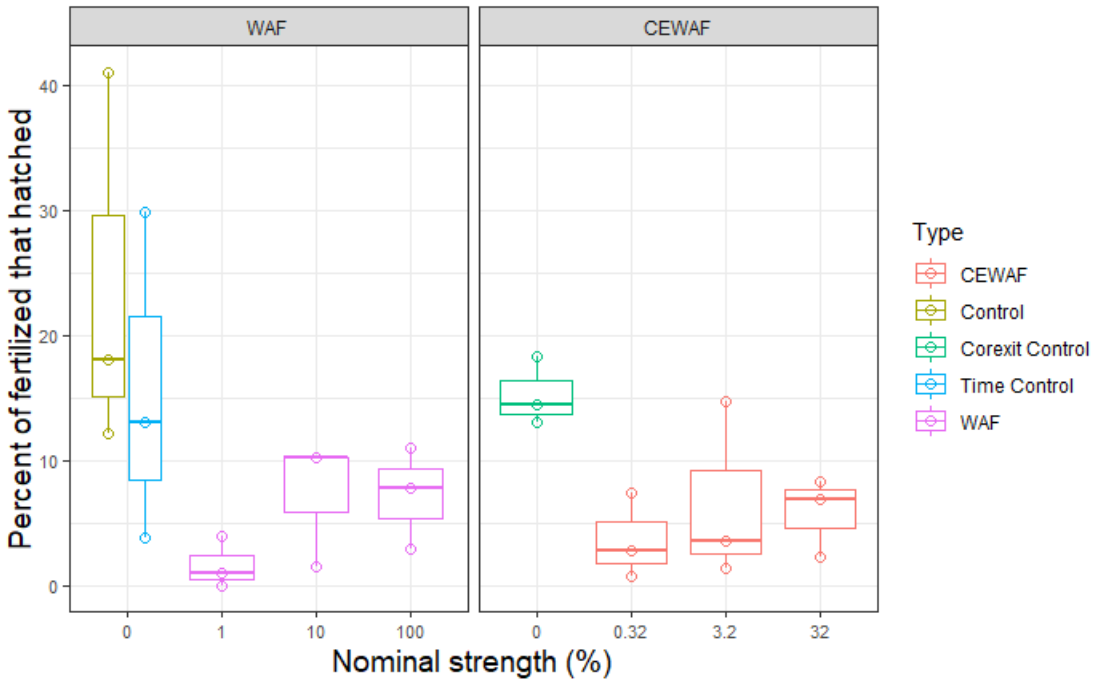


Figure 28: Percent of fertilized embryos that hatched from the egg only exposures

There was a significant effect of treatment on hatch (p = 0.049) with only the 1% WAF treatment significantly different from the pooled (time and seawater) controls (p = 0.025), and not the greater strength solutions, thereby demonstrating a lack of a consistent concentration-response relationship.

4.3.3 Fertilization Exposed

The accuracy of the ImageJ macro for counting the number of embryos was verified with manual counts. There was excellent agreement between the two measures (r^2 = 1, Figure 29) with the success of the method being highly dependent on the quality of the starting image.

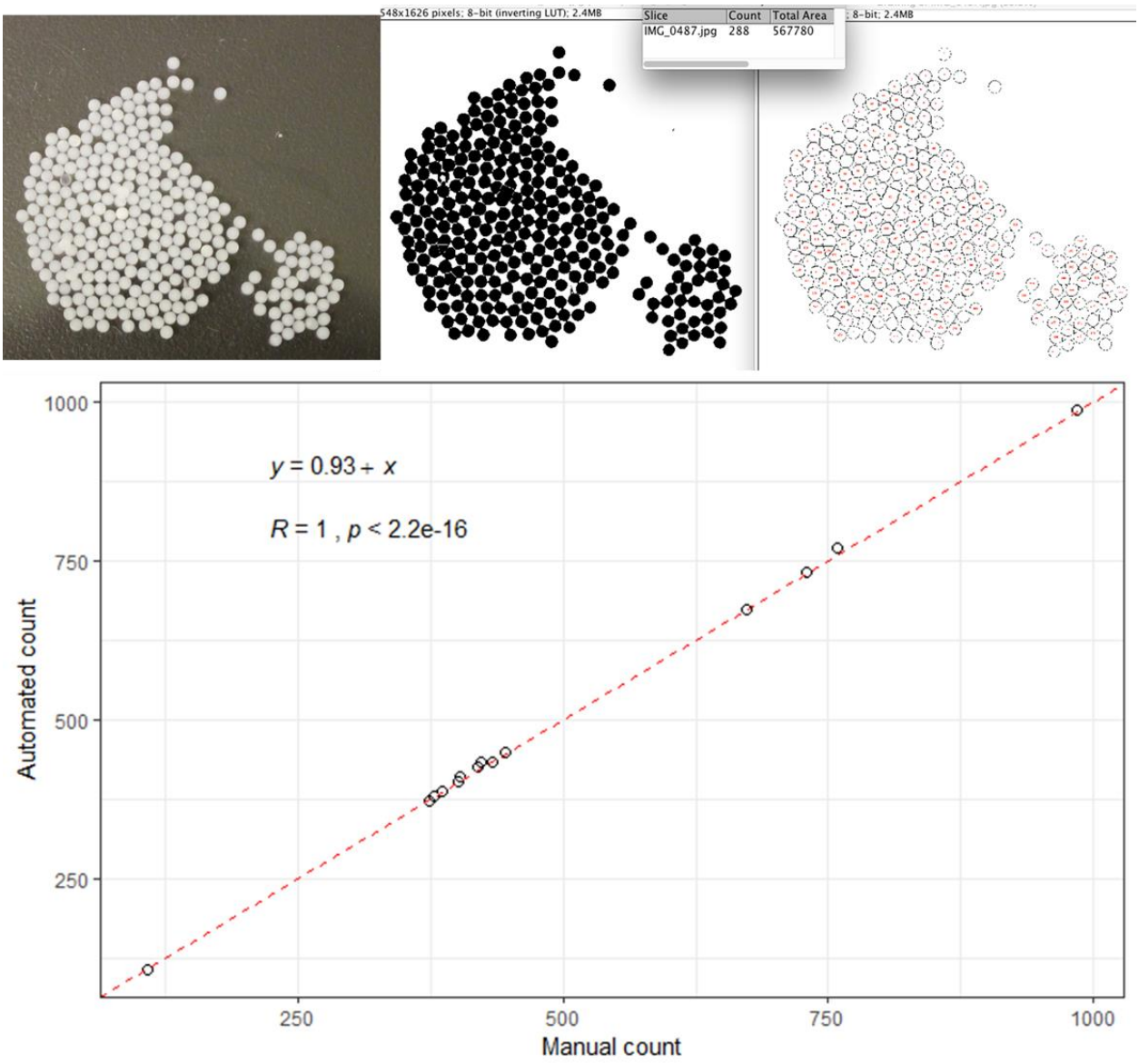


Figure 29: Validation of counting methods

There were four valid fertilization exposure trials (GM-004, GM-009, GM-013, and GM-015) with their specifics described in Table 12.

The results for GM-004 (female 20 x male 8) are summarized in Figure 30.

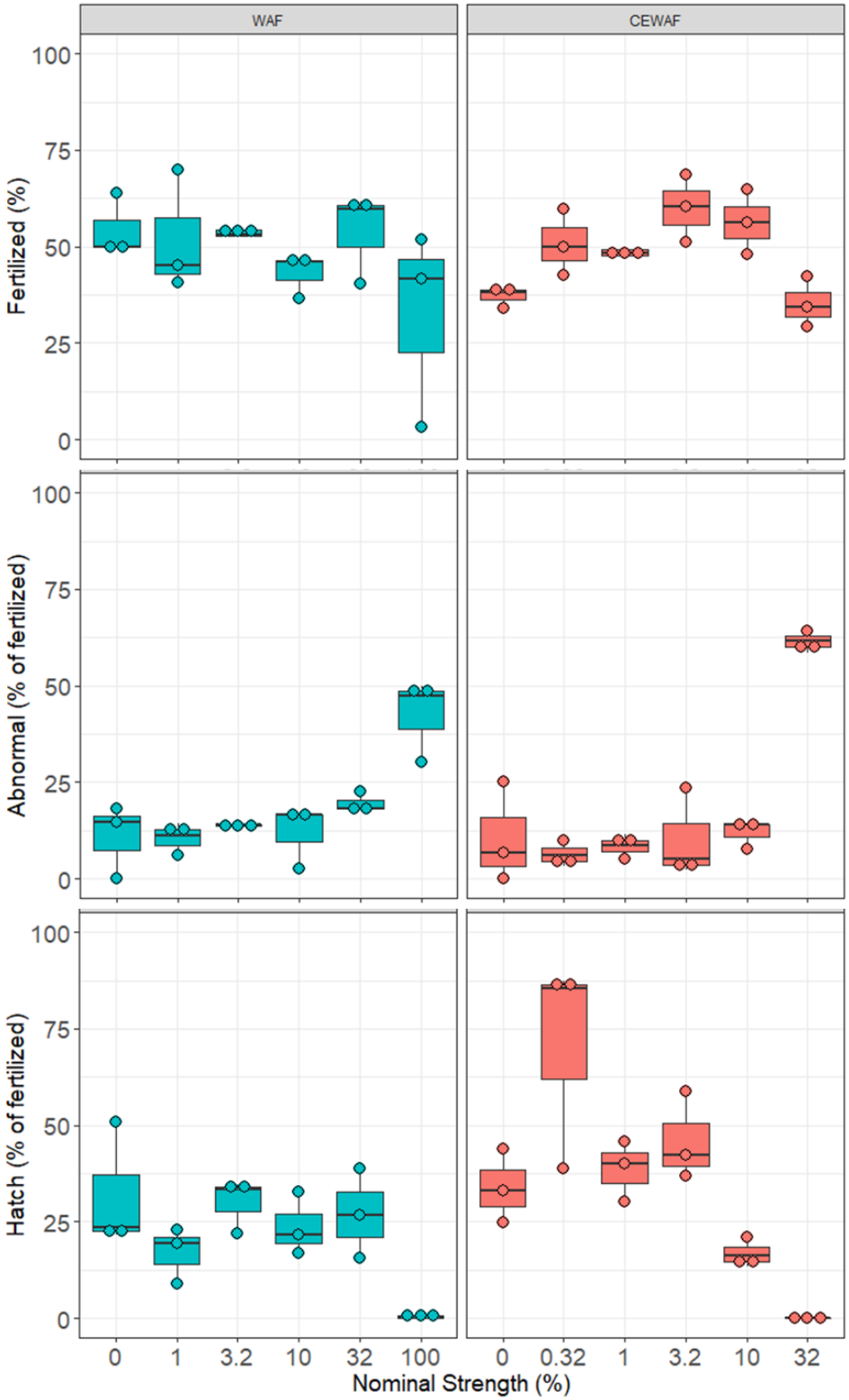


Figure 30: Summary of endpoints for GM-004 fertilization exposure, showing percent fertilization (top), percent of fertilized that are abnormal (middle) and percent hatch of fertilized (bottom), for the nominal concentrations of WAF (left) and CEWAF (right).

Fertilization was not significantly different between treatments (p = 0.066). There was a significant difference in the percent of fertilized embryos that were scored as abnormal (p = <<0.05) with 100% WAF (p = 0.00017) and 32% CEWAF (p = 4.5e-8) being significantly different from the control. Similarly, the percent of fertilized embryos that hatched was significantly different with 100% WAF (p = 0.023) and 32% CEWAF (p = 0.02) having significantly less hatch than the control, while 0.32% CEWAF had significantly more hatch (p = 0.004) than the control.

Abnormal fertilization and hatch each had significant differences in a concentration dependent manner so a concentration response analysis was performed. The concentration response model for abnormal fertilization with WAF and CEWAF exposure is shown in Figure 31.

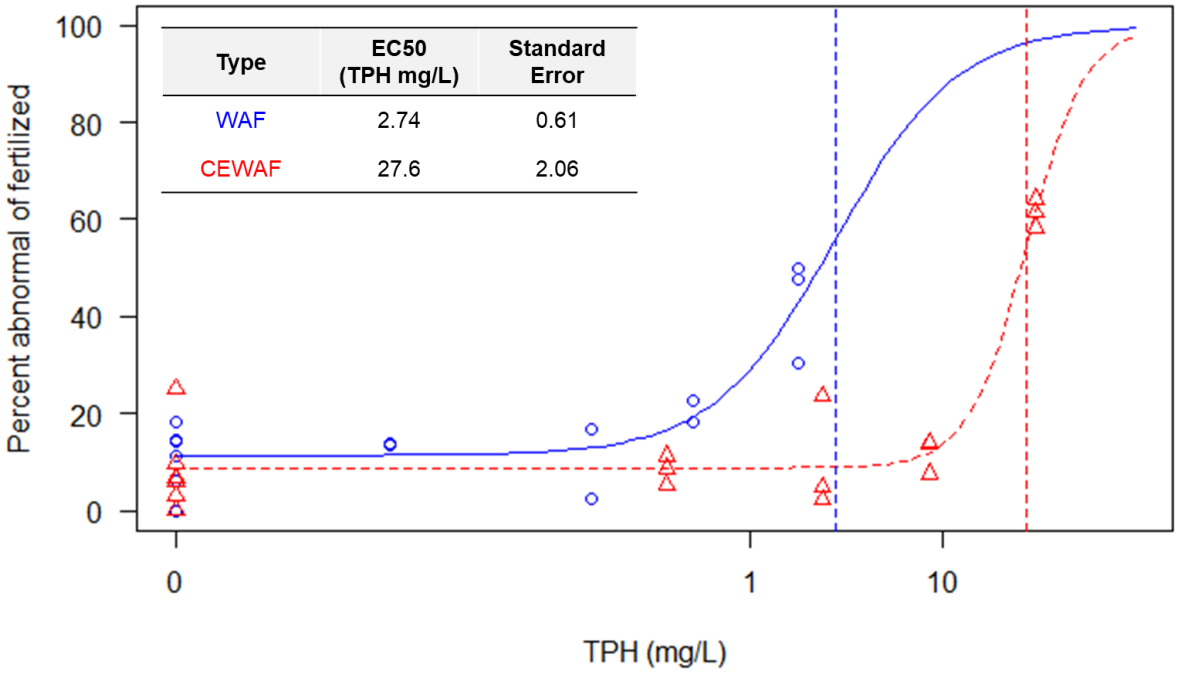


Figure 31: Concentration response relationship for abnormal fertilization following exposure to WAF (blue circles) and CEWAF (red triangles). The vertical dashed lines are the estimated EC50s for WAF (2.74 mg/L) and CEWAF (27.6 mg/L TPH).

A 3-parameter, Type-1 Weibull model was used to fit the percent of fertilized embryos that hatched data (Figure 32).

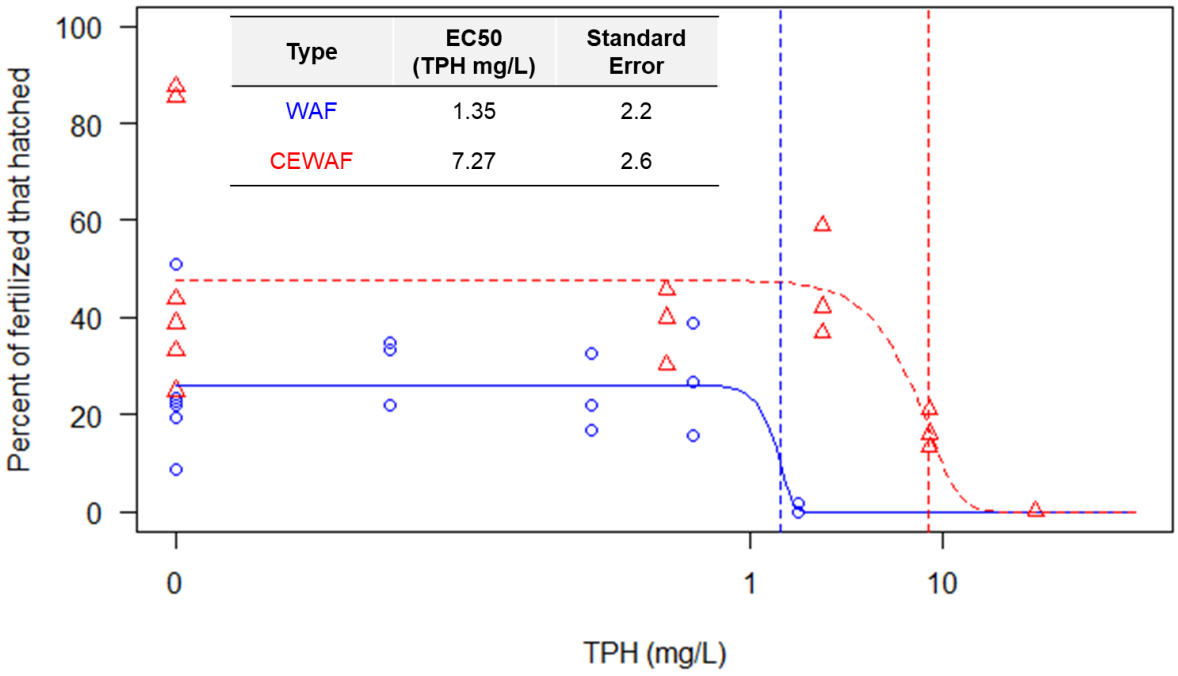


Figure 32: Concentration response relationship for percent of fertilized embryos that hatched

The EC50 for hatching was calculated for the WAF and CEWAF exposures as 1.4 and 7.3 mg/L TPH.

The percent of fertilized embryos that were abnormal was significantly correlated to the percent of fertilized embryos that hatched, when considered as WAF, CEWAF or combined (Figure 33).

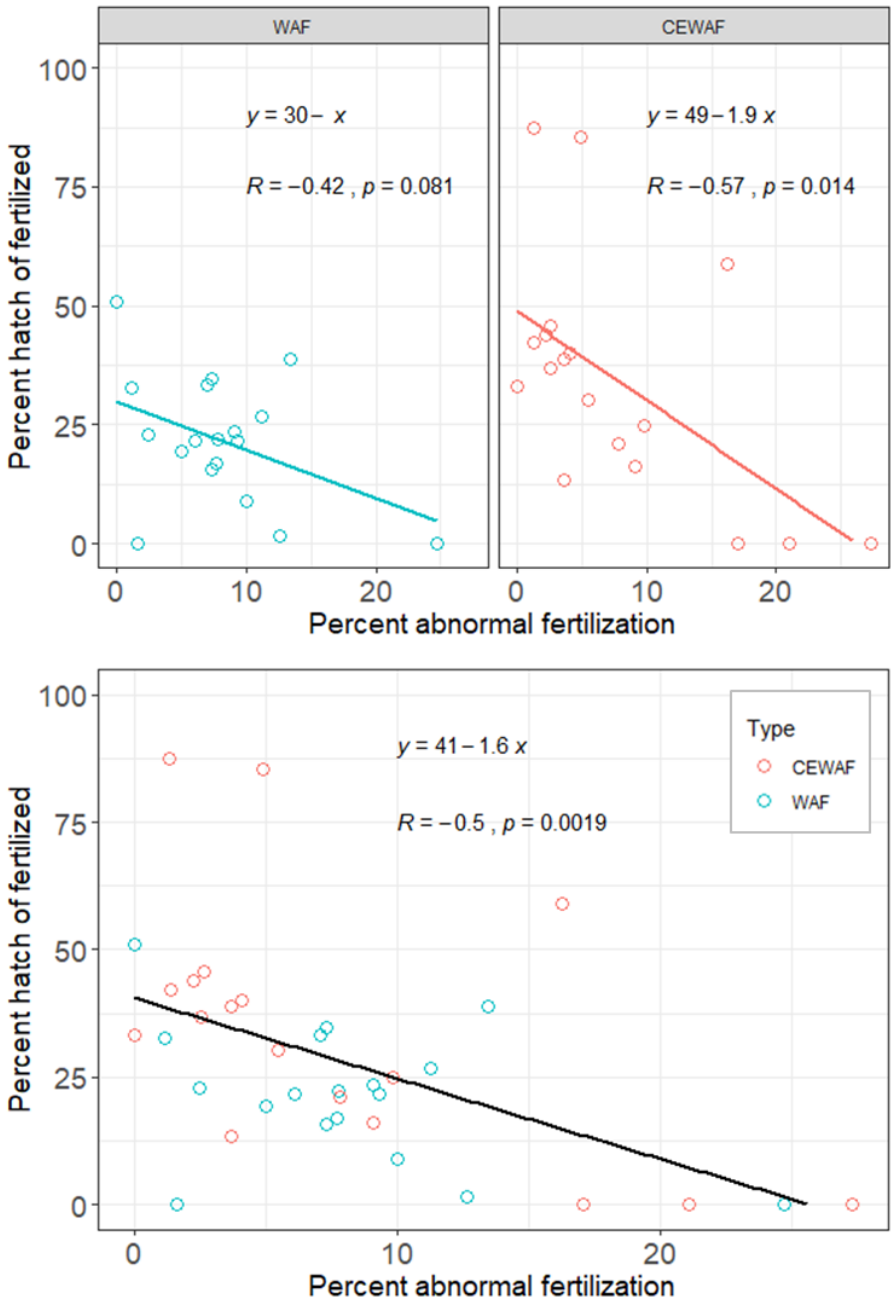


Figure 33: Relationship between abnormal fertilization and hatching

Abnormal fertilization was not significantly associated with post-hatch larval mortality (e.g., dead at hatch) for WAF (p = 0.55), CEWAF (0.36) or combined (p = 0.43).

The results from a second fertilization exposure (GM-009) with a different cross (female 12 x male 15) are summarized in Figure 34.

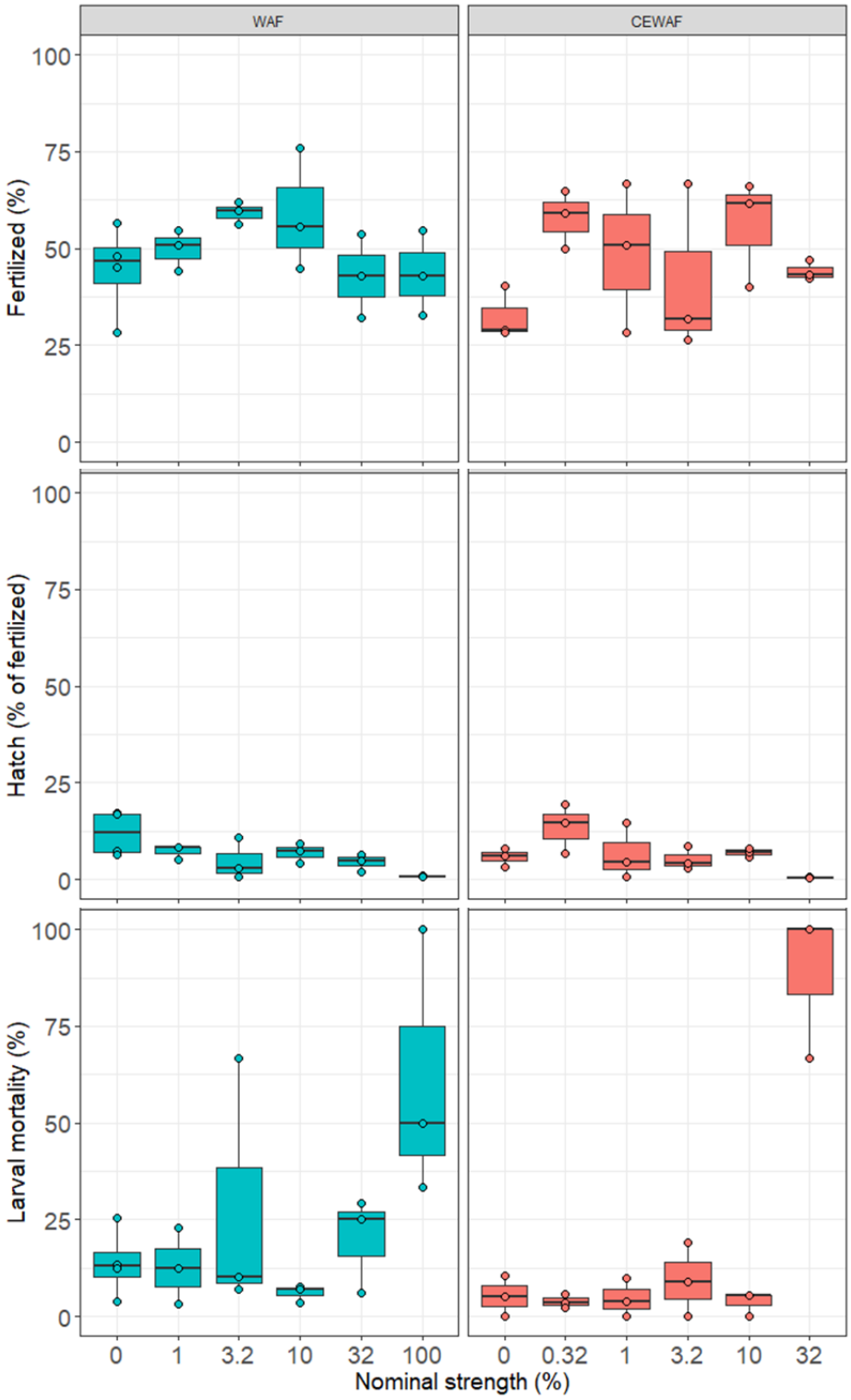


Figure 34: Summary of endpoints for GM-009 fertilization exposure, showing percent fertilization (top), percent of fertilized that hatched (middle) and percent larval mortality (bottom), for the nominal concentrations of WAF (left) and CEWAF (right)

Fertilization was not significantly different between treatments (p = 0.322). There was a significant difference in the percent of fertilized embryos that hatched (p = 0.014) with 100% WAF (p = 0.0105) and 32% CEWAF (p = 0.009) having significantly less hatch than the control. Mean time to hatch was not significantly different between treatments (p = 0.122). Hatching (as percent of fertilized) and post-hatch larval mortality had significant differences (p < 0.05), with the 100% WAF and the 32% CEWAF being significantly different from control for each endpoint. The low absolute values for the percent of fertilized that hatched limits the value of a concentration response relationship examination. The larval fish mortality (fish that died after hatch) concentration response relationship is shown in in Figure 35.

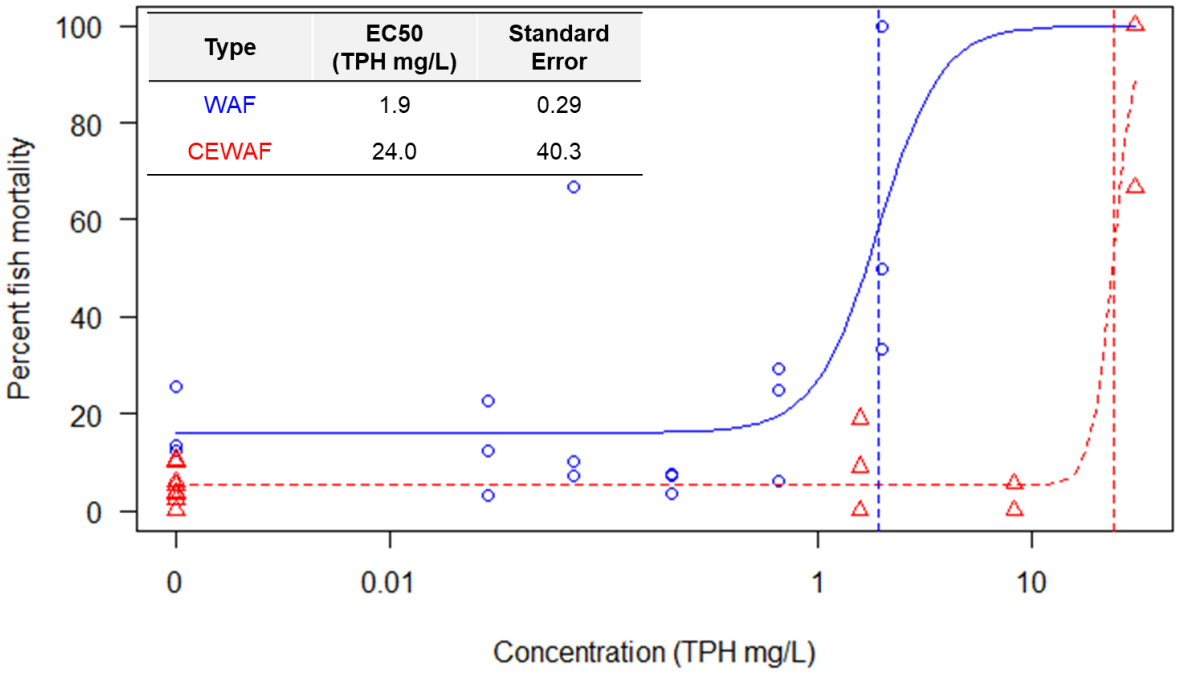


Figure 35: Concentration response relationship for post-hatch larval mortality from the WAF (blue circles) and CEWAF (red triangles) exposures.

The LC50 estimate for the CEWAF, 24.0 mg/L has a very large standard error (40.3, 95% CI = -58.3 - 106), and as such may be unreliable and should be interpreted with caution.

Additional fertilization trials were undertaken where each cross was only exposed to either WAF (GM015, female 12 x male 27) or CEWAF (GM013, female 12 x male 7) those results are summarized in Figure 36.

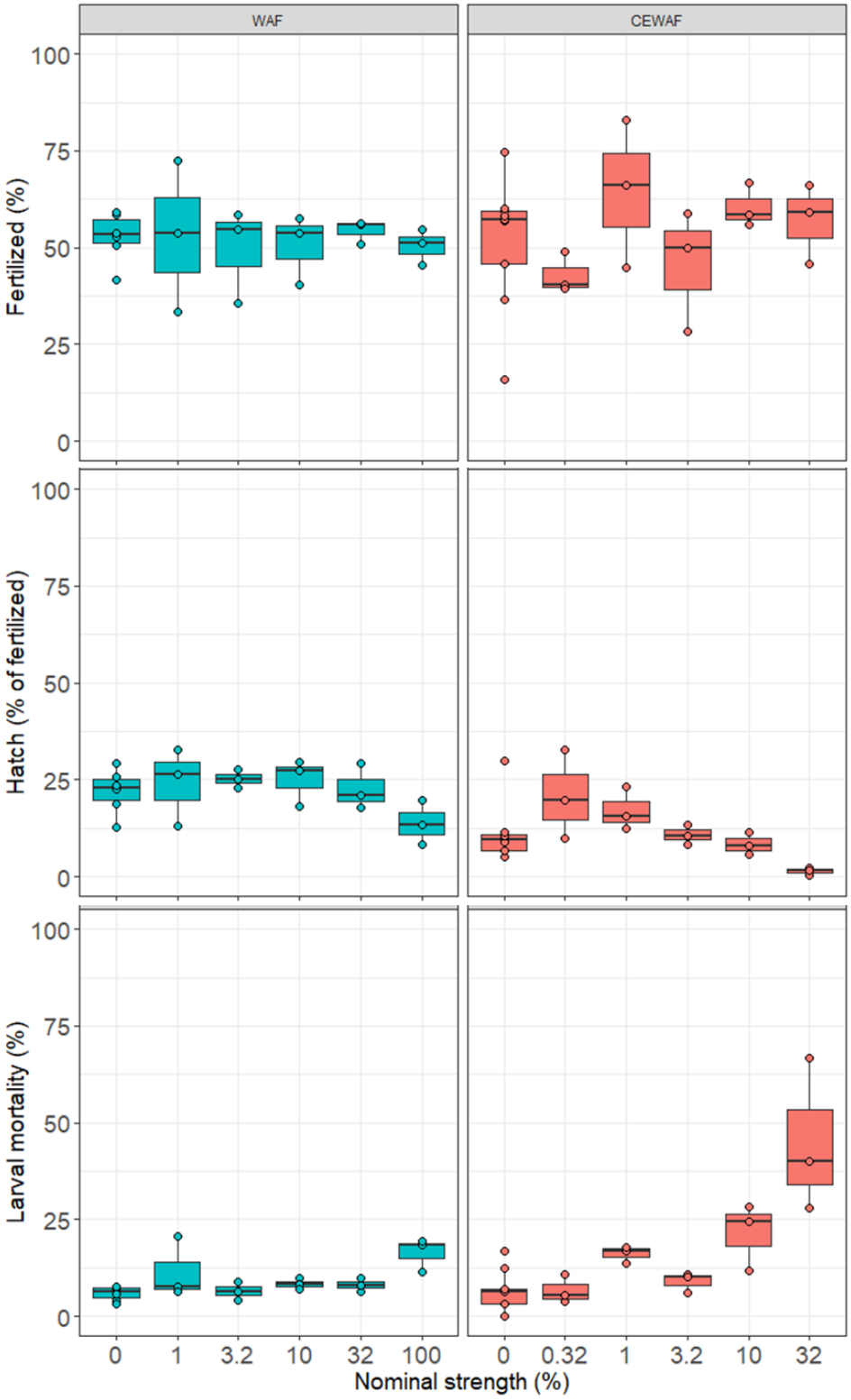


Figure 36: Summary of fertilization exposure endpoints for the WAF (GM-015, left) and CEWAF (GM-013, right) trials, showing percent fertilization (top), percent of fertilized that hatched (middle) and percent larval mortality (bottom) for the nominal concentrations.

These data were analyzed separately because the WAF and CEWAF exposures were conducted as separate trials with different crosses. Fertilization was not significantly different across treatments for either WAF (p = 0.988) or CEWAF (p = 0.485) exposures. There was a slight increase in abnormal fertilization in the WAF exposure in what appeared to be a concentration dependent manner, but the time control, revealed it to be a time dependent effect. Despite this, there was no significant difference between treatments (Figure 37).

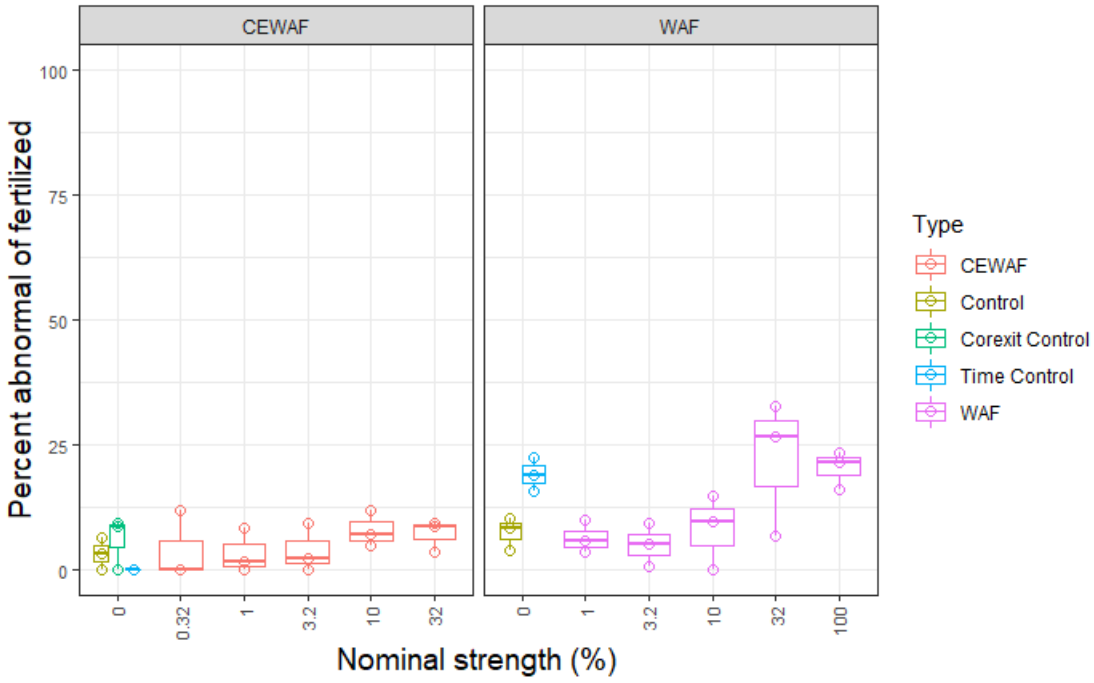


Figure 37: Percent abnormal fertilization in the CEWAF (left) and WAF (right) trials.

The percent of fertilized embryos that hatched was not significantly different in the WAF exposure (p = 0.279), however there was a significant difference in the CEWAF exposure (p = 0.038). The significant difference was not between the control and any treatment, but between 0.32% CEWAF and 32% CEWAF (p = 0.03, Tukeys HSD).

The percent larval mortality showed a significant treatment effect for WAF (p = 0.018) where 100% WAF (p = 0.0049) was significantly different from the control and for the CEWAF exposure (p = 0.0002) where the 32% CEWAF treatment (p = 4.2e-5) was significantly different from the control. The LOEC for the WAF exposure was 2.0 mg/L TPH and for the CEWAF exposure it was 30.1 mg/L TPH.

The data from the different fertilization trials were pooled and are summarized in Figure 38.

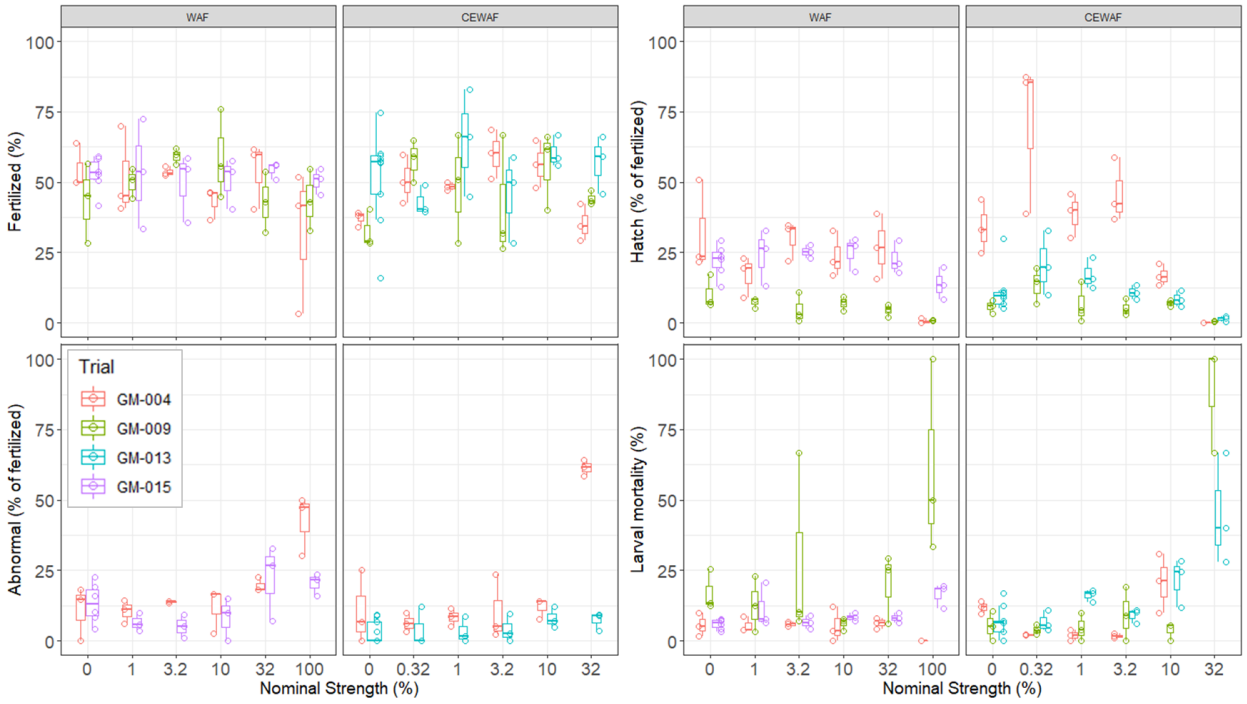


Figure 38: Summary of the results from the fertilization exposure trials (different colours)

4.4 Discussion

The gamete exposure represents “wet” fertilization (e.g., gametes are activated in seawater prior to coming into contact with each other), whereas the fertilization exposures are “dry,” which is expected to have greater fertilization success (internal Huntsman data).

We did not observe a significant effect of exposure to gametes alone at the concentrations (up to 30.7 mg/L TPH and 2.7 mg/L BTEX) and exposure durations (20 minutes) tested. This is in contrast with other gamete type exposures (e.g., echinoderms), which are able to notice effects within these exposure durations. Beirão et al. (2018) did not observe any effect of exposure to WAF (84 g of Hibernia oil per L of 15 psu saltwater), CEWAF (1:20 DOR) each tested at 1, 5, and 10% dilution, or dispersant alone (5 mL/L, nominally ~ 4.75 g/L) on capelin (*Mallotus villosus*) sperm motility or sperm swimming behaviour (at concentrations of 16.1 mg/L TPH). They did note a decrease in fertilization in the CEWAF and dispersant alone treatments, however a mechanism for this response was not clear.

The fertilization exposures had the same concentrations, but a duration of 7 hours, before the exposure was ended and the embryos rinsed. There were no effects on fertilization, however, there was an increase in abnormal fertilization/development with increasing concentrations of TPH in one of the crosses tested. In the first half hour post-fertilization there is a small and rapid increase in egg diameter caused by a cortical reaction and uptake of surrounding water by the colloidal material released to the perivitelline space (Kjorsvik and Lonning 1983). This influx of water may represent a small but significant exposure pathway resulting in an increased internal concentration of contaminants from the surrounding aqueous environment. This may help explain the differential effects observed between the gamete only exposures compared to exposures during fertilization.

Abnormal fertilization was assessed in three trials with crosses 20 x 8, 12 x 27 and 12 x 7. Only in the 20 x 8 cross was there a significant effect on abnormal fertilization, and given that the other two crosses had the same mother (“dam”), it is possible that there is a dam effect that resulted in increased susceptibility to abnormal fertilization. Further research would be required to elucidate this possible mechanism given the small numbers of crosses explored here. The assessment of all abnormal embryo patterns combined (rather than classified by type of abnormality) may confound estimates of embryonic mortality if more severe patterns of abnormality are responsible for a large proportion of the observed mortality, while less severe abnormalities may have little effect on embryogenesis and subsequent hatching success. Some patterns of abnormalities will be more serious and may cause immediate embryo death, while other patterns may be less severe and corrected thereby allowing development to proceed normally (Vallin and Nissling 1998). Hansen and Puvanendran (2010) demonstrated a significant correlation among blastomere morphology, fertilization and hatching success in Atlantic cod. Similarly, our results showed that when abnormal morphology was present it was correlated with reduced hatch and survival, however this relationship was not significant with all examined crosses. Vallin and Nissling (1998) similarly observed large variations in hatching success and noted that developmental success of irregular embryos was highly variable among females/batches.

These results demonstrate that a short (7 hours) but realistic exposure (in terms of duration and concentration) during a vulnerable (and specific) life stage can have adverse latent effects on Atlantic cod embryos. Hatching and post-hatch larval survival each showed a significant reduction effect with exposure (effects observed between 1-30 mg TPH/L), however these were not realized until ~ 20 days after a 7-hour exposure. The results were not consistent across all crosses and highlight the importance of examining population level effects.

Chapter 5 Effects of exposure to physically and chemically dispersed crude oil on the developing embryo of Atlantic cod

5.1 Introduction

Understanding the impacts of crude oil on the earliest life stages of ecologically and economically important native species is necessary to understand potential impacts to populations following an oil spill (Duffy et al. 2016; Echols et al. 2015; Hilborn 1996). Developing fish embryos are perhaps the most sensitive aquatic organisms to PAHs in the aquatic environment (Cherr et al. 2017) and hydrocarbon exposure during embryogenesis can lead to reduced growth and survival, as well as morphological abnormalities that may prove lethal (Short et al. 2003). The composition of crude oil varies greatly over spatial and temporal scales. Despite this variation, the effects of different crude oils on developing fish embryos are quite consistent (Incardona 2017). These effects are characterized by the accumulation of fluid around the heart and in the yolk sac with occasional defects in craniofacial structure and body axis in more severe cases of exposure. The embryonic toxicity of PAHs in fish appears to occur because of sensitivity to planar polycyclic aromatic hydrocarbons, high bioaccumulation and limited biotransformation, and exposure during critical developmental periods (Barron et al. 2004).

Upon fertilization, the embryonic life stage of Atlantic cod (*Gadus morhua*) is still vulnerable to contaminant exposure. The period of cod embryogenesis can be divided into six subperiods: (1) fertilization, (2) cleavage, (3) blastulation, (4) gastrulation, (5) somitogenesis, and (6) prehatching and hatching (Gorodilov et al. 2008). Early embryogenesis consists of a series of mitotic divisions, producing equally sized, symmetrical cells known as blastomeres. Embryogenesis can be affected by various external and internal factors, which may result in disturbances of the development of the embryo and its structures. Work with other pelagic fish species has found that there are developmental periods of enhanced sensitivity to oil exposures and these periods surround the hatching phase (Mager et al. 2017).

The Atlantic cod embryo is planktonic and floats in the water column near the surface. This increases the potential exposure opportunity of the embryos to the dissolved phase of oil, as well as to possible surface slicks. The objective of these exposure trials was to characterize the windows of sensitivity to exposure of physically and chemically dispersed crude oil.

5.2 Methods

5.2.1 Experimental Animals

Fertilized embryos (female 11 x Male 18, relatedness = -0.0156, sperm:egg ratio 1000000:1) were transferred to 45-L incubation pots post fertilization and held at 5°C (+/- 1.5 °C). Embryos were monitored daily with dead embryos (as indicated by sinking to the bottom of the pot) purged from the system every two days. Daily temperatures were recorded and used to calculate accumulated thermal units, or degree days (dd), which more accurately align with developmental milestones in fish. Embryos were removed from the incubation pots at select developmental intervals for use in toxicity studies (Figure 39).

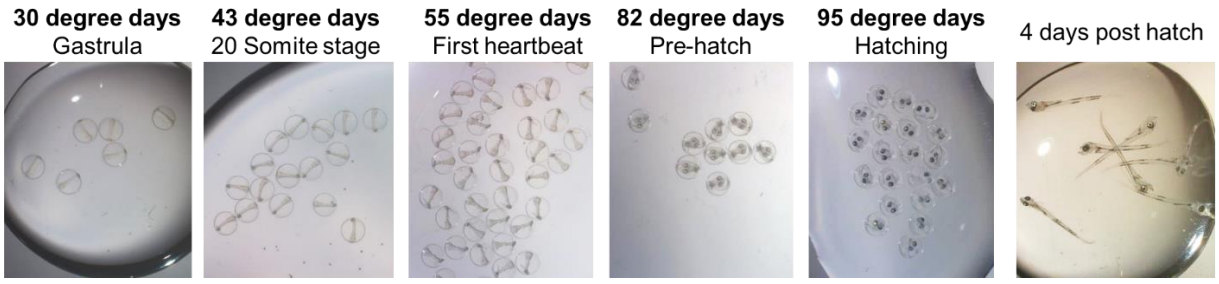


Figure 39: Reference images of the test organisms at the start of each exposure

5.2.2 Preparation of Exposure Media

Exposure waters were prepared to test the toxicity of oil to aquatic organisms by contacting the oil and water such that a mixture is generated consisting of oil components in both the dissolved phase and in the non-dissolved phase, following the methods described in Section 3.3.2, Table 10. Briefly, the benchtop mixing method we employed is a modification of the baffled flask method for dispersant effectiveness used by Environment Canada and the United States Environmental Protection Agency. Using a

gastight syringe, 1.6 g of oil is drawn up and dispensed onto the central surface of the water (1.6 L in a 2-L baffled flask) in the flask at a loading of 1 g of oil per L of water. If a dispersant is used, it is added to the centre of the surface slick at a dispersant to oil ratio of 1:20. The flask is sealed with DuraSeal, then secured on an orbital shaker, where it is shaken at 150 rpm for 60 minutes, followed by a settling time of one hour. After the settling time, the required volume of stock is poured out from the spout on the baffled flask and is then used to prepare exposure media following gradient dilutions.

5.2.3 Toxicity Testing

On a set interval corresponding to key developmental stages (Figure 39; e.g., days 5 [gastrula period], 8 [20 somite stage], 11 [period of first heartbeat], 17 [pre-hatch period], 20 [hatching period] and 23 [post hatch period] post fertilization) a 400 mL beaker was used to collect a sample of embryos that were floating at the top of the incubation pot that has been still for 1 hour.

From each beaker, an aliquot of embryos (20 embryos per vial) was added to a labelled scintillation vial. A sub-sample of 20 embryos were imaged to assess and confirm the developmental stage prior to exposure.

Transfer seawater was removed as excess volume from the vials using a transfer pipette in a manner to leave enough volume so that the embryos remained submerged. Each scintillation vial was replicated three times and brought up to 20 mL using the appropriate exposure media. Exposure media concentrations followed a log based dilution scheme of WAF (100%, 10.01%, and 1.0%) and CEWAF (31.6%, 3.17%, 0.32%) stock, a dilution water control (seawater), and a dispersant control (Corexit 9500A applied at the same rate as the 31.6% CEWAF based on a volume/volume percentage, nominally 15 mg/L). The scintillation vials were stored in the environmental room maintained at 5 +/- 2^{o}C and photoperiod of 16-hrs light and 8-hrs dark in a randomized design with their caps loosely attached.

Approximately 80% of the exposure media was exchanged with new, clean seawater at 24 and 48 hours post exposure. After 48 hours, 80% renewal of solutions occurs daily until hatch. At renewal, the organisms were assessed for development stage (e.g.,

embryo, larva, dead) then all live organisms were transferred back into the vial and brought back up to 20 mL in new seawater. This procedure was repeated until 10 days post hatch from the incubator pot. Percent hatch, percent larval survival, percent overall survival, time to hatch, yolk absorption, and time to starvation were evaluated.

5.3 Results

The hatching results (embryo survival) are shown in Figure 40 for each trial.

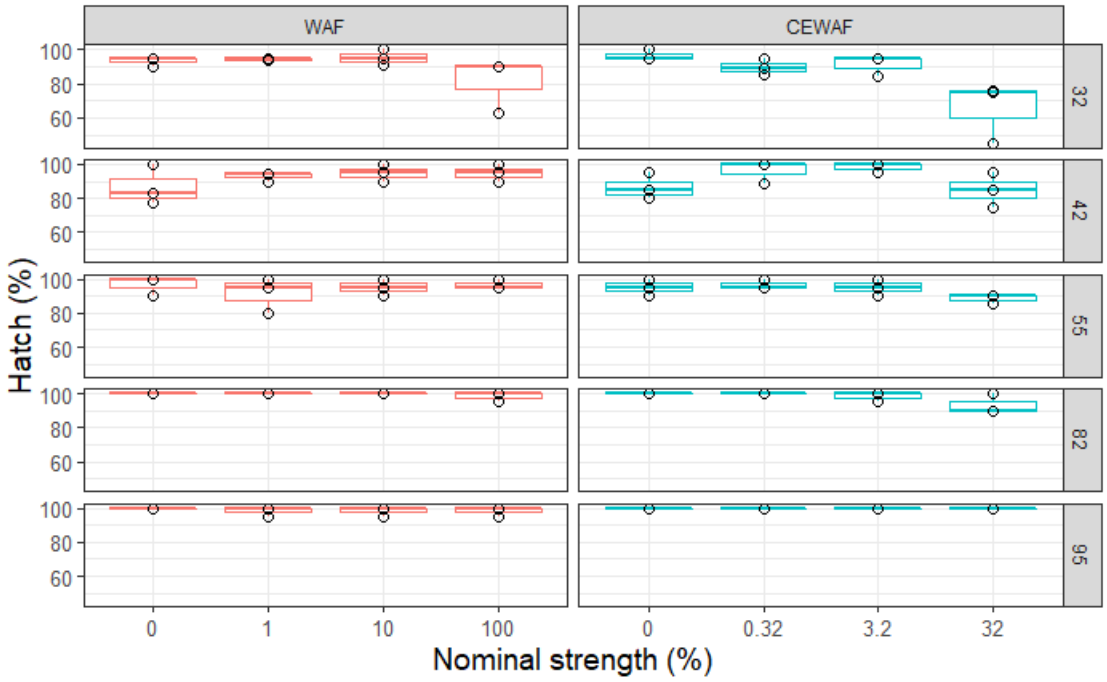


Figure 40: Percent hatch in each treatment (columns) by age in degree days (rows) at time of exposure. The 0% CEWAF represents the dispersant only control, nominal concentration 15 mg/L.

Two outliers were identified using Grubbs test ($p < 0.05$) from the 32 dd exposure, one from the 100% WAF treatment (hatch = 63%, other two replicates each had 90% hatch) and one from the 32% CEWAF treatment (hatch = 45%, other two replicates had 75 and 76% hatch). Subsequent analysis was conducted with and without the inclusion of these outliers to determine the impact they have on the interpretation of results.

There was a significant effect of treatment on hatching success in the 32 dd exposed embryos with the 32% CEWAF treatment being significantly different from the controls both with ($p = 0.0088$) and without ($p = 0.00085$) outliers. None of the later exposure days

showed a significant difference in hatching success: 42 dd (p = 0.229), 55 dd (p=0.555), 82 dd (p=0.0558) and 95 dd (0.661).

The other hatch related endpoints are presented in Table 15.

Table 15: Summary of hatch related endpoints from the different exposures

Endpoint (days)	Age at time of exposure (degree days)	Treatment							
		Control	Corexit Control (15 mg/L)	0.32% CEWAF	1% WAF	10% WAF	3.2% CEWAF	100% WAF	32% CEWAF
		Concentration TPH mg/L							
		0	0	0	0.029	0.21	1.57	2.01	30.08
Time to mean hatch	32	20.4 (0.2)	20.5 (0.3)	20.5 (0.1)	20.5 (0.3)	19.9 (1.0)	20.5 (0.4)	19.8 (0.1)	20.2 (0.4)
	42	23.8 (4.9)	22.1 (3.0)	21.8 (1.1)	21.9 (2.3)	26.9 (5.4)	20.6 (2.7)	21.2 (0.9)	21.6 (0.7)
	55	22.9 (0.4)	22.5 (0.6)	22.7 (0.9)	22.4 (0.8)	22.6 (0.3)	22.3 (0.5)	22.6 (0.4)	22.3 (0)
	82	23.8 (1.8)	22 (0.3)	23.6 (1.3)	23.5 (0.9)	23.4 (1.6)	22.5 (0.6)	22.1 (0.1)	23.4 (0.3)
	95	23.2 (0.1)	22.4 (0.6)	24.7 (2.3)	22.5 (0.8)	24.2 (1.9)	22.9 (0.8)	22.2 (0.5)	22.9 (1.4)
Hatching duration	32	6.3 (2.1)	4.3 (1.2)	6.3 (4.9)	6.7 (2.3)	9.7 (4.9)	8.7 (0.6)	4.7 (3.1)	4.7 (1.5)
	42	8.0 (2)	8.7 (2.5)	6.0 (1.7)	8.3 (1.5)	5.7 (2.1)	5.7 (0.6)	7.0 (2.6)	5.7 (1.5)
	55	6.7 (0.6)	9.7 (2.5)	5.7 (2.3)	6.7 (0.6)	7.7 (2.1)	7.0 (0.0)	9.0 (2.6)	7.3 (0.6)
	82	5.3 (2.3)	5.0 (0.0)	5.0 (0.0)	4.0 (0.0)	5.0 (1.7)	5.3 (0.6)	4.3 (0.6)	4.7 (0.6)
	95	1.7 (1.2)	1.7 (1.2)	2.3 (1.2)	2.3 (1.2)	2.3 (1.2)	1.7 (1.2)	3.0 (0.0)	2.3 (1.2)
Time to succumb	32	9.1 (0.8)	10.9 (0.9)	9.8 (1.9)	9.2 (2.1)	7.4 (3.3)	9.8 (0.7)	10.0 (1.5)	3.3* (4.8)
	42	4.1 (3)	3.1 (2.2)	6.5 (2)	5.2 (3)	1.8 (3.6)	6.4 (2.9)	7.2 (3.2)	1.6 (1.8)
	55	8.8 (1.0)	7.5 (0.8)	8.8 (1.7)	8.6 (1.4)	8.9 (1.9)	8.0 (0.4)	10.1 (2.4)	4.5* (0.3)
	82	8.8 (1.5)	11 (0.7)	9.2 (2.2)	9.1 (1.5)	7.5 (0.5)	8.9 (0.9)	9.4 (1.3)	7.6 (1.0)
	95	7.4 (0.9)	8.5 (1.2)	6.4 (3.0)	9.4 (0.5)	6.3 (2.5)	8.7 (1.0)	9.2 (0.7)	8.0 (1.8)

*significantly different (p <0.05) from control

Time to mean hatch and hatching duration showed no difference between treatments for any of the trials. Time to starvation did see an effect for both the 32 dd (p = 0.03) and 55dd (p = 0.01) exposures where the 32% CEWAF treatment had significantly shorter times to succumb (death by starvation) (p = 0.44 and p = 0.01 respectively).

5.4 Discussion

We saw few latent effects of exposure with only the 32 and 55 dd (curiously not the 42 dd exposure) showing less resistance to starvation when exposed to the 32% CEWAF treatment (~30 mg/L TPH), and no significant effects of exposure to the 100% WAF solution (2.0 mg/L TPH), or the dispersant only control (nominal 15 mg/L) at any developmental time point. The results presented in this study support findings in other fish species that have shown susceptibility to contaminants early in development, which then diminishes over time. In Atlantic herring, McIntosh et al. (2010) found that embryo sensitivity was greatest during the first 24-hrs, then declined rapidly as embryos developed, with no effect on the 11d embryo. This change in sensitivity is presumably due to a decrease in the permeability of the chorion as it water hardened. McIntosh et al. (2010) did note that there was increased sensitivity of embryos immediately after hatch, further supporting the protective role of the chorion, and indicating a later developmental window that may be more susceptible than the earlier embryo stage.

Chapter 6 Sublethal effects of exposure to WAF and CEWAF on Atlantic cod

6.1 Introduction

Sublethal effects caused by embryonic PAH exposures include edema of the yolk sac and pericardium, hemorrhaging, disruption of cardiac function, binding to the aryl hydrocarbon receptor (AhR) and CYP1A induction, mutations and heritable changes in progeny, craniofacial and spinal deformities, neural cell death, anemia, reduced growth, and impaired swimming (Barron et al. 2004). Many of these symptoms resemble the blue-sac disease (BSD) caused by exposure to planar halogenated compounds such as TCDD (Brinkworth et al. 2003; Scott and Hodson 2008). BSD presents as an assemblage of developmental abnormalities characterized by vertebral abnormalities, edemas, hemorrhaging, reduced growth, and survival (Boudreau et al. 2009; McIntosh et al. 2010; Scott and Hodson 2008). A major clinical sign of BSD is typically a swelling of the yolk-sac as a result of accumulation of serous fluid in the abdominal cavity between the inner and outer walls of the sac (referred to as 'hydrocoele embryonalis'). This swelling may often lead to the fish becoming immobile, or unable to swim or respire correctly, and usually results in death prior to transitioning to exogenous feeding (Gunasekera et al. 1998). Reduced growth, while not captured in the BSD, is an important sublethal endpoint that has implications for individual fitness. The trials described herein sought to explore these sublethal effects in Atlantic cod embryos and larvae exposed to dilutions of WAF and CEWAF and dispersant alone.

6.2 Methods

6.2.1 Hatching window exposures

Embryos from specific crosses were held in aerated incubators (60-L conical) until they approached the hatching window (~95 degree days). At one-day pre-hatch (D-1), embryos were collected from the surface of the incubator into a 400 mL beaker after the aeration was suspended for 1 hour. From the beaker, 20 embryos were randomly allocated into the exposure vessels (250-mL flasks), which were then filled with exposure media equal to 80% of the exposure vessel volume (e.g., 10, 32, and 100% WAF, 3.2,

10, and 32% CEWAF, seawater control, or Corexit 9500A control [nominally 15 mg/L]; or dispersant alone series: 5, 15, 45, 135, 405 mg/L). The flasks were covered with DuraSeal then placed and secured on an orbital shaker set at 120 rpm for 24 hours. After the 24 hours, the flasks were removed from the shaker, the contents of the flask were gently poured into a shallow dish, and the organisms were assessed for development (e.g., embryo, larva, dead). All live embryos were transferred into a new 600 mL beaker (filled with 500 mL seawater) and monitored until hatch. Each larval fish was counted and assessed as live or dead. All live fish had sublethal endpoints assessed using a blue-sac disease (BSD) rating system modified from Scott and Hodson (2008). Scores for swimming ability (SA) (scored as 0 = no movement; 1 = twitches/lethargic; or 2 = complete/normal swimming ability), spinal curvature (SC) (scored as 0 = no defect/straight spine; 1 = slight defect; 2 = moderate defect; or 3 = severe defect), and yolk sac edema (scored as 0 = no defect; or 1 = edema present) were integrated to form a modified BSD severity index (SI) as follows (Figure 20):

BSD SI = Sum YE + Sum SC - (2X SA) + (max score * #Dead)/ max possible score

Dead organisms were not assessed for sublethal phenotypes and were assigned a score of 0.5 higher than the maximum sublethal score.

Percent hatch, percent larval survival, percent overall survival, and percent BSD SI were evaluated for each exposure.

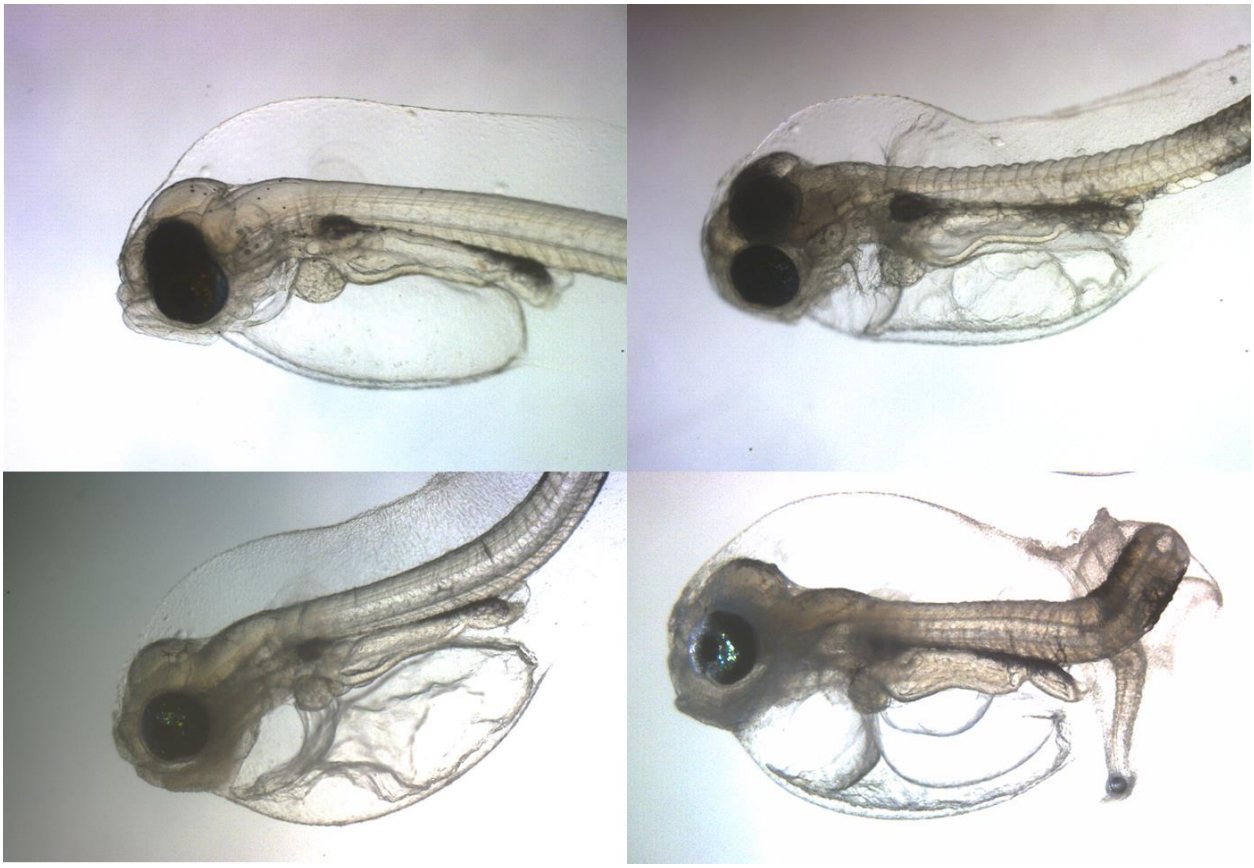


Figure 41: Gradation of severity of BSD presentation in freshly hatched Atlantic cod larvae

6.2.2 Growth assay

The larval cod growth assay follows the methods modified from *EC Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows* (EPS 1/RM/22 Second Edition- February 2011). Larval fish (~200 degree days; post-yolk sac absorption, confirmed to be feeding, with inflated swim bladders) were fasted for 18 hours prior to collection from the 250-L ponding tanks (initially stocked at 300 larva/L). Fish were allocated in a round-robin scheme where three fish were allocated into each of 35 unlabeled pill cups with 20 mL seawater (32 + 3 additional units for replacements) until all units were stocked with 10 fish. Pill cups were then chosen at convenience and added to the exposure vessels containing 175 mL of the appropriate solution (e.g., 10, 32, 100% WAF, 1.8, 5.6, 18% CEWAF, seawater control, or Corexit 9500A control [nominal 8.6 mg/L]). The test concentrations were based upon previous 24-hr exposures with larval cod and selected as they were not expected to cause more than 20-30% mortality in the highest test concentrations. Each concentration (n=8) was replicated four times for a total

of 32 experimental units (500-mL Pyrex crystallizing dishes with 175 mL exposure media covered with Dura-Seal for the duration of the exposure). Exposure duration was 24-hours followed by transfer into monitoring vessels (600-mL beakers) filled with 500 mL of clean, UV sterilized 0.22 µm filtered seawater with daily 80% renewal for an additional 6 days. Dead organisms were counted and removed during daily observation. The exposure and subsequent monitoring was conducted in an environmentally controlled room with temperature maintained at 7 +/- 2^{o}C and a photoperiod for the entire duration of 16-hrs light and 8-hrs dark. Water quality measurements (e.g., dissolved oxygen, salinity, temperature, pH) were taken from one unit per treatment, at pre- and post-renewal, during the 6-day monitoring period. Each unit was fed twice daily (feeding rate: 2 rotifers, (*Brachionus plicatilis*, Reed Mariculture) per mL) with the afternoon feeding occurring after water renewal. There was no feeding during the final 12 hours of the trial or during the exposure period. Each unit was assessed at the end of the 7-day period and the surviving fish from each unit were euthanized with a lethal dose of MS-222 (200 mg/L, Tricaine-S/Aqualife TMS), rinsed with distilled water then placed on pre-weighed aluminium weigh boats and dried at 60^{o}C for 24 hours prior to dry weight determination. Survival and growth (dry weight and dry weight normalized to biomass) were compared across treatments.

6.2.3 Flow-through Assay

In 2020, two exposures were conducted with WAFs prepared using a variable oil loading approach (following the same procedures as described in Table 10, with varying loadings of loadings of 1000, 100, 10, 1 mg oil/L) to assess the effects on earlier exposure to the sublethal BSD presentation and growth. These WAFs were used as the exposure media for a 24-hr exposure trials to mid-developmental stage embryos (47.6 dd [BSD trial] and 61.1 dd [growth trial]) along with control seawater and a positive control of 1-methylnapthalene (3.08 +/- 0.88 mg/L), applied using a passive dosing technique modelled after Butler et al. (2016). The embryos were transferred into clean seawater in custom flow-through vessels after the exposure and monitored until hatch.

6.3 Results

6.3.1 Hatching window exposures

The results from five separate crosses (female x male) exposed during the hatching window are summarized in Figure 42 for BSD presentation and mortality.

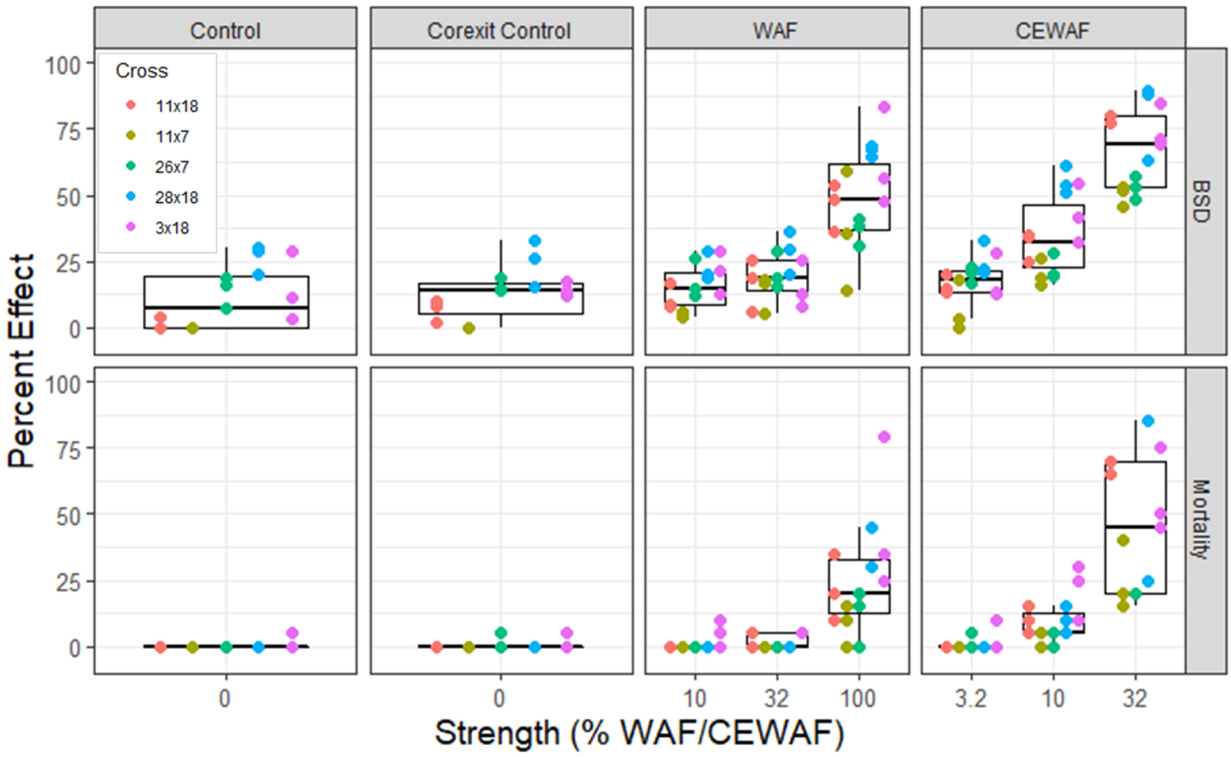


Figure 42: Blue sac disease (BSD) presentation (top row) and survival (bottom row) by cross (colour) for each exposure media. The boxplots are the pooled response.

The presentation of BSD and occurrence of mortality each increased with increasing nominal strength of WAF and CEWAF. The responses were modelled against measured concentration, with WAF and CEWAF combined, with the crosses pooled and treated individually (Figure 43 and Figure 44).

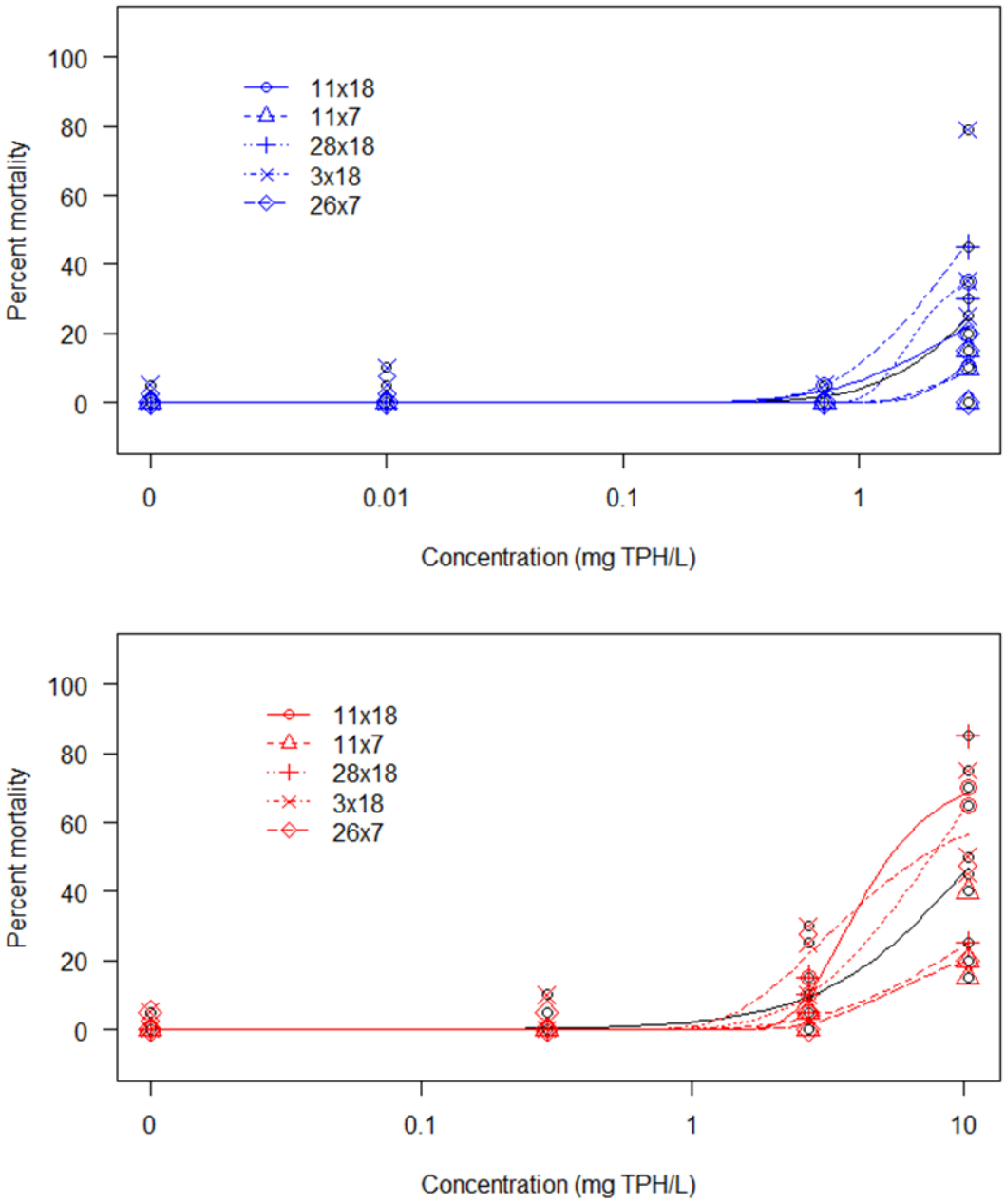


Figure 43: Concentration response relationship for mortality from the WAF (top) and CEWAF (bottom) exposed organisms. Individual crosses are shown in blue (WAF) and red (CEWAF) with the response of the pooled crosses modelled in black.

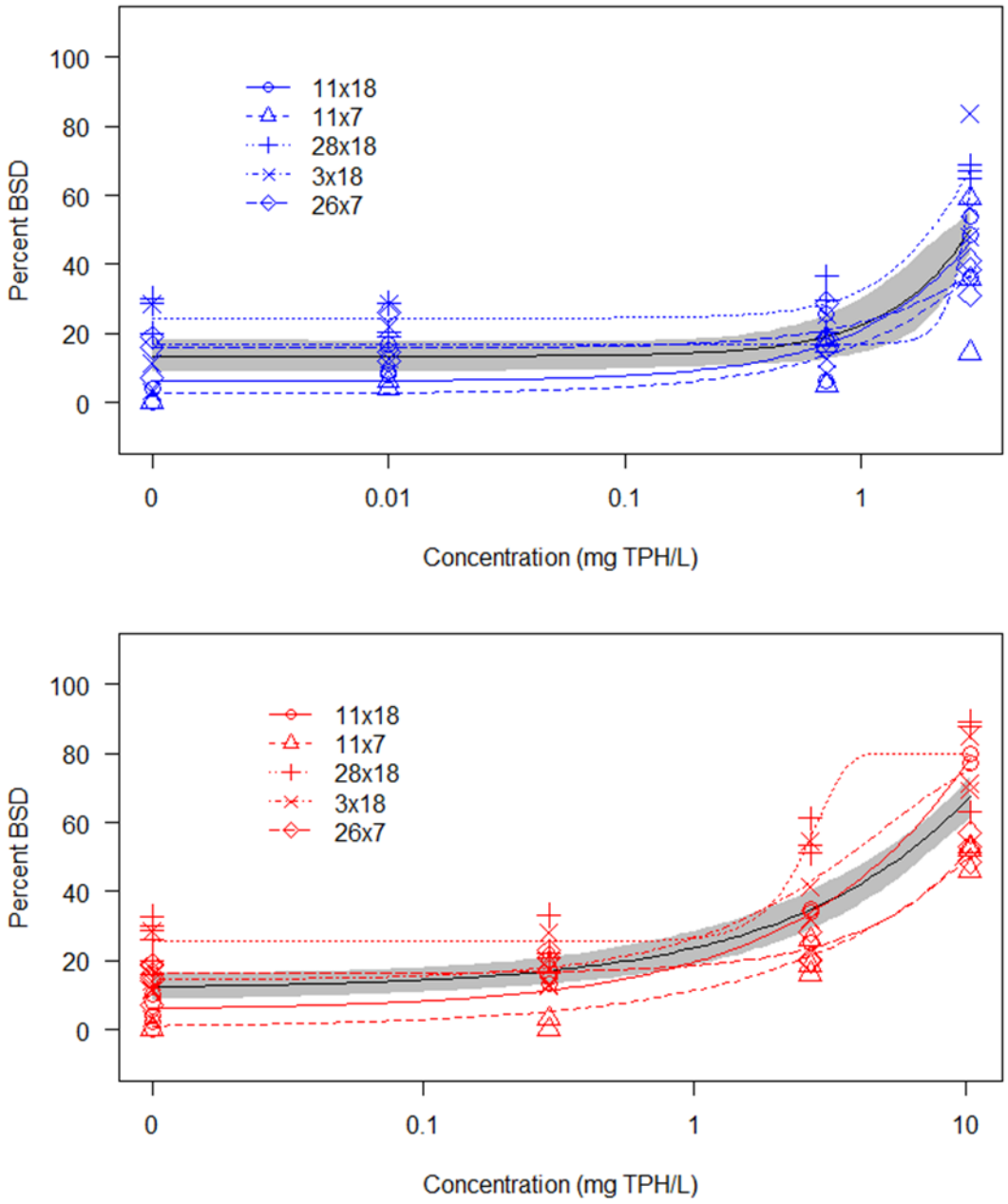


Figure 44: Concentration response relationship for blue sac disease (BSD) presentation from the WAF (top) and CEWAF (bottom) exposed organisms. Individual crosses are shown in blue (WAF) and red (CEWAF) with the response of the pooled crosses modelled in black.

The effect concentrations derived from the concentration response models are presented in Table 16.

Table 16: Summary of BSD and lethal effect concentrations (EC50 and LC10) for Atlantic cod exposed one day pre-hatch to WAF)and CEWAF. 95% confidence intervals are represented in brackets. LC10 values reflect both failed embryonic hatch and larval death)

			WAF (mg/L TPH)		CEWAF (mg/L TPH)	
Trial	**Cross** (♀ x ♂)	**Relatedness**	**EC50** (95% CI)	**LC10** (95% CI)	**EC50** (95% CI)	**LC10** (95% CI)
GM-058	3 x 18	-0.0249	2.81 (1.7 - 3.9)	1.59 (-1.3 - 4.5)	4.85 (2.6-7.1)	1.38 (0.2 - 2.5)
GM-052	11 x 18	-0.0156	3.65 (2.0 - 5.3)	1.56 (-0.1 - 3.2)	4.09 (3.3-4.9)	2.67 (1.3 - 4.1)
GM-058	28 x 18	-0.0961	2.58 (1.8 - 3.3)	2.08 (-10.7 - 14.9)	3.98 (1.8-6.2)	2.67 (1.2 - 4.2)
GM-059	26 x 7	-0.1106	7.97 (-7.5 - 23.4)	2.74 (0.7 - 4.8)	12.26 (10.1-14.5)	8.8 (-23.4 - 41.1)
GM-052	11 x 7	0.1552	5.35 (0.9 - 9.8)	3.09 (0.4 - 5.8)	9.75 (6.2-13.3)	9.60 (-29.5 - 48.7)
	Pooled		3.50 (2.71 - 4.29)	1.89 (0.53 - 3.24)	6.69 (5.17 - 8.21)	2.95 (1.38 - 4.51)

The results of the Corexit alone exposure during the hatching window, which followed the same procedure as the WAF and CEWAF exposure, are shown in Figure 45.

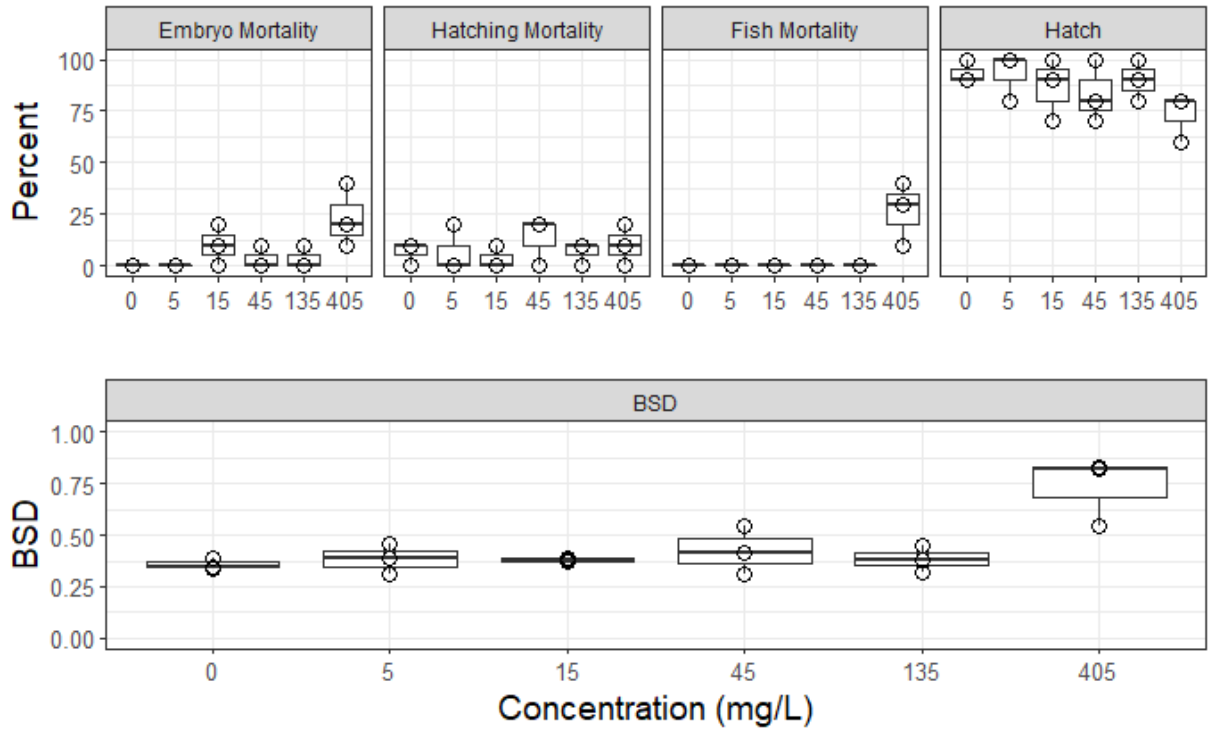


Figure 45: Hatching (top) and BSD (bottom) results from Corexit 9500 exposure during hatching window

There was no significant difference in percent embryo mortality (p = 0.066), percent mortality while hatching (p = 0.83), or percent hatch (p = 0.366) between the treatments and control. There was a significant effect on fish mortality (p = 0.0048), with the 405 mg/L treatment being significantly different from the control. There was a significant effect of exposure to Corexit on the presentation of BSD (p = 0.002), where hatched fish in the 405 mg/L treatment presented significantly greater BSD scores than the control (p = 0.003).

6.3.2 Growth assay

The growth assay did not meet validity criteria for survival (≥80%) as the control mortality in the negative seawater replicates exceeded 20% over the course of the trial. This was not necessarily unexpected given the delicate nature of this early Atlantic cod life stage. However, the results displayed a logical concentration response and still informative for determining the effects of exposure on growth. The mortality results for each day post exposure are shown in Figure 46.

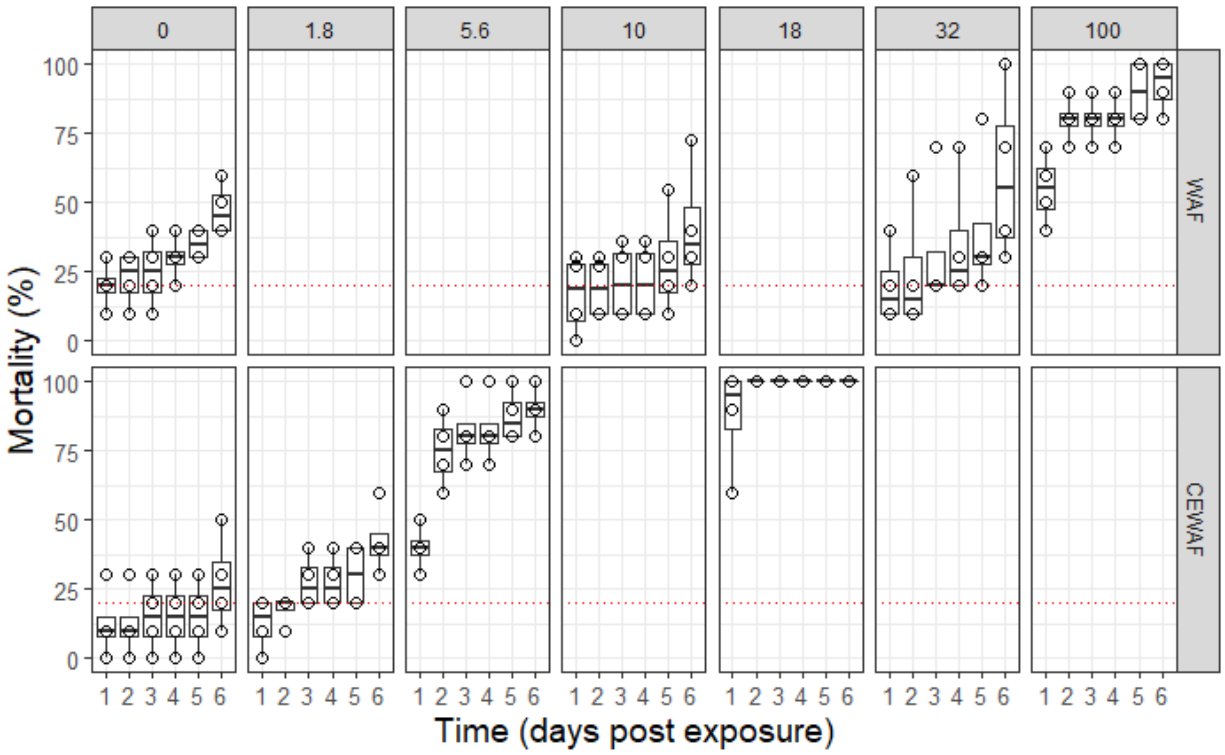


Figure 46: Percent mortality over time, dotted red line is the validity criteria of 20% control mortality.

The overall mortality and growth measures (dry weight and biomass) are presented in Figure 47.

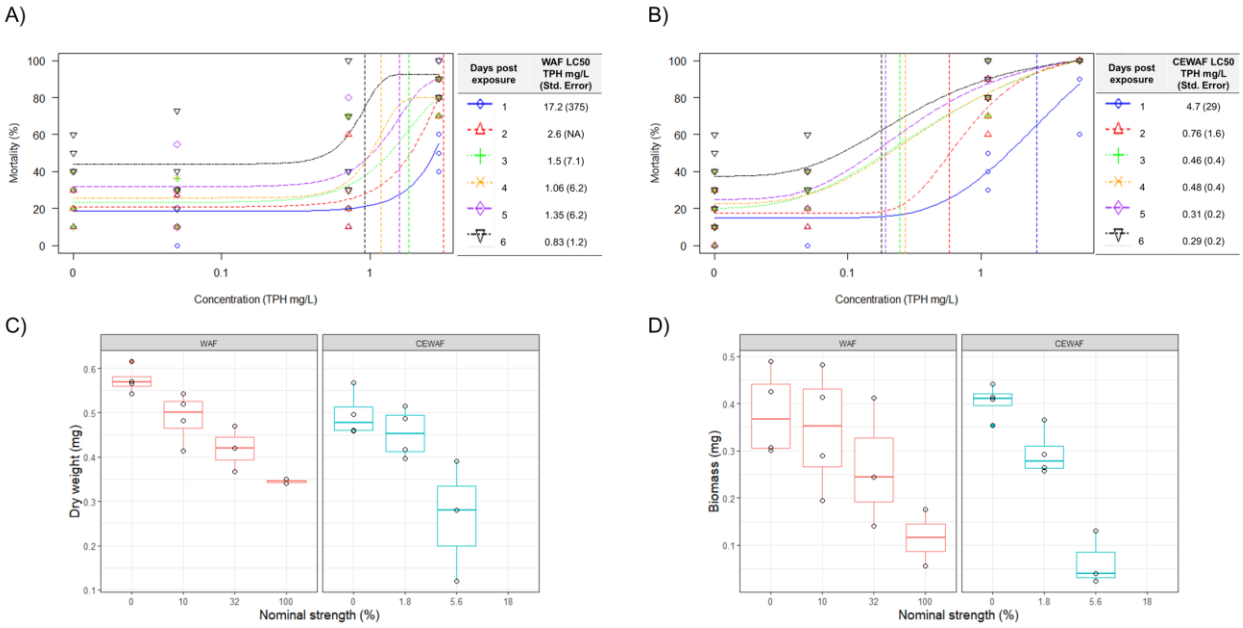


Figure 47: Summary of results from 24-hr exposure followed by 6 days in clean conditions. A) Percent mortality on each day post-WAF exposure, B) percent mortality on each day post-CEWAF exposure, C) dry weight at 7 days post-exposure, D) normalized biomass at 7 days post-exposure

As the growth assay did not meet validity criteria for survival at 7 days post exposure (<20% mortality), the LC50 results (Figure 47) should be interpreted with caution. However, the models do account for the control response, and the validity criteria are based upon more standard laboratory organisms, and as such the results are still considered relevant. The results show that a 24-hr exposure to concentrations of WAF (100%, 2.9 mg TPH/L) and CEWAF (5.6%, 1.13 mg/L TPH) can result in a significant decrease in growth.

6.3.3 Flow-through assay

The two trials in the flow-through vessels had similar results with no adverse effects of oil exposure observed on hatching, BSD presentation or growth (Figure 48).

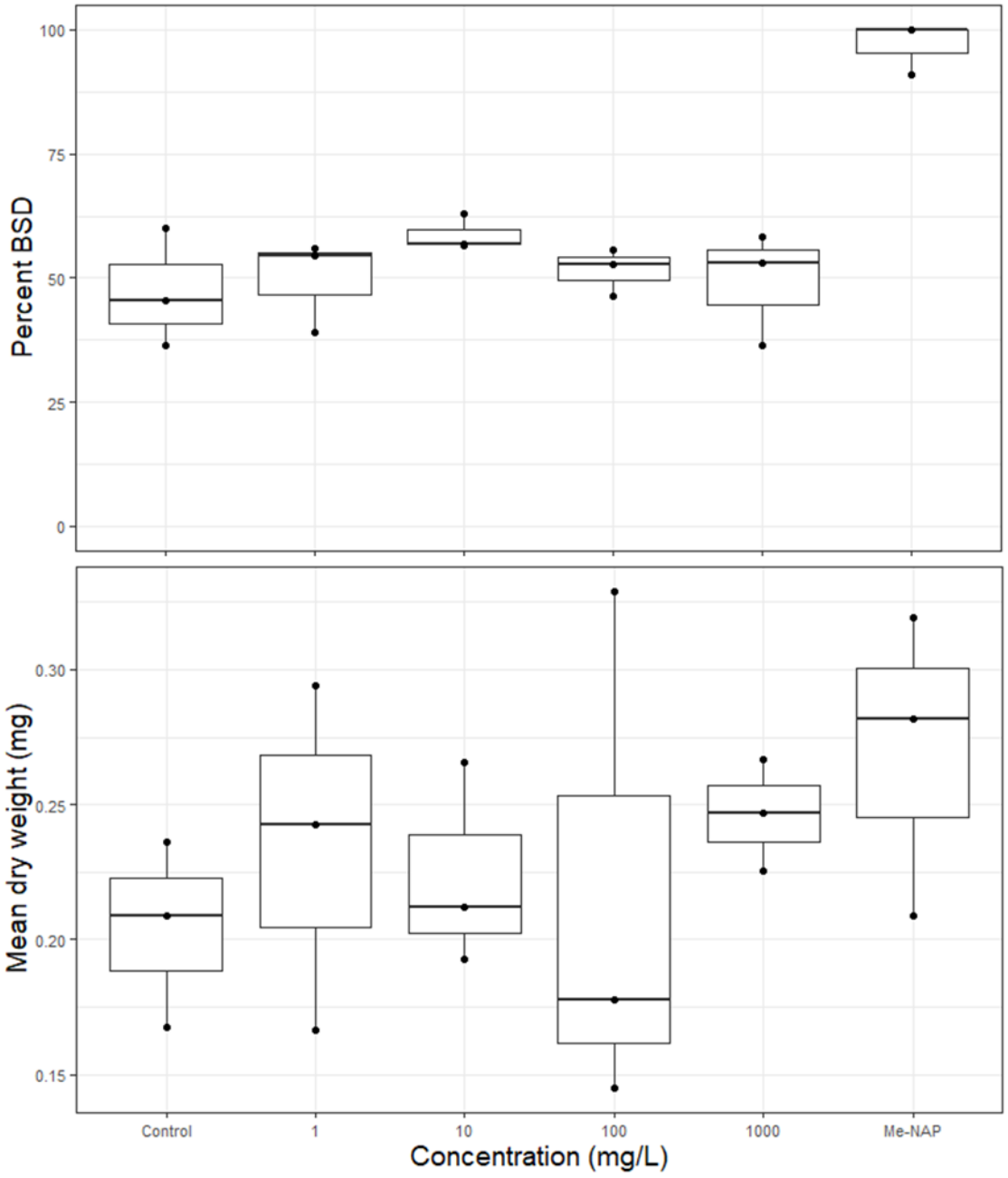


Figure 48: Summary of the growth and blue sac disease (BSD) endpoints from the variable loading trials.

The methylnaphthalene positive control (Me-NAP, 3.08 +/- 0.88 mg/L) induced significant BSD presentation in two of the 60 embryos that hatched. None of the variable loadings (NOEC = 1000 mg/L loading, estimated TPH = 3.0 mg/L) exhibited a BSD or growth response that was different from the control seawater.

6.4 Discussion

There was significant variation in response to the different exposure concentrations across the five cod families tested in the experiment. For example, embryos that were products of the 11x18 and 3x18 crosses had a significantly higher BSD scores following exposure to 10% CEWAF compared to the dispersant control. Whereas embryos that were products of the 28x18 cross had no significant difference in BSD between control CEWAF and 10% CEWAF. As these results were obtained with fish exposed under the same conditions, and in some cases to the same exposure media, it is likely that the differences observed in responses is the result of variation between families, which may have a heritable genetic component that could have population level effects.

At the larval stage, *G. morhua* undergo many essential physiological processes, such as swim-bladder inflation, jaw formation, and juvenile fin formation. It is possible that effects of acute exposure during the embryonic stage may not fully manifest until later stages of development. However, in our flow-through trials we did not see any impact of exposure on later development (e.g., hatching, yolk sac edemas, spinal curvature, or weight).

In fish, fecundity is both a logarithmic function of size (length) and a linear function of body weight. Thus, if growth rates of adults are supressed then this will lead to lower overall egg production (NASEM 2020). Bay anchovy (*Anchoa mitchilli*) larvae exposed to CEWAF did not result in significant mortality over 24 hours, but did result in a reduction in survival (25-77%) and weight specific growth rate (12-34%) after 6 days of post-exposure growth following the initial exposure (Duffy et al. 2016). With a 24-hour exposure, we observed a significant effect on growth at 0.7 mg/L TPH, which could have longer term consequences for fitness of the exposed individuals. These results suggest that acute responses (e.g., lethality) may not accurately reflect potential population level mortality and impacts to growth and development.

Chapter 7 Variation in survival among half-sibling families of Atlantic cod exposed to physically and chemically dispersed crude oil, implications for population level effects modelling

7.1 Introduction

Variability, defined as an observable diversity in biological sensitivity or response and in exposure parameters (IPCS, 2004), is often considered the bane of ecotoxicology studies. At best it is considered as illustrative information and at worst as noise that prevents the revelation of true biological effects (Devin et al. 2014). Literature detailing the variability of ecotoxicological responses has traditionally sought to understand the mechanisms underlying the variability and propose solutions to limit the variability, rather than explore the potential of the variability data to better understand the effect of a contaminant on the studied system (Calow 1996).

The experimental and natural variability must be properly characterized and attributed to appropriately interpret the observed effects on the studied system (Simmons et al. 2015). Controlling and addressing experimental variability is well understood and steps have been taken to improve it – through following validated standard methods, reporting all conditions (e.g., GLP), and participating in ring tests. Biological variability proves more challenging to address as there are multiple contributing sources including seasonal and temporal variation, genetic variation among individuals, life history, trophic interactions, and physiological status (varying according to age, reproduction or feeding status for example) to name a few. Further, variability in the toxicological response to chemicals is determined by the fate of the chemical within the body (toxicokinetics) and the toxicity of the chemical and its metabolites (toxicodynamics). Each of these also exhibit inherent variability related to the individual (or organism) and other factors that relate to the physiology and environment of the individual and which change over time (IPCS 2009).

Our project efforts made an initial attempt to control environmental variability experienced amongst specific crosses within their rearing and exposure conditions to elucidate the importance of familial variability in exposure response to population level effects for the Atlantic cod after exposed to physically and chemically dispersed crude oil. The

implications of this variability are discussed with reference towards environmental risk assessments associated with oil spill response operations.

7.2 Methods

7.2.1 Husbandry and test animals

Atlantic cod (n = 30; 20 females, 10 males) were longline caught offshore Nova Scotia, Canada in October 2016 and held within Huntsman wetlab facilities (ambient water temperatures and simulated natural photoperiod) with a wild food diet consisting primarily of squid, shrimp, and herring. After acclimatization to culture conditions, individual cod were PIT tagged in the left side of the dorsal region to allow individual fish tracking and recording of morphometric data. Fin clips were also taken and sent for genotyping to estimate the relatedness of individuals based on six microsatellite markers. Maturity status was assessed and tracked through visual and physical examination leading up to the spawning season. This also involved biopsy of the oocytes in the case of females. Gametes were collected from ripe and running male and female cod according to Huntsman Standard Operating Procedures and had their quality assessed. For the collected eggs, quality was assessed through a float test to determine the fraction of viable eggs, microscopic examination of uniform size and clarity (non-viable eggs are often discolored and/or dimpled), and qualitative judgement of the technical staff based on ease of collection, flow of eggs, color, and smell. The quality of milt was assessed through visual confirmation of motility under a microscope (qualitatively scored as none, poor, good, or excellent), spermatocrit readings, and again the judgement of technical staff. Fertilizations were then performed using gametes from a single male and a single female cod based on volumes collected, quality of gametes, and preference given to crosses that minimize relatedness of parents. Post-fertilization, embryos were transferred to 45-L aerated incubation pots and were monitored daily with dead embryos (as indicated by sinking to the bottom of the pot) purged from the system every two days. Upon hatch (~95-100 degree days), the yolk-sac larvae were gently transferred to 250-L circular ‘ponding tanks’, fed *Artemia* and rotifers, and monitored until use in bioassays (at approximately 200 degree days).

7.2.2 Test material

Exposure waters were prepared to test the toxicity of oil to aquatic organisms by contacting the oil and water such that a mixture is generated consisting of oil components in both the dissolved phase and in the non-dissolved phase, following the methods described in Section 3.3.2, Table 10. We employed a modification of the baffled flask method for dispersant effectiveness used by Environment Canada and the United States Environmental Protection to generate water accommodated fractions (WAFs) and chemically enhanced water accommodated fractions (CEWAFs) of offshore Newfoundland crude oil to provide an environmentally realistic oil microdroplet size profile and distribution. Briefly, 1.6 L of 0.5 µm filtered seawater was poured into a clean 2-L baffled flask to maintain a 20% headspace. An aliquot of oil was drawn up and dispensed onto the central surface of the water in the flask at a rate of 1 g of oil per L of water (equivalent to 1.6 g) using a gastight syringe. For the CEWAF preparation, the dispersant Corexit 9500A was then added to the centre of the surface slick at a dispersant to oil ratio of 1:20 (equivalent to 80 µL). The flask was sealed with DuraSeal then secured on an orbital shaker where it was shook at 150 rpm for one hour followed by a settling time of one hour. After the settling time, the stock was poured out from the spout of the baffled flask and used to prepare exposure media following gradient dilutions. The exposure media solutions were then physically characterized using a Laser *in Situ* Scattering and Transmissometry instrument (LISST-100X, Sequoia Scientific Inc., Seattle, WA) and select samples were preserved and shipped for analytical characterization.

7.2.3 Bioassays

The bioassay involved exposing groups of larvae to eight treatments (three concentrations of WAF, three concentrations of CEWAF, one dispersant control, and one dilution seawater control) for 24 hours. The exposures were conducted in 25-mL borosilicate scintillation vials with 20 mL of exposure media. The caps were screwed tight then loosened by a ¼ turn. Atlantic cod larvae from a specific cross were collected from the ponding tank and fasted (ensures that digestion does not consume energy, and limits the ammonia output during the exposure) while held in aerated water in buckets for 18 hours prior to exposure. Prior to exposure, 10 representative larval siblings were selected

and imaged under magnification (Leica Wild M420 microscope with a Leica MD190 camera at 10x magnification). These reference organisms had their eye diameter (EYED), jaw length (J), anal-body depth (ABD) and standard length (SL) measured using LAS software version 4.8. Ten larval siblings were allocated into each unlabeled scintillation vial following strict allocation criteria (e.g., active and regular swimming, normal morphology). Two rounds of replacements occurred after the initial fish allocation to ensure that all allocated fish continue to meet allocation criteria. Vials were then chosen at convenience for solution allocation and the vial assigned a corresponding label. Test concentrations for the cod bioassays were chosen after a range finding test and were 100, 56 and 32% WAF and 56, 32 and 18% CEWAF, plus a dispersant and dilution control, with each concentration replicated three times. The exposure was conducted in an environmental chamber with temperature maintained at 5 +/- 2^{o}C and a photoperiod of 16-hrs light and 8-hrs dark. The trial cod siblings were assessed as live or dead after the exposure. The bioassays were considered valid only if the following criteria were met: control mortality less than 20%, dissolved oxygen was greater than 60% saturation, and temperature variation was less than 1.5^{o}C amongst units. Blinding and masking technical staff was practiced during the post-exposure assessment to remove any likelihood for observational bias. A total of 24 family-based bioassays were conducted with crosses involving 12 different females and 8 males (Table 17).

Table 17: Summary of larval cod crosses

Year	Dam	Sire	Relatedness	Degree Days at Exposure
2017	11	18	-0.0156	225.8
2018	2	18	-0.1604	222.2
	3	25	-0.1106	186.3
	4	15	0.1644	176.8
		29	-0.0946	181.7
	6	8	0.0334	217.3
		18	0.0334	169.2
	11	7	0.1552	241.3
		9	-0.095	242.8
		18	-0.0156	239.9
	12	25	0.1021	207.8
	16	18	-0.1263	230
	20	27*	-0.0658	209.9
		27	-0.0658	202.9
	23	18	0.0033	184.6
		27	0.1049	176.9
	24	7	0.1427	203.5
		8	0.1584	162.4
		18	0.0497	169.3
	28	8	-0.0961	196.4
2019	10	7	-0.2201	272.85
		9	-0.0787	266.2
	24	9	-0.2201	270.45
		15	-0.0013	268.8

*held under different rearing conditions in a smaller bucket

7.2.4 Chemical analyses

Water samples from each test concentration from one trial were sent to RPC (Fredericton, NB) for analytical characterization. Each trial had all test solutions characterized using a LISST-100x to verify consistency of droplet size between preparations.

7.2.5 Statistical analyses

Toxicological data analyses were performed in R using version 3.1.3 (R Development Core Team, 2015). Percent mortality was obtained at the conclusion of the test and concentration-response curves were estimated by fitting log-logistic functions (*drc*

package: (Ritz et al. 2016; Ritz and Streibig 2005; Ritz 2016)). Median lethal effect concentration (LC50) estimates accounted for control condition and calculated values from each cross were compared using the EDcomp function available in the *drc* package. The LC50 values were used to generate a distribution curve using bootstrapping methodology.

Narrow-sense heritabilities (h^2) were estimated for survival of each individual within the 2018 family crosses, as the most robust dataset for this type of analysis, using animal models that are used within livestock breeding by fitting the following linear mixed model:

$$\boldsymbol{y = \mu 1 + X_1 t + Z a + e}$$

where, $\boldsymbol{y}$ is the vector of the phenotypic measurements; μ is the overall mean effect; $\boldsymbol{t}$ is the fixed effect of TPH; $\boldsymbol{a}$ is the random effects vector of animal effects, with $\boldsymbol{a}$~N($\mathbf{0}$, $\sigma_a^2\boldsymbol{A}$); and $\boldsymbol{e}$ is the random vector of errors, with $\boldsymbol{e}$~N($\mathbf{0}$, $\sigma_e^2\boldsymbol{I_e}$). $\boldsymbol{X_1}$ and $\boldsymbol{Z}$ are incidence matrices, and $\boldsymbol{A}$ is the numerator relationship matrix obtained from pedigree information. The binary trait of survival was assumed continuous normal for simplicity and helping to fit models. Significance of the factor TPH was evaluated with a Wald test, and narrow-sense heritabilities were calculated for each trait as: $h^2 = {\sigma_a}^2/({\sigma_a}^2 + {\sigma_e}^2)$, and their standard errors were approximated with the Delta method. Full-sibling family effects were not included as data does not include multiple generations, ages within year classes are similar, and no significant effects had occurred prior to challenging. All variance components and genetic parameters were estimated by fitting the previously described linear mixed models using the library ASReml-R 3.0 (Butler et al. 2009) as implemented for the R statistical package (R Core Team 2015).

7.3 Results

7.3.1 Reference organisms

The condition of test organisms from each cross was assessed by measuring 10 reference organisms for anal body depth, eye diameter, jaw length and standard length (Figure 49). There were no statistical differences amongst the families when considering

these key morphometric measurements indicating that siblings from all families were of the same overall size and condition before entering the respective challenges.

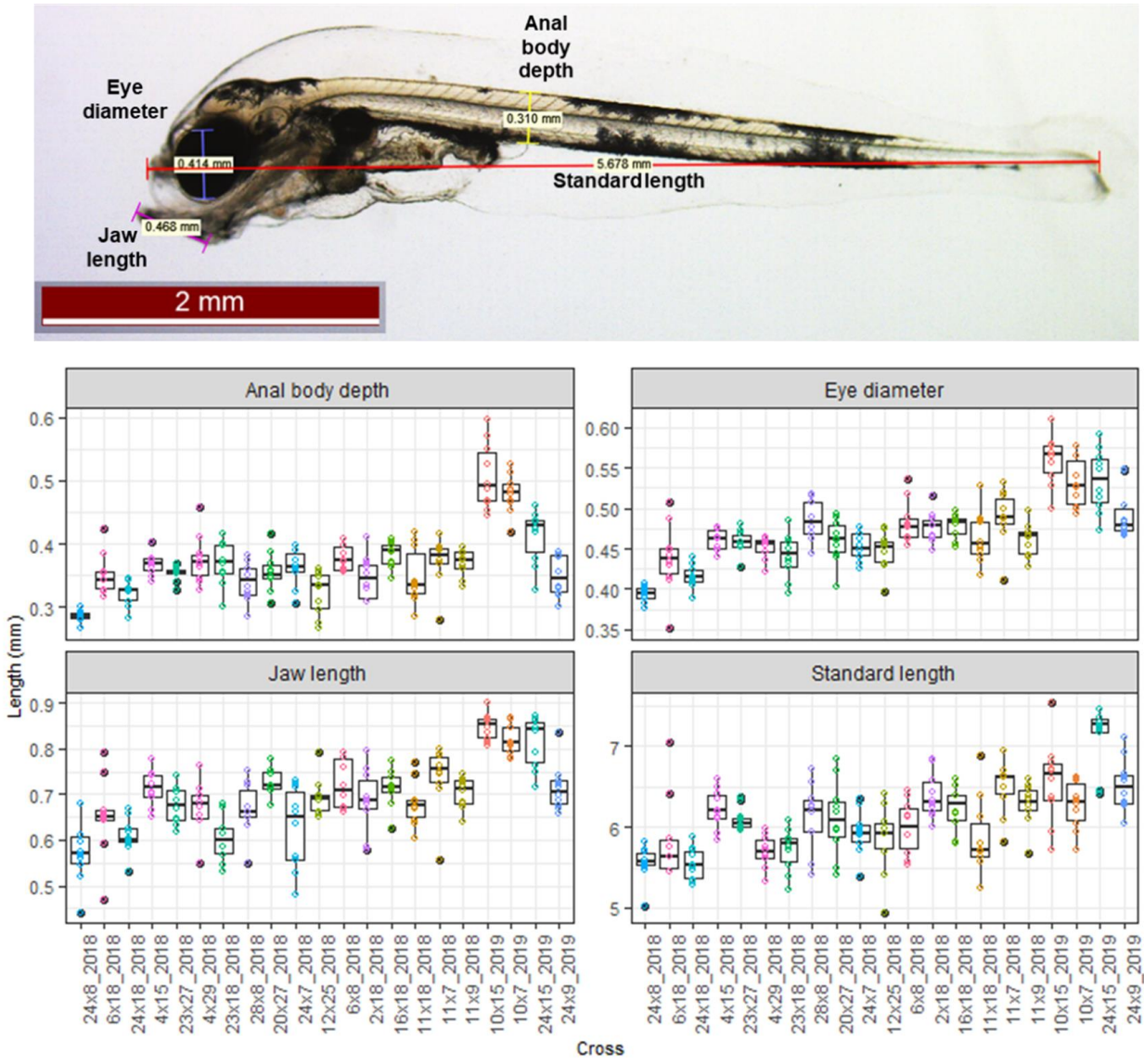


Figure 49: Summary of the reference organism measurements (top) from each cross (x-axis) ordered left to right by age (as degree days) at time of exposure.

7.3.2 Lethality

The results from the bioassay for each family cross is summarized in Figure 50.

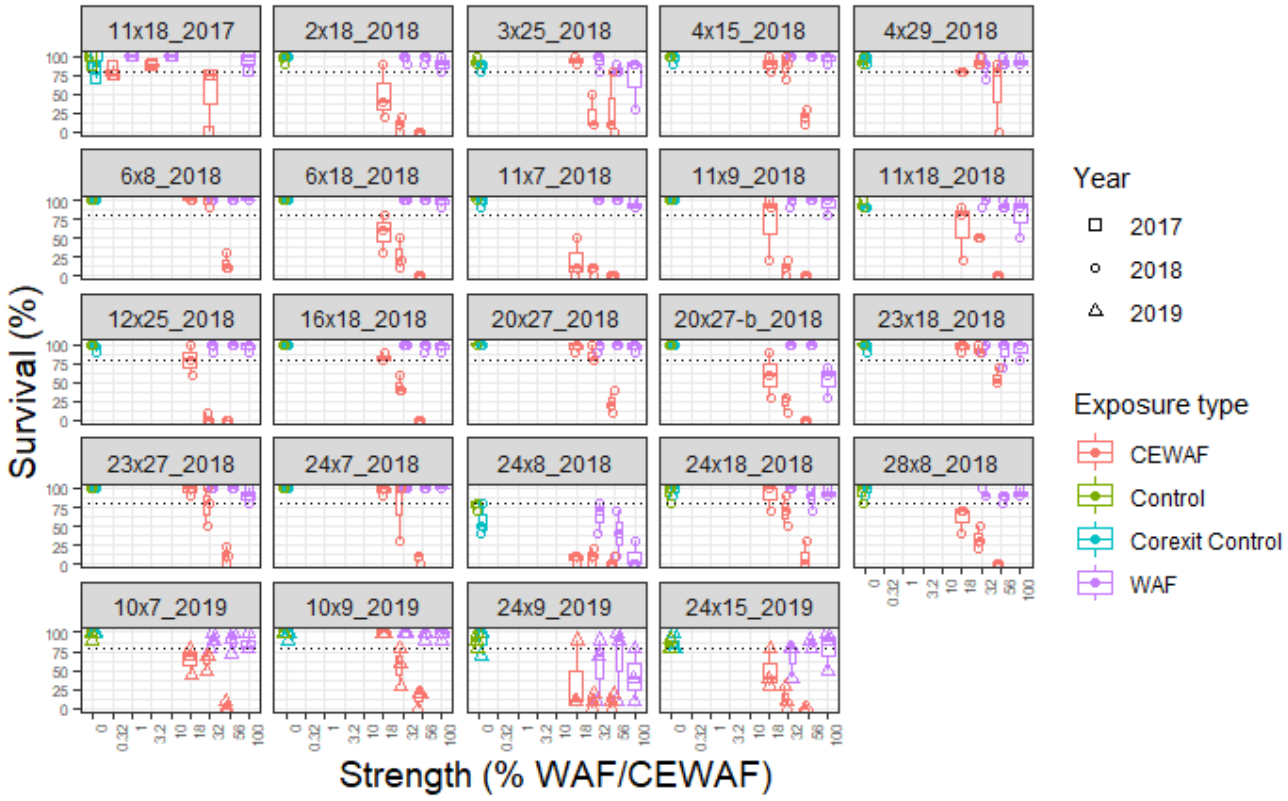


Figure 50: Summary of 24-hr bioassay results with each panel representing an individual cross (Female x Male_Year) and the nominal strength of each the WAF (purple) and CEWAF (red) on the x-axis.

The variability in responses at each of the exposure concentrations was visually examined by pooling the results from family all trials (Figure 51). There was much more variability in the CEWAF exposed organisms, especially between the 18 and 32% nominal dilution, where responses ranged from 0 to 100% survival. Both the seawater and dispersant only control demonstrated greater than 80% survival in most crosses.

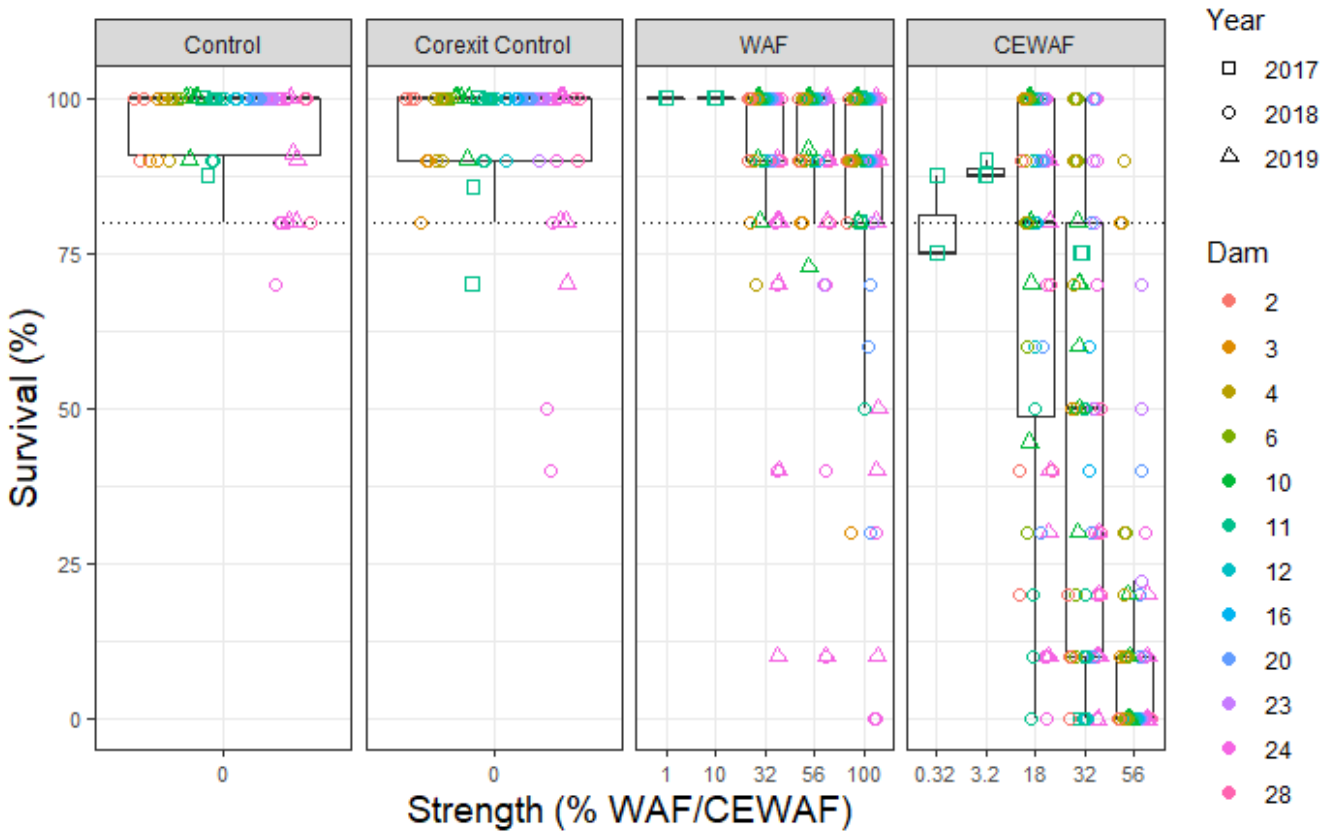


Figure 51: Variability in responses at each exposure treatment. The colour indicates the maternal contribution and the year of the bioassay is indicated by the shape (2017 = square, 2018 = circle, and 2019 = triangle).

The response data was combined by family cross and WAF or CEWAF treatments to model against TPH concentration using a 4 parameter log-logistic model and determine the LC50 values for each individual family cross and the entire pooled population (Figure 52).

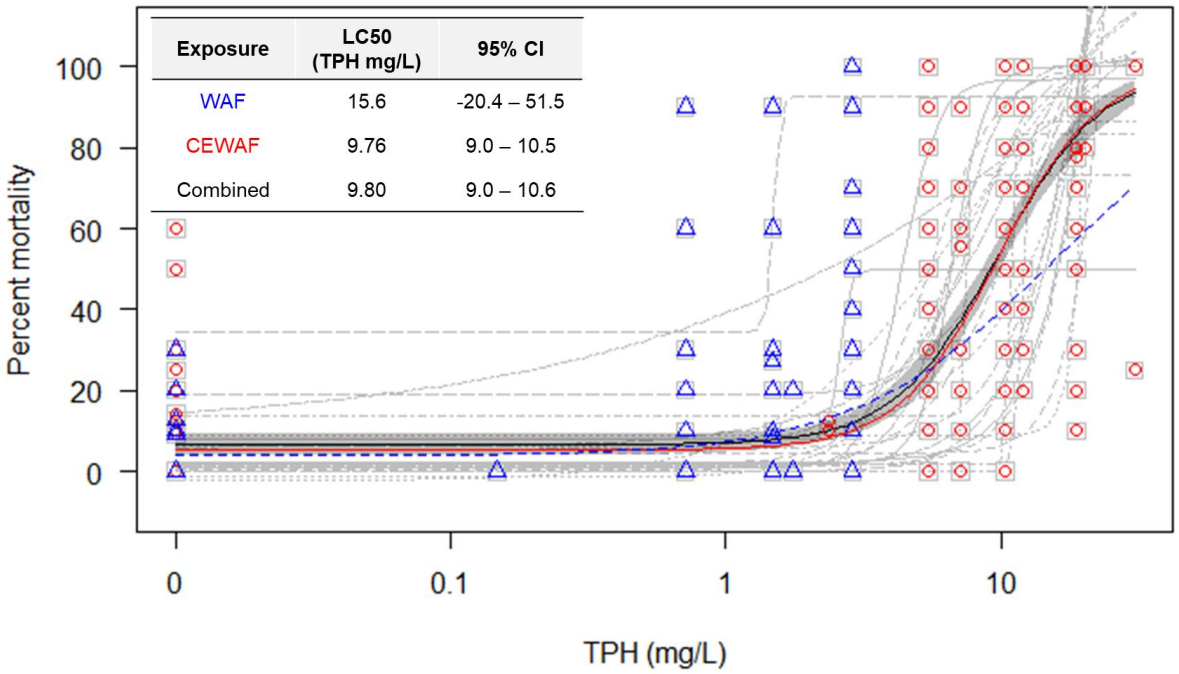


Figure 52: Concentration (TPH) response relationship showing each individual cross (grey lines), with the WAF (blue triangles), CEWAF (red circles) and combined (black line) exposures modelled. The estimated LC50s for the pooled population are shown in the insert for the WAF, CEWAF and combined exposures.

The 24-hr LC50s were not able to be calculated from the WAF exposure for each cross, however the LC50s for individual family crosses from the CEWAF exposure ranged from 0.02 - 31.3 mg/L TPH, and the pooled combined response had an LC50 = 9.8 mg/L TPH (9.0 - 10.6 95% CI). The LC50 values from the combined WAF CEWAF response pooled from each cross were compared using the EDcomp function within the *drc* package. Significant differences ($p<0.05$) between family crosses are identified in Table 18.

Table 18: Comparison of LC50 values of each family cross

	10x7 (19)	10x9 (19)	11x18 (17)	11x18 (18)	11x7 (18)	11x9 (18)	12x25 (18)	16x18 (18)	20x27 (18)	20x27b (18)	23x18 (18)	23x27 (18)	24x15 (19)	24x18 (18)	24x7 (18)	24x8 (18)	28x8 (18)	2x18 (18)	3x25 (18)	4x15 (18)	4x29 (18)	6x18 (18)	6x8 (18)
10x7 (19)																							
10x9 (19)	0.00																						
11x18 (17)	0.00	1.00																					
11x18 (18)	0.99	0.00	0.00																				
11x7 (18)	0.00	1.00	1.00	0.00																			
11x9 (18)	0.00	1.00	1.00	0.00	1.00																		
12x25 (18)	0.00	1.00	1.00	0.00	1.00	1.00																	
16x18 (18)	0.99	0.04	0.11	0.17	0.09	0.39	0.03																
20x27 (18)	0.91	0.00	0.00	1.00	0.00	0.00	0.00	0.06															
20x27b(18)	0.59	0.00	0.00	1.00	0.00	0.00	0.00	0.01	1.00														
23x18 (18)	0.03	1.00	1.00	0.00	1.00	1.00	1.00	0.79	0.00	0.00													
23x27 (18)	0.02	1.00	1.00	0.00	1.00	1.00	1.00	0.76	0.00	0.00	1.00												
24x15_(19)	0.03	1.00	1.00	0.00	1.00	1.00	0.99	0.82	0.00	0.00	1.00	1.00											
24x18 (18)	0.04	0.99	1.00	0.00	1.00	1.00	0.99	0.85	0.00	0.00	1.00	1.00	1.00										
24x7 (18)	0.01	1.00	1.00	0.00	1.00	1.00	1.00	0.61	0.00	0.00	1.00	1.00	1.00	1.00									
24x8 (18)	0.00	1.00	1.00	0.00	1.00	1.00	1.00	0.03	0.00	0.00	0.99	1.00	0.99	0.99	1.00								
28x8 (18)	1.00	0.01	0.04	0.37	0.03	0.19	0.01	1.00	0.16	0.04	0.53	0.49	0.56	0.61	0.35	0.01							
2x18 (18)	0.01	1.00	1.00	0.00	1.00	1.00	1.00	0.45	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.22						
3x25 (18)	0.00	1.00	1.00	0.00	1.00	1.00	1.00	0.03	0.00	0.00	1.00	1.00	0.99	0.99	1.00	1.00	0.01	1.00					
4x15 (18)	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.99	0.95	0.69	0.02	0.02	0.02	0.03	0.01	0.00	1.00	0.00	0.00				
4x29 (18)	0.00	1.00	1.00	0.00	1.00	1.00	1.00	0.03	0.00	0.00	1.00	1.00	0.99	0.99	1.00	1.00	0.01	1.00	1.00	0.00			
6x18 (18)	0.26	0.77	0.94	0.00	0.92	1.00	0.73	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.69	0.97	1.00	0.73	0.20	0.74		
6x8 (18)	0.00	1.00	0.94	0.00	1.00	1.00	1.00	0.04	0.00	0.00	1.00	1.00	1.00	0.99	1.00	1.00	0.01	1.00	1.00	0.00	1.00	0.77	

The 24-hr mortality response was also modelled using the PAH (sum 31) concentration in a 4 parameter log-logistic model (Figure 53).

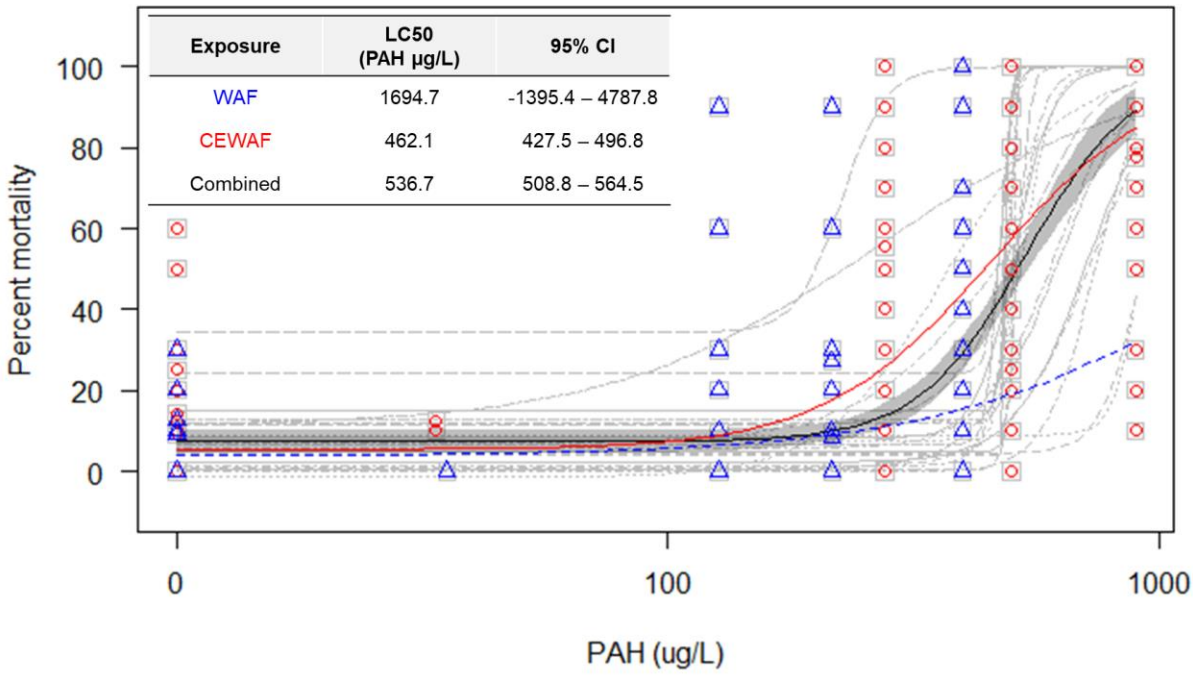


Figure 53: Concentration (PAH) response relationship showing each individual cross (grey lines), with the WAF (blue triangles), CEWAF (red circles) and combined (black line) exposures modelled. The estimated LC50s for the pooled population are shown in the insert for the WAF, CEWAF and combined exposures

The 24-hr LC50s from the pooled crosses was not relaible for the WAF exposure (large confidence interval). For the CEWAF exposure the LC50 was 462.1 µg/L PAH31 (427.5 - 496.8 95% CI), while the pooled combined response had an LC50 = 536.7 µg/L PAH31 (508.8 - 564.5 95% CI).

The 24-hr LC50 (TPH, combined WAF and CEWAF exposure) values from each cross were ranked and fit using a bootstrap methodology (a statistical method of resampling with replacement from a dataset to make an inference about an estimate for a population parameter) to generate a sensitivity distribution (Figure 54). The HC5 was calculated as 3.5 mg/L TPH with the 95% confidence interval of 2.4 - 5.3 mg/L TPH. This hazardous concentration is assumed to be protective of 95% of the larval cod population.

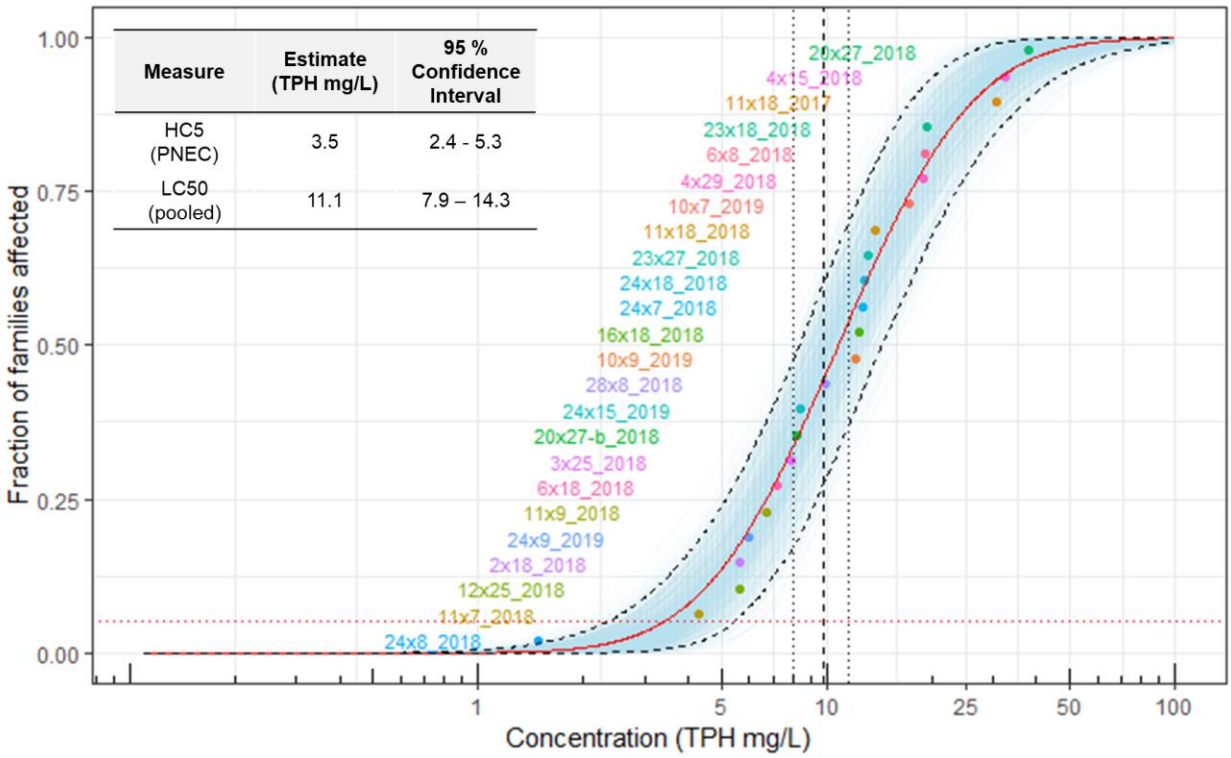


Figure 54: The distribution of LC50 values for each individual cross. The dashed vertical black line is the pooled LC50 and the dotted horizontal red line is the HC5.

7.3.3 Variability and Heritability

The variability in LC50 values was not explained by relatedness value (p = 0.89) or age at time of exposure (Figure 55, p = 0.64). The variability was also not signficantly explained by the reference organism parameters (Figure 56).

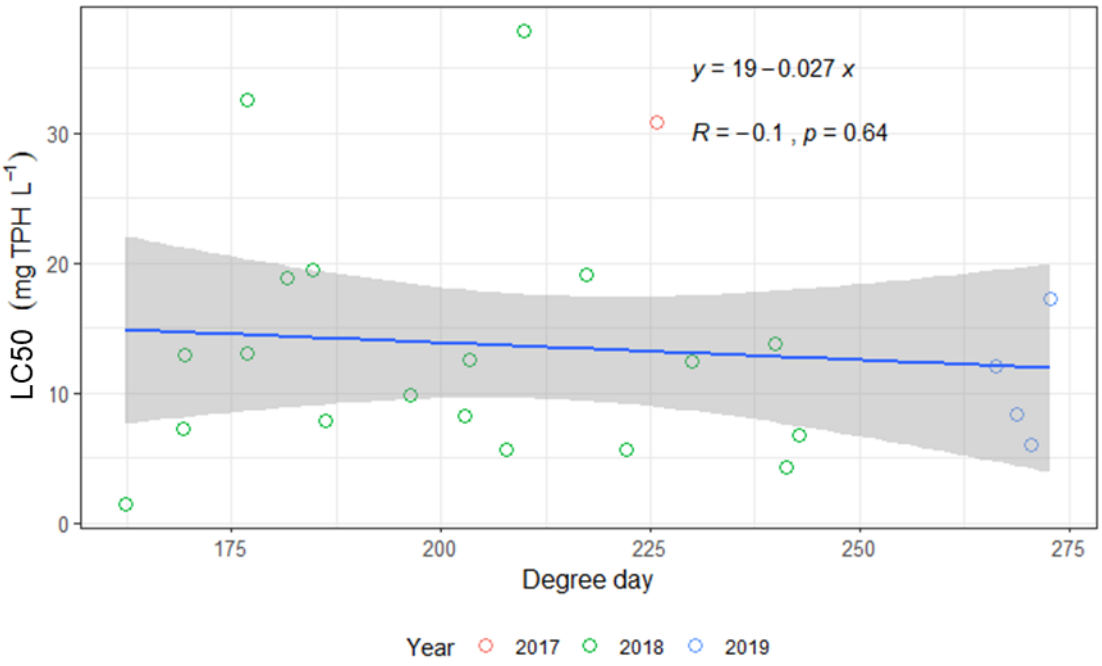


Figure 55: Relationship between age (degree day) at time of exposure and LC50 value.

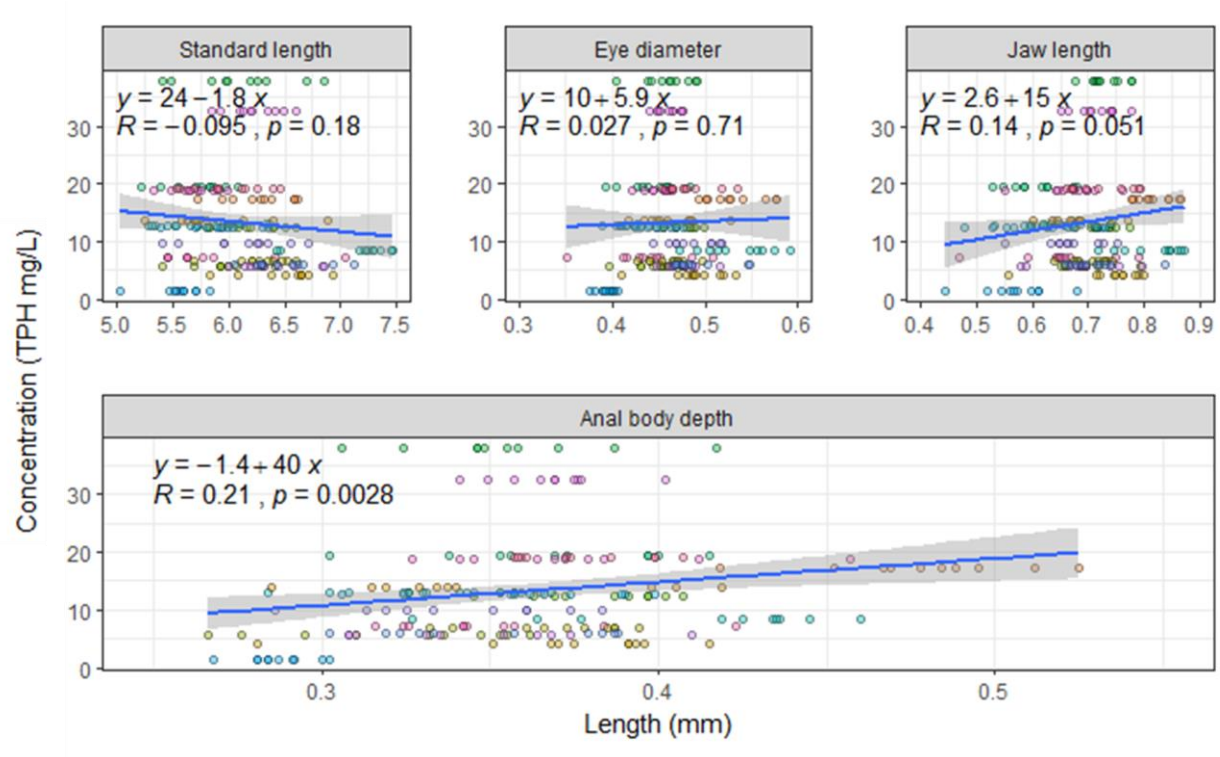


Figure 56: Reference organism measurements and LC50 responses

Anal body depth measurement did show a significant relationship with LC50 values with a larger body depth resulting in a larger LC50 value (less sensitive). However, this relationship was quite weak (r^2 = 0.21) and largely driven by a single cross that could be a statistical artefact rather than a true biological effect.

The narrow-sense heritability for survival as calculated with TPH was 0.40±0.17 (significance of TPH p<0.0001). However, a more typical approach to estimate heritability would evaluate performance of an individual. This means that survival is not calculated but an individual is either considered as alive or dead (referred to as fate here). The heritability of the results from the same experiments considering individual fate data with TPH was 0.28±0.09 (TPH p<0.0001).

7.4 Discussion

Calow (1996) highlights the two fold challenge for ecotoxicology in dealing with variability: the first is to understand its causes in affecting results from tests and the second is to understand its significance in relating observations from tests to an assessment of the risks of ecological systems in nature and damage from commercial chemicals. The results of our study show that there was significant variability in the lethal response of Atlantic cod to petroleum hydrocarbons with LC50s ranging from 1.5 to 37.9 mg TPH/L.

The causes of variability observed in any study can be attributed to experimental and/or biological factors. In this study, we sought to control and address as many of the experimental factors as possible but changing the parental contribution by exposing siblings from individual family crosses within each successive bioassay. We employed consistent methodologies in the husbandry of parents and progeny, preparation of exposure media, conduct of the tests, and maintanance of constant environmental conditions, which have been highlighted as a major contributor to variability (Hrovat et al. 2009). Those experimental factors that were outside of our control (e.g., exposure dates, age) did not explain the variation in response and, as such, the discussion focuses on potential biological factors that may have contributed to the observed variability. In particular, estimating heritability is a common method in livestock breeding programs to characterize the proportion of total observable (phenotypic) variance in a trait that is due

to genetics and degree of resemblance amongst relatives. Heritability may be estimated following experiments, such as our family cross exposures, that assess defined trait performance for multiple individuals from known families, multiple families are assessed under the same conditions, individual parent information is recorded, and (especially) when there is relatedness between families (half siblings).

Our estimated heritability of survival in terms of TPH ranged from 0.40±0.17 (calculated survival) and 0.28±0.09 (individual fate). These values indicate that the genetics component of siblings, families and relatedness of parents explains around 28-40% of the variation measured for the survival trait amongst families. The influence on these data from a specific sire (male parent), dam (female parent) or the specific combination of sire and dam (cross or family) are all possible factors that may be affecting our results. Heritability, and the ability to estimate it, is not only affected by the genetic variability of the individuals considered, the size of the dataset and the mating structure, but it is also affected by environmental, maternal and dominance effects. These effects include: differences in the environment that parents experienced prior to spawning (e.g., nutrition at critical points in egg development prior to spawning may affect later egg quality affecting fertilization rates, hatch survival and resulting strength of larvae), age of parents (e.g., virgin or first time spawners often have poorer egg quality), females that were spawned in successive years or skipped a year (e.g., skipped years may improve egg quality), reduced egg quality may affect the ability to hatch, and dominance deviation (interactions among alleles) associated with the particular combination of parents that may at worst cause an inability to create viable offspring.

As an example, maternal, paternal and half-sibling influences on early life history traits (e.g., fertilization, survival, hatching) of Baltic cod (*Gadus morhua*) was previously examined by Trippel et al. (2005). They found that of 13 early life history traits in Baltic cod (*Gadus morhua*), the relative contribution to variance from the female was greater than 50% for 11 of the traits measured. This contrasts with male and half-sibling pairing contribution of only greater than 5% in two (yolk area at day 5, and time to starvation) and four traits (egg survival days 0-4, egg survival days 5-9, yolk utilization rate, and time to starvation), respectively. Many of the traits that were dominated by female effects,

including standard length, yolk sac area, absorption, and usage, may be related to the differing egg sizes and quality among females. These differences in maternal traits are strongly influenced by many factors, including diet (especially during critical points in egg development), stress, genetics, egg over-ripening, age, and reproductive status (e.g., virgin or first time spawners often have poorer egg quality) (Avery et al. 2009; Hamoutene et al. 2009; Ouellet et al. 2001; Salze et al. 2005; Trippel and Neilson 1992). All of our families resulted from crossing gametes collected from the same small pool of male and female Atlantic cod parents within the same short timeframe to diminish many of these concerns within our collected dataset. Further, Petersen et al. (2016) found no significant relationship between female condition and size with survival rates of metamorphosed cod fry. They further noted that the female effect appears to be most apparent in early life stages. The cod in this study were aged ~200 dd and have already metamorphosed with some inherent selection pressures already surpassed (e.g., hatching, yolk-sac absorption, inflation of swim-bladder, first feeding) that may have been maternally driven. Therefore, we may have expected to observe a more uniform response given that these selection pressures have already been exerted upon the cod in contrast to our high variability noted with corresponding relatively high estimated heritability.

It is worthwhile to note that our estimated heritability for survival is less applicable to survival in a hatchery setting at a juvenile life stage and more applicable to heritability estimates of survival variation from a pathogen challenge. Ødegård et al. (2010) reported significant heritabilities of Atlantic cod survival to viral nervous necrosis of 0.75 ± 0.11 and 0.43 ± 0.07. The latter heritability estimate (0.43) or even a number such as 0.25 are more common heritabilities recorded in aquaculture production for these types of traits. In this project, we noted variations between crosses and the main desire to estimate heritability was to better quantify how those variations were related or unrelated to specific parents as variation based on genetic contribution when there are attempts to control the environment.

The discrepancy in response may in part be due to genetic variability associated with xenobiotic metabolism capability, specifically in the CYP1A family. Cytochromes P450 (CYPs) constitute a superfamily of monooxygenase enzymes that are involved in the

phase I of biotransformation of numerous endogenous and exogenous compounds, they are a major source of variability in biological responses (Zanger and Schwab 2013). The CYP1 family consists of four subfamilies, CYP1A, CYP1B, CYP1C, and CYP1D. The CYP1A subfamily plays an important role in metabolism and is a well established *in-vivo* biomarker of exposure to xenobiotics including, PAHs, PCBs, dioxins, pesticides (Uno et al. 2012). A bioinformatical analyses of the cod genome assembly was undertaken by Karlsen et al. (2012) to charaterize the full CYP-complement using historically archived liver microsomes of PAH (β-napthtoflavonene) treated Atlantic cod. They found 29 unique CYPs representing 9 different CYP gene families and 15 subfamilies, with the most prominent CYP isozymes coming from the CYP1A, CYP1C, CYP3A, and CYP4V families. Our preliminary screening of CYP1A activity for a different project demonstrated that there is variation between cod families in terms of the capacity and extent of induction when exposed to petroleum hydrocarbons. This variable level of induction, and thus biotransformation, could contribute to differences in sensitivity and susceptibility to petroleum hydrocarbons as manifested in differential survival of the exposed larval cod.

Our results demonstrate that a specific male Atlantic cod parent (sire), specific female Atlantic cod parent (dam), or the combination of specific male and female parents (crosses or families) may affect the resulting survival of siblings within a family. This observation makes the consideration of family important when performing toxicity tests, and providing insight into potential resilience and adaptation for an entire population (or at least the locally exposed population). We have demonstrated that tolerance/susceptibility to the effects of an oil spill is indeed a heritable trait within Atlantic cod, and through external pressures, selection of more resilient cod may occur.

We have conducted studies where biological variability could be assessed with respect to influences of parents (heritability). Our data show that choice of parents may affect experimental results and that some thresholds are heritable traits. This finding is important for interpreting results of other studies with cod and oil products where investigating sources of biological variability is impractical. This suggests that a consistent experimental population (lab populations) would be beneficial when assessing relative toxicity of oils, dispersants, etc. The use of the “lab rat” approach would likely eliminate

much of the variability but at the expense of assessing risk in the real world. By characterising variability as we have done in this study we are able to develop a threshold concentration (HC5) that is likely to be protective of the population should concentrations remain below this value.

Atlantic herring (*Clupea harengus*)

Definitions

- **BSD**: blue-sac disease; phenotypic presentation of an assemblage of developmental abnormalities characterized by vertebral abnormalities, edemas, hemorrhaging, reduced growth, and survival
- **Dam**: the female parent of an animal
- **Edema**: swelling caused by the buildup of fluid
- **Eggs**: fully ripe, unfertilized internal egg masses in the ovaries, or the released external egg masses of fish and certain marine animals
- **Embryo**: the fertilized, and developing egg
- **Family/Cross**: offspring that carry part of the genetic material from the same two individuals
- **Full-sibling**: individuals sharing the same dam and sire
- **Half-sibling**: individuals sharing one of either the same dam or sire
- **Heritability**: fraction of the total variation observed in a trait that can be accounted for by genetics
- **Lordosis**: an abnormal inward (forward) curvature of the vertebral column
- **Milt**: the semen of a male fish
- **Scoloiosis**: an abnormal lateral curve to the vertebral column
- **Sire**: the male parent of an animal

Background

Atlantic herring (~50 per collection) were acquired from fishers with activities in the Bay of Fundy. Recently caught (< 48-hours) herring were received wharf-side (Blacks Harbour, NB), packed on ice, and transported to the Huntsman. After successful biosecure entry into the laboratory, the gonads of each fish were excised and separated by sex (Figure 57).

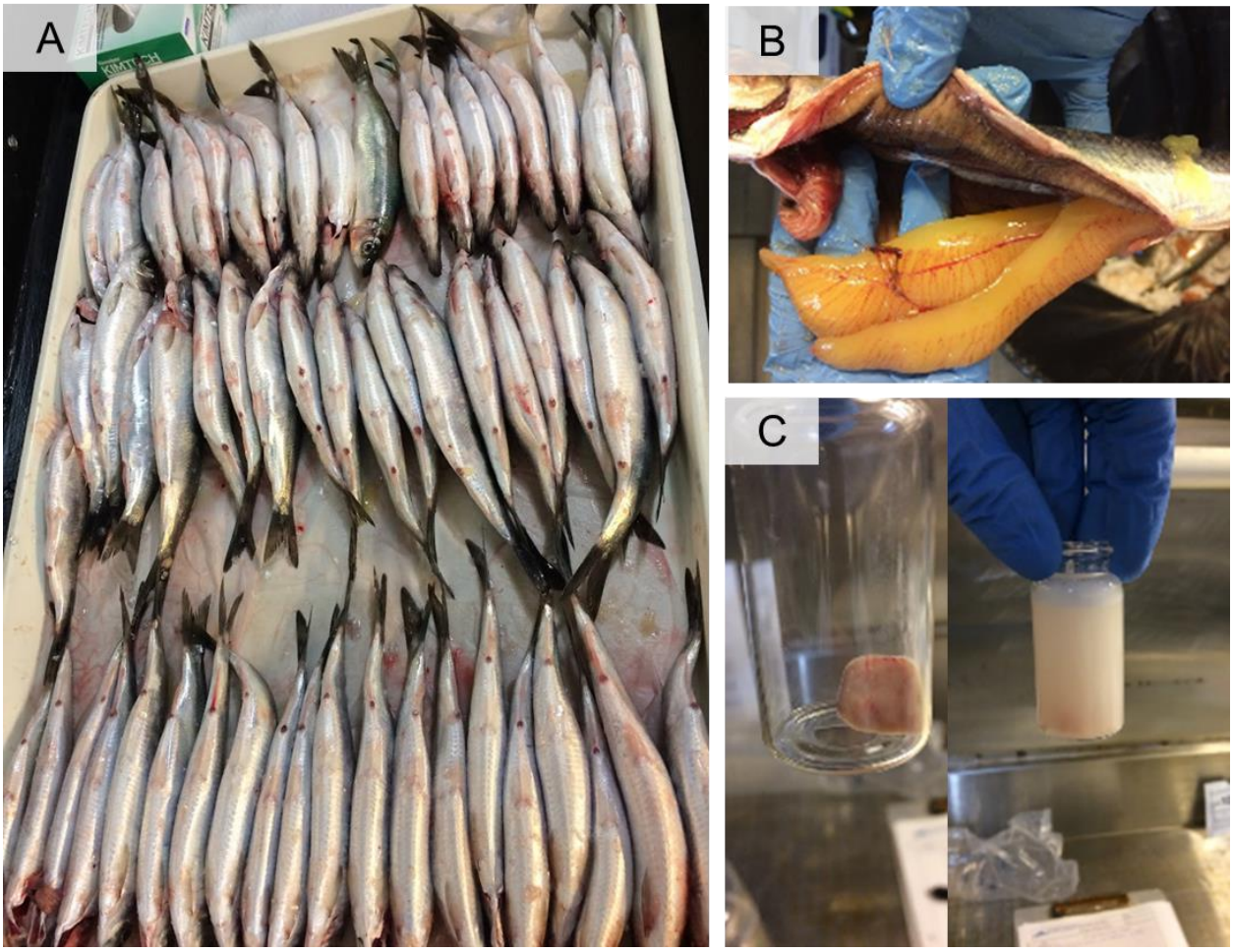


Figure 57: A) Atlantic herring collected from fishing activities, B) excised ovary, and C) piece of testes before (left) and after (right) maceration in seawater.

Fertilizations were performed by depositing eggs that had be extruded from the excised ovary into a thin layer on a substrate (e.g., glass microscope slide or petri dish) in a shallow layer of seawater. A sperm suspension, made by macerating a piece of testes in seawater, was then added to the submerged eggs for a period of 60 minutes then rinsed and placed in seawater until use in toxicology trials.

The toxicology trials involving Atlantic herring are described in Chapter 8.

Chapter 8 Effects on the early life stages of Atlantic herring

8.1 Introduction

Oil tanker routes in eastern Canada often pass through fish spawning grounds, particularly those of the Atlantic herring (*Clupea harengus*) around New Brunswick, Nova Scotia, and Newfoundland, Canada (Greer et al. 2012). Atlantic herring may be a useful model for other marine species and the results of research on Atlantic herring could be extended to other *Clupea* species in the North Atlantic and even to Pacific herring.

Along George's Bank off Eastern Canada and the United States, spawning of Atlantic herring occurs at 40 to 80 m depth, which is likely below the range of dispersed oil. However, on Canada's Scotian shelf herring spawn 15 to 60 m below the surface and off the coast of British Columbia Pacific herring (*Clupea pallasil*) spawn in shallow, near shore waters of 0.5 to 8 m below mean tidal depths. This range in depth highlights that exposure of vulnerable early-life stages to oil and dispersed oil is contingent on their spawning habitats (McIntosh et al. 2010).

We used a modification of the herring "sticky-egg" bioassay (Boudreau et al. 2009; Kocan et al. 1996) that gained prominence following the Exxon Valdez spill (Carls et al. 2002) to assess the toxicity of physically and chemically dispersed oil on Atlantic herring at several early life stages and across relatively short, realistic exposure durations. Results of these trials will help inform mechanistic oil exposure and toxicity models, like Spill Impact Model Application Package (SIMAP; developed by Applied Science Associates, Narragansett, RI, USA), which require high quality, reliable data for the sensitivity of exposed organisms over a range of time scales.

8.2 Methodology

8.2.1 Test Animals

Atlantic herring (~50 per collection) were acquired from fishermen active in the Bay of Fundy. Recently caught (< 48-hours) herring were received wharf-side (Blacks Harbour, NB), packed on ice, and transported to the Huntsman Marine Science Centre (HMSC). The gonads of each fish were excised and separated by sex after successful biosecure

entry into the laboratory. The gonads were then used to perform fertilizations and the embryos were then available for testing.

8.2.2 Media Preparation and Exposure Conditions

All WAFs and CEWAFs were prepared as discussed in Section 3.3.2, Table 10.

All trials were conducted in the environmental chamber with temperature maintained at 10+/- 2^{o}C, a photoperiod of 16-hrs light and 8-hrs dark for the entire duration, and in full salinity natural seawater (~32 PSU). Water quality parameters (temperature, dissolved oxygen, pH, salinity) were measured in all test and exposure solutions at pre- defined frequencies. All measures were within appropriate ranges for this species and did not differ between treatments.

8.2.3 Fertilization Exposure

Exposures were conducted during fertilization to examine the effects of physically and chemically dispersed crude oil on the earliest life stage. Eggs from the ovary of a single female (Figure 58A) were extruded and ‘painted’ onto glass microscope slides (~50 eggs per slide, Figure 58B) then submerged in seawater.

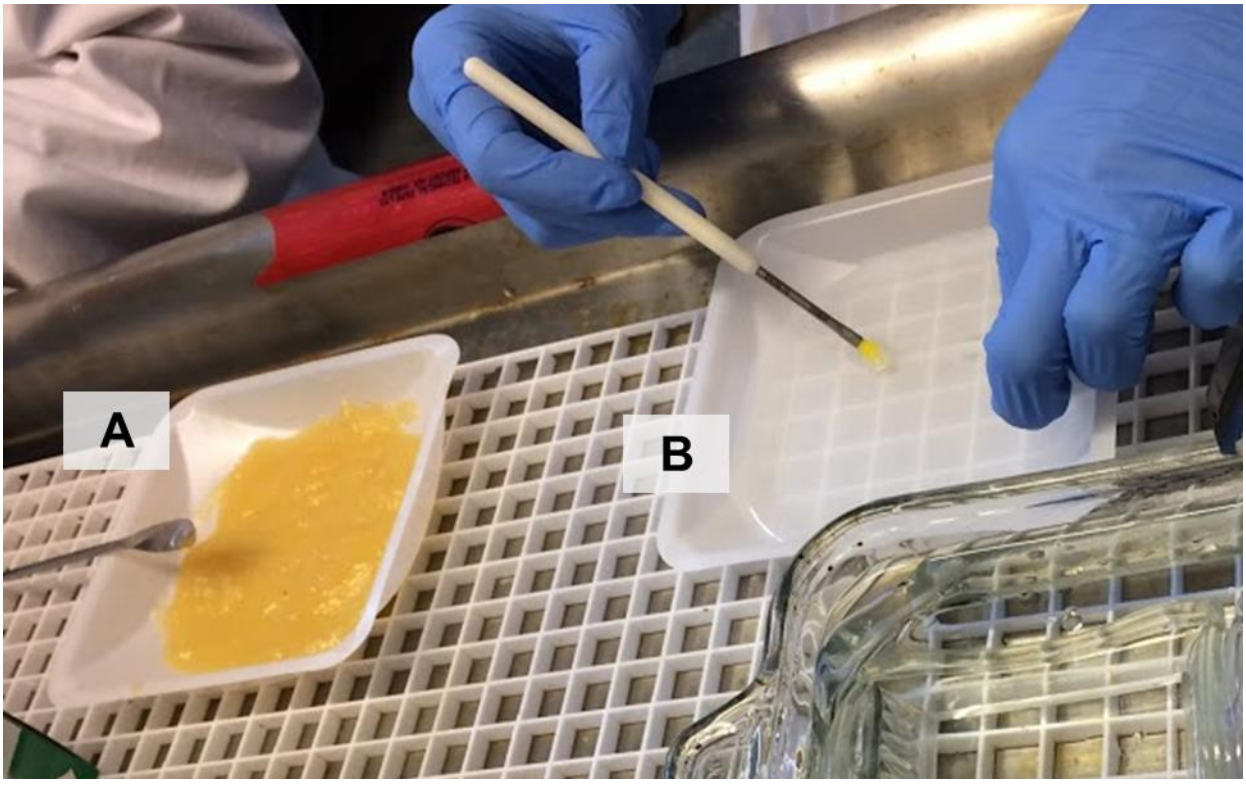


Figure 58: A) extruded eggs from an excised ovary; B) 'painting' a thin layer of eggs onto a submerged microscope slide.

Egg-loaded slides (n = 3 per treatment) were placed into a slide rack, which was then lowered into a glass staining dish (16.5 x 9.7 x 12.4 cm) filled with either a WAF (100, 56, 32, 18, or 10% strength), CEWAF (32, 18, 10, 5.6, 3.2, or 1.0% strength), negative control (0.22 µm seawater), dispersant control (Corexit 9500A at the same strength as the 32% CEWAF, nominally 15.2 mg/L), or positive control (10 mg/L sodium dodecyl sulfate [SDS]) solution. 10 mL of sperm solution (made from 3 cm^2 testis from a single male macerated into 100 mL seawater) was added to the ~500 mL of exposure solution and gently mixed just prior to the addition of the slide rack to the staining dish. The lid was placed on the staining dish after the slide rack was submerged and the slides were left in solution for 1-hour.

After the exposure period, the slides were removed, rinsed (0.22 µm seawater), then placed into 250-mL glass mason jars filled with 200 mL 0.22 µm filtered seawater and monitored with daily renewal of seawater until hatch. At 2 days post fertilization, all embryos on each slide were assessed (fertilized, non-fertilized, abnormally fertilized, non-

viable), then thinned (accomplished under a dissection microscope with forceps) until 20 well-spaced, fertilized embryos remained on each slide. Each day the slides were removed from the falcon tubes, placed in a shallow dish containing seawater, and assessed under the microscope with any dead embryos being removed. After assessment, the slides were returned to the falcon tube with new seawater. The slides were monitored until all embryos had hatched or died.

8.2.4 Embryo exposures

Embryos for use in trials were prepared as discussed in Section 8.2.3 but only in seawater. Two days post fertilization embryos were exposed to solutions of WAF (100, 32, or 10% strength), CEWAF (32, 10, or 3.2% strength), negative control (0.22 µm seawater), dispersant control (Corexit 9500A at the same strength as the 32% CEWAF), and positive control (10 mg/L SDS) solution for 1, 6, 18 or 24 hours to assess the role of exposure duration. Exposures were conducted by placing a slide rack with four slides into a staining dish containing exposure solution. The dish was covered then a single slide was removed, rinsed, and placed into a monitoring vessel (250-mL mason jar filled with 200 mL 0.22 µm filtered seawater) at each time interval (n = 1 for each concentration and time point). The units were monitored until hatch with daily assessment and renewal of seawater.

The sensitivity of different developmental stages at the time of exposure was assessed by performing a 24-hr exposure to solutions of WAF (100, 32, or 10% strength), CEWAF (32, 10, or 3.2% strength), negative control (0.22 µm seawater), and dispersant control (Corexit 9500A at the same strength as the 32% CEWAF, nominally 15.2 mg/L) on embryos at 2, 7 and 10 days post fertilization (Figure 59).

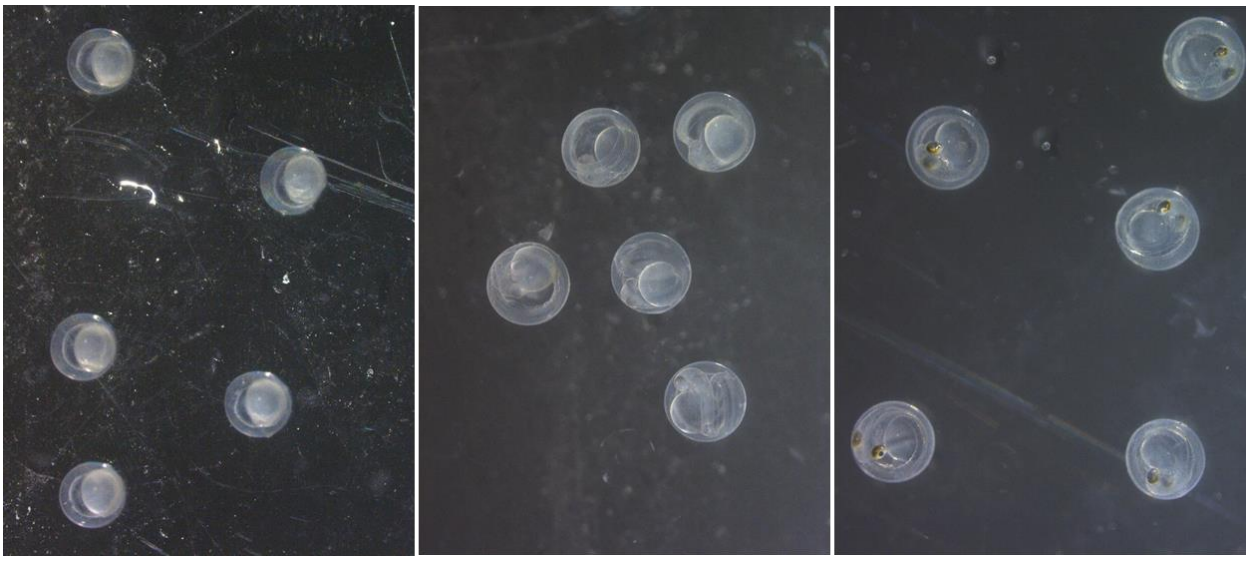


Figure 59: Representative embryos at the start of each exposure stage. Left to right = embryos at 2, 7 and 10 days post fertilization.

The slides (n=1 for each treatment and age) were transferred to 50-mL falcon tubes (filled with seawater) post-exposure and monitored until hatch.

Finally, the variability in response of the herring population was assessed by exposing herring embryos from 7 different half-sibling crosses (1 sire, 7 dams) to solutions of WAF (100, 56, 32, 18, or 10% strength), CEWAF (32, 18, 10, 5.6, 3.2, or 1.0% strength), negative control (0.22 µm seawater), and dispersant control (Corexit 9500A at the same strength as the 32% CEWAF, nominally 15.2 mg/L) for 24-hrs. After exposure, the slides (N=91) were transferred to individual falcon tubes (50-mL filled with 0.22 µm seawater) and monitored with daily renewal until hatch.

8.2.5 Assessments

In all trials, hatching occurred over several days and newly hatched larvae were assessed (normal or abnormal; live, dead or dead while hatching), removed and preserved in Stockard's solution each day. Larvae were classified as normal larvae if they were quite straight with little to no curvature of the head or spinal regions, had normal facial features, and were active swimmers. Abnormal larvae had moderate to severe curvature of the spine (scoliosis or lordosis), abnormal facial features, and reduced or erratic swimming behavior. The trial validity criteria were control mortality less than 20%, dissolved oxygen

saturation greater than 60%, and temperature variation less than 1.5ºC amongst units on a given time point.

8.3 Results

8.3.1 Fertilization in WAF/CEWAF

There was an overall low fertilization rate with a high degree of variability between replicates of the same treatment, especially the control (Figure 60).

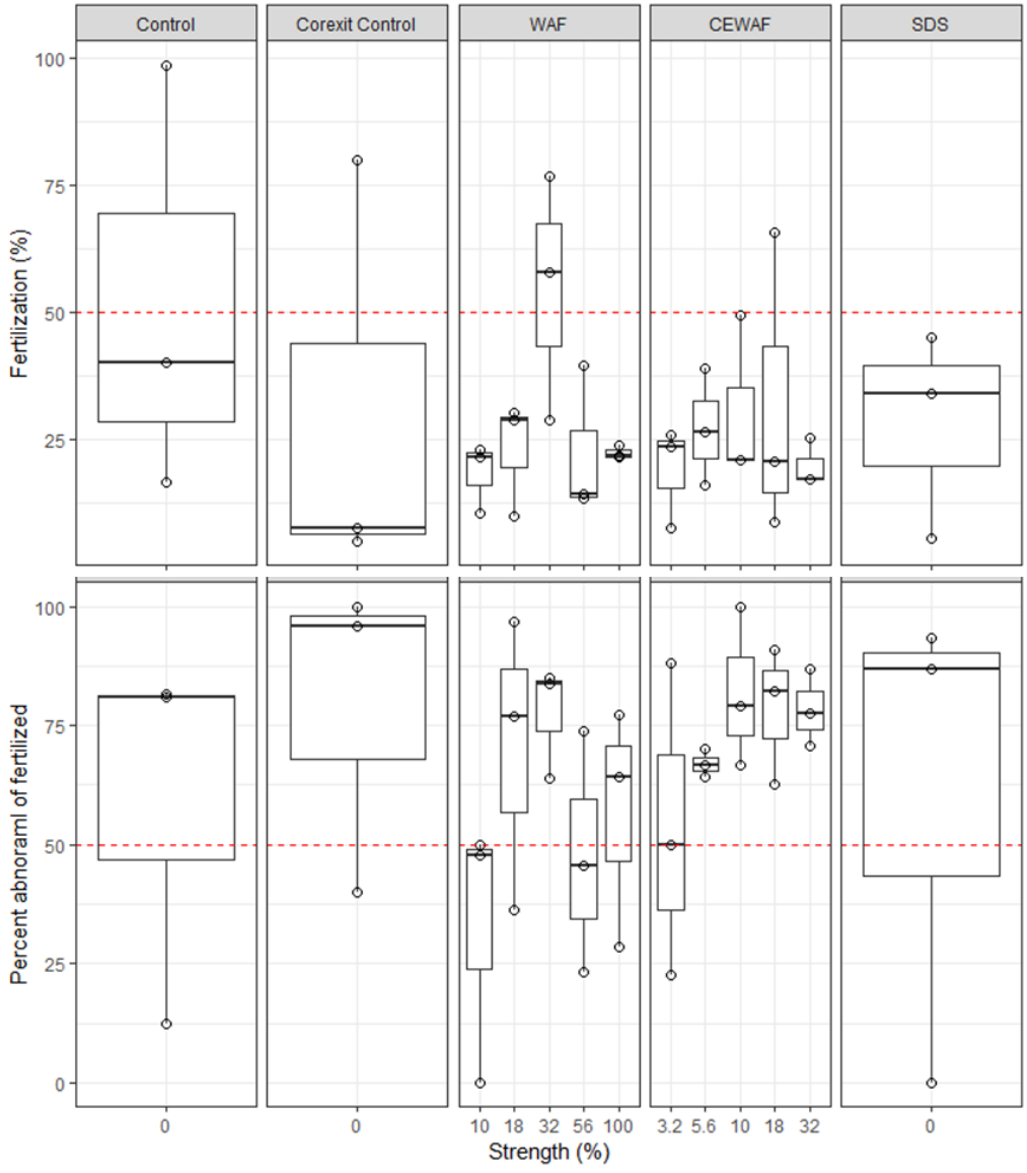


Figure 60: Percent fertilization (top) and percent of fertilized embryos that were abnormal (bottom)

The low fertilization rate, combined with the variability between replicates, precluded the detection of any significant differences between treatments (p = 0.63).

The low fertilization rate also meant that after thinning there were no slides that had 20 embryos remaining and the number of fertilized embryos remaining per slide ranged from 0 to 17. The embryos were followed through to hatch despite the uneven numbers after thinning (Figure 61).

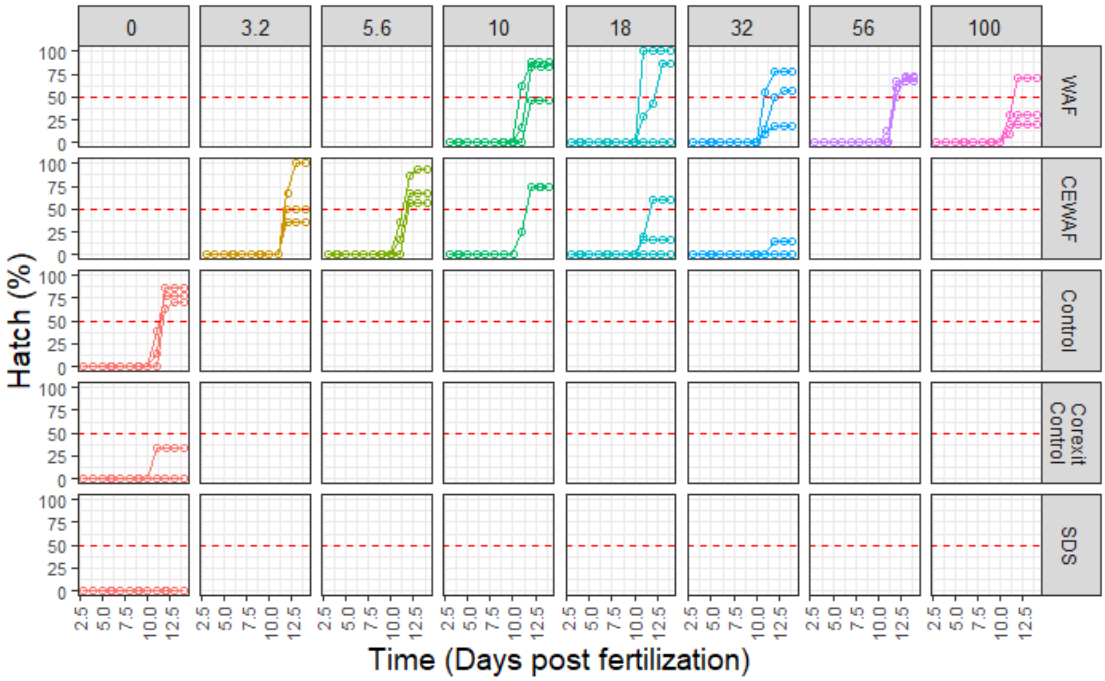


Figure 61: Percent overall hatch by each exposure type (rows) and strength (columns) with each line an individual replicate.

There was a concentration response relationship with both the WAF and CEWAF treatments, where increasing strength of solution saw decreasing hatching success. However, the response was variable between replicates, possible owing to the unequal distribution of fertilized embryos. The exposure to the positive control (10 mg/L SDS) resulted in 0% hatching as expected.

The overall survival was modelled using TPH concentrations considering the WAF and CEWAF responses alone and combined using a 4 parameter log-logistic model with only those slides that had five or greater fertilized embryos (Figure 62).

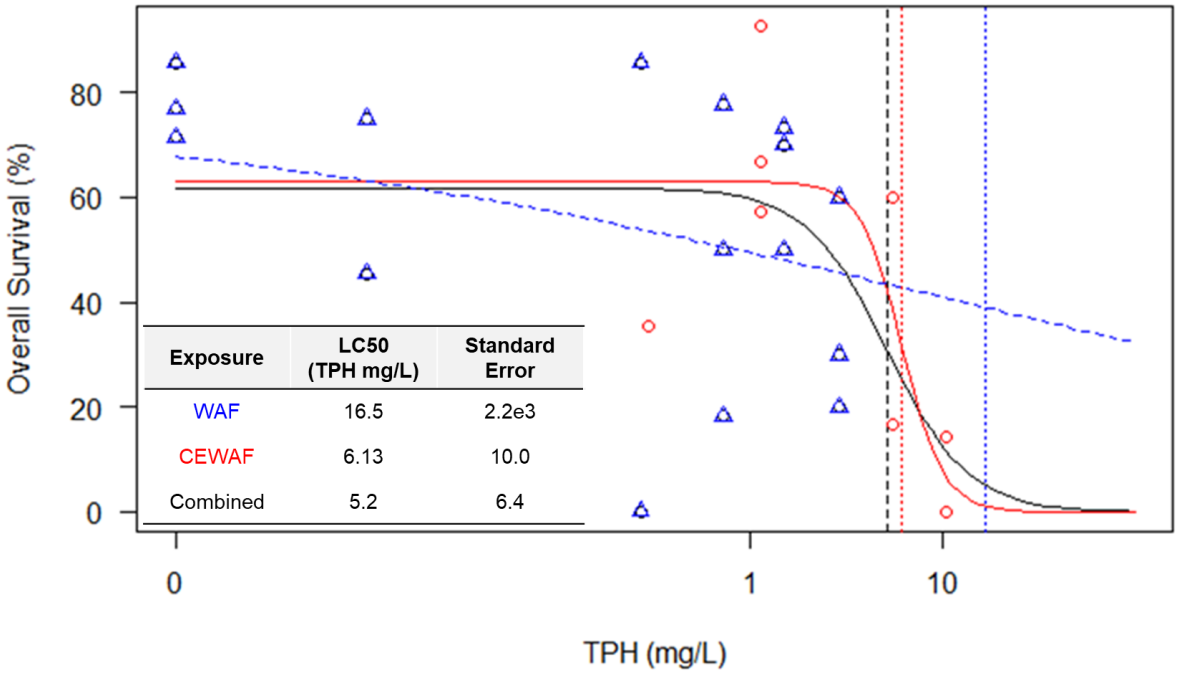


Figure 62: Modelled concentration response relationship for overall survival for the WAF (blue triangles), CEWAF (red circles), and combined (black line). The vertical lines are the estimated LC50 values (insert).

The *drc* model was not significant (p = 0.18) and the effect concentrations should be interpreted with caution.

The larvae that did successfully hatch were visually examined to identify possible trends in morphological abnormalities (Figure 63).

Figure 63: Post hatch larvae from the 100% WAF treatment with one of the three hatched larvae showing yolk sac abnormality.

Yolk sac abnormalities were identified sporadically in the larvae (<20%) but without treatment related effect or pattern.

While individual embryo and fish numbers were low, the results do highlight that an acute, 1-hour exposure during fertilization is able to exert significant latent effects in the form of reduced hatching.

8.3.2 Variable exposure duration

With increasing exposure duration there was decrease in the percent hatch (Figure 64).

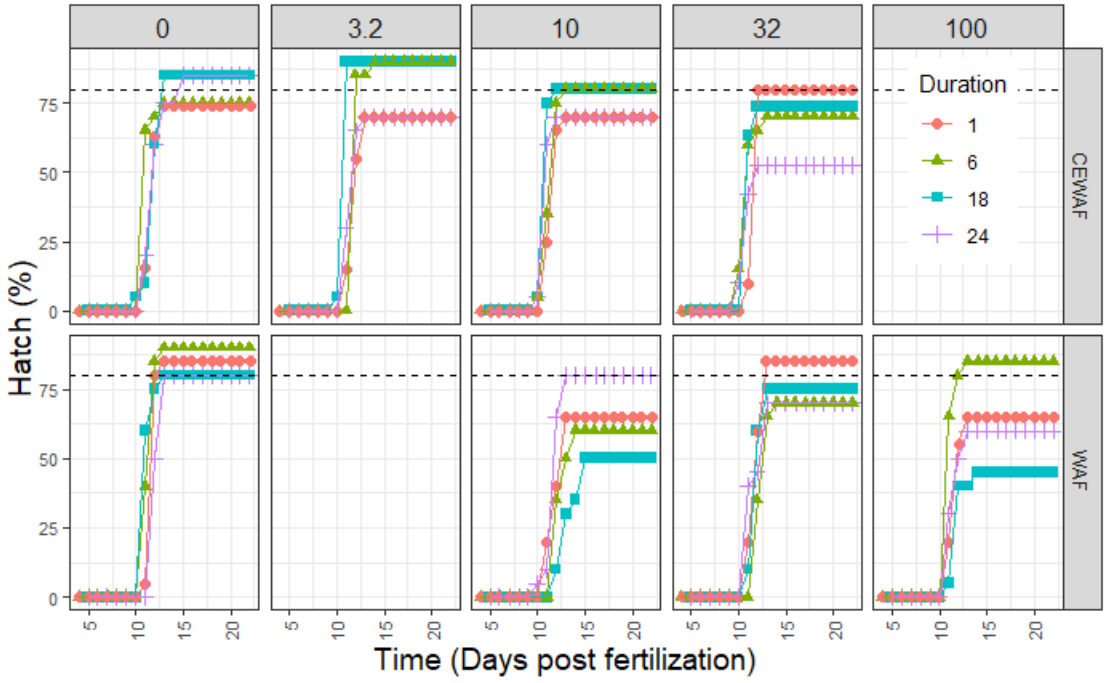


Figure 64: Percent hatch following exposure to different strength (columns) of CEWAF (top row) and WAF (bottom row) for exposure durations of 1 (red circle), 6 (green triangle), 18 (blue square) and 24 hours (purple cross). The dashed horizontal line is the control acceptability criteria of 80% hatch.

The single replicate for each concentration and duration made statistical comparisons difficult, but there was a trend towards reduced hatch with increasing concentration and exposure duration. There was no hatch in the positive control for any duration greater than 1-hr (not shown).

There was a concentration response trend with an increase in concentration resulting in decreased hatch and an increase in abnormal presentation at hatch following the 24-hr exposure duration (Table 19).

Table 19: Hatch outcomes from the 24-hr exposed embryos

Type	Strength (%)	Percent Hatch	Percent Abnormal of Hatch
Seawater	0.0	80.00	6.67
WAF	10.0	80.00	7.14
	32.0	70.00	7.14
	100.0	60.00	16.67
Corexit control	0.0	85.00	6.25
CEWAF	3.2	70.00	15.38
	10.0	70.00	66.67
	32.0	52.63	62.50

The percent of hatched fish that were abnormal from the 24-hr exposed embryos was modeled against TPH concentration (WAF and CEWAF exposure combined) to estimate an EC50 (Figure 65).

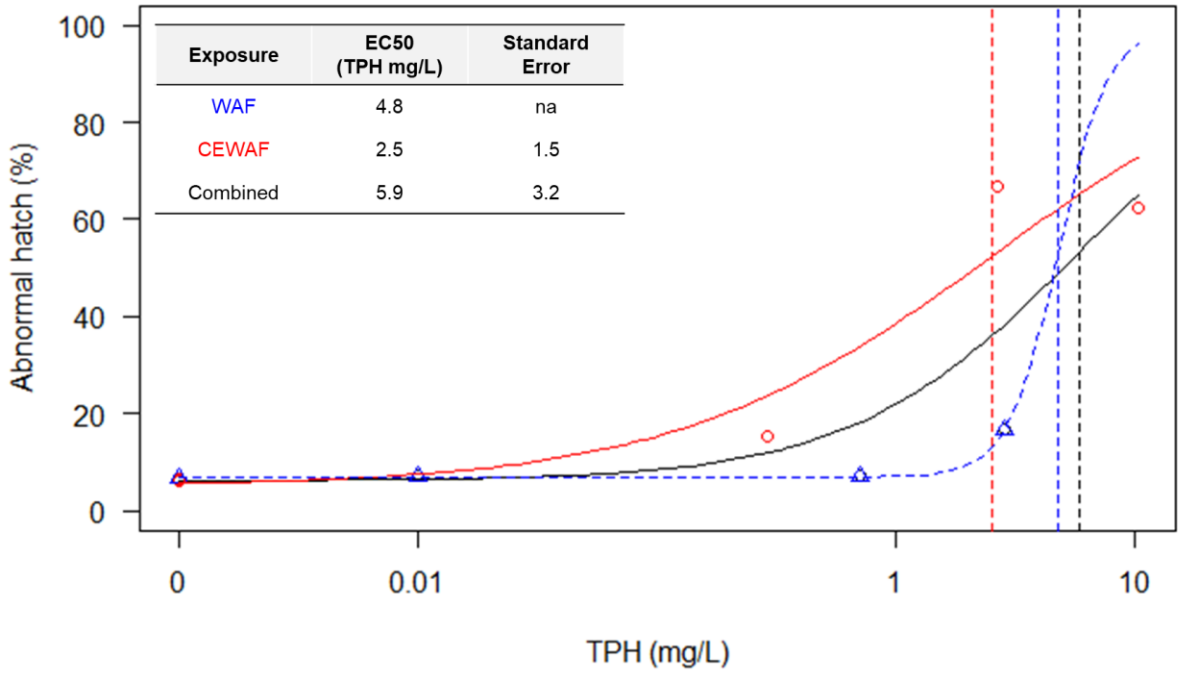


Figure 65: Concentration response relationship for percent of hatched fish from the 24-hr exposure to WAF (blue triangle), CEWAF (red circles) and combined (black line) that were abnormal. The dashed vertical lines are the EC50s.

The EC50s for abnormal hatch were calculated as 4.8, 2.5, and 5.9 mg/L TPH (for the WAF, CEWAF, and combined exposure respectively. These values should be considered preliminary as they were calculated from single replicate trials.

The overall survival (embryo and hatched fish) from the 24-hr exposed embryos was modeled against TPH concentration to estimate an LC50 (Figure 66).

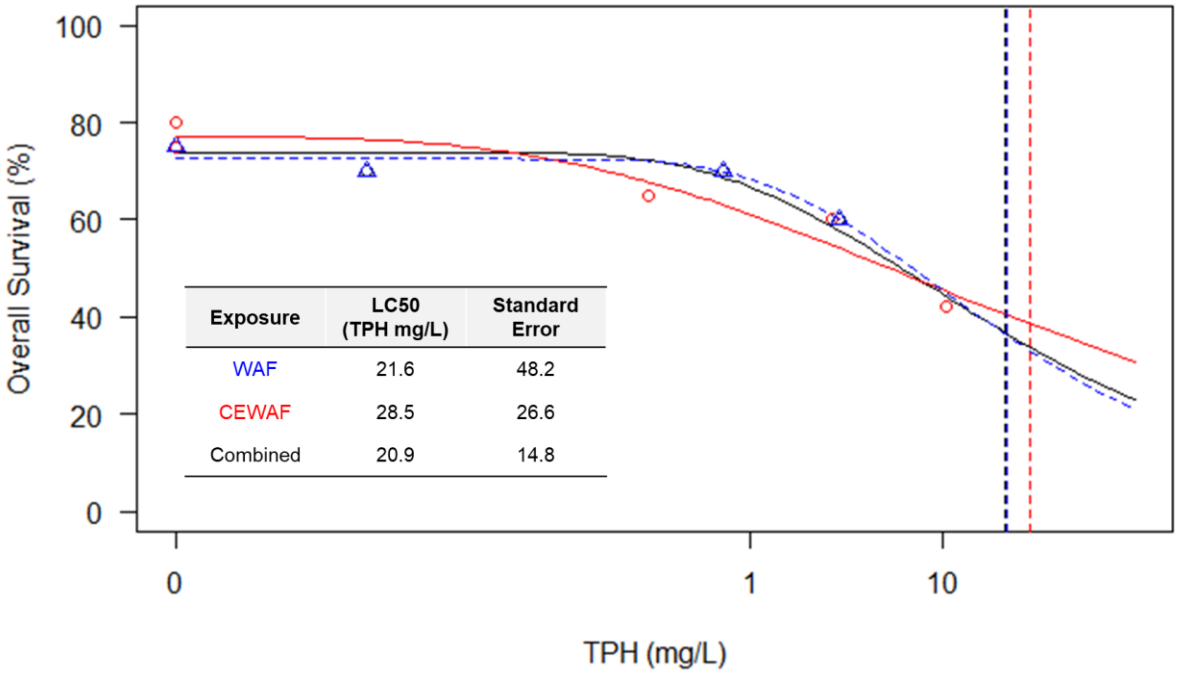


Figure 66: Concentration response for overall survival following 24-hr exposure embryonic to WAF (blue triangle), CEWAF (red circles) and combined (black line). The dashed vertical lines are the estimated LC50 values from three parameter type-2 weibull model.

The LC50 was estimated to be between 20.9 and 28.5 mg/L TPH depending on exposure type, however this value was greater than the tested concentrations and should be interpreted with caution given that only a single replicate was tested.

8.3.3 Developmental time series: expose the same cross at set developmental time points

There was poor control survival in the exposures of the 2 and 7 days post fertilization embryos, which hampered our ability to draw significant conclusions (Figure 67).

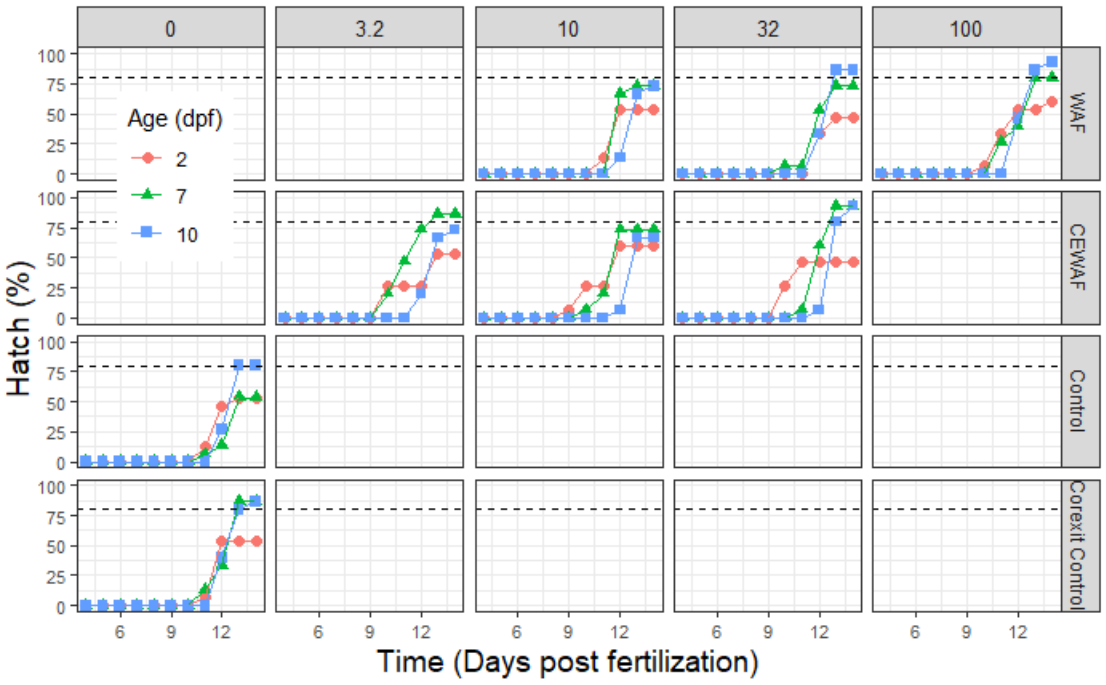


Figure 67: Percent hatch in each treatment (rows) and concentration (columns), by age (as days post fertilization, dpf) at time of exposure (red circle = 2 dpf, green triangle = 7 dpf, and blue square = 10 dpf). The dashed horizontal line is 80% hatch.

There was a noticeable trend across nearly all treatments despite the control mortality/low hatch rate whereby the embryos exposed at 10 dpf (blue squares in Figure 67) were less sensitive than the 2 and 7 dpf exposures and had greater overall survival (Figure 68).

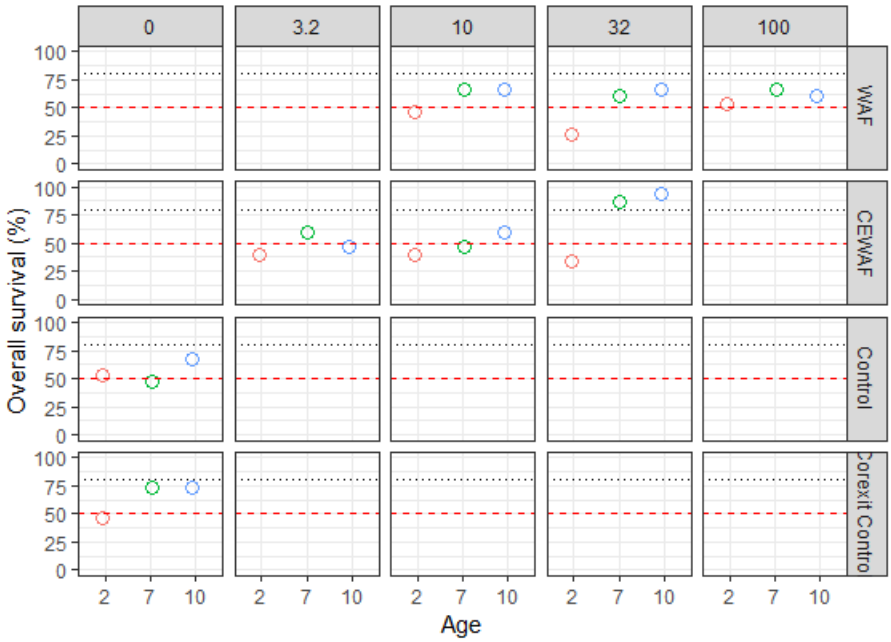


Figure 68: Percent overall survival by age (days post fertilization) at time of exposure for each concentration (columns) of the treatment solution (rows)

These results do show that effects are not likely to be equally distributed across the embryonic life stage and that earlier exposures are more likely to result in adverse outcomes.

8.3.4 Variation in survival across half-sibling families

There was considerable variability in the hatching success rate between the different half-sibling crosses even in the absence of exposure to WAF or CEWAF (Figure 69).

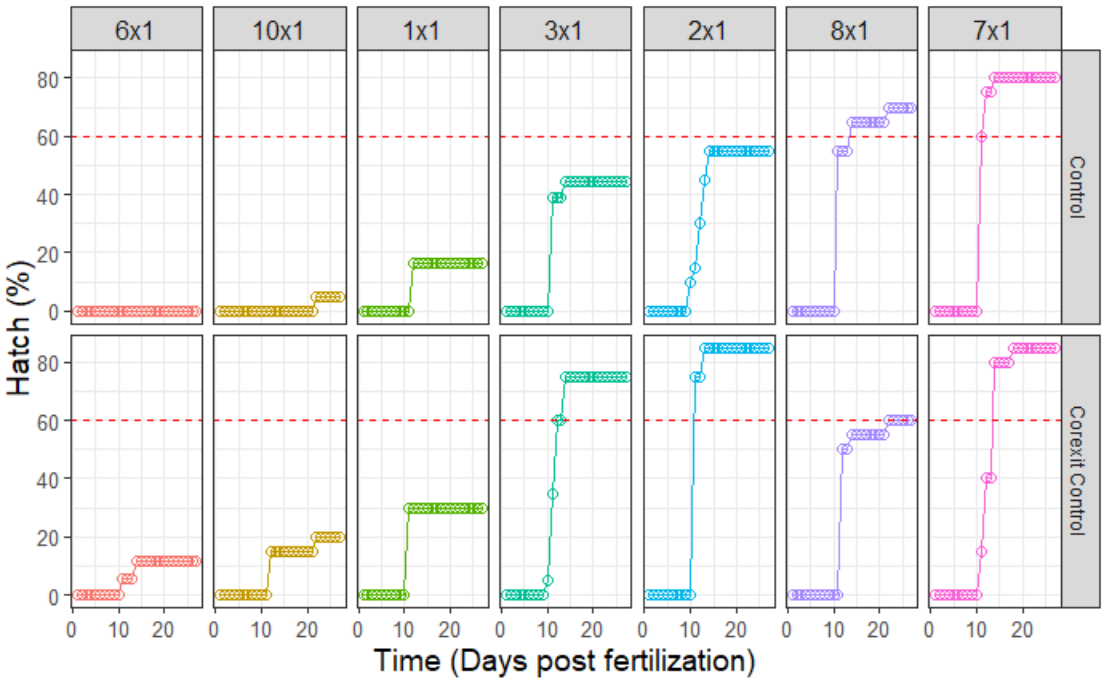


Figure 69: Percent hatch from the different crosses (columns) exposed to control seawater (top row) or Corexit only (bottom row).

The embryos that did hatch were assessed for abnormalities and larval mortality. The full results are summarized in Table 20 with select crosses and concentrations visually summarized in Figure 70.

Table 20: Hatching outcomes from the different crosses

	♀	Controls		WAF (%)					CEWAF (%)				
		0	0*	10	18	32	56	100	3.2	5.6	10	18	32
Percent Hatch	6	0.0	11.8	10.0	15.0	5.0	5.0	10.0	12.5	0.0	5.9	0.0	25.0
	10	5.0	20.0	5.0	15.0	25.0	10.0	20.0	20.0	15.0	0.0	20.0	15.0
	1	16.7	30.0	28.6	23.1	45.0	15.0	20.0	50.0	15.0	50.0	30.0	20.0
	3	44.4	75.0	66.7	75.0	54.5	75.0	50.0	66.7	60.0	66.7	70.6	28.6
	2	55.0	85.0	55.0	45.0	55.0	37.5	25.0	60.0	52.9	45.0	50.0	40.0
	8	70.0	60.0	65.0	75.0	85.0	70.0	55.0	60.0	65.0	75.0	50.0	55.0
	7	80.0	85.0	75.0	95.0	80.0	95.0	70.0	95.0	5.0	90.0	65.0	80.0
Percent Abnormal of Hatch	6	-	50.0	100.0	0.0	100.0	0.0	0.0	0.0	-	0.0	-	75.0
	10	0.0	0.0	0.0	50.0	75.0	50.0	75.0	0.0	100.0		100.0	100.0
	1	0.0	50.0	50.0	50.0	28.6	66.7	100.0	37.5	0.0	42.9	60.0	50.0
	3	12.5	33.3	25.0	7.1	33.3	7.1	20.0	0.0	41.7	25.0	41.7	100.0
	2	62.5	25.0	0.0	22.2	50.0	25.0	20.0	50.0	77.8	57.1	85.7	83.3
	8	33.3	9.1	15.4	6.7	26.7	25.0	45.5	66.7	25.0	38.5	60.0	63.6
	7	0.0	6.3	13.3	5.6	0.0	10.5	21.4	21.1	68.8	52.9	66.7	100.0
Percent Larval Survival	6	-	100.0	50.0	100.0	100.0	0.0	100.0	50.0	-	0.0	-	80.0
	10	80.0	-	100.0	100.0	50.0	50.0	66.7	75.0	66.7	-	-	-
	1	66.7	66.7	100.0	66.7	77.8	100.0	75.0	80.0	66.7	70.0	83.3	100.0
	3	100.0	100.0	100.0	93.3	100.0	93.3	100.0	50.0	100.0	100.0	100.0	100.0
	2	72.7	94.1	100.0	100.0	54.5	66.7	100.0	83.3	100.0	77.8	70.0	75.0
	8	85.7	91.7	100.0	100.0	88.2	92.9	100.0	75.0	92.3	93.3	100.0	100.0
	7	100.0	94.1	100.0	94.7	100.0	100.0	100.0	100.0	100.0	94.4	92.3	81.3

*Dispersant only control applied at the same concentration as the highest tested CEWAF treatment (32%).

Figure 70: Visual overview of the hatched fish from the different crosses (columns) to the different treatments (rows)

From all of the crosses, only two (8x1 and 7x1) were considered to be valid (>60% hatching in the control) and were examined for the concentration response relationship with embryo survival using a 4-parameter log-logistic model individually and pooled (Figure 71).

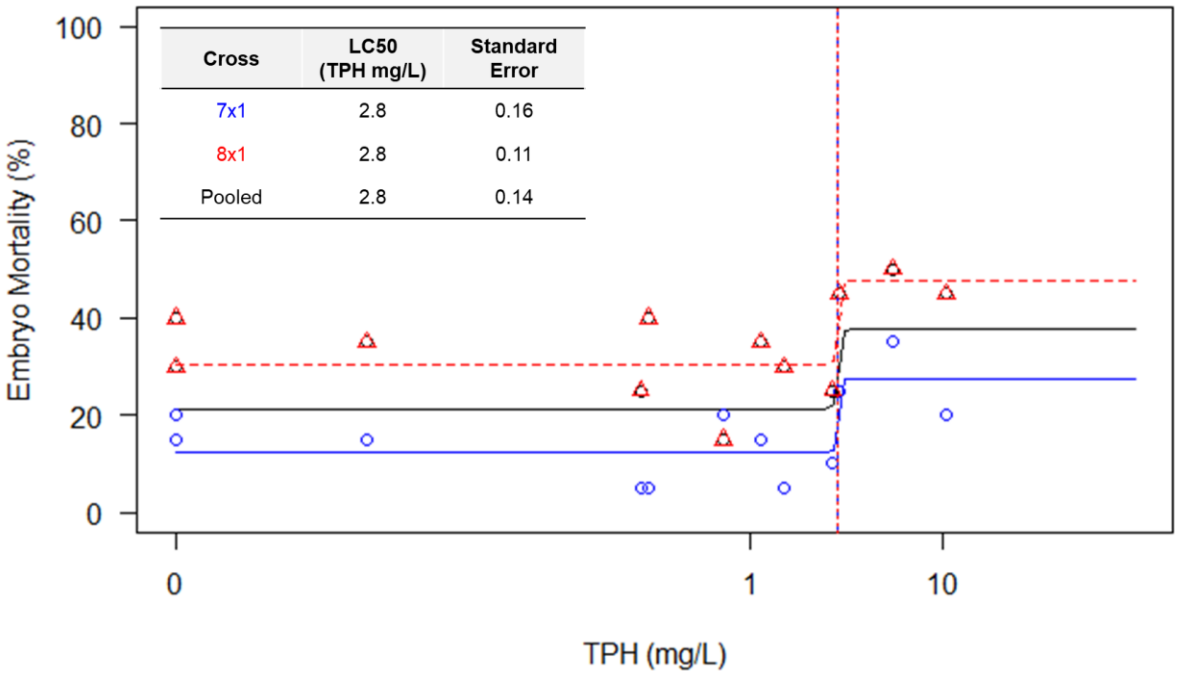


Figure 71: Concentration (TPH mg/L) and response (embryo mortality) relationship from the two half sibling crosses (blue circles = 7x1; red triangles = 8 x1) that had greater than 60% hatch, and their pooled response (black line). The vertical line is the LC50 = 2.8 mg/L TPH.

The two crosses were pooled to examine the concentration response relationship for abnormal hatching using a 4-parameter Type 2 Weibull model given the similar results in embryo mortality (hatching) and calculated LC50s (2.8 mg/L TPH for each cross) (Figure 72).

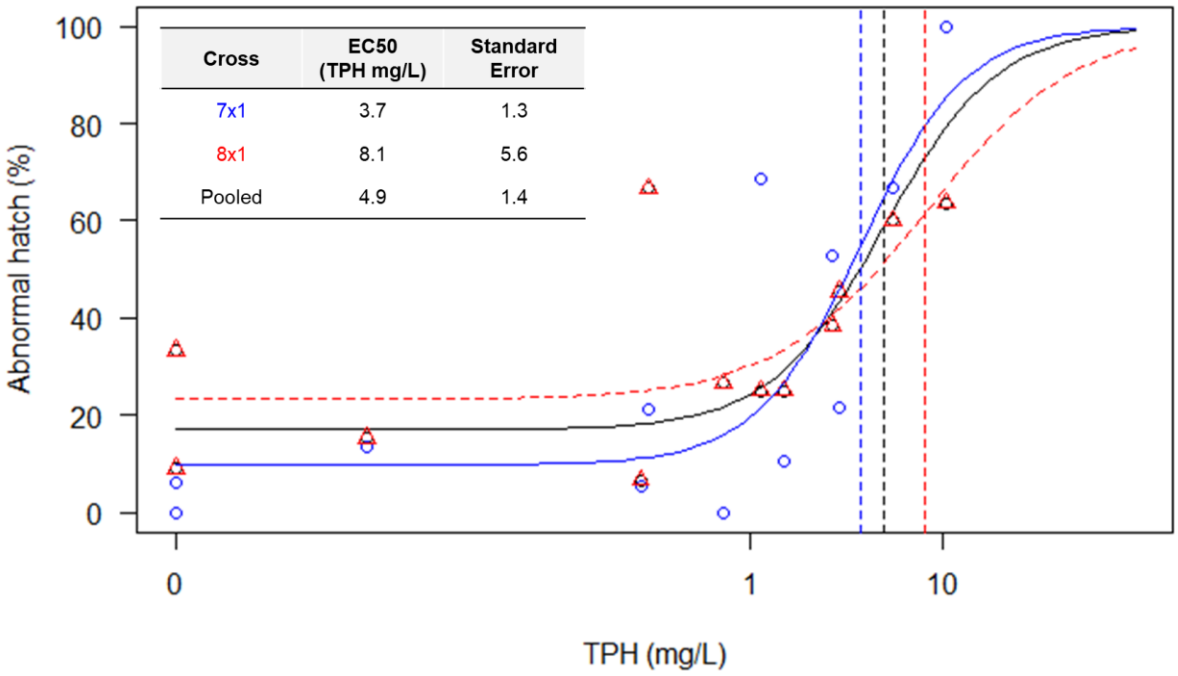


Figure 72: Concentration (TPH mg/L) response relationship for crosses 7x1 (blue circles), 8x1 (red triangles), and pooled (black line) for abnormal hatch.

The EC50 for abnormal hatch was estimated to be 4.9 mg/L TPH from the pooled response. The EC50 values from the individual crosses (3.7 and 8.1 mg/L for 7x1 and 8x1) are in line with the EC50 for abnormal hatch calculated from a single replicate from the variable exposure duration trial (EC50 = 2.3 mg/L, Figure 65).

8.4 Discussion

Many of the trials in 2017 were designed for method development and range finding with the intent to perform definitive trials (e.g., additional replicates, refined concentrations) with the next batch of viable herring that we received. Unfortunately, we were only able to receive two collections in 2018 and none in 2019 due to the sporadic availability and variable quality of the fish. As such, we were not able to re-visit some of these trials with increased number of replicates. However, we are still able to make some tentative Atlantic herring conclusions from these preliminary and range finding trials that can be used to inform future studies, risk assessments and response operations.

The results demonstrated that a 1-hour, static exposure, at a critical developmental window (fertilization to 2 days post fertilization) is sufficient to observe significant latent effects, such as embryo mortality and reduced hatch success. With longer 24-hour exposures durations we observed increased abnormal hatch (EC50s ranging from 2.3 - 8.1 mg/L). Greater effects from longer exposure duration is not surprising and has been previously shown in this species following exposure to dispersed crude oil from 1 to 4 days with a more than 25-fold increase in toxicity (McIntosh et al. 2010). Our intent was to examine more realistic shorter exposure durations to see if similar increases in effect took place from 1 to 24 hours. There were negligible differences in effect between exposure durations up to 18 hours, at which point there was a noticeable difference in hatching success compared to the control in the higher concentrations.

The timing of exposure proved to be a significant factor in whether the negative effects on hatching were observed. There was greater survival with later developmental stage exposures, which has been similarly observed in other studies (McIntosh et al. 2010). This variable sensitivity during the embryonic life stage highlights that the effects will not be distributed equally across year classes that may be present at the specific time of exposure to spilled or dispersed crude oil.

All these trials were conducted with gametes collected from wild caught organisms. We observed significant variability in responses based on the specific pairing or cross that was made. This variability could be attributed to genetic and/or environmental factors. In Atlantic cod, Garber et al. (2010) reported differential survival and loss of specific families could have been a result of variability in initial egg quality stemming from maternal or other environmental effects. This could further affect development, including additive genetic effects of the individual and dominance deviation (interactions among alleles) associated with the particular combination of parents. The natural variability and condition of field caught organisms needs to be considered, as the magnitude of response can be quite variable depending on the condition (both genetic and environmental) of the organisms. The use of appropriate controls (positive and negative) is a means of assessing some of the variability associated with field collected organisms. The variability in responses amongst individual families should not be ignored as this gives a sense of

the range of sensitivity of a population and may speak towards the resilience of the population to withstand an intermediate disturbance.

American lobster (*Homarus americanus*)

Definitions

- **Abdomen:** the section commonly referred to as the “tail”.
- **Antennae:** tactile organs, having a sense of touch.
- **Batch**: Collection of larvae released during a 24-hr period
- **Carapace**: the outer shell of the cephalothrax
- **Cephalothorax**: contains the head and thorax sections: together they are commonly called the “body”
- **Clutch**: Batch of eggs extruded during a single spawning season
- **Molt**: Cyclical process of preparing for, undergoing, and recovering from ecdysis. The molt cycle in crustaceans is divided into 5 main phases (A to E), which characterize post-molt (A-C), pre-molt (D), and ecdysis (E) phases.
- **Ovigerous**: carrying or bearing eggs
- **Pereiopods/‘walking legs’**: The two sets of walking legs immediately behind the claws are also used for catching and eating food and have many “taste” sensors; the last two sets of legs are used primarily for walking.
- **Pleopods**: commonly known as “swimmerets”. with tiny hairs. In females the hairs are somewhat longer and are the attachment point for eggs.
- **Telson**: the central tail fin
- **Uropods**: the outer pairs of tail fins

Background

American lobster was chosen as a test species due to its economic importance in Atlantic Canada and its ecological importance in nearshore ecosystems. Lobster larvae production methods were modified from the large-scale hatchery production procedures of Homarus Inc. (Shediac, NB) to ensure best practices. These methods (e.g., broodstock management, larval production, staging, and morphometric measurements) were adapted into Huntsman specific SOPs.

The test organisms in this study were larval stages of the American lobster (*Homarus americanus*) obtained from ovigerous (“berried”) female adult lobsters (n = 30 in each of2017 and 2018) (Figure 73) sourced from the wild fishery from LFA 36 (Bay of Fundy, NB, Canada). Berried females were acquired under special federal license and delivered to the Huntsman where they were held in ambient communal tanks until assessed to be ready for larval release. Collected lobsters were visually assessed following transfer to the Huntsman for abnormalities (e.g., missing limbs), size (i.e., carapace length, weight)

and clutch (i.e., stage, size, embryo size) measurements were taken. Lobster wet weights ranged between 500 - 1100 g (mean = 823.9 g) with carapace lengths between 8.2 - 10.6 cm (mean = 9.5 cm). The number of embryos in each clutch was quite variable (3,000 - 26,000) and was correlated with carapace length (r^2 = 0.75). The development of the clutch was assessed using a 5-point scoring scheme.

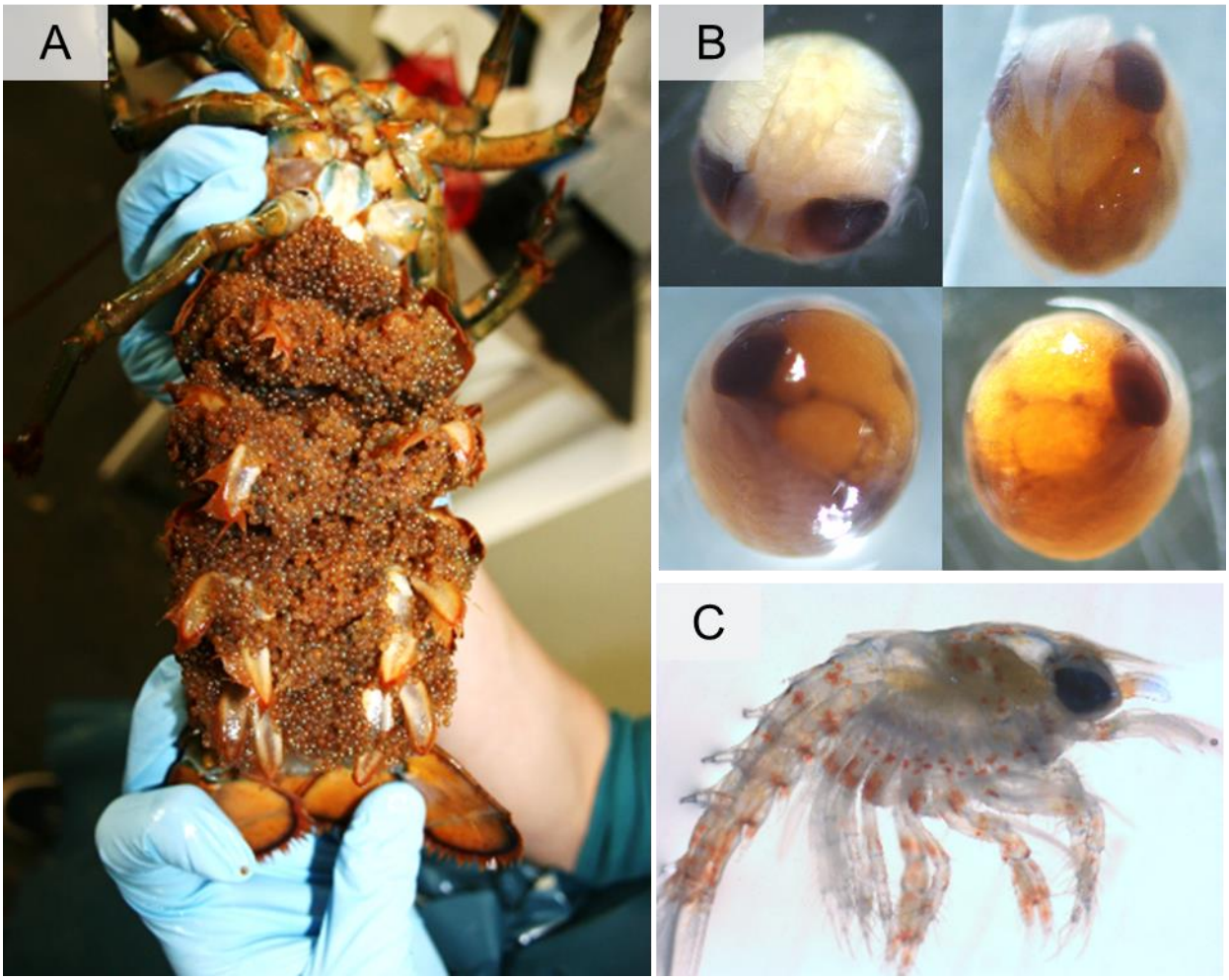


Figure 73: A) Berried female lobster, B) developing lobster embryos, C) less than 24-hr old hatched stage I larval lobster.

Stage 1 are recently spawned embryos that are dark green or black in colour with the embryos not visible within the eggs. Stage 2 are developing eggs, which are two-toned in colour due to the growth of the larva and its consumption of the yolk. This is the first stage where the eyes of the embryos are visible. Stage 2.5 is quite rapid and defined as the time when the eggs begin to change colour towards tan or orange. Stage 3 are the mature

eggs with an overall appearance of orange or light tan (no longer two-toned) and generally lasts about one week before the eggs hatch. Stage 4 is the hatching stage where the color of the clutch will change back towards a darker color (singled toned as the larva is filling the egg and the yolk is consumed). The distribution of the lobsters across the developmental stages at the time of receipt (Figure 74), along with careful temperature manipulation, allowed us to stagger the hatching of larval lobsters to ensure that sufficient numbers were available to complete all trials.

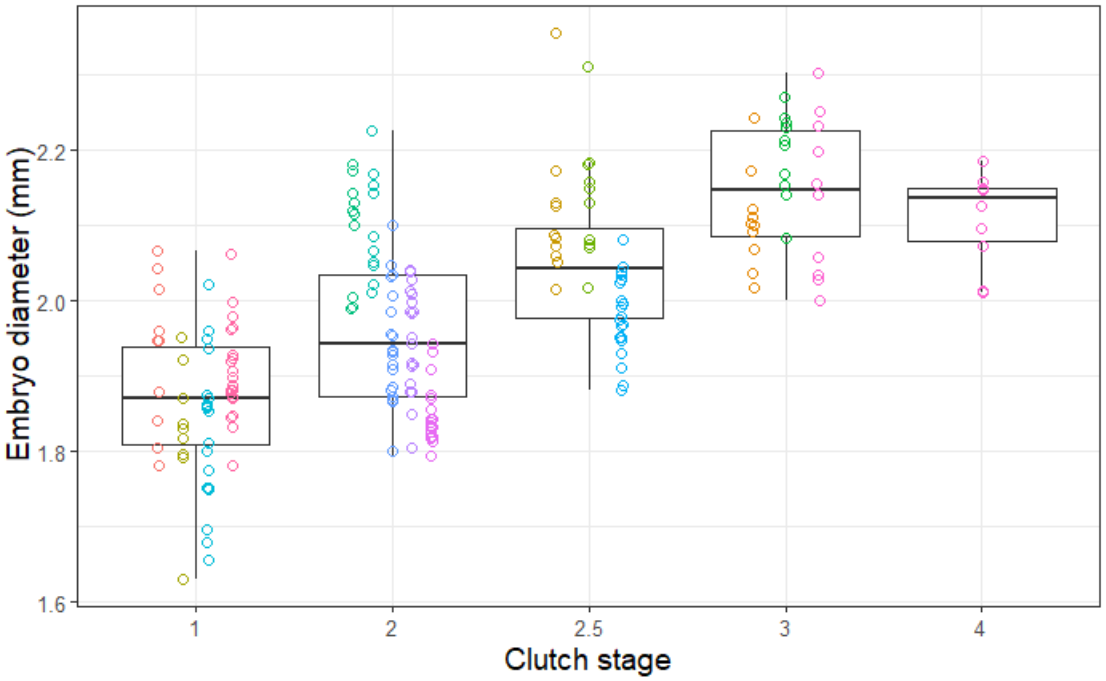


Figure 74: Average embryo diameter from 16 lobsters (colour; n=16) by clutch developmental stage at the time of receipt in June 2018.

The post-embryonic development of marine decapod crustaceans, like the American lobster, is characterized by a sequence of morphologically distinct larval forms, called stages or instars. Each stage is subjected to a series of internal morphological and physiological alterations ending with the process of ecdysis, thus producing another larval stage (Anger 1987). The first larval stages (I–III) of the lobster are truly pelagic and the first post-larval stage (stage IV, metamorphosis) spends at least some of its time in the water column prior to settling to the bottom (Burridge and Haya 1997). Planktonic crustacean larvae are considered sensitive to contaminants and other stressors and lack the ability to avoid exposures through swimming long distances. Unlike fish, lobsters (and

other crustaceans) may have a limited capacity to metabolize the polycyclic organic compounds found in oil and petroleum (Payne et al. 1983) thereby increasing the potential for bioaccumulation and/or toxic effects. Further, lobster larvae are quite sensitive when compared to other crustaceans (Wells and Sprague 1976).The American lobster planktonic stages were the focus of this research given these considerations.

The toxicology trials involving American lobster are described in Chapters 9-10.

Chapter 9 Variation in survival after exposure to physically and chemically dispersed crude oil across newly hatched stage I American lobsters

9.1 Introduction

Assessing the impact of an oil spill to an aquatic species requires an understanding of the biological life-history traits of an organism and its likely crucial life stages that may experience the exposure. The American lobster (*Homarus americanus*) is a species for which the life-history is well understood, but the toxicological responses to petroleum hydrocarbons are less well understood, especially when compared to other crustaceans. The first larval stages (I–III) of the American lobster are truly pelagic and the first post-larval stage (stage IV) spends at least some of its time in the water column prior to settling to the bottom (Burridge and Haya, 1997). Planktonic crustacean larvae are considered sensitive to contaminants and other stressors while lacking the ability to swim long distances to avoid exposures. The sensitivity of pelagic lobster larvae (i.e., stages I-III) to environmental pollution, particularly to crude oil, has been previously demonstrated (Wells and Sprague 1976). In laboratory studies, Wells and Sprague (1976) found the acute toxicity of 0.86 mg/L and 4.9 mg/L of crude oil to stage I and stages III-IV lobster larvae, respectively. More recently, in 1996, the tank barge *North Cape* grounded off the southwestern coast of Rhode Island (USA) spilling an estimated 2700 metric tons of fuel oil into the shallow near shore waters (Reddy and Quinn 2001). A total of approximately 9 million lobsters were killed (French-McCay 2003) further demonstrating the sensitivity of this culturally and economically important species to crude oil.

Experimental and natural parameters that may potentially cause variability in sensitivity of species to the compounds should be taken into account to appropriately investigate the impact of hazardous compounds to aquatic species. The variability of data derived from laboratory-based toxicity tests introduces uncertainty to further extrapolation for environmental risk assessment purposes. Thus, it is crucial to properly characterize the experimental and natural variabilities that may influence the results of toxicity tests (Simmons et al. 2015). Mayer and Ellersieck (1986) demonstrated that physical conditions of the test environment, such as pH and temperature, and chemical properties of the test compound, such as solubility, can significantly influence the result of toxicity tests. Biological variability proves more challenging to address as there are multiple contributing sources, including seasonal and temporal variation, genetic variation among individuals, choice of test species, and life stage of test species (Hrovat et al. 2009; Simmons et al. 2015).

In the present study, we investigated the sensitivity of American lobster larvae exposed to physically and chemically dispersed crude oil and whether the response was consistent amongst different batches released from the same lobster and amongst different lobsters. A series of acute toxicity trials were conducted with less than 24-hr old stage I larvae to address these objectives.

9.2 Methods

9.2.1 Test Organism: American lobster (*Homarus americanus*)

This study was undertaken using the first planktonic life stage (i.e., stage I) of American lobster (*Homarus americanus*) (Factor, 1995). Adult commercial size (0.5 - 2.0 kg) ovigerous (egg carrying or ‘berried’) females were obtained from local fishers following acquisition of a special permit from Fisheries and Oceans Canada and captured in the Bay of Fundy Lobster Fishing Area 36. The collected berried female lobsters were transferred to the Huntsman Marine Science Center (St. Andrews, NB) where they were held under controlled environmental conditions to promote egg mass development. Berried female lobsters were transferred to an individual holding tank that received seawater of 18ºC ± 2ºC when visual inspection of embryo development indicated that

larval release was imminent. Larval lobsters released from first, second or third single overnight hatches were separately collected and held for single batch toxicity tests. Twenty lobster larvae were collected following release of the first batch then imaged as reference organisms using a Leica Wild M420 microscope and Leica MD190 camera at 12.5x magnification. Carapace length (mm) of reference lobster larvae was measured using the LAS software version 4.8. Weight and carapace length of berried female lobsters were also recorded.

9.2.2 Preparation of WAF and CEWAF Stock Solutions

Exposure waters were prepared to test the toxicity of oil to aquatic organisms by contacting the oil and water such that a mixture is generated consisting of oil components in both the dissolved phase and in the non-dissolved phase, following the methods described in Section 3.3.2, Table 10. The background water for preparing the WAF and CEWAF stock solutions was 0.22 µm filtered seawater (i.e., salinity of ~ 30 psu) received from the Bay of Fundy. Briefly, two 2-L glass baffled flasks with a hose bib at the base were used. The volume of crude oil (i.e., weathered offshore Newfoundland crude oil) was loaded to 1.6 L of filtered seawater into each baffled flask (20% headspace) using a 5 mL Hamilton® gastight syringe. Target loading was 1 g of crude oil per 1 L of seawater therefore resulting in the addition of 1.6 g of crude oil. The flasks containing dilution water and oil were sealed with DuraSeal®. The CEWAF solution was prepared by adding the Corexit 9500A dispersant at this step with a dispersant to oil ratio (DOR) of 1:20. The baffled flasks were then placed on the MaxQ SHKE2000 digital shaker (Thermo Scientific) in an environmental chamber with controlled temperature at 15ºC ± 2ºC. The solutions were mixed at 150 rpm for 1 hour followed by a 1 hour settling time before used to generate the exposure media. The dispersant only control was prepared at a concentration that was equal to the volume of dispersant in the highest tested CEWAF concentration (32% strength) and was nominally 15.8 mg/L in the range finding test (equal to the highest CEWAF concentration tested, 32%) and 4.75 mg/L in the definitive tests (equal to the highest CEWAF concentration test, 10%).

9.2.3 Acute Toxicity Test

The preparation of test solutions, as well as performing the definitive toxicity tests, were undertaken in an environmental chamber with temperature maintained at 15ºC ± 2ºC and a photoperiod of 16h light and 8h dark throughout all of the experimental trials. The dilution water for toxicity tests was the same filtered seawater (i.e., 0.22 µm) that was used to prepare WAF and CEWAF stock solutions. The preliminary nominal range of WAF included 100%, 56%, 32%, 18%, and 10% of WAF stock solution while the range of CEWAF was 32%, 18%, 10%, 5.6%, and 3.2%. The definitive toxicity tests followed with 100%, 56% and 32% of the WAF and 10%, 3.2% and 1% of the CEWAF stock solutions. Appropriate volume of WAF/CEWAF stock solution was added to the required volume of filtered seawater followed by a gentle stirring to make a gradient dilution of test solutions. Control seawater and control Corexit 9500A were also included in the experimental set up. Test solutions were used in less than 1 hr of preparation for the toxicity tests. A randomized experimental setup was employed to minimize the variability of unexpected effects on the observed response. Water quality variables, including dissolved oxygen (DO%) and temperature (ºC), were measured using a YSI model ProSolo Digital Water Quality Meter (Yellow Springs Instruments, Yellow Springs, OH, USA) in three replicates per treatment before (i.e., 0 hr ± 1 hr) and after (i.e., 24 hr ± 1 hr) exposure. Salinity (psu) and pH were also measured using the YSI model MultiLab 4010-2 (Yellow Springs Instruments, Yellow Springs, OH, USA) calibrated with standard pH buffer solutions. The tests were conducted in static baths with no renewal of test solution. The lobster larvae from the first day post-release (stage I) were individually placed in scintillation vials containing 20 mL of test solution with 10 replicates per concentration. Lobsters were not fed during the toxicity tests. The toxic effects were recorded as numeric categories as described in Table 21 at the end of the 24 hr exposure period. Test solutions were sampled with three replicates per concentration for water quality analysis at the end of toxicity tests (i.e., 24h).

Table 21: Endpoints observed in lobster larvae exposed to physically and chemically dispersed crude oil for 24h.

Score	Description
0	**No observed effect**: vigorously swimming, active internal organ movement
1	**Affected**: passive swimming, erratic swimming, positioned on side or back, rigid body position, exopodites/pereiopod beating in coordinated motion
2	**Moribund (mortally affected)**: no swimming activity, twitching, sporadic movement of mouthparts and exopodites/pereiopod, positioned on side or back, faint heartbeat, slight internal organ movement
3	**Dead**: no swimming, no visible heartbeat, change in coloration towards brown/opaqueness, absence of movement after gentle prodding

Validity criteria for the toxicity tests were less than 20% mortality in negative controls, greater than 60% dissolved oxygen saturation, and less than 1.5ºC variation of temperature amongst treatment vials. In total, 19 toxicity tests were conducted between June to September 2018 using 14 female lobsters.

9.2.4 Analytical Chemistry Measurements

The concentration of WAF and CEWAF in different treatments was characterized as the concentration of different analytes of the total petroleum hydrocarbons (TPH), including the analytes of the polycyclic aromatic hydrocarbons (PAHs). Chemical characterization included solvent extraction followed by gas chromatography coupled with mass spectroscopy (GC-MS) performed at RPC in Fredericton, New Brunswick. Water chemistry characterization was based on the method described in the USEPA 3510C/8270C document (Edgell & Wesselman, 1989). The volume concentrations (VC; µL/L) of oil droplets were also measured in the test solutions using a LISST-100x (Sequoia Scientific, Inc) at the Huntsman. The concentrations of TPH for the CEWAF were estimated in the rest of the experimental trials based on the relationship between

measured VC and the measured analytes of TPH for the same experimental trial. The measured and estimated concentrations were used to model immobilization response and determine the effect concentration to 50% of the population (i.e., EC50). The measured concentrations of TPH were used to calculate the 24h EC50 value of the water accommodated fraction of oil to lobster larvae.

9.2.5 Statistical Design and Data Analysis

Water quality parameters (i.e., dissolved oxygen, pH, and temperature) at pre- and post-toxicity tests, carapace length of reference lobsters per each trial, and the wet weight of female lobsters were all tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. Analysis of variance (ANOVA) using R program was performed to investigate any significant differences within and between data sets. The significance criterion was set at $p < 0.05$. In case of any significant differences, the post-hoc tukey test was performed to find the trial(s) with significantly different effect concentration(s). If normality and homogeneity of variance were not met then the non-parametric test of Mann-Whitney U (also called Wilcoxon Rank Sum Test) was performed.

The *drc* package (v 3.0-1; Ritz et al., 2015) in R (v 3.6.1; R Core Team, 2019) was used to fit the appropriate model to the acute toxicity results. The best fitted model with the smallest AIC value was selected to represent the concentration-response curve for each experimental trial and used to calculate the 24-hr EC50. The variability in sensitivity of toxic responses of lobster larvae was assessed within female lobsters with more than one released batch used in toxicity tests, as well as amongst female lobsters across experimental trials. The precision of acute toxicity results for both within and amongst females was evaluated using the coefficient of variation (CV) and standard deviation (Burton et al., 1996). The standard deviation of acute toxic responses across all experimental trials in this study was calculated by implementing the meta-analysis method in the *metafor* package (v 2.1-0; Veichtbauer 2010), which were then used to calculate the CVs. Using meta-analysis for calculating the standard deviation would not weigh all of the EC50 values equally as they were not equally precisely estimated.

A species sensitivity distribution (SSD) type approach was used with the cumulative distribution of EC50 values to assess the potential risk to the lobster larvae population. A log-normal distribution function and resampled randomly 1000 times were used to generate the SSD mean value and the 5th percentile hazard concentration (HC5) with corresponding 95% confidence interval (95% CI). The concentration of HC5 at the lower limit was considered to provide the maximum protection of lobster larvae in a given oil contaminated aquatic ecosystem, essentially the predicted no-effect concentration (PNEC).

9.3 Results

9.3.1 Water quality

No significant differences were observed for water quality parameters between experimental trials at both pre- and post-toxicity measurements. However, the DO (%) was significantly different from control seawater at chemically dispersed treatments (1% and 3.2% CEWAF) across all trials at post-toxicity measurements. The *p* values were equal to 0.04 for 1% CEWAF and 0.01 for 3.2% CEWAF, which the latter also showed a significantly different level of DO with the *p* value of 0.01 (%) as compared to the dispersant-only control ($p < 0.05$). The mean value of DO declined from 93.9% (± 2.3%) to 83.3% (± 8.5%) across all trials between pre- and post-toxicity measurements but never dropped below 60% saturation. pH ranged between 7.33 and 7.90 at the pre-toxicity test measurement with the mean value of 7.76 (± 0.12). Its range was slightly wider at the post-toxicity test measurement with the minimum of 6.78, maximum of 7.94, and mean value of 7.75 (± 0.16), which was not significantly different from the pH mean of pre-toxicity test measurement ($p < 0.05$). The variability of temperature in the test solutions was not significantly different between the two measurements with an average temperature of 14.4℃ (± 0.6℃) and 14.5℃ (± 0.5℃) at the pre- and post-toxicity test measurements, respectively.

Water quality parameters were also assessed for precision using the coefficient of variation (CV) between experimental trials (i.e., inter-trial variability) as significantly different levels of DO (%) were obtained across all trials at the post-toxicity measurement.

The inter-trial variability of parameters is shown in Table 2. More variability of DO was determined within the post-toxicity test measurements. The CV (%) of DO level for the WAF treatments changed from low variability of 2.3-2.4% CV at pre-toxicity measurements to higher variation of 10.5-12.0% CV at post-toxicity measurements. The CEWAF treatments had a close range of CV (%) as WAF treatment at the pre-toxicity measurements (i.e., 2.1-2.5% CV). The two highest concentrations of CEWAF (3.2% and 10%) had close estimates of CV (%) with the values of 8.9% for the 3.2% CEWAF and 8.7% for the 10% CEWAF as compared with the 1% CEWAF with a variability of 2.9% CV at post-toxicity measurements. The higher variabilities of DO at the post-toxicity test measurements might be due to the closed scintillation vials used as the exposure medium container. No significant reduction of DO was identified across the concentrations and experimental trials that would violate the validity criteria of toxicity tests (i.e., DO ≤ 60%) despite more variability of DO observed at the post-toxicity test measurements. On the other hand, pH had low inter-trial variability at both pre- and post-toxicity test measurements. The pH variability within pre-toxicity tests were 1.6% and 1.8% for the CEWAF and WAF treatment, respectively. The variation of pH did not change considerably at the post-toxicity test measurement for these treatments as the CV was as low as 1.7% for the CEWAF and 2.1% for the WAF. The inter-trial coefficient of variations of temperature at the pre-toxicity test measurement were almost exactly the same for the CEWAF and WAF treatments with the range of 0.0%-2.0% and 0.0%-1.9%, respectively.

Table 22: The inter-trial coefficient of variation (CV) of water quality parameters (dissolved oxygen, pH, and temperature) at the pre- and post-toxicity test measurements for the CEWAF and WAF treatments.

Treatment	Pre-trial DO (%)			Post-trial DO (%)		
	Mean	STD	CV%	Mean	STD	CV%
Control	94.81	2.03	2.1	83.22	7.80	9.4
Control Corexit	92.66	1.73	1.9	84.30	10.86	12.9
WAF 10%	92.84	2.19	2.4	79.07	9.45	12.0
WAF 32%	92.91	2.24	2.4	80.85	8.46	10.5
WAF 100%	93.22	2.12	2.3	82.77	9.72	11.7
CEWAF 1%	94.89	2.36	2.5	85.89	7.64	2.9
CEWAF 3.2%	94.51	2.00	2.1	80.20	7.14	8.9
CEWAF 10%	94.29	2.19	2.3	82.22	7.15	8.7
Treatment	Pre-trial pH			Post-trial pH		
	Mean	STD	CV%	Mean	STD	CV%
Control	7.74	0.13	1.7	7.72	0.22	2.8
Control Corexit	7.77	0.11	1.5	7.74	0.14	1.8
WAF 10%	7.76	0.13	1.6	7.76	0.16	2.0
WAF 32%	7.77	0.12	1.6	7.75	0.15	2.0
WAF 100%	7.77	0.13	1.6	7.76	0.13	1.7
CEWAF 1%	7.77	0.12	1.6	7.73	0.15	1.9
CEWAF 3.2%	7.77	0.12	1.5	7.74	0.11	1.5
CEWAF 10%	7.78	0.12	1.5	7.76	0.12	1.5
Treatment	Pre-trial Temperature (°C)			Post-trial Temperature (°C)		
	Mean	STD	CV%	Mean	STD	CV%
Control	14.5	0.54	3.7	14.5	0.48	3.3
Control Corexit	14.5	0.58	4.0	14.5	0.44	3.0
WAF 10%	14.6	0.69	4.7	14.5	0.49	3.3
WAF 32%	14.6	0.69	4.7	14.5	0.50	3.4
WAF 100%	14.7	0.74	5.1	14.6	0.49	3.4
CEWAF 1%	14.5	0.59	4.1	14.6	0.41	2.8
CEWAF 3.2%	14.4	0.60	4.2	14.6	0.46	3.2
CEWAF 10%	14.5	0.59	4.1	14.6	0.47	3.2

9.3.2 Chemistry

The chemical characterization of the TPH analytes for the WAF and CEWAF treatments are shown in Table 23.

Table 23: Chemical characterization of the total petroleum hydrocarbon (TPH) of nominal concentrations of physically dispersed oil (32% WAF) and chemically enhanced fraction of oil (1%, 3.2% and 10% CEWAF) dispersed using Corexit 9500A.

Analytes	Unit	MDL	Control Seawater	Control Corexit 9500A	32% WAF	1.0% CEWAF	3.2% CEWAF	10% CEWAF
Naphthalene	µg/L	0.05	< MDL	< MDL	47	3.7	9.2	28
Acenaphthylene	µg/L	0.01	< MDL	< MDL	0.08	0.04	0.12	0.31
Acenaphthene	µg/L	0.01	< MDL	< MDL	0.18	0.09	0.22	0.42
Fluorene	µg/L	0.01	< MDL	< MDL	1.1	0.55	1.5	4
Phenanthrene	µg/L	0.01	< MDL	< MDL	1	1.1	3.2	8.8
Anthracene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Fluoranthene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	0.08	0.16
Pyrene	µg/L	0.01	< MDL	< MDL	< MDL	0.04	0.12	0.29
Bz(a)anthracene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Chrysene/Triphenylene	µg/L	0.01	< MDL	< MDL	< MDL	0.06	0.25	0.55
Bz(b)fluoranthene	µg/L	0.01	< MDL	< MDL	< MDL	0.02	0.07	0.19
Bz(k)fluoranthene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Bz(e)pyrene	µg/L	0.01	< MDL	< MDL	< MDL	0.04	0.12	0.3
Bz(a)pyrene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	0.04
Indenopyrene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	0.04
Bz(g,h,i)perylene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	0.08
Dibz(a,h)anthracene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
C1-Naphthalenes	µg/L	0.1	< MDL	< MDL	19	4.3	12	37
C2-Naphthalenes	µg/L	0.1	< MDL	< MDL	7.9	4.3	11	36
C3-Naphthalenes	µg/L	0.1	< MDL	< MDL	1.3	2.2	5.7	16
C1-Phenanthrenes	µg/L	0.1	< MDL	< MDL	0.5	0.9	2.5	7.4
C2-Phenanthrenes	µg/L	0.1	< MDL	< MDL	0.4	0.9	3	7.2
C3-Phenanthrenes	µg/L	0.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Dibenzothiophene	µg/L	0.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
C1-Dibenzothiophenes	µg/L	0.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
C2-Dibenzothiophenes	µg/L	0.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
C3-Dibenzothiophenes	µg/L	0.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
1-methylnaphthalene	µg/L	0.05	< MDL	< MDL	23	5.3	13	40
2-methylnaphthalene	µg/L	0.05	< MDL	< MDL	20	4.7	11	33
Perylene	µg/L	0.01	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Biphenyl	µg/L	0.05	< MDL	< MDL	3.1	0.95	2.2	7.1
ΣPAH 31	µg/L	_	0.00	0.00	124.56	29.49	75.57	227.16
Benzene	mg/L	0.001	< MDL	< MDL	0.015	0.0005	0.001	0.004
Toluene	mg/L	0.001	< MDL	< MDL	0.31	0.01	0.03	0.12
Ethylbenzene	mg/L	0.001	< MDL	< MDL	0.06	0.003	0.009	0.034
Xylenes	mg/L	0.001	< MDL	< MDL	0.3	0.015	0.049	0.18
VPH C6-C10 (Less BTEX)	mg/L	0.01	< MDL	< MDL	0.52	0.05	0.14	0.45
EPH >C10 - C16	mg/L	0.05	< MDL	< MDL	0.33	0.49	1.6	5.1
EPH >C16 - C21	mg/L	0.05	< MDL	< MDL	0.07	0.4	1.3	3.8
EPH >C21-C32	mg/L	0.1	< MDL	< MDL	< MDL	0.6	1.8	5.8
Modified TPH Tier 1	mg/L	0.1	< MDL	< MDL	0.9	1.5	4.8	15
TPH	mg/L	_	0.00	0.00	1.61	1.57	4.93	15.49

**MDL: Method Detection Limit*

Values ≤ MDL were replaced with $\frac{1}{2}$ MDL for calculating sums.

The concentrations of each analyte increased as the nominal concentration of the chemically dispersed oil elevated (i.e., 1%, 3.2% and 10%). The measured concentrations of TPH analytes were below the method detection limit (MDL) of the GC-MS in the control seawater and control Corexit 9500A.

9.3.3 Bioassay

A total of 19 bioassays with 13 different females contributing larvae were performed. No significant differences were observed between carapace length (mm) of lobster larvae released from different batches and/or different females ($p < 0.05$; Figure 1). The carapace length of reference larvae ranged from 1.74 – 2.21 mm with the average length of 1.99 mm (± 0.087 mm).

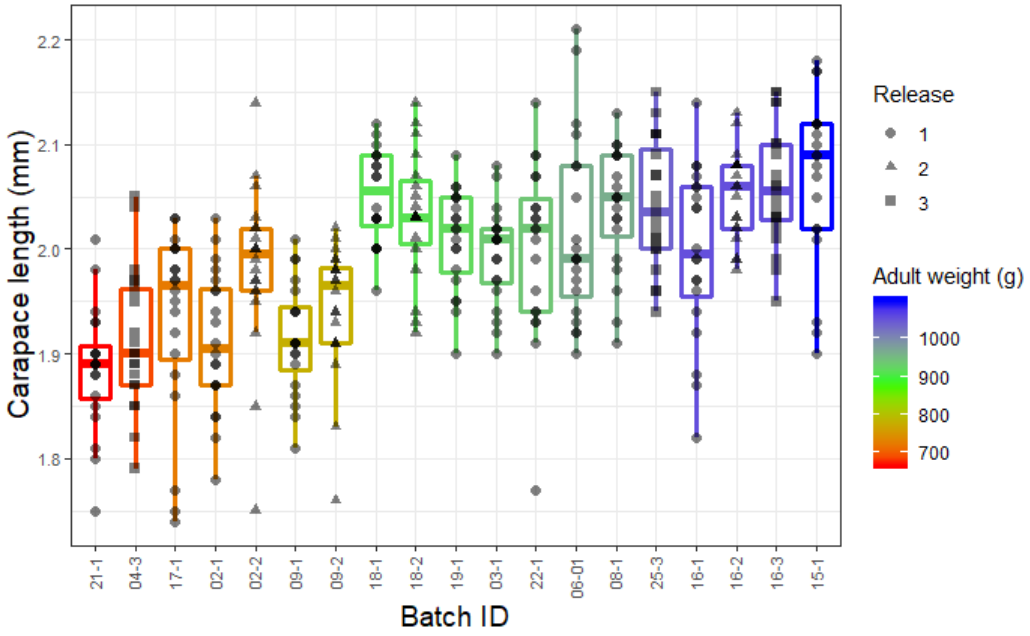


Figure 75: The average carapace length (mm) of reference lobster larvae (boxes; n = 20 per trial) on the y-axis. The increasing weight (g) of adult female lobsters (color gradient red to blue) on the x-axis. The shape of points indicates whether the batch is the first (circle), second (triangle), or third (square) release from each lobster.

The acute toxic endpoint was immobilization as characterized by lack of movements. The performance of lobster larvae in the control seawater and the control Corexit 9500A met the validity criteria of greater than 80% survival for all the sets of toxicity tests in this study. The results of the bioassays are visually summarized in Figure 76

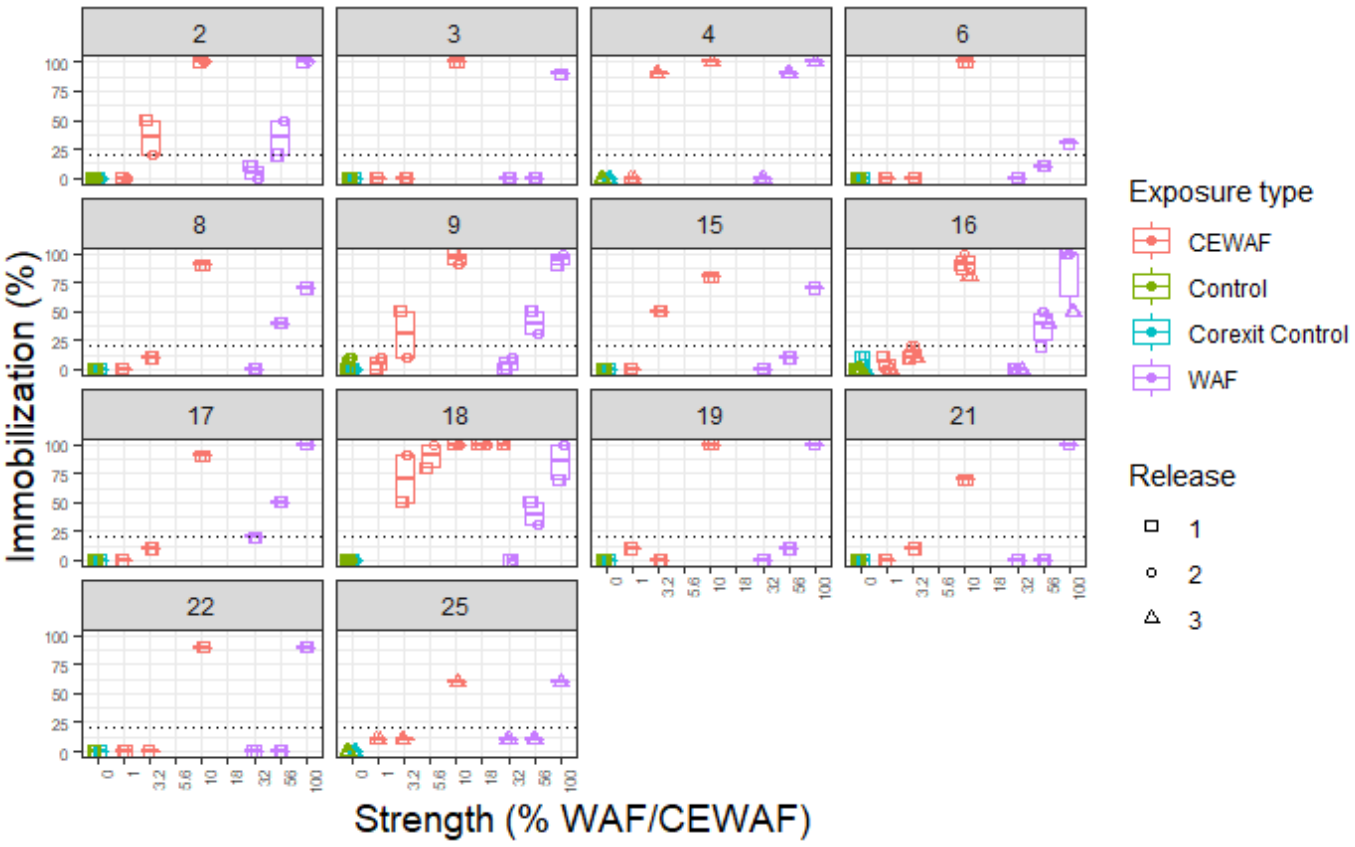


Figure 76: Visual summary of the 24-hr immobilization results for larvae from each lobster (individual panels) exposed to dilutions of WAF (purple) and CEWAF (red). The dotted horizontal line is the validity criteria of <20% immobilization in the controls.

The variability in responses at each of the exposure concentrations was visually examined by pooling the results from all trials (Figure 77).

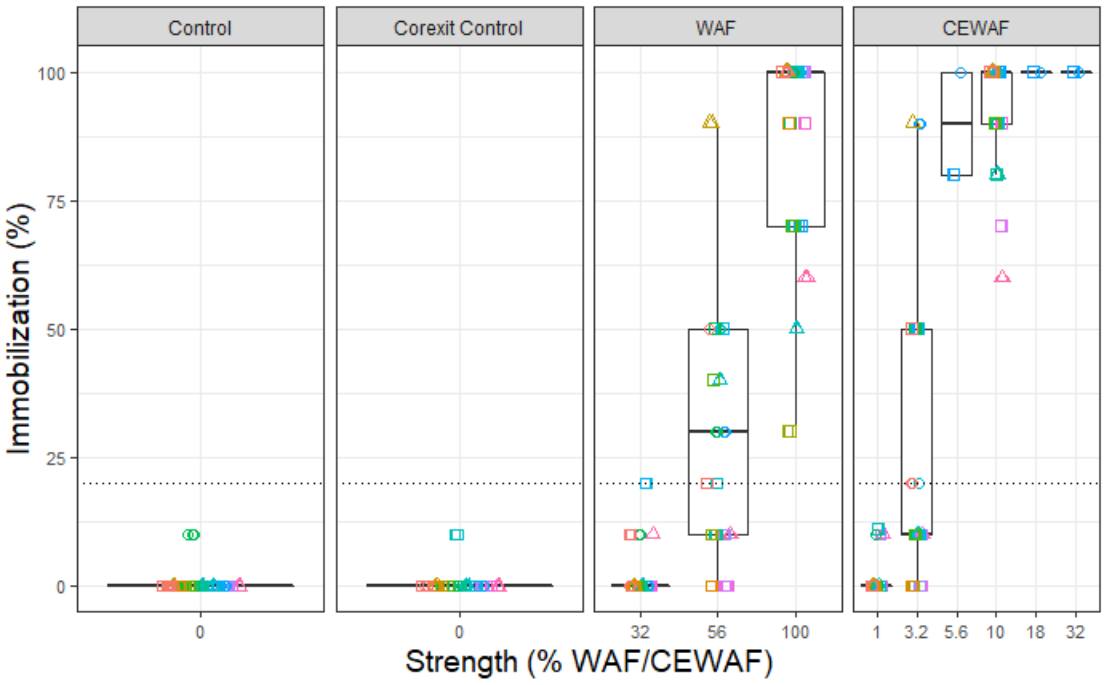


Figure 77: Combined immobilization data. Each colour is a release from the same female and the shape of the point is the release (square = first, circle = second, triangle = third). The dotted horizontal line is the validity criteria of <20% immobilization in the controls

There was near equal variability in the WAF and CEWAF exposed organisms with a large spread in response (0-90% immobilization) observed for each the 56% WAF and the 3.2% CEWAF treatments. The Control and Corexit Control treatments each showed a very consistent lack of immobilization response across all trials.

Comparing the best fitted model for the TPH-based effective concentration for different experimental trials supported the Weibull1.3 model as the best fitted model in most cases (Table 4). The best fitted model was then used to calculated the EC50 values and 95% confidence intervals.

Table 24: The best fitted model based on the AIC criterion and calculated 24h EC50 values (mg/L) with lower and upper limit (95% confidence limit) calculated on the basis of WAF and CEWAF alone and combined. The lowest EC50 value in each column is bolded and the highest values are bold and italicized.

Lobster	Batch	WAF		CEWAF		Combined	
		Model	24h EC50 (95% CI) (TPH mg/L)	Model	24h EC50 (95% CI) (TPH mg/L)	Model	24h EC50 (95% CI) (TPH mg/L)
2	1	W2.3	3.60 (2.60-4.61)	W1.3	4.71 (4.71-4.71)	LL.4	4.72 (4.49-4.95)
	2	W1.3	2.93 (2.91-2.94)	W1.3	2.28 (2.26-2.31)	W1.3	2.91 (2.56-3.26)
3	1	W1.4	4.07 (3.87-4.27)	W1.3	3.24 (3.15-3.33)	W1.3	4.08 (4.07-4.10)
4	3	W1.3	**2.30** (2.21-2.40)	W1.3	5.46 (4.98-5.94)	W1.3	**2.54** (-6.47-11.56)
6	1	W1.3	3.35 (3.07-3.64)	W1.3	3.10 (3.02-3.19)	W1.3	5.35 (1.90-8.79)
8	1	W1.3	2.81 (2.80-2.81)	W1.3	5.33 (5.33-5.33)	W1.3	4.54 (2.94-6.13)
9	1	W1.3	2.83 (2.82-2.84)	W1.3	3.44 (3.44-3.44)	W1.3	3.19 (2.68-3.70)
	2	LL.4	3.15 (2.89-3.41)	LL.4	4.53 (0.30-8.75)	LL.4	3.10 (1.36-4.85)
15	1	W1.3	3.93 (3.93-3.94)	W1.4	**2.15** (2.15-2.15)	W1.3	3.90 (2.30-5.50)
16	1	W1.3	4.09 (3.05-5.12)	W2.3	8.98 (6.10-11.86)	W1.3	4.79 (1.20-8.39)
	2	W1.3	2.93 (2.91-2.94)	W1.3	6.07 (6.02-6.12)	W1.3	3.69 (1.02-6.37)
	3	W1.3	2.46 (2.37-2.54)	W1.3	7.84 (7.84-7.85)	W1.3	5.93 (3.02-8.84)
17	1	W2.4	2.84 (0.33-5.36)	W1.3	5.33 (5.33-5.33)	W1.3	3.52 (1.38-5.66)
18	1	W1.3	2.58 (2.52-2.62)	W1.3	8.06 (6.91-9.20)	W1.3	4.77 (-1.09-10.63)
	2	W1.3	3.45 (3.17-3.73)	W1.3	7.33 (7.15-7.51)	W1.3	3.03 (1.60-4.46)
19	1	LL.4	3.04 (2.99-3.08)	LL.4	4.37 (4.37-4.37)	W1.3	3.15 (-3.25-9.55)
21	1	LL.4	3.86 (3.23-4.50)	W1.3	5.41 (3.51-5.31)	W1.3	3.38 (-21.29-28.05)
22	1	W1.4	4.07 (3.87-4.27)	W1.4	9.54** (9.50-9.58)	W1.3	4.99 (4.99-5.00)
25	3	W2.3	***4.77**** (3.80-5.73)	LL.4	***12.85*** (6.46-19.25)	LL.4	***9.73*** (-1.16-20.62)

*Significant difference between 24h EC50 value of WAF with CEWAF and Combined ($p < 0.05$).

**Significant difference between 24h EC50 value of CEWAF with WAF and Combined ($p < 0.05$).

The TPH-based 24h EC50 values ranged from 2.30 - 4.77 mg/L for the WAF exposures, 2.15 - 12.8 mg/L for the CEWAF exposures, and 2.54 - 9.73 mg/L when the exposures

were considered together. In each case, the least sensitive batch was the third release from female 25. The most sensitive batch was the first release from female 15 when considering the CEWAF only exposure but the third release from female 4 when considering the WAF only and the combined data. There were few differences between the EC50 estimates from a given batch regardless of the method of calculating the effect concentration. This was also seen when the data from all trials were pooled and analyzed on the basis of WAF and CEWAF exposures alone and combined (Figure 78).

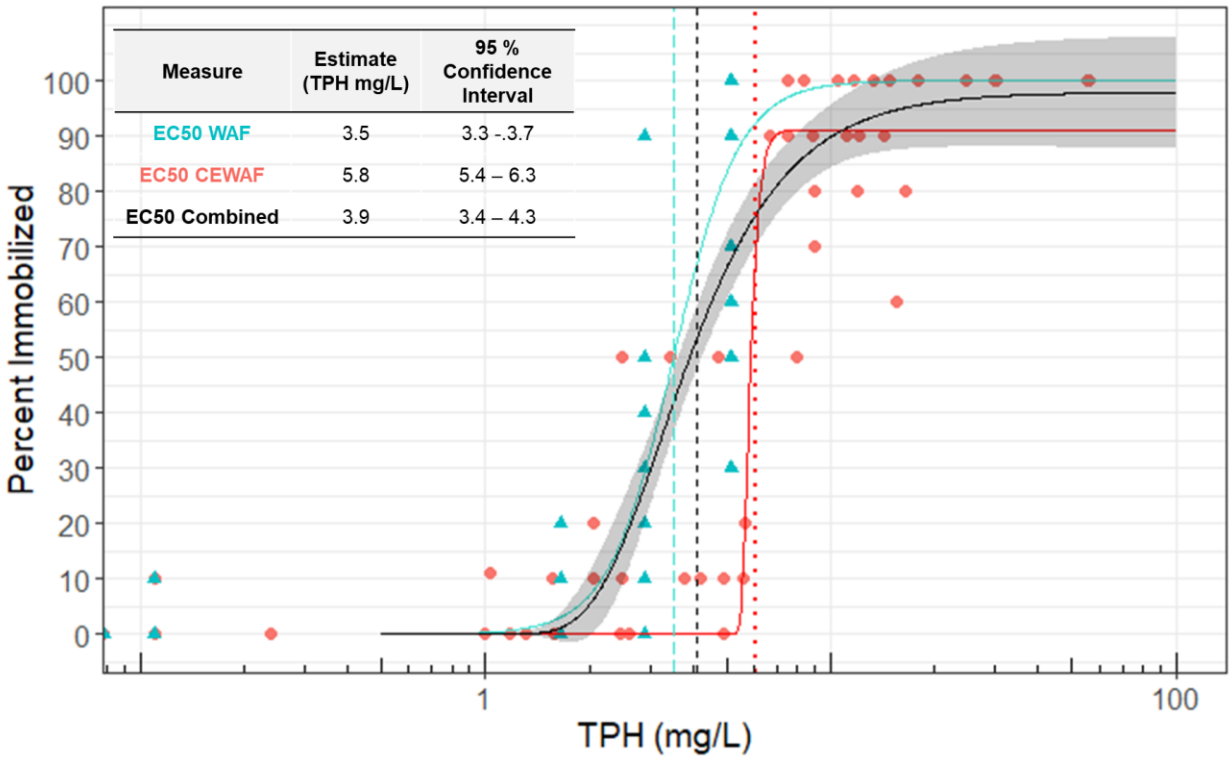


Figure 78: Concentration immobilization response models considering the WAF (blue triangles), CEWAF (red circles), and combined (black line) data. The dashed vertical lines are the EC50 values, which are reported in the inset.

The EC50 values calculated from the combined responses (Table 24) were used to generate a cumulative distribution from which the HC5 and PNEC could be calculated (Figure 79).

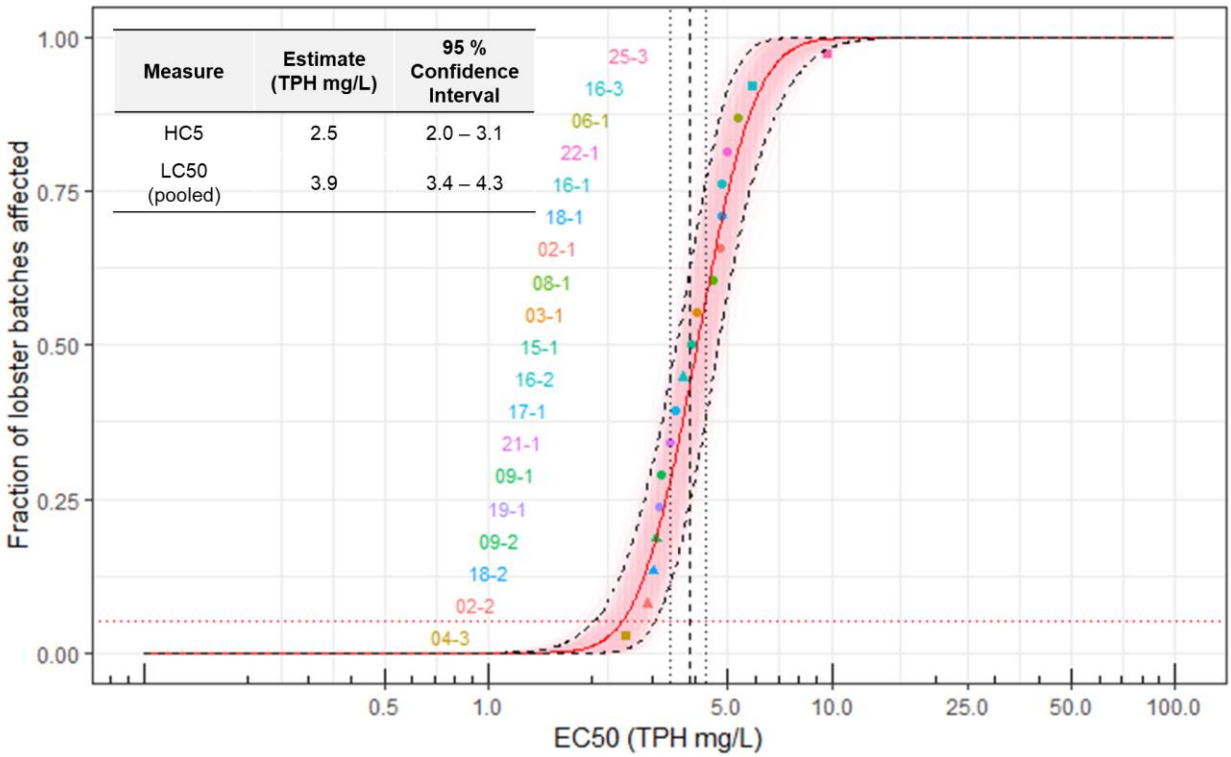


Figure 79: Distribution of lobster toxicity values where each point is an individual batch. The horizontal, dotted, red line is the HC5 estimate of 2.5 mg/L and the vertical dashed black line is the pooled LC50 estimate of 3.9 mg/L.

The HC5 based on the 24-hr EC50 values for immobilization was equal to 2.52 mg/L and is considered to be the PNEC for this population of larval lobsters. The difference between the LC50 and the PNEC is quite narrow and reflects that an increase in concentration above the HC5 will quickly translate into significant effects. When considering these values for risk assessment, application factors (e.g., 10) could be applied to the PNEC estimate to add a larger degree of conservatism.

9.3.4 Variability

The inter-trial variability between the acute toxic response of lobster larvae to the total petroleum hydrocarbons was assessed using EC50 values from the combined responses. The coefficient of variation (CV) was 17.1%, demonstrating low variability in toxic response of lobster larvae when considering the total petroleum hydrocarbons. There was no significant difference in response between successive larval batches from the same female. The within female variability was estimated from lobster 16, which had three larval

batches tested. The CV was estimated to be 23.3%, demonstrating a slightly higher within female/batch variability than amongst batches from different females (17.1%).

The variation in acute toxicity results of lobster larvae was not explained by carapace length of reference lobster larvae, wet weight of female lobsters, or the variability of DO (%) at post-toxicity measurements across all trials.

The inter-trial variability of the 24 hr EC50 values derived from acute toxicity tests of lobster larvae was compared with the acute toxicity of standard test species of mysid shrimp (*Americamysis bahia*) and inland silversides (*Menidia beryllina*). A total of 91 data points were obtained from the United States Environmental Protection Agency (US EPA) National Contingency Plan (NCP) Product Schedule (US EPA, 2019) showing the toxicity of No. 2 Fuel oil as 48 hr EC50 (mysid shrimp) and 96 hr LC50 (inland silverside) on a TPH basis (Figure 80).

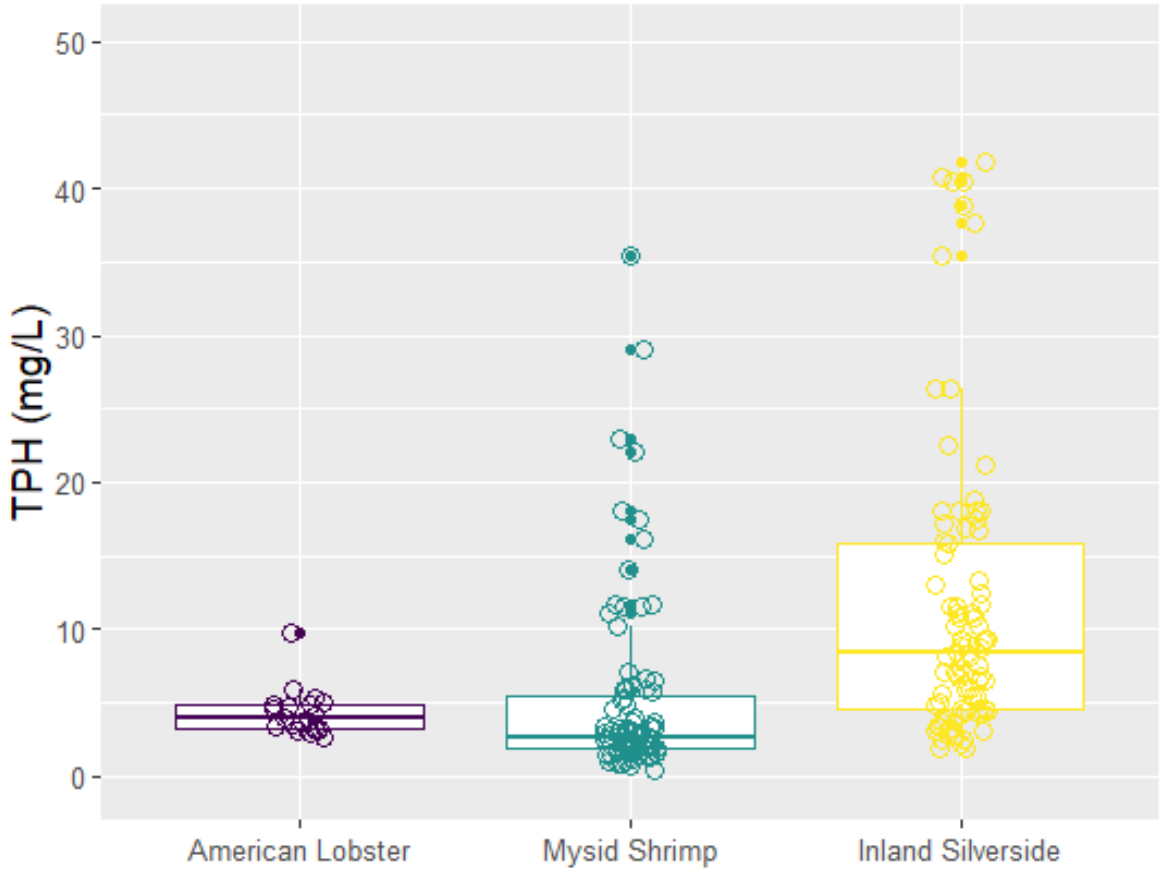


Figure 80: Comparing the inter-trial variability of 24 hr EC50 of American lobster larvae (Homarus americanus) (n = 14) with 48 hr EC50 of mysid shrimp (Americamysis bahia) (n = 91) and 96 hr LC50 of inland silversides (Menidia beryllina) (n = 91) exposed to petroleum products.

The lobster data generated in this study is comparable to the effect levels observed with Mysid shrimp. The dispersion of toxicity data points from the mean value of these species was calculated using the variance of toxicity results to account for the variability in sensitivity of toxic responses of American lobster. The variance was 2.62, 302.48 and 1779.93 for American lobster larvae, mysid shrimp, and inland silverside, respectively.

9.4 Discussion

The variability of toxic responses in laboratory-based experiments should be determined to assess the potential hazard of chemicals to species in the natural ecosystem, which also yields the precision of toxicity estimates. Two aspects should be identified to account for the variability of species sensitivity in ecotoxicology: first, the potential causes that can result in variable toxic responses, such as inherent natural variability between different

batches of a test species or temporal and spatial variabilities; and second, the significance of those variabilities that may mirror the observed toxic responses (Calow 1996). In this study, a well-detailed and consistent methodology was followed to conduct the toxicity tests to limit the number of variables that may lead to significantly different toxic responses. For example, the water quality parameters in the test solutions or the preparation of exposure medium are considered as the interfering causes of experimental variabilities (Hrovat et al. 2009). In this study, the inter-trial variability amongst water quality parameters had a narrow range in both pre- and post-toxicity test measurements demonstrating precision of data points. Although the post-toxicity DO (%) showed more variation (i.e., higher CV) there was no significant effect of post-toxicity DO (%) on variability of acute toxic effects of total petroleum hydrocarbon to lobster larvae.

The procedure to prepare the exposure medium for toxicity tests can alter the sensitivity of test species to the oil/dispersed oil, irrespective of type of dispersant or oil product. Therefore, determining the concentration of oil constituents is required in order to account for the subsequent variability in the observed toxic responses. In the current study, combining the WAF and CEWAF exposures along the measured concentration continuum exhibited nearly equal precision when estimating the oil toxicity to lobster larvae than when the WAF and CEWAF were considered alone. The CV of the EC50 values calculated from the combined WAF and CEWAF was equal to 17% as compared with the WAF and CEWAF alone with CVs of 20% and 43%, respectively. The lack of variability in toxic responses between lobsters could be justified considering the wild female lobsters all had been caught from the same area with unknown relatedness. This narrow range of response in the larval lobsters suggests a reduced capacity to adapt to exposure to petroleum hydrocarbons as compared to fish or other crustaceans, such as shrimp.

Lack of variation in sensitivity of early life stages of lobster to acute exposure of oil can be attributed to narcotic mechanism of action of oil products that is not taxa specific (Barron et al. 2004; Russom et al. 1997)(Barron et al. 2004; Russom et al. 1997). The less variability in the toxic response of lobster larvae might be due to less variation in the ability of crustacea to metabolize xenobiotics. There is no consensus on the presence

and inducibility of cytochrome 1A (CYP1A) enzymes in invertebrates (Koening et al. 2012). James and Boyle (1998) reviewed cytochrome P450 in crustacea and found evidence that lobsters (*Panulirus argus* and *Homarus americanus*) do not perform biotransformation of benzo-a-pyrene (B[a]P) in a CYP1 manner. Most remaining B[a]P was untransformed accumulating in the muscle and hepatopancreas of the American lobster. The authors also noted that the aryl hydrocarbon receptor (Ah-receptor) is absent in American lobster. Altogether, it suggests that the lobster does not have the same ability to metabolize and ameliorate exposure to petroleum hydrocarbons as do fish or mammals. The absence, or reduced capacity, for xenobiotic metabolism, is compounded by the potentially relatively low level of genetic variability in lobsters, which limits the ability for selection and adaptation. Tracey et al. (1975) employed starch-gel electrophoresis followed by selective enzyme assay to quantify the amount of genetic variation in geographically distinct (three offshore and five nearshore) natural populations of the American lobster. They found there to be rather low levels of genetic variability within the 300 animals surveyed, with the average proportion of heterozygous loci per individual being 3.8%. The small and nearly constant variance in EC50 values observed for lobsters in this study may be indicative of low phenotypic variability, which can be associated to lower genetic variability (Devin et al. 2014).

Exposure conditions of the current study were static non-renewal and thus the derived toxicity values (LC50 = 3.9, HC5 or PNEC = 2.5 mg/L TPH) may overestimate the real-world exposure scenario of oil/dispersed oil to lobster larvae, which is expected to be dynamic. However, the exposure duration was short (24 hours) and thus may be reflective of conditions experienced during certain spill scenarios.

Chapter 10 Time-dependent toxicity of physically and chemically dispersed crude oil to the planktonic stages of American lobster.

10.1 Introduction

Aquatic organisms are not often exposed to oil products for long periods of time during a marine oil spill. Generally, crude oil enters the aquatic environment in pulses and episodic events then rapidly begins to experience changes in bulk properties (e.g., density and viscosity) and composition due to weathering (e.g., differential evaporation, dissolution and degradation). The toxic effects that an organism may experience are directly related to the duration of exposure, which itself is related to environmental fate processes and the behavior of the organisms. The application of a dispersant will also drastically change the environmental fate of the crude oil, leading to more rapid dissolution and degradation. Bejarano et al. (2014) noted that high oil concentrations (<54 mg/L) declined in less than 4 hours to <1 mg/L following a survey of field measurements.

Mechanistic oil exposure and toxicity models, like Spill Impact Model Application Package (SIMAP; developed by Applied Science Associates, Narragansett, RI, USA), require high quality, reliable data for the sensitivity of exposed organisms over a range of time scales.

In the present study, the toxic effect of chemically and physically dispersed oil to early life stages of American lobster larvae (*Homarus americanus*) were characterized at different time intervals. The objective was to determine if there is a relationship between oil concentration (i.e., as cumulative concentrations of both chemically and physically dispersed oil), exposure time, and acute toxicity of oil (i.e., immobility). The incipient lethal level was also determined to accommodate oil spill risk management.

10.2 Methods

10.2.1 Test Organism: American lobster (*Homarus americanus*)

Adult lobsters were captured in the Bay of Fundy Lobster Fishing Area 36 upon receiving a special permit from Fisheries and Oceans Canada. Adult commercial size berried females (0.5-2 kg) were obtained from local fishers. The berried females were maintained at Huntsman Marine Science Center in St. Andrews, New Brunswick. The egg mass

development of female lobsters was monitored under controlled environmental conditions. Larval lobsters at stages I, II and III each were used in bioassays. Prior to using each batch, 20 lobster larvae were imaged as reference organisms using a Leica Wild M420 microscope and Leica MD190 camera at 12.5x magnification. Carapace length (mm) of reference larvae was measured. The LAS software version 4.8 was used to measure the larval carapace length.

10.2.2 WAF and CEWAF Preparation

Exposure waters were prepared to test the toxicity of oil to aquatic organisms by contacting the oil and water such that a mixture is generated consisting of oil components in both the dissolved phase and in the non-dissolved phase, following the methods described in Section 3.3.2, Table 10. The background water for preparing the WAF and CEWAF stock solutions was 0.22 µm filtered seawater (i.e., salinity of ~ 30 psu) received from the Bay of Fundy. The stock solutions in this study were prepared using an orbital mixing technique. Briefly, two 2-L glass baffled flasks with a hose bib at the base were used. The volume of crude oil (i.e., weathered offshore Newfoundland crude oil) was loaded after adding 1.6 L of filtered seawater into the flasks (20% headspace) using a 5 mL Hamilton® gastight syringe to each baffled flask. Target loading was 1 g of crude oil per 1 L of seawater resulting in the addition of 1.6 g of crude oil. The flasks containing dilution water and oil were sealed with DuraSeal®. The CEWAF solution was prepared by adding the Corexit 9500A dispersant at this step with a dispersant to oil ratio (DOR) of 1:20. The baffled flasks were then placed on the MaxQ SHKE2000 digital shaker (Thermo Scientific) in an environmental chamber with controlled temperature at 15ºC ± 2ºC. The solutions were mixed at 150 rpm for 1 hour followed by a 1 hour settling time before being used to generate the exposure media. The dispersant only control was prepared at a concentration that was equal to the volume of dispersant in the highest tested CEWAF concentration (32% strength) and was nominally 15.8 mg/L.

10.2.3 Bioassay

A controlled environmental chamber was used for preparing the test solutions and performing the toxicity tests. The chamber was set at 15ºC ± 1ºC and equipped with light

(~ 232.3 lux) for 16 h light and 8 h dark photoperiod. The required volume of WAF and CEWAF stock solutions were added to an appropriate volume of filtered seawater (0.22 µm) to make 100%, 10.0% and 1.0% WAF test solution and 32%, 3.2% and 0.32% CEWAF test solution. Then, 10 x 20-mL scintillation vials were filled within 1 h upon preparation with each concentration of WAF or CEWAF, plus control seawater and control Corexit 9500A (nominally 15.2 mg/L, same as highest tested concentration of CEWAF, 32%), which were randomly placed for the toxicity tests. Lobster larvae at stages I, II or III were each used in separate 48h static toxicity tests with no test solution renewal. The toxic effects were recorded as numeric categories (0 = no observed effects, 1 = partially affected, 2 = mortally affected, and 3 = dead with no swimming or visible heartbeat as described in Table 21) at 6, 18, 24 and 48 h after launching each exposure. The validity criteria of toxicity test included control mortality ≤ 20%, dissolved oxygen ≥ 60% saturation, and change of temperature ≤ 1.5℃. Water quality parameters, including dissolved oxygen (DO; %), pH, salinity (psu), and temperature (^{o}C) were measured at 6, 18, 24 and 48 h concurrently with the intervals for toxicity assessment of lobster larvae.

A dispersant only trial was conducted following the same methodology, however only with stage I larvae. The larvae were exposed to Corexit 9500A at concentrations of 40, 63, 100, 250, 400, 630 and 1000 mg/L with assessments at 24 and 48 hrs.

10.2.4 Statistical analysis

Percent immobilization and mortality were obtained at each time interval for each stage and the concentration-response curves were estimated by fitting log-logistic functions (*drc* package in R, Ritz et al. 2016; Ritz and Streibig 2005; Ritz 2016). Median lethal effect concentration (LC50) estimates were compared across time points and stages. Analyses were performed in R using version 3.1.3 (R Core Team 2012).

10.3 Results

The results from all bioassays are summarized in Table 25 and the immobilization results are modelled by TPH in Figure 81.

Table 25: Immobilization results for each time point and larval stage.

Duration (hours)	Stage	Treatment							
		Control	Corexit Control (nominally 15.2 mg/L)	0.32% CEWAF	1% WAF	10% WAF	3.2% CEWAF	100% WAF	32% CEWAF
		Concentration TPH mg/L							
		0	0	0	0.029	0.21	1.57	2.01	30.08
6	I	0.0	0.0	0.0	0.0	0.0	0.0	80.0	70.0
	II	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
	III	0.0	0.0	0.0	0.0	0.0	20.0	100.0	100.0
	Mean (St. dev.)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6.7 (11.5)	93.3 (11.5)	90 (17.3)
18	I	0.0	0.0	0.0	0.0	0.0	50.0	80.0	100.0
	II	10.0	10.0	10.0	10.0	20.0	10.0	90.0	100.0
	III	0.0	0.0	20.0	0.0	20.0	20.0	100.0	80.0
	Mean (St. dev.)	3.3 (5.8)	3.3 (5.8)	10.0 (10.0)	3.3 (5.8)	13.3 (11.5)	26.7 (20.8)	90.0 (10.0)	93.3 (11.5)
24	I	10.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
	II	20.0	0.0	10.0	10.0	10.0	40.0	100.0	100.0
	III	0.0	20.0	0.0	0.0	0.0	60.0	100.0	100.0
	Mean (St. dev.)	10.0 (10.0)	6.7 (11.5)	3.3 (5.8)	3.3 (5.8)	3.3 (5.8)	66.7 (30.6)	100.0 (0.0)	100.0 (0.0)
48	I	0.0	0.0	0.0	0.0	10.0	70.0	100.0	100.0
	II	10.0	20.0	30.0	10.0	0.0	80.0	100.0	100.0
	III	20.0	0.0	20.0	20.0	40.0	100.0	100.0	100.0
	Mean (St. dev.)	10.0 (10.0)	6.7 (11.5)	16.7 (15.3)	10.0 (10.0)	16.7 (20.8)	83.3 (15.3)	100.0 (0.0)	100.0 (0.0)

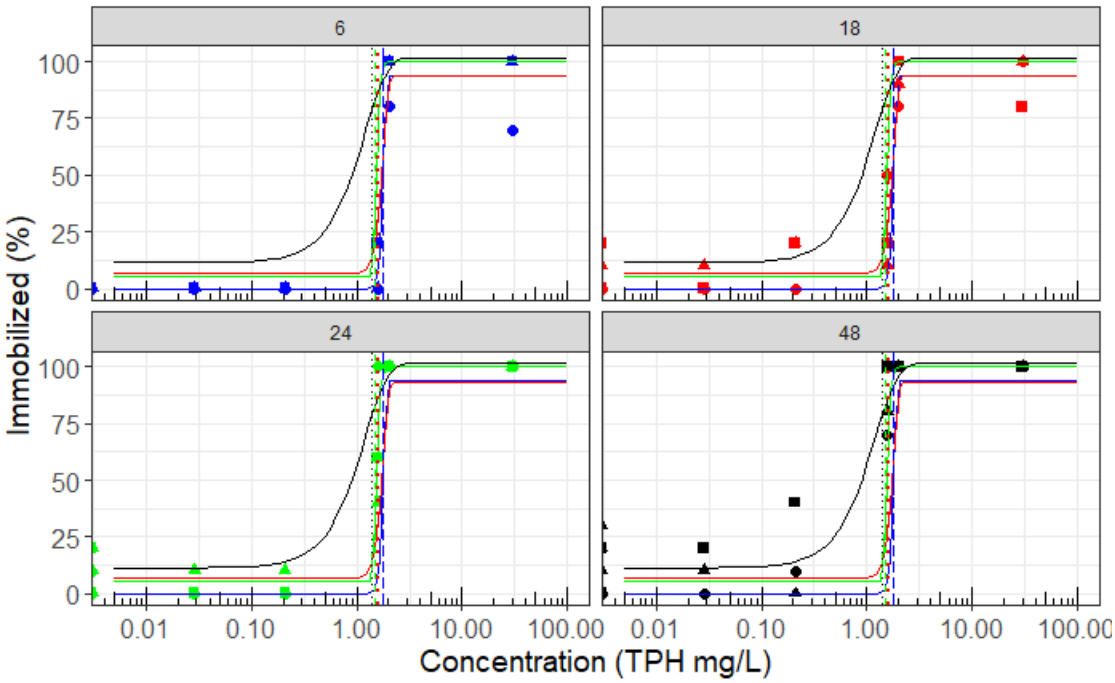


Figure 81: Concentration response relationship at each time point (individual panel) by stage (Stage I = circle, Stage II = triangle, Stage III = square).

The data from each stage was combined given the consistent response across stages in order to produce a concentration response relationship with TPH and immobilization (Figure 82).

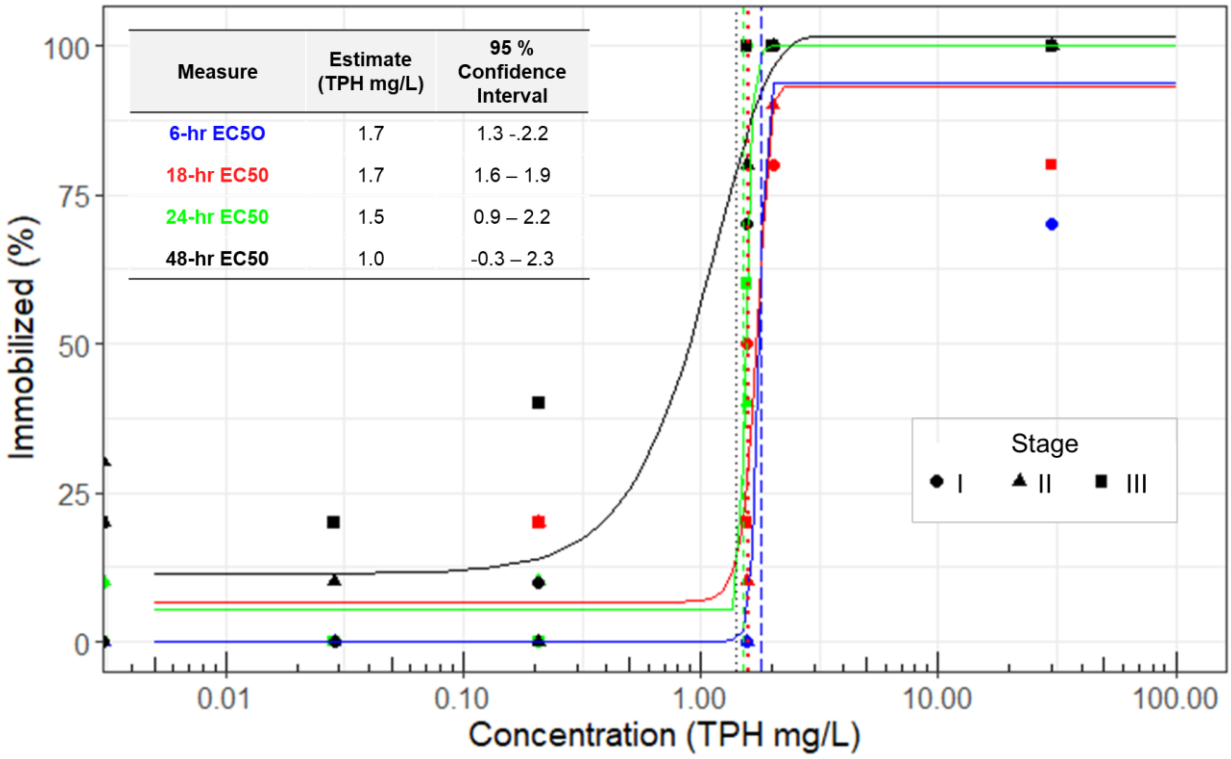


Figure 82: Concentration response relationship by stage (shape) and duration (colour)

There was no difference between the calculated EC50 values at any time point when the data was combined by stage.

The control immobilization was at or above the validity criteria at each time point for the dispersant alone testing and, as such, these results should be interpreted with caution (Figure 83).

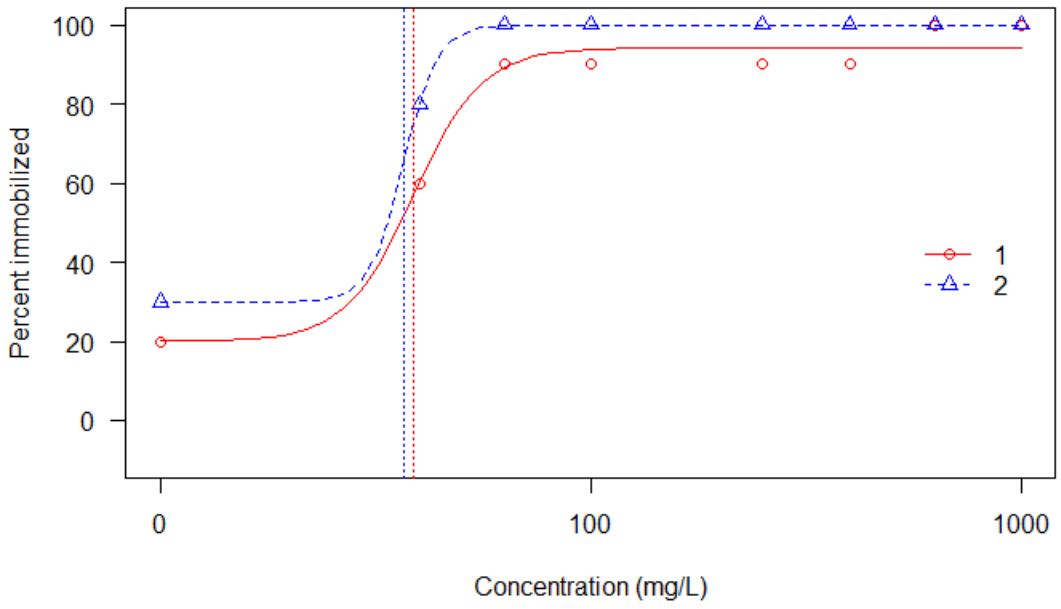


Figure 83: Concentration response relationship for Corexit 9500A after 1 (red circles) and 2 days (blue triangle) of exposure.

The nominal EC50s were 38.8 mg/L (95% CI 34 - 42) and 36.8 mg/L (95% CI 20.6 - 52.9) for the 24 and 48-hr exposures, respectively.

10.4 Discussion

The planktonic larval stages of the American lobster did not show a significant difference in response to exposure to petroleum hydrocarbons. All three stages had near identical responses at each time point observed in this study and the corresponding EC50 values were not significantly different from each other. The rapid and significant onset of immobilization following with 6 hours of exposure highlights the importance of early time point assessments.

The toxicity of Corexit 9500A to the stage I larvae is low with the 24-hr EC50 value (38.8 mg/L) greatly above expected environmental concentrations in a spill response scenario, which is estimated at worst case to be between 2.7 to 9.0 mg/L based on application rates (Bejarano 2018).

Collectively, these data will be useful inputs into mechanistic oil exposure models that help to predict environmental impacts and inform spill response decision making.

Northern shrimp (*Pandalus borealis*) and Snow Crab (*Chionoecetes opilio*)

Definitions

- **Gravid**: carrying developing young or eggs (see also ovigerous)
- **Megalopae**: Transitional stage between a crab larva and a juvenile.
- **Molt**: Cyclical process of preparing for, undergoing, and recovering from ecdysis. The molt cycle in crustaceans is divided into 5 main phases (A to E), which characterize post-molt (A-C), pre-molt (D), and ecdysis (E) phases.
- **Ovigerous**: carrying or bearing eggs
- **Pre-zoae**: Short developmental period (on the order of hours) immediately post-hatch, prior to Zoea stage I.
- **Zoea**: The common larva of decapods. Characterized by a large cephalothorax that is covered with a helmet-like carapace that also sports spines and is protruded into a rostrum in front

Background

The initial proposal had called for the use of Northern shrimp (*Pandalus borealis*). After adapting and modifying protocols from the International Research Institute of Stavanger (Norway), for the collection ("barrel trawling") and husbandry of berried female shrimp, we were unable to successfully return healthy individuals to the lab, and as such this species was not pursued further. We did have an opportunity to complete preliminary toxicity exposures with the economically important snow crab (*Chionoecetes opilio*). Berried female snow crab (n = 30) were obtained from DFO Gulf Region Survey on 15-September-2016 and communally held within Huntsman wetlab facilities (ambient water and simulated natural photoperiod with sufficient shading over the tanks) with a wild food diet primarily of squid, shrimp and herring. Each crab had clutch status (e.g., egg biopsy for measurements and embryo staging) assessed to determine when the embryos were nearing the final developmental stage, as indicated by an average egg diameter (720 µm and dark orange to dark brown colouration). The snow crab were transferred into individual containers with the sides replaced by 500 µm (~50% open) Nitex mesh so as to retain larvae hatched by the female upon reaching the final egg development stage (Figure 84).

Figure 84: Snow crab showing early (A; orange eggs) and late (B; brown, mossy eggs) egg development. As the eggs neared hatch the snow crabs were transferred to holding containers (C) to retain the released larvae.

The planktonic larvae were available for toxicity testing following release from the female clutch. The toxicology trials involving snow crab are described in Chapter 11.

Chapter 11 Effects on the early life stages of Snow crab

11.1 Introduction

Northern shrimp (*Pandalus borealis*) toxicology trials proved difficult over the course of the project given difficulties to live capture, transport and hold berried female individuals for later hatching of larvae to support exposures. However, we did take an opportunity to work with larvae from a collection of snow crab (*Chionoecetes opilio*) obtained from a partnership with Fisheries and Oceans Canada research staff. Snow crab have planktonic larvae that undergo successive molts (pre-zoea, zoea I [Z1], zoea II [Z2] and megalopodae [M]) before settling on the benthos as crab stage [C1]. The larval stages are particularly vulnerable to contaminant exposure and were the focus of these trials.

After performing numerous method development trials, we were able to conduct three exposure tests with Z1 snow crab larvae.

11.2 Methods

Exposure waters were prepared following the methods described in Section 3.3.2, Table 10. Exposures were conducted on Z1 larvae that were less than 24-hrs old. Briefly, Individual organisms (5 or 10 organisms per concentration) were held in its own test vessel to prevent cannibalism (Figure 85).

Figure 85: Z1 snow crab larvae

Images were collected from 30 reference organisms prior to exposure to determine carapace width as distance between lateral spines. Organisms that were actively swimming in the water column were allocated to test vessels (25-mL scintillation vials) containing the exposure solutions according to the details in Table 26.

Table 26: Test conditions for the three exposure trials with Z1 larvae of snow crab (Chionoecetes opilio)

	CO-001	CO-003	CO-002
Treatments	**WAF**: 100, 32, 10.0, 3.2, 1.0% **CEWAF**: 32, 10.0, 3.2, 1.0, 0.32% **Negative control**: 0.22 µm filtered seawater **Dispersant control**: Corexit 9500 applied at the same rate as 32% CEWAF	**WAF**: 100, 32, 10.0, 3.2, 1.0% **CEWAF**: 32, 10.0, 3.2, 1.0, 0.32% **Negative control**: 0.22 µm filtered seawater **Dispersant control**: Corexit 9500 applied at the same rate as 32% CEWAF	**WAF**: 100, 10.0, 1.0% **CEWAF**: 32, 3.2, 0.32% **Negative control**: 0.22 µm filtered seawater **Dispersant control**: Corexit 9500 applied at the same rate as 32% CEWAF
Replicates	10	10	6
Organisms /replicate	1	1	3
Exposure Duration	24	48	48
Monitoring duration	7	5	5

Organisms were transferred to new scintillation vials with clean seawater after the exposure period. Subsequently 80% of the water was renewed daily for up to 7 days post exposure. Mortality determination with invertebrates, particularly at the larval stages, is often difficult to determine definitively. The effect of immobilization or loss of equilibrium, as characterized by an inability to maintain a 'normal' position, is more readily assessed and often considered equivalent to mortality for larval crustaceans. Organisms were assessed daily according to the health categories listed in Table 27 with dead organisms removed upon observation.

Table 27: Health evaluation categories for snow crab larva as determined by observation with microscope (adapted from Perkins et al. 2003)

Category	Description
Alive	Vigorously swimming; tail bent under in a normal position; active internal organ movement; good phototactic response (e.g., successfully swimming to water's surface); swims away when prodded
Affected	Passively swimming; phototactic response diminished; tail cocked or flipped backwards; organ movement detectable; reduced response to being prodded
Mortally Affected	Not swimming, but twitching; slight organ movement; no phototactic response; no response to being touched
Dead	No internal organ movement; opaque beige in color

11.3 Results

For the trial CO-001, the concentration response relationship was modelled at each day with response across WAF and CEWAF combined on the basis of TPH concentration (Figure 86).

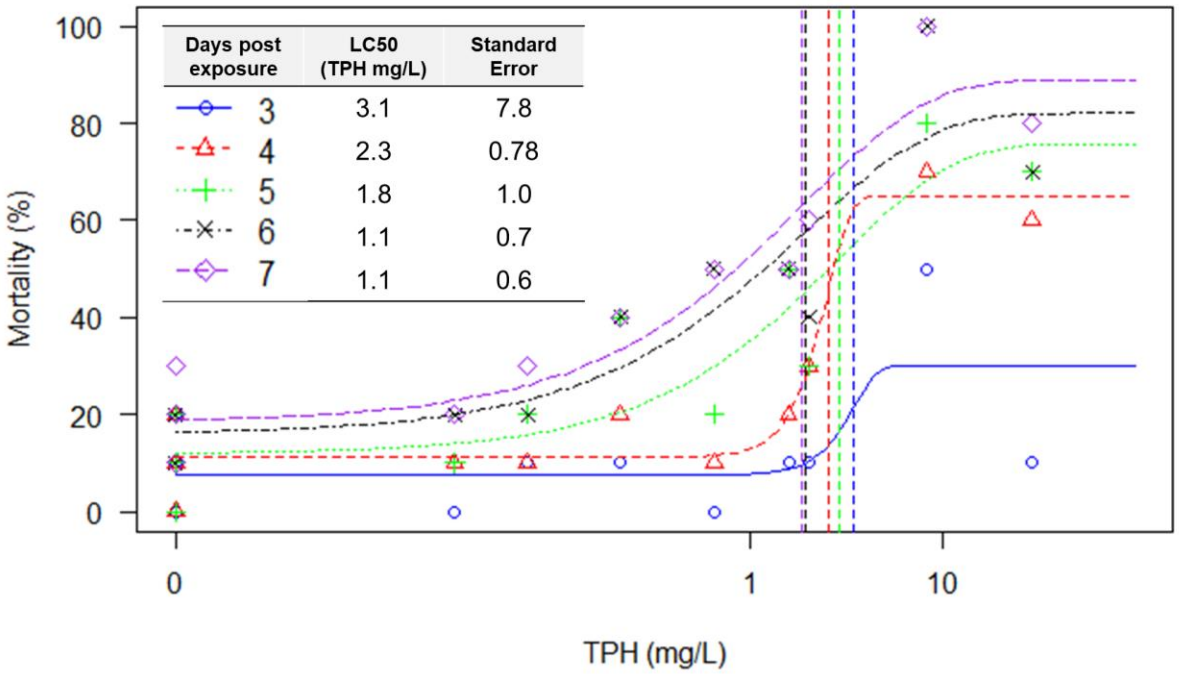


Figure 86: Concentration response relationship by day post exposure (3 = blue circles, 4 = red triangles, 5= green cross, 6 = black x, and 7 = purple diamond) with the dashed vertical lines being the corresponding LC50 values (insert).

The concentration response relationship only became significant at 3 days post exposure, at which point LC50s were able to be calculated for each subsequent day. The LC50s calculated after a 24-hour exposure indicated that exposure to concentrations of TPH between 1 to 3 mg/L are sufficient to cause latent mortality effects that were not observed within the first 48-hours post exposure. The concentration response relationship at day 7 for each the WAF and CEWAF exposed larvae is shown in Figure 87, and the calculated LC50 values were similar, 1.1 and 1.9 mg/L TPH respectively.

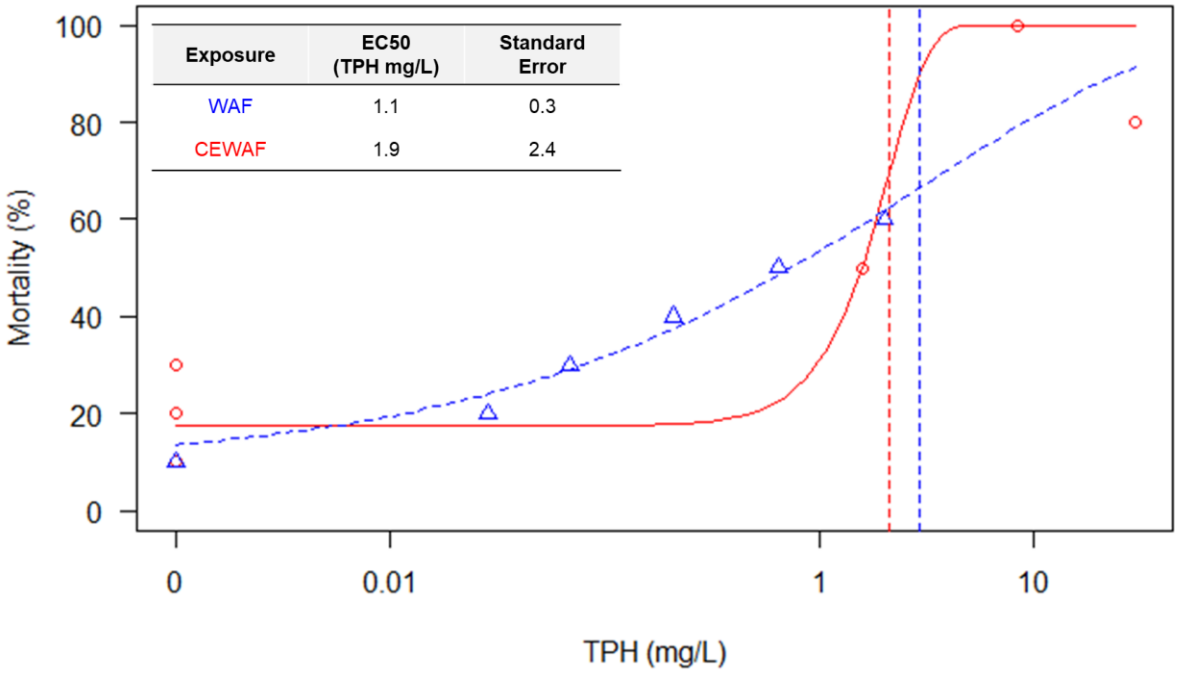


Figure 87: Concentration response relationship at 7 days post exposure to WAF (blue triangles) and CEWAF (red circles). The dashed vertical lines are the corresponding LC50 values.

The 48-hr exposure (CO-003) to the same concentrations as CO-001 similarly revealed latent effects on mortality, with the concentration response model shown for 5 days post exposure in Figure 88.

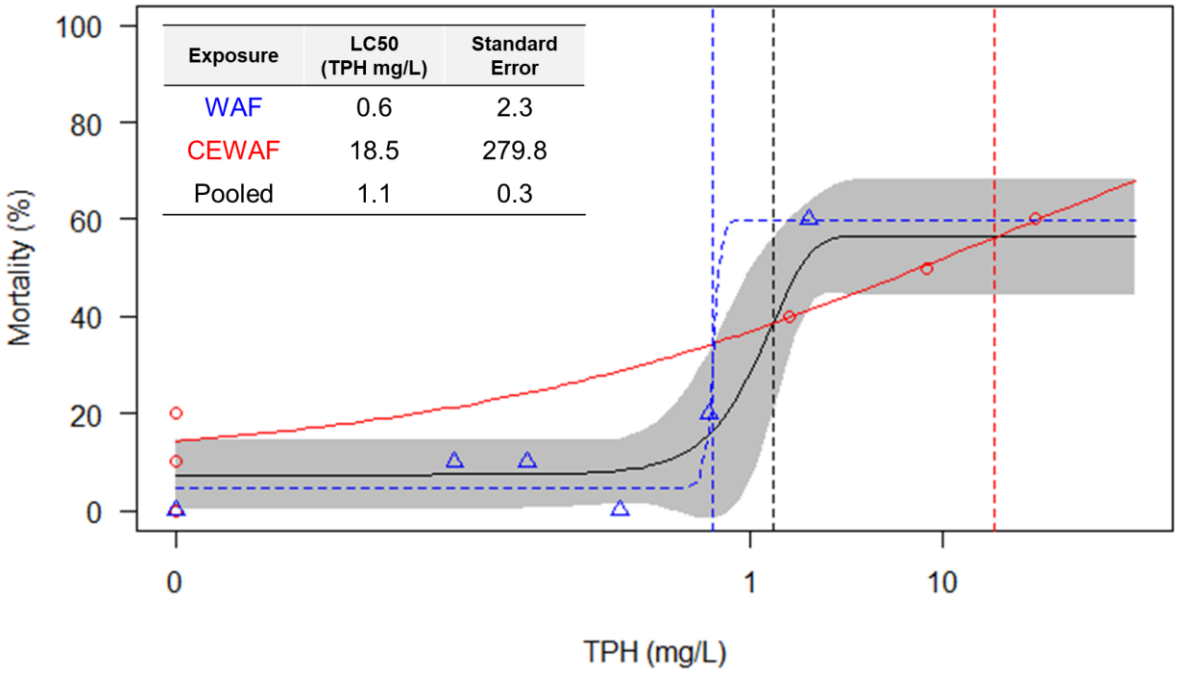


Figure 88: Concentration response model at 5 days post 48-hr exposure to WAF (blue triangles), CEWAF (red circles), and combined (black line). The dashed vertical lines are the corresponding LC50 values (insert).

CO-002 was also a 48-hr exposure, however with reduced concentrations and a different loading of organisms (e.g., 3/replicate, 6 replicates/treatment). The responses were pooled across replicates to model the concentration response relationship (Figure 89).

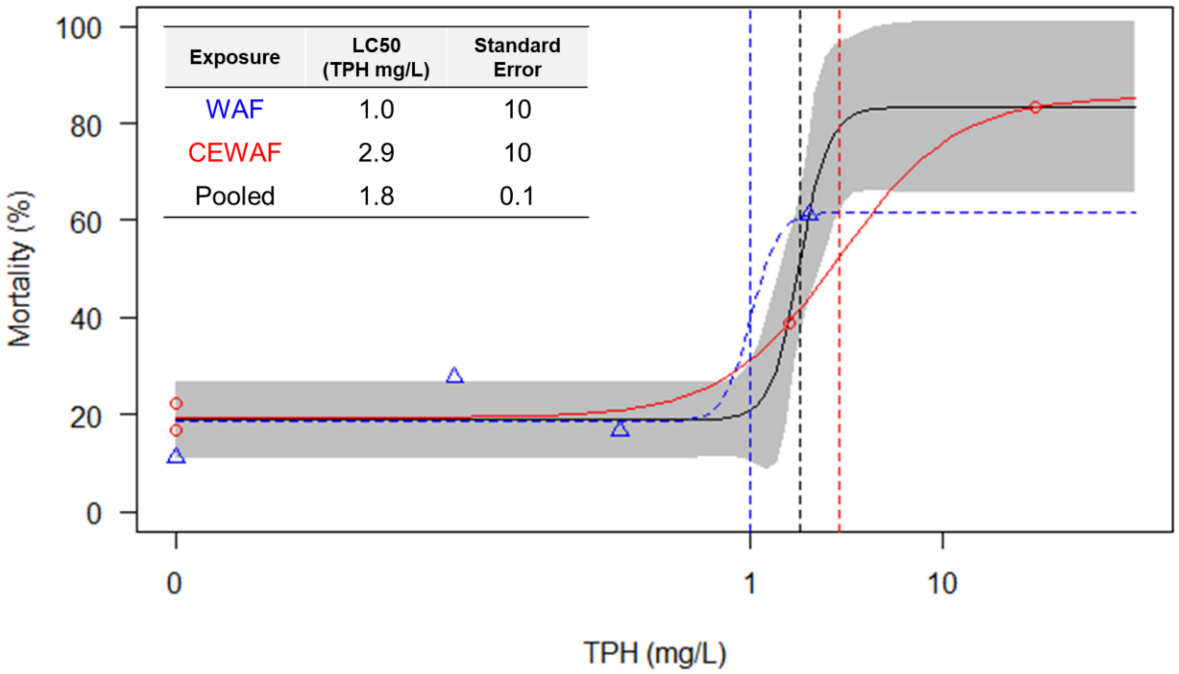


Figure 89: Concentration response model at 5 days post 48-hr exposure to WAF (blue triangles), CEWAF (red circles), and combined (black line). The dashed vertical lines are the corresponding LC50 values (insert).

The similar responses between CO-002 and CO-003 (LC50s of 1.8 and 1.1 mg/L, respectively, when WAF and CEWAF are considered together) support combining these two trials to increase the confidence in the LC50 estimate (Figure 90).

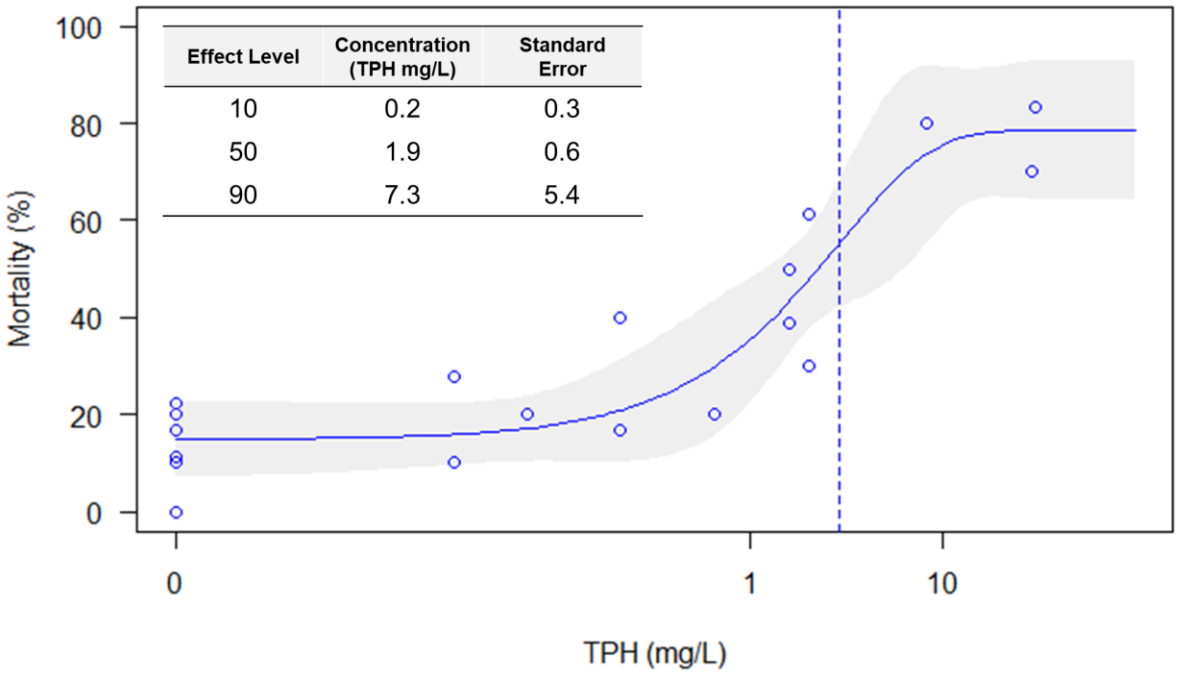


Figure 90: Concentration response relationship from the combined CO-002 and CO-003 trials with WAF and CEWAF exposures considered on a continuum based on TPH concentrations. The dashed vertical line is the LC50, with it, and other effect concentrations listed in the insert.

11.4 Discussion

The results presented here provide valuable information for a less commonly studied species of great economic importance, especially from the offshore Newfoundland environment. The estimated LC50 values were comparable to those generated for American lobster larvae in this project (Chapter 9) and Northern shrimp data acquired from the published literature (Arnberg et al. 2019) (Figure 91).

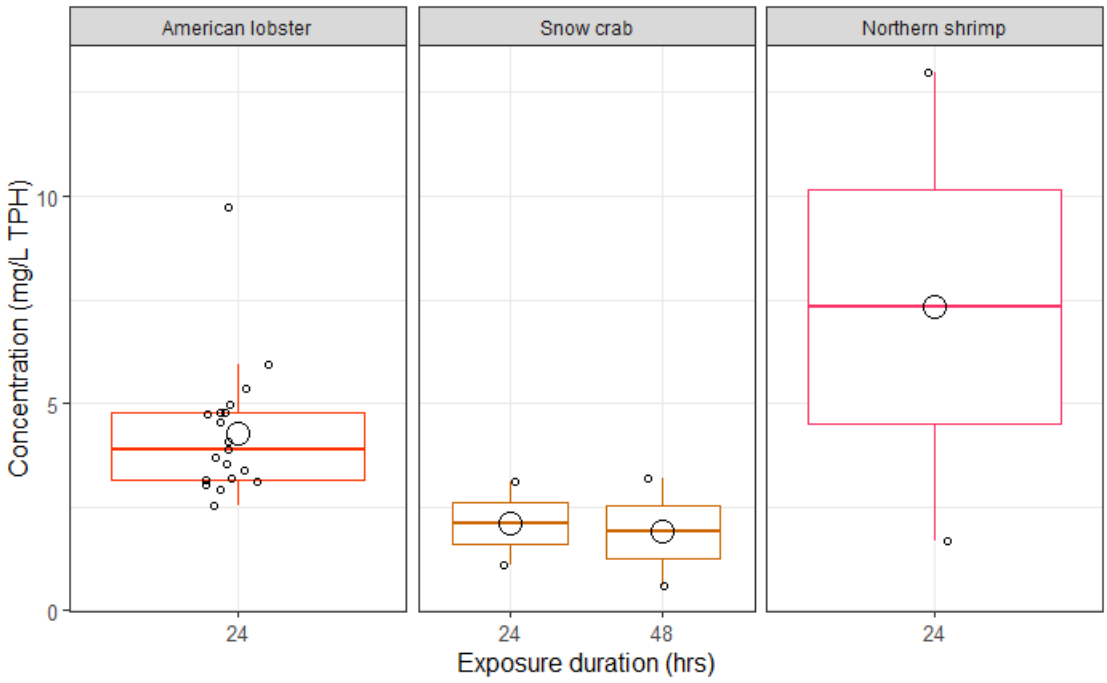


Figure 91: Summary of the effect concentrations for northern species of crustaceans. American lobster and snow crab data from this study while the Northern shrimp data is extrapolated from Arnberg et al. (2019).

These data support the limited variability observed in crustacean responses to crude oil and suggests that effect concentrations derived from American lobster and/or snow crab larvae would be reflective of the expected response from Northern shrimp.

Summary

Chapter 12 Overview of Results

12.1 Exposures

The observation of effects is dependent on the life stage, exposure concentration and duration. The toxicity effects observed in this study were more pronounced for the CEWAF exposures when strictly reported on a nominal dilution basis (e.g., 32% CEWAF more toxic than 32% WAF). Crucially, when the effects were reported on a constituent concentration basis (e.g., TPH), essentially normalizing the responses, there was little difference between exposure to WAF or CEWAF. The increased response in the nominal CEWAF preparations is a reflection of the increased concentrations of dissolved hydrocarbons and microdroplets that act as a reservoir in the CEWAF solution. The concentrations used for testing in this study are compared to those measured in the field during the Deepwater Horizon spill response in Figure 92.

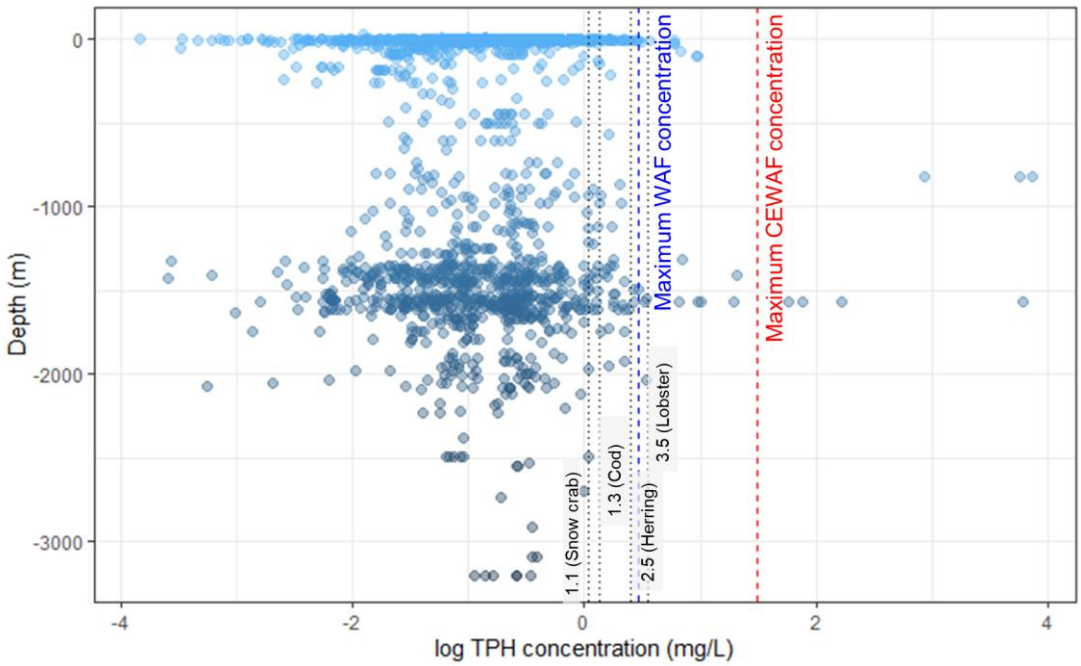


Figure 92: Overview of 13172 TPH measurements (Wade et al. 2016) taken during the Deepwater Horizon oil spill response collected from various depths. The dashed vertical lines are the maximum measured concentrations in WAF (blue) and CEWAF (red) exposure solutions while the dotted lines are the lowest effect concentrations observed for each species.

84% of the field observations (11,023) were below the lowest threshold value from Snow crab. Our upper concentrations were on the higher end of what may be considered realistic exposure concentrations under certain scenarios, but these concentrations must also be considered in the context of exposure duration and TPH composition.

Exposure durations used in this study ranged from 1 to 48 hours. These relatively short exposure durations are expected to be more realistic for a spill scenario, with the possible exception of exposure of non-motile organisms to a continuous blow-out. Dispersion efficacy tests suggest that appreciable concentrations of waterborne hydrocarbons persist for only 1 to 48 hours post-dispersion, and not several days (McIntosh et al. 2010). The results from the SL Ross wave tank to support this project demonstrated a 75% reduction in TPH concentrations within 10 minutes of dispersion (Chapter 3**,** Figure 9). This rapid dissipation has also been confirmed during field trials and real world spill operations where surface water samples (<10 meters) demonstrated that peak total hydrocarbon concentrations (THC) of 30–50 mg/L decreased to <1–10 mg/L within a few hours (typically $\leq$ 4 hours) (Bejarano et al. 2014; Lessard and DeMarco 2000).

Here, we were able to observe significant lethal effects with exposure durations of 1 (herring; Chapter 8, Figure 61), 6 (lobster; Chapter 10, Table 25), 7 (cod; Chapter 4, Figure 30) and 24 hours (snow crab; Chapter 11, Figure 86). Select results are summarized in Table 1.

12.2 Dispersants

Surfactants diffuse the oil/water interface as they align themselves so that the lipophilic (hydrophobic) end of the molecule is attached to the oil phase and the hydrophilic end extends into the water phase when dispersants are applied to an oil slick (Lessard and DeMarco 2000). This action allows the oil to mix into the top 5 - 10 m of the water column as small (<70 µm) microdroplets. This process makes the oil microdroplets highly accessible to hydrocarbon-degrading bacteria, thereby promoting removal from the environment by natural biodegradation (Lee et al. 2013; Prince et al. 2015). Recent reviews of dispersant, specifically Corexit 9500A, toxicity have found that the toxicity of the dispersant is negligible relative to the toxicity of the crude oil and that thresholds of

toxicity are in the low mg/L range (Echols et al. 2019; 5.9 mg/L). In our studies, we found that cod embryos exposed to Corexit 9500A for 24-hr during the vulnerable hatching window had significant sublethal effects (increase in blue-sac disease presentation) occurring at 405 mg/L, but there was no significant effect on hatching. In larval lobsters, the 24 and 48-hr LC50s were 38 and 36 mg/L, respectively. For herring, a full concentration range of the dispersant was not tested (due to the difficulty in obtaining and maintaining good quality herring embryos), however there were some notable responses in the Corexit control (16 mg/L when tested with 32% CEWAF) with certain crosses and developmental points having reduced hatching and increased post-hatch malformations suggesting a particular sensitivity of this species to dispersants. These nominal effect concentrations for the dispersant alone and exposure duration of 24-hrs are well above those expected to be observed in the environment following a spill response with dispersants. The highest dispersant-only concentrations in field applications are expected to range between 3 and 10 mg/L in the first minute to several hours following successful application based on operational dispersant application rates at the surface (NASEM 2020). Our results support the low inherent toxicity of the dispersant alone.

12.3 Variability

Many ecotoxicological studies focus on the mean response of the population of test organisms and measures of variability are calculated and reported just to provide confidence estimates with the assumption that values far removed from the mean are atypical and do not reflect the response of the population (Bennett 1987). However, it is insufficient to only find an explanation for the responses of the majority of organisms in a population to understand the mechanisms underlying ecological change and its biological significance, for example the mean +/- standard deviation or 68.3% of the population (Depledge 1990). In this case, it is also vital to know something of the responses of the remaining 31.7% of the population so that the resiliency and potential for recovery of the population can be understood. This has consequences for population modelling and re-bound in the face of an exposure/spill.

Our results show significant variability within the life history of a species, with specific developmental points (e.g., hatching) and early life stages being more vulnerable. In our

fish trials, we observed increased sensitivity with the earliest life stage (e.g., fertilization, up to 2 days post fertilization) then reduced sensitivity until the hatching window. In the lobster trials, we did not observe a significant difference in sensitivity between the three planktonic larval stages. We observed significant intra-species variation (e.g., the degree of variability within the tested population) between different individuals at the same life stage. The intra-species variability for cod was a factor of 5.2 and for lobster this was 4.0, each within a typically recommended intra-species assessment factor of 10 (Chapman et al. 1998). Despite that recommendation, in practice, intra-species assessment factors are often ignored or minimized. In a review of environmental risk assessments (ERAs), Duke and Taggart (2000) found that none of the 24 examined ERAs used an uncertainty factor greater than 1 to account for intra-species variation. The results of our study and others (e.g., Roubeau Dumont et al. 2019) strongly suggest that intra-species variability should be considered as an assessment factor in ecological risk assessments. These assessment factors could be species or contaminant specific based on the mode of action and whether it acts on a pathway that has high potential for adaptability/variation. The variation in response across life stages and between individuals of the same population mean that the impacts of an oil spill are not likely to be equally distributed across a population. We can account for some of the species specific variation (e.g., temporal and genetic) by collecting data from different life stages and from mixed populations to more accurately perform a spill impact mitigation evaluation.

12.4 Significance of Results

12.4.1 Modelling

"*All models are wrong; the practical question is how wrong do they have to be to not be useful but some are useful*" - George Box, 1976.

The usefulness of any model largely depends on the quality of the input data and the degree of uncertainty surrounding those values. Toxicity inputs based on laboratory data generated for multiple relevant species (e.g., local) and life stages is required for biological effects models to have meaningful output and effectively predict potential population level effects. The Spill Impact Model Application Package (SIMAP) developed

by Applied Science Associates (Narragansett, RI, USA) provides insight into the fate of oil and its components by using variable exposure duration toxicity data to predict population level effects following a spill (French-McCay 2002; French-McCay et al. 2018). The use of surrogate species data (e.g., warm water, standard test species) and longer exposure (typically 96-hours) are employed in the absence of appropriate species or exposure duration data thereby introducing an additional layer of uncertainty surrounding the prediction of effects. Other models, such as a fecundity-hindcast, equally rely on quality toxicity data, but also the general biological and life history data for the species of interest/concern. For instance, Gallaway et al. (2017) combined a fecundity-hindcast model incorporating acute toxicity data with the life history data for Arctic cod larvae to predict the effect of dispersing a large oil spill on the regional cod population in Alaska Beaufort Sea. The model predicted the effect of dispersing the oil to be insignificant and highlighted that effects at the individual level do not necessarily translate into population level impacts owing in part to the unequal distribution of effects across a population due to different developmental stages and resiliency.

The data generated in this report will serve as valuable inputs into these models and better help predict and mitigate effects from oil spills and response measures.

12.4.2 Biomimetic Extraction and Solid Phase Microextraction

The SPME-BE data (Chapter 3, Figure 17) was used to model the toxicity responses of the four test species on the basis of measured fiber concentrations and extrapolate the critical BE value (Critical BE value is closely related to the critical target lipid body burden value, with a systematic offset between the two values based on differences in partitioning between PDMS and the target lipid. The critical BE value is a species specific value for predicting narcosis mediated toxicity, which can be used to rank species in terms of sensitivity) values. The concentrations for the other trials/species were estimated by regression as the SPME-BE data was only collected for one trial. The derived critical BE values were compared to a compilation of critical BE values taken from (Redman et al. 2018) and used to create a cumulative distribution of the CTLBB effect values (Figure 93).

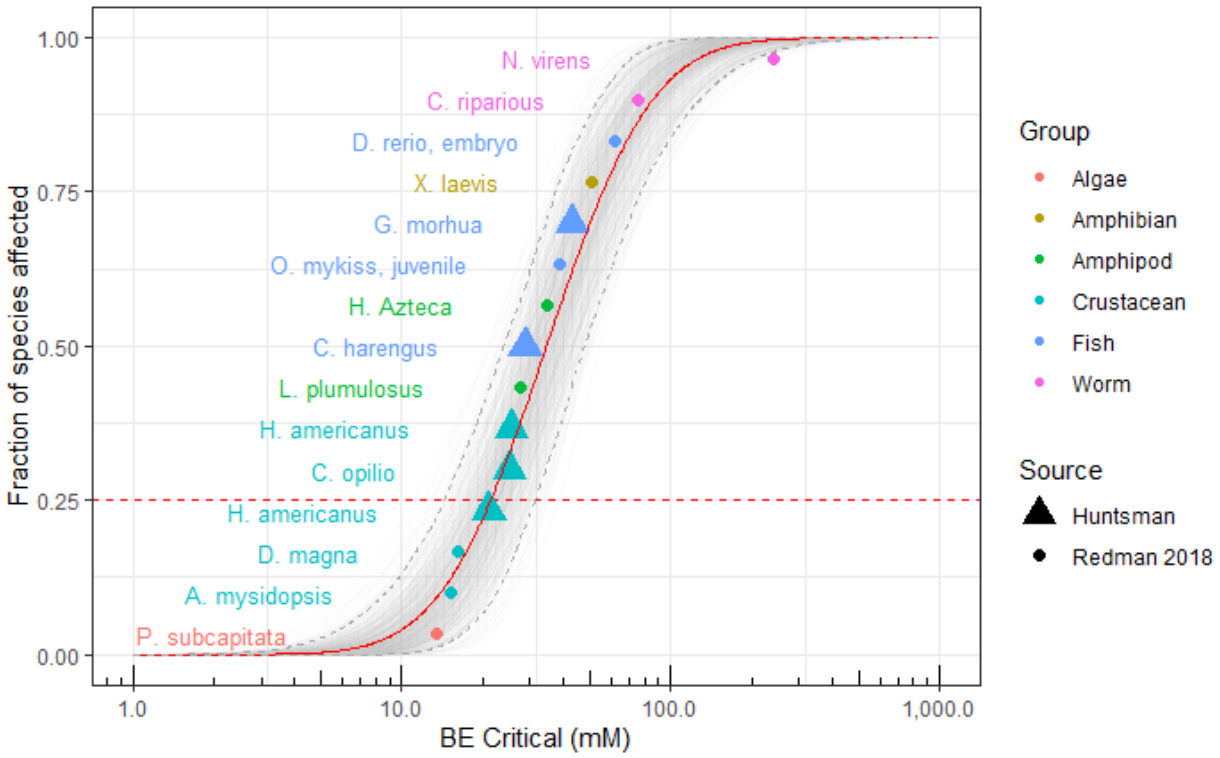


Figure 93: Species sensitivity distribution of critical BE data collected for different species (colours) from Redman et al 2018 (circles) and this research program (triangles). The dashed red line is at 25%.

The cold water commercially important species examined in this research program are within the range of values reported for other species. While it is important to have data for local or an appropriate surrogate species when conducting risk assessments or weighing response options as part of a spill impact mitigation assessment (SIMA), using modelled relationships with critical BE (or CTLBB) values from literature to derive protective thresholds (e.g., HC5s) would likely be protective for commercial Atlantic species.

12.4.3 SIMA (NEBA)

It is prudent to consider dispersant application as a possible spill response option considering the history of, and potential for future, oil spills and that response times with conventional methods are likely to be slow for some high-risk areas offshore Newfoundland and Labrador. The question as to under what spill scenarios could

dispersants be used to improve the response capacity in the offshore Newfoundland and Labrador environment was the subject of a previous ESRF report (Trudel 2004). Those authors determined a number of scenarios where dispersant application could be warranted, including:

- very small to very large spills at offshore platforms;
- all transportation-related spills of Grand Banks crude oil;
- all spills occurring in either highly sensitive areas (e.g., near Cape St. Marys seabird colonies) or in high spill risk areas (e.g., Cape Race); and
- all spills occurring where high sea states complicate mechanical recovery operations.

A spill impact mitigation assessment (SIMA) would need to be undertaken prior to the use of dispersants under those scenarios. The SIMA process is meant to support aspects of the oil spill response decision-making process, not replace them. SIMA (like its analogous predecessor NEBA [net environmental benefit assessment]) is a process by which relevant data (such as those generated in this report) are evaluated, outcomes are predicted, and an attempt at weighing and balancing the trade-offs of response measures is performed before selecting the best response options. Certain questions need to be asked and answered with respect to determining whether the usage of dispersant is warranted:

Will mechanical response be sufficient?

- Mechanical recovery is often the preferred option during an oil spill response (Prince et al. 2017) and in some jurisdictions, such as the current situation in Canada, it is the only available option. However, mechanical response has specific operational limitations (e.g., high wind speed and waves) that will limit its effectiveness.

Is the spilled oil known to be dispersible under the ambient conditions of the spill?

- The composition of the oil (e.g., viscosity, asphaltene content) and its weathering state proportional to the time elapsed since the spill will dictate whether a particular dispersant will even be effective.

Are sufficient chemical response assets available in the right time frame?

- Dispersants may be applied to surface oil from vessels or aircraft. Subsea injection may be employed in the event of a continuous release at depth, such as a blowout. Dispersant application requires specialized equipment and expertise that may require special approvals and meeting regulatory requirements (NASEM 2020). Having the required response assets prepared in advance of a spill will ensure that this option is available should the need arise.

Are the environmental conditions conducive to successful application and dispersion? (e.g., operational conditions, sea-state, mixing energies)

- Working with the operational conditions for the application of dispersant ensures more effective dispersion and maintains the safety of the responders. Dispersant application has a wider range of operational conditions than conventional mechanical recovery, which make it a favorable response option under certain conditions. Wind and wave data was collected for the South West Grand Banks region from the DFO Canadian Wave database from the period of 1988 to 2013 (http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/waves-vagues/index-eng.htm). A total of 104,045 data points were available after applying quality filters. These data points were compared against suggested dispersant application operational cut-offs for wind speed (18 m/s; 35 knots) and wave height (5 m) from the Oil Spill Response Limited Dispersant Application Field Guide (2015). Weather conditions on the Grand Banks are favorable to dispersant application for 86.7% of the time analyzed (Figure 94).

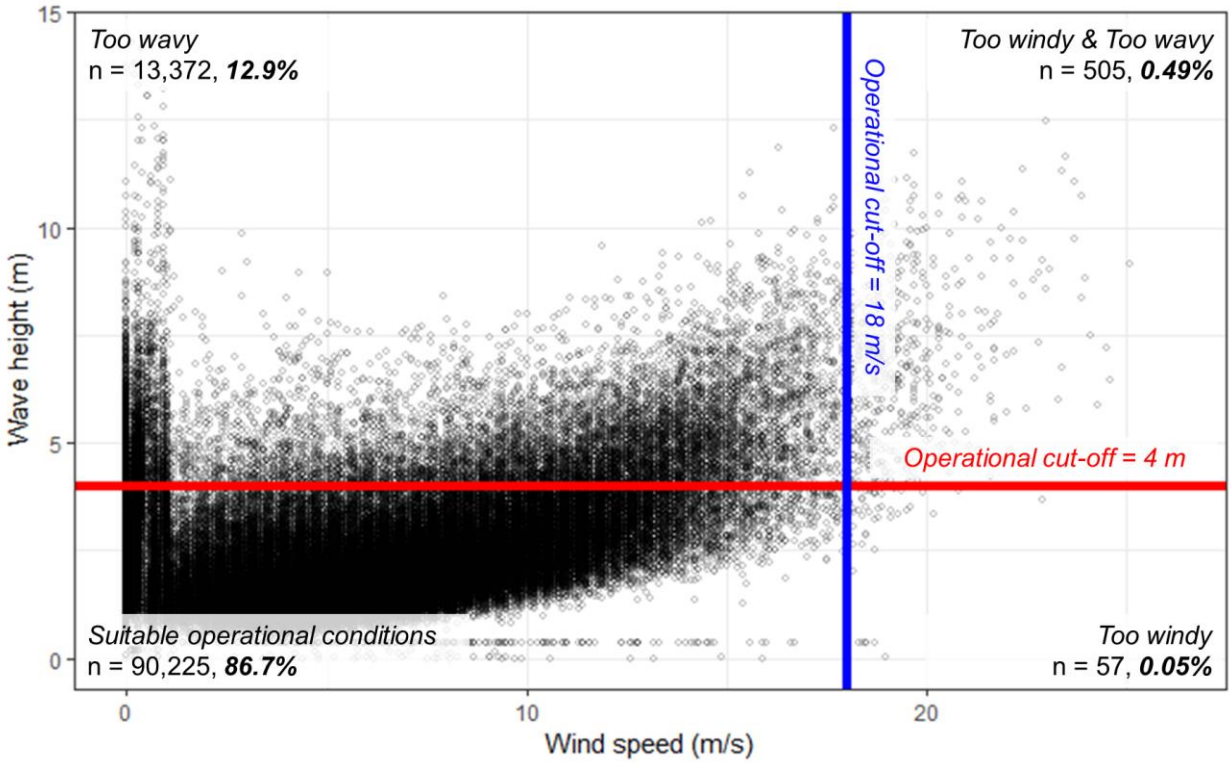


Figure 94: Operational conditions favourable to surface application of dispersants.

- Water quality parameters, such as salinity and temperature, will also have an impact on the efficacy of dispersants. The SL Ross wave tank results and our trials at different temperatures (e.g., 5, 10 and 15ºC) highlighted a reduced efficacy in dispersion of this crude oil at lower temperatures. There may be 6 months of the year where temperatures are below 5ºC when examining the surface water temperatures on the Grand Banks, which may limit the efficacy of Corexit 9500A on an offshore Newfoundland crude oil during these times (Figure 95).

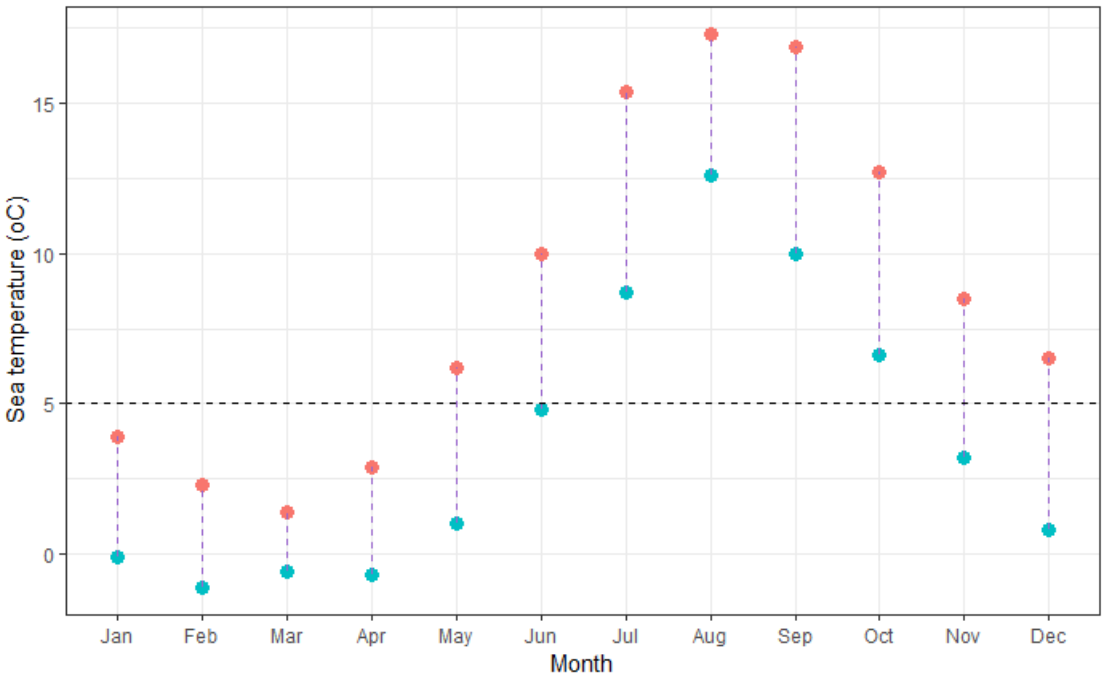


Figure 95: Maximum and minimum monthly sea temperatures on the Grand Banks. Source: https://www.seatemperature.org/north-america/canada/grand-bank.htm

Will application reduce water surface and shoreline impacts without significantly increasing aquatic life and impacts?

- This question represents the crux of most SIMA considerations. Decision makers and responders need to balance the trade-offs between the amount of damage to oil-sensitive coastal habitats and resources compared to the highly localized and short term increase in effects to the marine environment. Large presence of marine birds in the Grand Banks area also coincides when seawater temperatures are above 5^{o}C (June-November) and dispersant efficacy is expected to be greatest (Figure 96). Seabird diversity peaks in the spring and summer months because of a combination of northern hemisphere breeding birds and southern hemisphere migrants, while significant numbers of over-wintering alcids, gulls and Northern fulmars (*Fulmarus glacialis*) use these waters during fall and winter (Brown et al. 1986; Fifield et al. 2009).

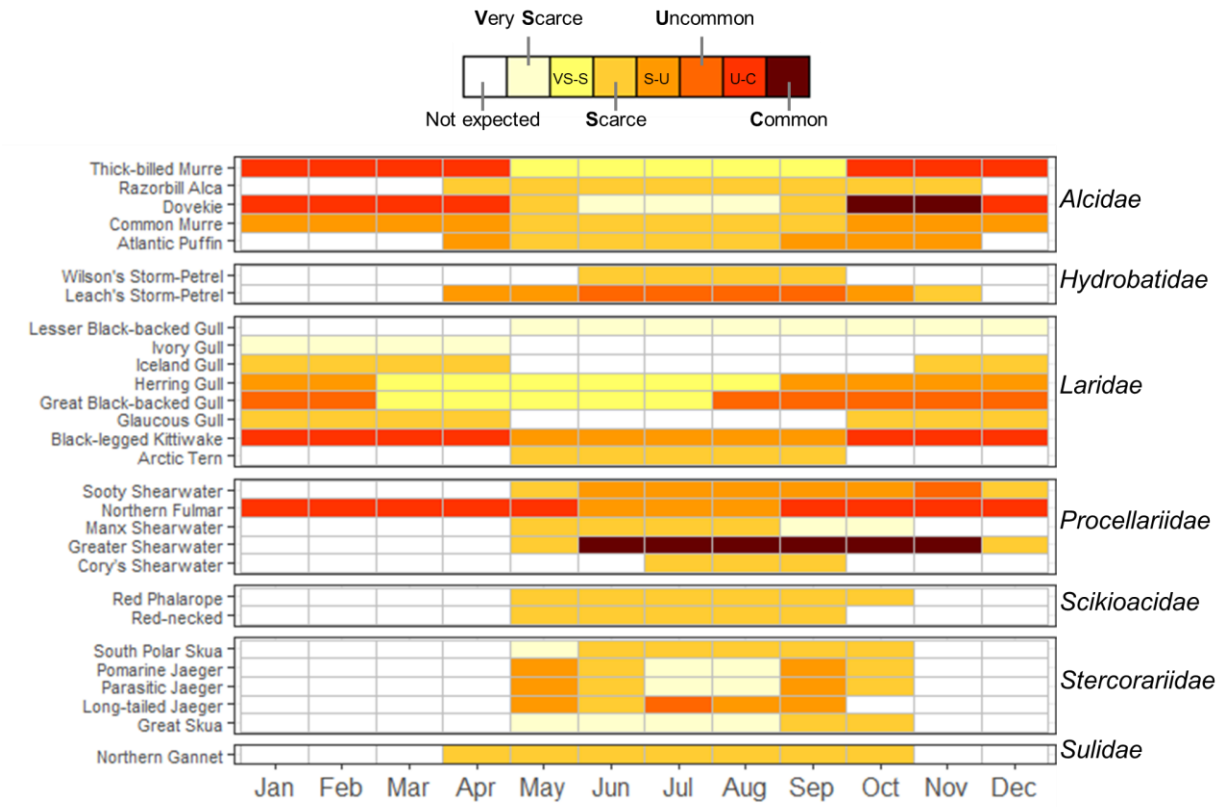


Figure 96: Monthly relative abundance of bird species (vertically grouped by family) that likely occur in the pelagic waters around the Grand Banks area. Common = present daily in moderate to high numbers; Uncommon = present daily in small numbers; Scarce = present regularly in very small numbers; Very Scarce = very few individuals or absent. Modified from C-NLOPB Southern Newfoundland Strategic Environmental Assessment (Chapter 3, Part 4, LGL Limited 2010).

- The decision to apply dispersants to break up a surface slick and minimize effects to seabirds and mammals is balanced against the potential impacts to pelagic organisms, including commercially important fish and crustacean species. Fish and crustacean species are particularly vulnerable during their early life stages when they are planktonic and have limited capacity to avoid or respond to spills. The estimated peak spawning times for several commercially important fish and crustacean species are shown in Figure 97, representing times where these species may be more vulnerable to oil spills.

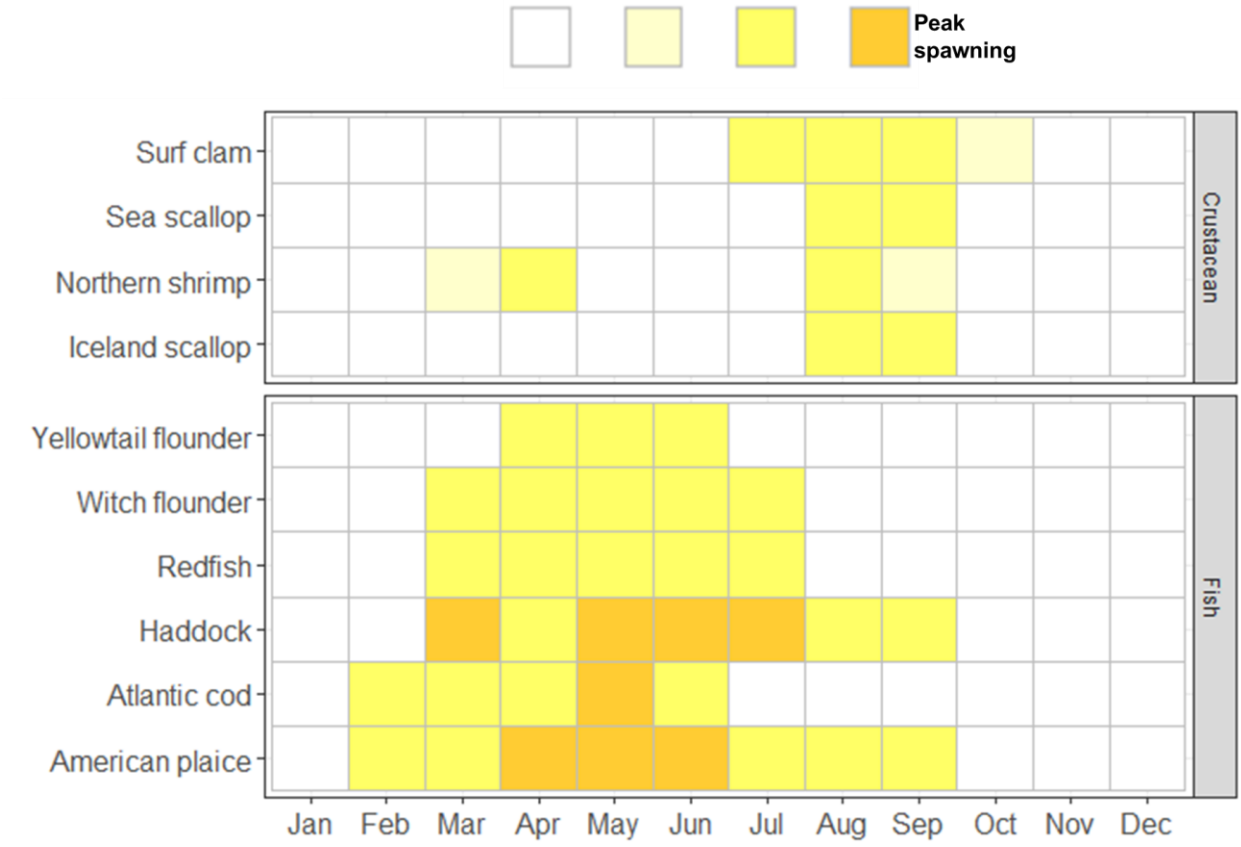


Figure 97: Approximate spawning times for commercially important fish and crustacean species on the Grand Banks. Colour gradation reflects spawning intensity with orange being the estimated peak spawning times (modified from Ollerhead et al. (2004)

- A previous ESRF report (Hurlbut et al. 1991) modelled the impact of different spill scenarios on commercial fisheries, using LC50 values of 14.3 ppb, 143 ppb, and 1.43 ppm total aromatic hydrocarbons. In the worst-case situation of a 90-day blowout at the maximum daily flow rate occurring in summer the totals in lost catch of cod and plaice were estimated to be 21 and 7 metric tonnes, respectively, representing 0.02% of the total annual Grand Banks catch for both species. If a worst-case tanker spill happened to coincide with the spring peak in larvae abundance then the total in lost catch would be 15.7 and 6.1 metric tonnes for cod and plaice, respectively, or 0.01% of the total annual catch (assuming the catch losses per larvae killed were 17.15 kg/106 cod larvae, and 0.95 kg/106 plaice larvae). These results suggest that under those scenarios the impact on the commercial fishery would be negligible.

- The decision to use dispersants as a spill response option is likely to differ throughout the year as environmental parameters and biological assemblages vary along with efficacy and potential impact of dispersant application.

12.5 Conclusion

The results of this research program will greatly improve the ability of researchers to predict effects and for responders to employ the best available and most appropriate response strategies based on the particular scenario. Toxicity data alone will not provide great insight into the environmental risk from an oil spill or the possible response measures. The complex interaction of the local environmental and biological factors, coupled with the dynamic fate of the spilled oil, all need to be considered to accurately evaluate the effects of a real, or hypothetical, spill. Oil spill response strategies that incorporate more detailed and local biological knowledge, including toxicity, are imperative and will result in better accounting of natural biological processes and variability to better mitigate effects.

Our activities directly addressed a data gap related to the toxicity of dispersed and non-dispersed crude oil on vulnerable early life stages of commercially harvested fish and invertebrate species. Access to this information will enhance the public confidence towards offshore oil exploration and extraction activities in the event that an oil spill occurs in the region and must be dealt with through the use of chemical mitigation measures.

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Appendices

Appendix 1: SL Ross Report

May 25, 2016

Chris Bridger
The Huntsman Marine Science Centre
1 Lower Campus Road
St. Andrews, NB
E5B 2L7

Dear Mr. Bridger

I am pleased to provide you with the results of the wave tank tests with White Rose crude oil. The test procedures and results are discussed below.

Background

It is understood that the Huntsman Marine Science Centre (Huntsman) intends to conduct toxicity testing of the water accommodated fraction (WAF) and chemically enhanced water accommodated fraction (CEWAF) of White Rose crude oil. Huntsman contracted SL Ross Environmental Research Ltd. (SL Ross) to conduct wave tank tests with White Rose crude oil to determine the characteristics of dispersed and dissolved oil under breaking wave conditions.

Test Oil and Dispersant

Three liters of weathered White Rose crude oil was supplied by Huntsman. It was reported that the crude oil had been artificially evaporated to 10% loss by mass, by stripping with nitrogen. Corexit 9500 dispersant, supplied by SL Ross, was used in the preliminary and tank tests with dispersant.

Preliminary Dispersant Effectiveness Assessment

Field effectiveness tests were conducted on the weathered White Rose crude oil to assess the performance of Corexit 9500 at the two test temperatures. The FET is a quick and simple qualitative method to evaluate the potential effectiveness of a dispersant on a given oil. The method compares the behaviour of a sample of oil dosed with dispersant against a blank sample of oil alone when they are agitated gently. The test procedure is as follows:

- Two 100-ml glass cylinders were filled with 80 mL of seawater, to which was added 1.5 mL of the test oil.
- Sixty micro-liters of dispersant was added to one of the cylinders (1:25 dispersant to oil ratio).
- No dispersant is added to the second cylinder (it was used as a reference to assess natural dispersion).
- After one minute of contact time between oil and dispersant, both cylinders were gently inverted and returned upright thirty times over one minute (i.e., 30 rpm).

After the agitation, the cylinders were observed and the resulting dispersion was characterized visually. The following general criteria for dispersant effectiveness are used:

S L Ross Environmental Research Limited

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Tel: (613) 232-1564 • Fax: (613) 232-6660 • Email: James@SLRoss.com

- Good Dispersion: formation of small oil droplets (brown dispersion) that will only very slowly rise to the surface at a standstill.
- Reduced Effectiveness: formation of dark/black large oil droplets that quickly rise to the surface.
- Poor Effectiveness: little or no difference from the untreated oil (reference oil). Fast rising of large oil droplets to the surface.

The tests were conducted at 4°C and 13°C. Corexit 9500 produced a good dispersion of the White Rose crude oil sample at 13°C. The dispersant showed reduced effectiveness at 4°C. Photos of the test apparatus and resulting dispersions are provided in Appendix A.

Tank Tests

Larger-scale tests were completed in the SL Ross indoor wind/wave tank. The test tank is 11 meters long by 1.2 meters wide by 1.2 meters deep and is fitted with a computer controlled wave-generating paddle at one end, and wave-dissipating beaches at both ends of the tank. The tank is equipped with sand and activated carbon filters. A photo of the test tank looking toward the wave-paddle end is shown below.

The tank was filled with municipal water to a depth of 85 cm prior to each test. Salt (either Windsor Feed Salt or Windsor Pool Salt) was added during filling to bring the salinity to 35 ppt. The final salinity was verified using a salt refractometer (Sper Scientific, model 300011). The sand filters were operated for 48 to 72 hours after filling to remove sediment and suspended solids.

Figure 1: SL Ross Wind/Wave Tank

A rectangular air curtain bubble barrier was used to contain the test oil slicks in the center of the tank. The barrier was constructed of ½-inch copper tubing, and measured 2 m long by 1 m wide. The barrier was submerged to a depth of approximately 55 cm below the water surface. The top of the piping was perforated with 2-mm diameter holes. Compressed air was supplied to the barrier at approximately 25

psi. The air bubbles that rose from the submerged piping system induced a water flow to the surface that turned inward, and oil placed inside the containment area was held inside the barrier. 500 mL of weathered White Rose crude oil was used for each test. The oil was introduced to the containment area by gently pouring the measured volume onto the water surface from a glass beaker.

The air flow from a small box fan was used to herd the surface oil slick towards the wave paddle end of the containment zone, which helped to counteract the imparted movement down the tank from the breaking waves. The box fan produced an air current of 2.2 m/s, measured approximately 2 cm above the water surface.

The computer-controlled wave paddle was programmed to generate a train of waves that combined to form a breaking wave in the middle of the containment barrier, a distance of 4.2 m from the wave paddle. The breaking waves pushed the oil toward the beach end of the containment zone but the surface oil remained contained. Breaking waves with a total height of 18 cm at a frequency of 2 waves per minute were used in the tests. Each test was run for a period of 30 minutes so the slicks were subjected to a total of 60 breaking waves.

For two of the tests, dispersant was sprayed onto the test slick through a Spraying Systems Company 80015 nozzle using compressed air. The apparatus was rinsed with water and then dispersant prior to each test to ensure that no contamination or dosage errors from residual hold-up occurred. The spraying apparatus was then loaded with a pre-measured volume of dispersant in order to achieve a dispersant-to-oil ratio of 1:20, by volume (i.e., 25 mL of dispersant). The discharge nozzle was moved continuously during spraying at a height of approximately 30 cm above the water surface to ensure uniform application of dispersant to the test slick.

The water quality in the tank was monitored using a LISST laser particle size analyzer (Sequoia Scientific Inc. Model 100x Type C) suspended 40 cm below the water surface. The LISST measures particles in the size range from 2.5 to 500 µm. The oil concentration and droplet size distribution of the dispersed oil below the containment area was sampled approximately every 1.5 s for the duration of the tests. The data was analyzed to characterize the droplet size distribution and concentration in the dispersed oil cloud for each test.

Water grab samples were collected during the tests from a tube submerged beneath the oil slick at a depth of 40 cm, at the same location as the LISST. Water samples were collected immediately prior to introducing the oil (i.e., background) and at 2, 8, 15 and 30 minutes following the first wave to impact the test slick. The samples were analyzed for petroleum hydrocarbons (F1- through F4-fractions), and Benzene, Toluene, Ethylbenzene and Xylenes (BTEX), by Maxxam Analytics Inc.

Oil remaining in the containment area after the 30 minutes of waves was collected using a plastic separatory funnel and pre-weighed sorbent pad. The dispersant effectiveness for each test was calculated by comparing the known mass of oil placed on the water surface before the waves, to the mass of oil remaining on the water surface in the containment area after completion. The effectiveness was calculated as follows:

$$Effectiveness = 100\left(1 - \frac{m_{final}}{m_{initial}}\right)$$

Test Matrix

Tests were conducted at two temperatures: nominally 4°C and 13°C. One test at each temperature was done with no dispersant (i.e., a control), while the second test was done with Corexit 9500 applied at a 1:20 dispersant to oil ratio.

Results

The results of the tank tests are provided in Appendix B. The dispersed oil concentrations, as measured by the LISST over the duration of the tests, are presented. Histograms showing dispersed oil droplet size distribution are presented over 15 s intervals at five times during the test: test start; 2 minutes; 8 minutes; 15 minutes; and, 30 minutes. As well, the following descriptive statistics were calculated over the same intervals: mean droplet size; 90th percentile droplet size; 50th percentile droplet size; and. % by volume of oil droplets less than 75 µm (droplets less than 75 µm are unlikely to resurface).

The dissolved-phase analysis of petroleum hydrocarbons and BTEX compounds conducted by Maxxam Analytics Inc. is presented in Appendix C.

Please contact me if you have any questions concerning this report.

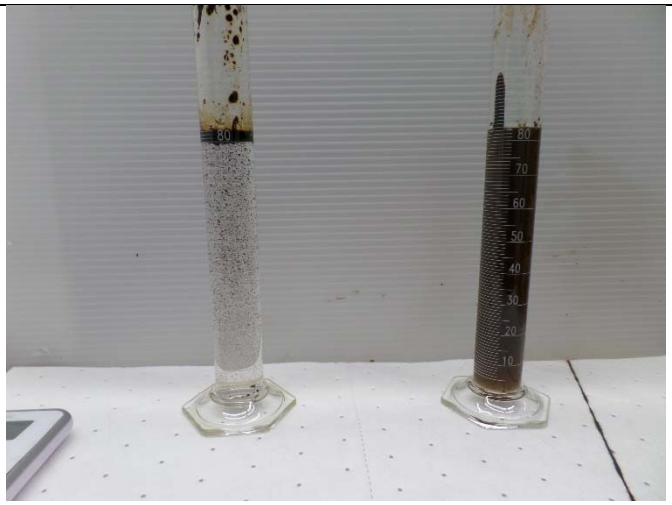
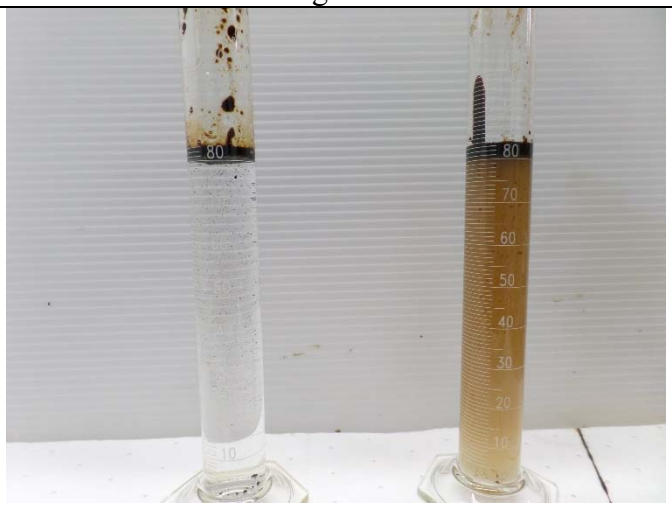
Regards,

James L. McCourt, P.Eng.

Appendix A

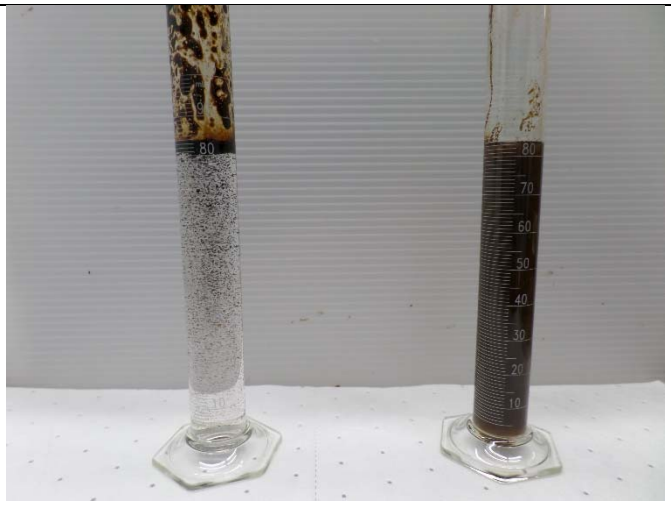
Field Effectiveness Test Photos

T=4°C

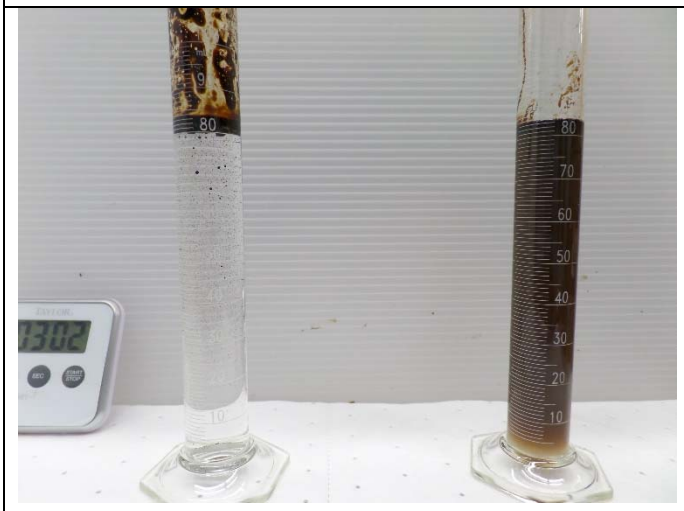
 Initial	 After 1 minute of mixing
 After 1 minute of settling	 After 5 minutes of settling

T=13°C

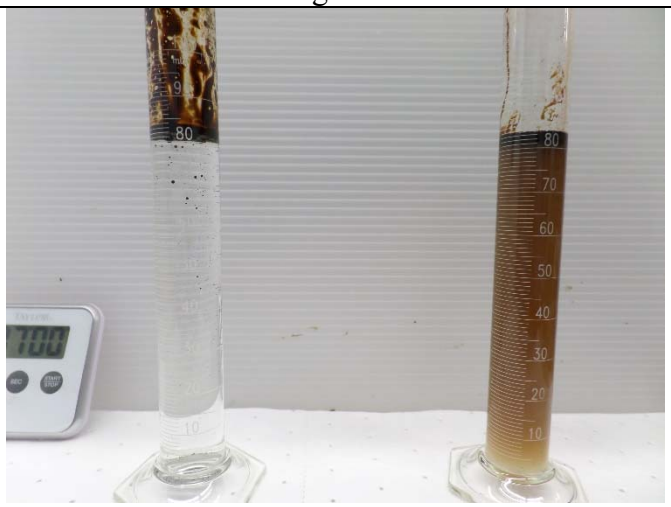
Initial



After 1 minute of mixing



After 1 minute of settling



After 5 minutes of settling

Appendix B

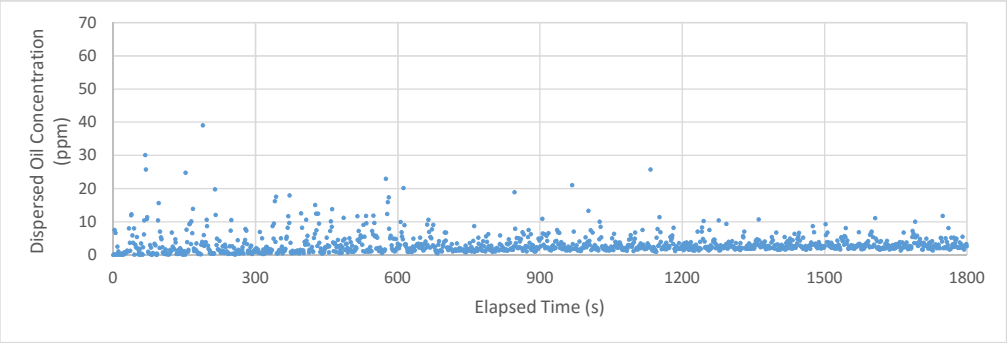
Tank Test Results

Test Number 1 **Date** 21/4/2016

Oil and Dispersant	
Oil Type	White Rose
Weathering	10%
Mass Oil Spilled	440.4 (g)
Mass Oil Recovered	434.33 (g)
Dispersion Efficiency	1%
Dispersant	Control
Dispersant:Oil Ratio	N/A

Tank Conditions	
Temperature	4.31 (°C)
Salinity	36 (ppt)
Wave Height	18 (cm)
Wave Period	30 (s)

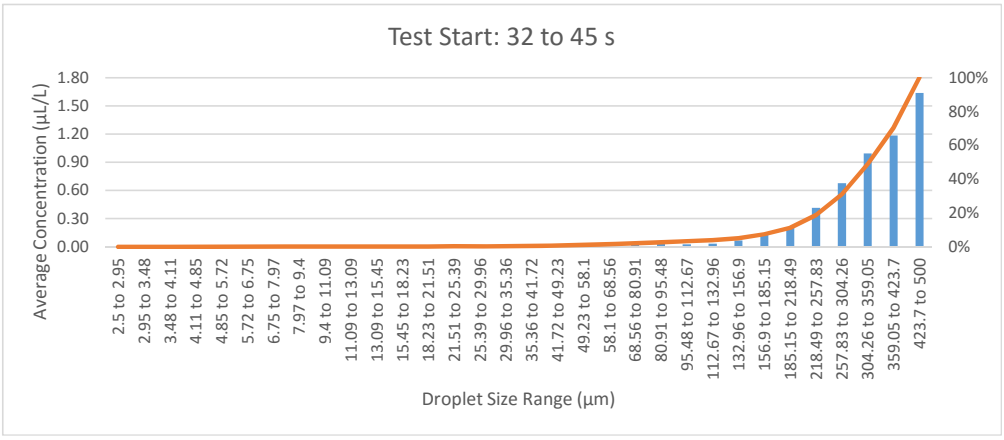
Dispersed Oil Concentration over Time

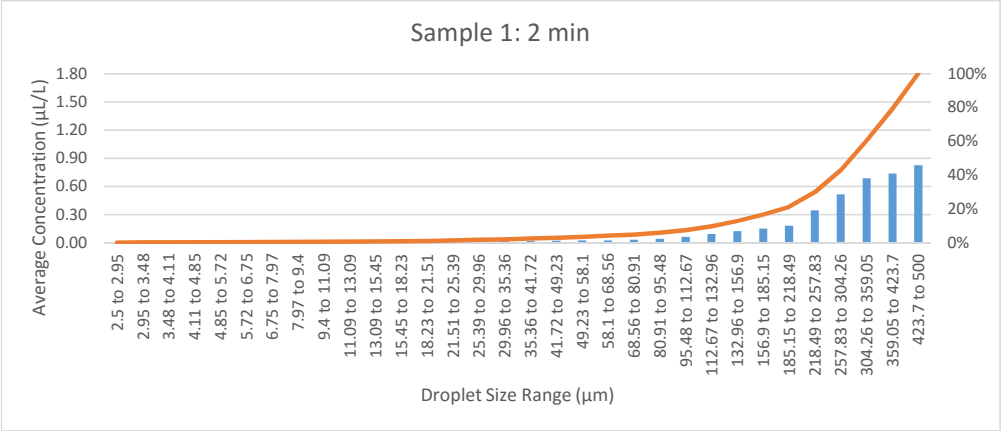


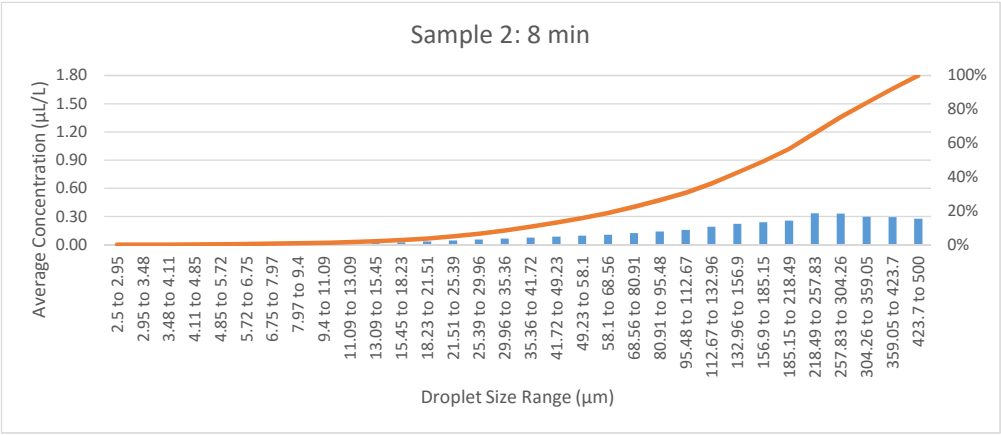
Water Samples

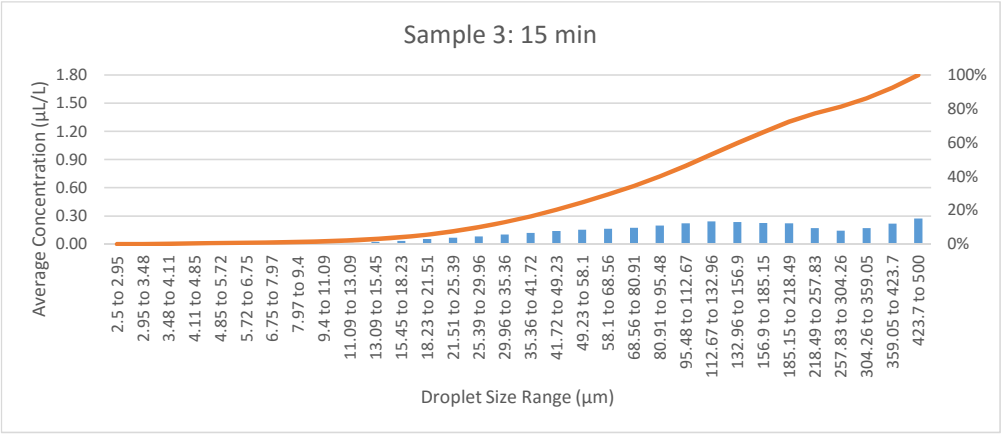
#	Elapsed Time (min)	F1 (mg/L)	F2 (mg/L)	F3 (mg/L)	F4 (mg/L)	B (mg/L)	T (mg/L)	E (mg/L)	X (mg/L)
0	Background	ND	0.062	0.07	0.18	ND	0.0053	ND	ND
1	2	ND	0.055	0.07	0.019	ND	0.056	ND	ND
2	8	ND	0.06	0.082	0.22	ND	0.0059	ND	ND
3	15	ND	0.061	0.092	0.24	ND	0.063	ND	0.002
4	30	ND	0.062	0.093	0.24	ND	0.064	ND	0.0031

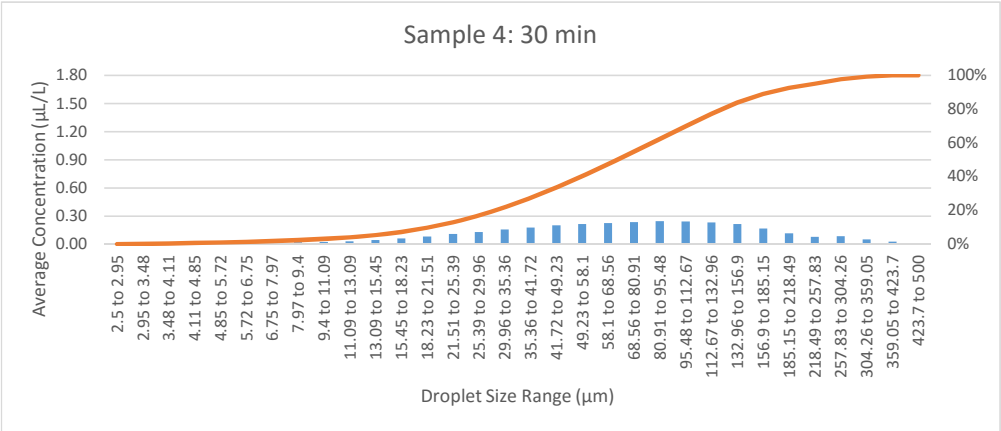
Oil Droplet Distribution




Sample 1: 2 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 2: 8 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 3: 15 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 4: 30 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)

Average Concentration by Bin

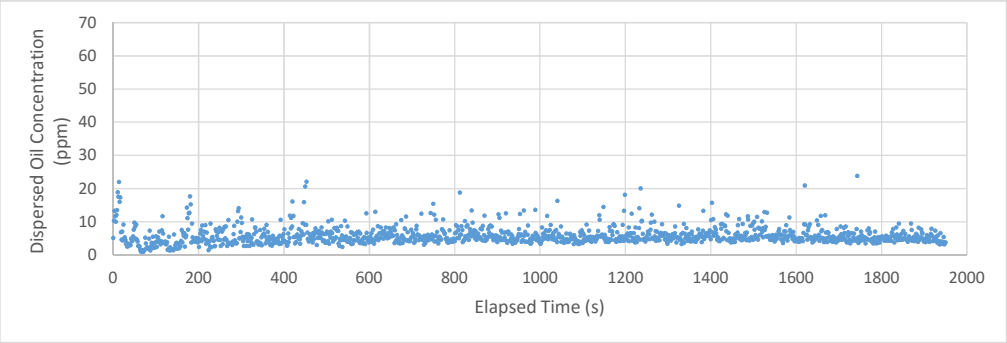
Bin	Droplet Diameter Range (µm)	Start Waves Average Concentration (µL/L)	Sample 1 Average Concentration (µL/L)	Sample 2 Average Concentration (µL/L)	Sample 3 Average Concentration (µL/L)	Sample 4 Average Concentration (µL/L)
1	2.5 to 2.95	0.0000981	0.0025342	0.0003380	0.0030778	0.0022542
2	2.95 to 3.48	0.0000865	0.0015080	0.0006422	0.0035221	0.0030324
3	3.48 to 4.11	0.0001629	0.0006077	0.0014817	0.0043900	0.0055006
4	4.11 to 4.85	0.0020810	0.0009563	0.0035647	0.0059714	0.0079075
5	4.85 to 5.72	0.0035936	0.0026835	0.0054267	0.0072161	0.0099961
6	5.72 to 6.75	0.0026893	0.0026902	0.0061330	0.0080719	0.0120321
7	6.75 to 7.97	0.0014677	0.0021071	0.0064947	0.0094683	0.0146020
8	7.97 to 9.4	0.0016630	0.0024368	0.0078741	0.0122225	0.0186508
9	9.4 to 11.09	0.0016854	0.0026464	0.0094578	0.0136115	0.0234280
10	11.09 to 13.09	0.0009543	0.0025058	0.0113867	0.0170917	0.0293121
11	13.09 to 15.45	0.0009231	0.0036365	0.0168806	0.0266488	0.0412035
12	15.45 to 18.23	0.0008509	0.0055545	0.0243590	0.0359590	0.0589110
13	18.23 to 21.51	0.0014718	0.0081314	0.0344503	0.0566051	0.0819002
14	21.51 to 25.39	0.0022621	0.0106797	0.0462871	0.0720048	0.1068289
15	25.39 to 29.96	0.0030465	0.0120844	0.0553883	0.0848558	0.1275570
16	29.96 to 35.36	0.0052441	0.0143866	0.0672827	0.1054029	0.1556828
17	35.36 to 41.72	0.0086697	0.0167095	0.0760623	0.1210855	0.1764715
18	41.72 to 49.23	0.0119986	0.0197426	0.0871519	0.1424350	0.1993937
19	49.23 to 58.1	0.0158902	0.0227117	0.0970802	0.1549656	0.2133381
20	58.1 to 68.56	0.0254197	0.0240268	0.1079721	0.1663841	0.2233670
21	68.56 to 80.91	0.0331250	0.0280628	0.1226305	0.1767976	0.2345252
22	80.91 to 95.48	0.0352644	0.0404139	0.1398397	0.2010140	0.2448158
23	95.48 to 112.67	0.0280517	0.0603860	0.1575571	0.2223405	0.2400632
24	112.67 to 132.96	0.0335955	0.0912620	0.1919471	0.2431927	0.2308706
25	132.96 to 156.9	0.0676847	0.1221222	0.2228749	0.2368982	0.2118023
26	156.9 to 185.15	0.1283575	0.1502693	0.2383897	0.2284310	0.1648257
27	185.15 to 218.49	0.2052931	0.1808663	0.2580899	0.2240723	0.1149564
28	218.49 to 257.83	0.4157760	0.3428002	0.3340902	0.1739875	0.0776266
29	257.83 to 304.26	0.6776603	0.5144544	0.3308666	0.1455926	0.0829588
30	304.26 to 359.05	0.9936985	0.6848439	0.2965733	0.1739841	0.0513918
31	359.05 to 423.7	1.1864390	0.7357951	0.2924664	0.2210465	0.0252278
32	423.7 to 500	1.6376659	0.8229334	0.2760638	0.2738149	0.0000000
	Mean droplet size (µm)	466.5	397.4	168.6	136.7	80.5
	90th percentile droplet size (µm)	500.0	423.7	390.0	390.0	185.2
	50th percentile droplet size (µm)	330.5	304.3	170.4	122.4	68.6
	Volume percent < 75 µm	2%	5%	22%	34%	55%
	Dispersed Oil Concentration (ppm)	5.53	3.93	3.53	3.57	3.19

Test Number 2 **Date** 25/4/2016

Oil and Dispersant	
Oil Type	White Rose
Weathering	10%
Mass Oil Spilled	430.3 (g)
Mass Oil Recovered	378.76 (g)
Dispersion Efficiency	12%
Dispersant	Corexit 9500
Dispersant:Oil Ratio	1 to 20 (by vol.)

Tank Conditions	
Temperature	4.59 (°C)
Salinity	36 (ppt)
Wave Height	18 (cm)
Wave Period	30 (s)

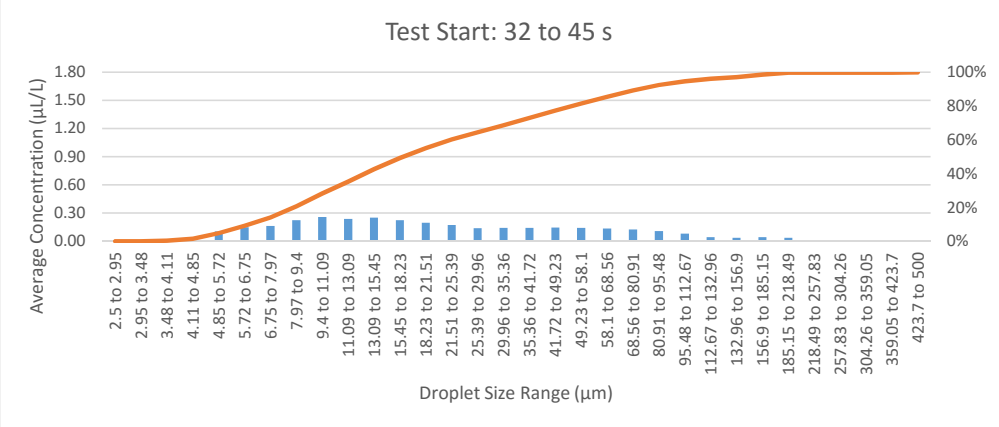
Dispersed Oil Concentration over Time

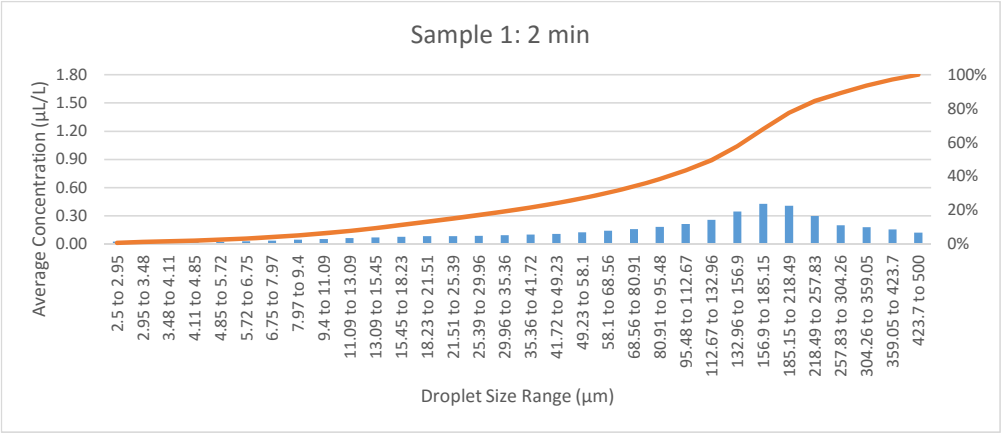


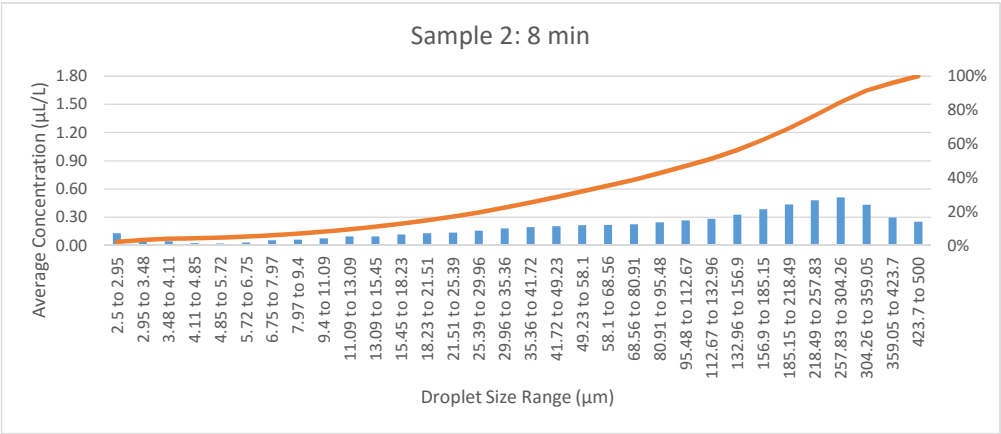
Water Samples

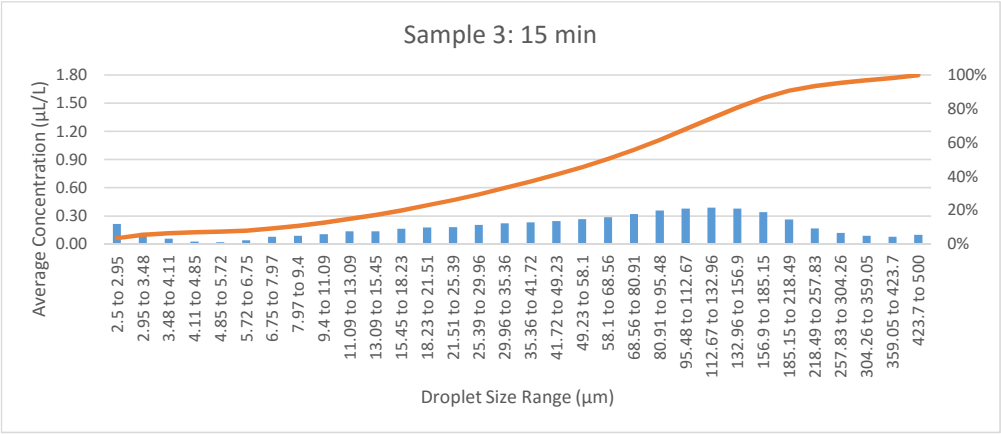
#	Elapsed Time (min)	F1 (mg/L)	F2 (mg/L)	F3 (mg/L)	F4 (mg/L)	B (mg/L)	T (mg/L)	E (mg/L)	X (mg/L)
0	Background	ND	ND	ND	ND	ND	0.0034	ND	ND
1	2	0.022	0.71	0.34	0.55	ND	0.0092	0.002	0.011
2	8	0.03	0.68	0.42	0.67	ND	0.0099	0.0022	0.013
3	15	0.034	0.85	0.61	0.99	ND	0.012	0.0028	0.016
4	30	0.038	0.77	0.56	0.86	ND	0.013	0.0031	0.018

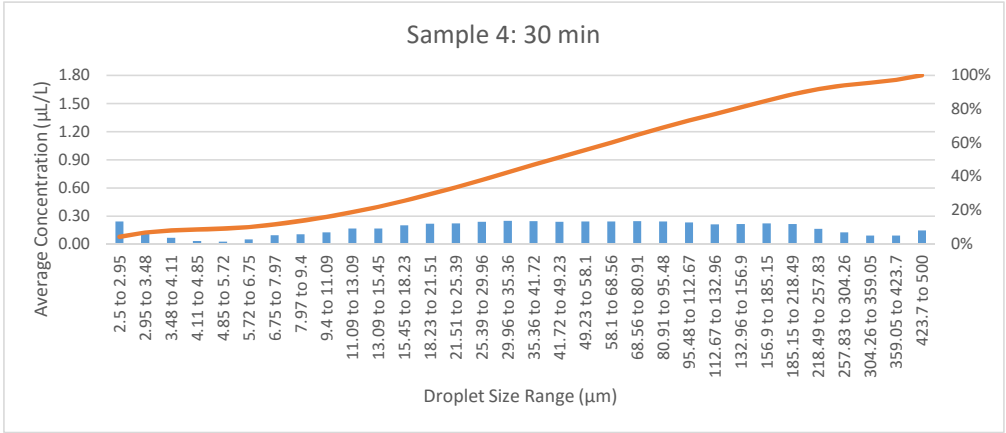
Oil Droplet Distribution




Sample 1: 2 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 2: 8 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 3: 15 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 4: 30 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)

Average Concentration by Bin

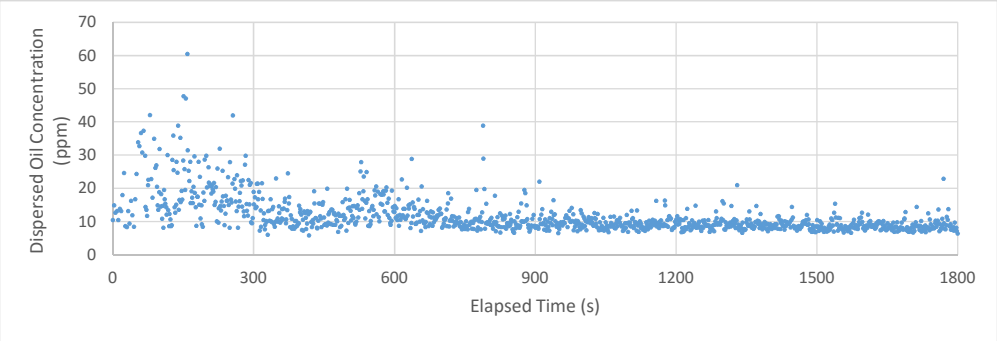
Bin	Droplet Diameter Range (µm)	Test Start Average Concentration (µL/L)	Sample 1 Average Concentration (µL/L)	Sample 2 Average Concentration (µL/L)	Sample 3 Average Concentration (µL/L)	Sample 4 Average Concentration (µL/L)
1	2.5 to 2.95	0.0000000	0.0248193	0.1301590	0.2154222	0.2404838
2	2.95 to 3.48	0.0011160	0.0218719	0.0792209	0.1212227	0.1343866
3	3.48 to 4.11	0.0104079	0.0195088	0.0429078	0.0598654	0.0658708
4	4.11 to 4.85	0.0422699	0.0181156	0.0228642	0.0295773	0.0326318
5	4.85 to 5.72	0.1077959	0.0199771	0.0189538	0.0242682	0.0271769
6	5.72 to 6.75	0.1518753	0.0264848	0.0317294	0.0436480	0.0496844
7	6.75 to 7.97	0.1653117	0.0355441	0.0544459	0.0801984	0.0928908
8	7.97 to 9.4	0.2258209	0.0433114	0.0613848	0.0899496	0.1052408
9	9.4 to 11.09	0.2598391	0.0513978	0.0730366	0.1071796	0.1261435
10	11.09 to 13.09	0.2388554	0.0605510	0.0941753	0.1382380	0.1644509
11	13.09 to 15.45	0.2522114	0.0670082	0.0962321	0.1375225	0.1656486
12	15.45 to 18.23	0.2251718	0.0762654	0.1170889	0.1655851	0.2001652
13	18.23 to 21.51	0.1994081	0.0814005	0.1303872	0.1802506	0.2173593
14	21.51 to 25.39	0.1738840	0.0818598	0.1365257	0.1835895	0.2192599
15	25.39 to 29.96	0.1410283	0.0838100	0.1577693	0.2064770	0.2381972
16	29.96 to 35.36	0.1436753	0.0910576	0.1797802	0.2243510	0.2490769
17	35.36 to 41.72	0.1455319	0.0990775	0.1942268	0.2353020	0.2445588
18	41.72 to 49.23	0.1466538	0.1063973	0.2026265	0.2477028	0.2387023
19	49.23 to 58.1	0.1431141	0.1222536	0.2133714	0.2682910	0.2411354
20	58.1 to 68.56	0.1375741	0.1383023	0.2160469	0.2894715	0.2397212
21	68.56 to 80.91	0.1263122	0.1580499	0.2258031	0.3234338	0.2450350
22	80.91 to 95.48	0.1086252	0.1801356	0.2440351	0.3592106	0.2409976
23	95.48 to 112.67	0.0813739	0.2111302	0.2639307	0.3819091	0.2291773
24	112.67 to 132.96	0.0465785	0.2543309	0.2832830	0.3910523	0.2085250
25	132.96 to 156.9	0.0382950	0.3428647	0.3251165	0.3807673	0.2139313
26	156.9 to 185.15	0.0457155	0.4267731	0.3835428	0.3418151	0.2192214
27	185.15 to 218.49	0.0398945	0.4042563	0.4368780	0.2651608	0.2131588
28	218.49 to 257.83	0.0033937	0.2971149	0.4813355	0.1699263	0.1627748
29	257.83 to 304.26	0.0000000	0.1974527	0.5106514	0.1199788	0.1233792
30	304.26 to 359.05	0.0000000	0.1759073	0.4306032	0.0913432	0.0889713
31	359.05 to 423.7	0.0000000	0.1531868	0.2944631	0.0796980	0.0912092
32	423.7 to 500	0.0047848	0.1177916	0.2502864	0.1016362	0.1459318
	Mean droplet size (µm)	28.3	114.9	93.7	76.4	57.8
	90th percentile droplet size (µm)	74.5	280.1	330.5	201.1	201.1
	50th percentile droplet size (µm)	16.8	122.4	122.4	63.1	45.3
	Volume percent < 75 µm	89%	34%	39%	56%	65%
	Dispersed Oil Concentration (ppm)	3.41	4.19	6.38	6.05	5.48

Test Number 3 **Date** 4/5/2016

Oil and Dispersant	
Oil Type	White Rose
Weathering	10%
Mass Oil Spilled	426.8 (g)
Mass Oil Recovered	244.26 (g)
Dispersion Efficiency	43%
Dispersant	Corexit 9500
Dispersant:Oil Ratio	1 to 20 (by vol.)

Tank Conditions	
Temperature	12.63 (°C)
Salinity	35 (ppt)
Wave Height	18 (cm)
Wave Period	30 (s)

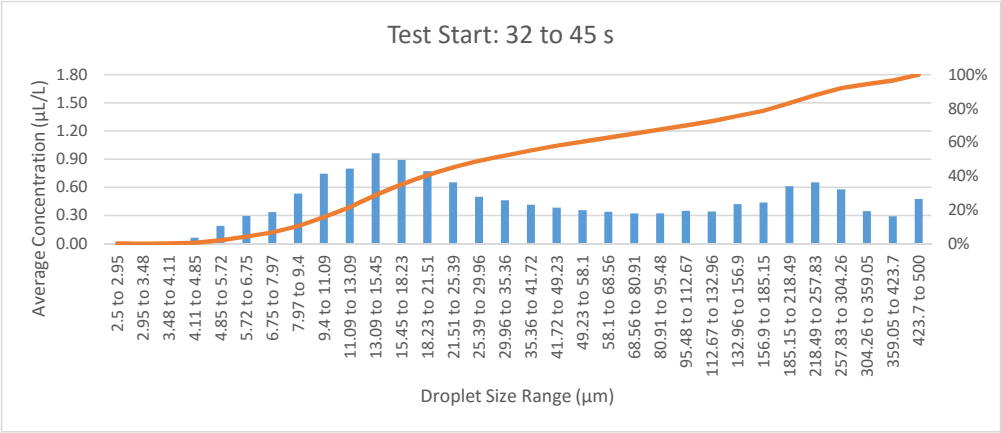
Dispersed Oil Concentration over Time

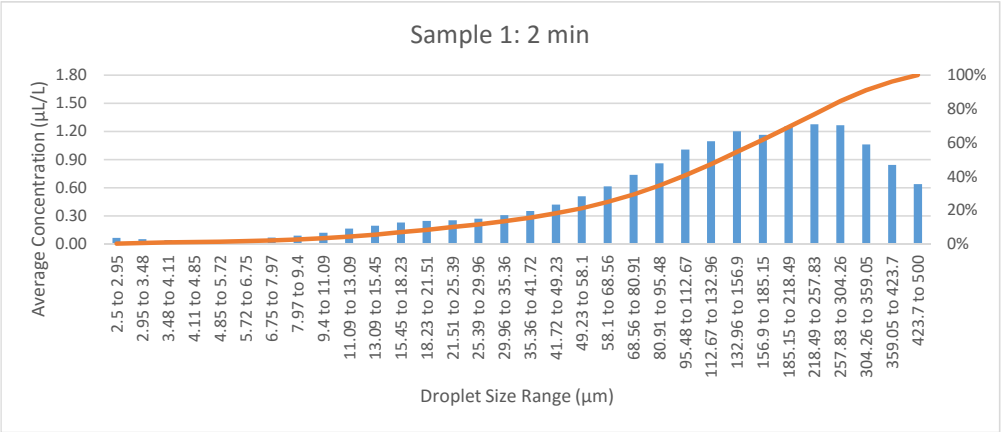


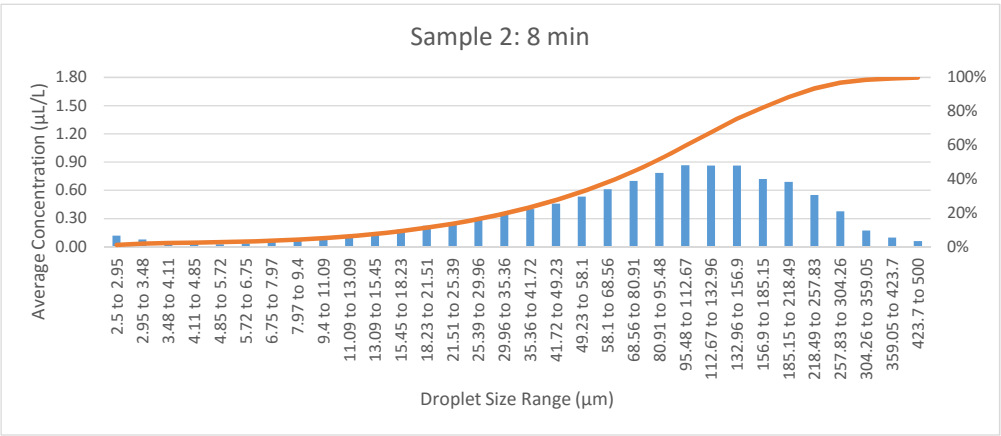
Water Samples

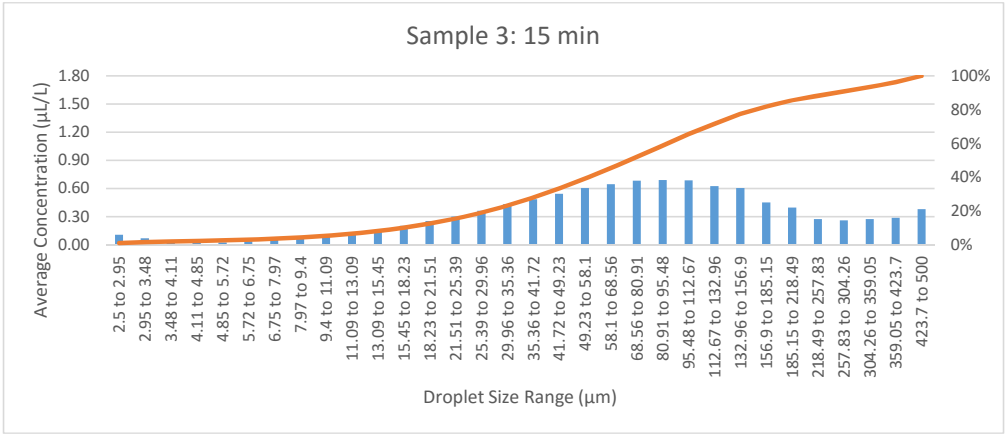
#	Elapsed Time (min)	F1 (µg/L)	F2 (µg/L)	F3 (µg/L)	F4 (µg/L)	B (µg/L)	T (µg/L)	E (µg/L)	X (µg/L)
0	Background	ND	ND	ND	ND	ND	0.0035	ND	ND
1	2	0.23	4.3	3.2	4.5	0.0025	0.0650	0.0210	0.0120
2	8	0.076	1.3	1.0	1.4	0.0015	0.0310	0.0071	0.0410
3	15	0.07	1.2	0.97	1.4	0.0012	0.0290	0.0068	0.0400
4	30	0.066	1.3	1.1	1.6	0.0011	0.0260	0.0067	0.0380

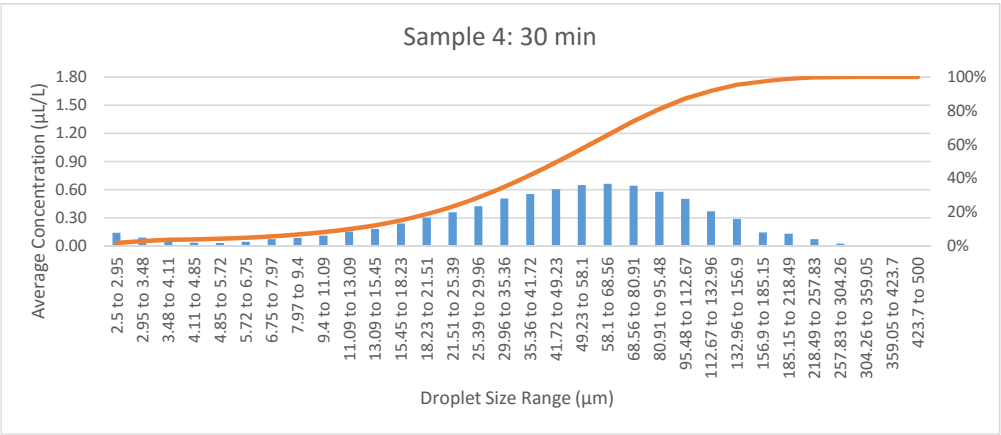
Oil Droplet Distribution




Sample 1: 2 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 2: 8 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 3: 15 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 4: 30 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)

Average Concentration by Bin

Bin	Droplet Diameter Range (µm)	Start Waves Average Concentration (µL/L)	Sample 1 Average Concentration (µL/L)	Sample 2 Average Concentration (µL/L)	Sample 3 Average Concentration (µL/L)	Sample 4 Average Concentration (µL/L)
1	2.5 to 2.95	0.0006265	0.0679595	0.1202249	0.1093699	0.1373985
2	2.95 to 3.48	0.0036761	0.0542662	0.0767578	0.0722247	0.0857545
3	3.48 to 4.11	0.0163684	0.0437834	0.0465624	0.0459036	0.0505472
4	4.11 to 4.85	0.0641247	0.0369740	0.0297773	0.0309046	0.0315682
5	4.85 to 5.72	0.1906732	0.0367178	0.0257185	0.0277991	0.0275457
6	5.72 to 6.75	0.2975284	0.0487053	0.0371269	0.0403742	0.0415581
7	6.75 to 7.97	0.3374804	0.0707264	0.0594626	0.0641337	0.0693824
8	7.97 to 9.4	0.5340428	0.0915192	0.0709824	0.0770341	0.0842418
9	9.4 to 11.09	0.7469254	0.1221997	0.0903386	0.0975034	0.1086375
10	11.09 to 13.09	0.8021261	0.1640995	0.1228998	0.1317715	0.1511694
11	13.09 to 15.45	0.9655097	0.1955349	0.1422510	0.1561784	0.1794940
12	15.45 to 18.23	0.8926046	0.2289859	0.1833233	0.2054858	0.2378844
13	18.23 to 21.51	0.7741785	0.2470808	0.2233837	0.2560183	0.2990814
14	21.51 to 25.39	0.6544755	0.2555344	0.2586726	0.3042639	0.3559329
15	25.39 to 29.96	0.4990169	0.2700927	0.3032105	0.3639443	0.4228128
16	29.96 to 35.36	0.4631956	0.3072047	0.3584564	0.4371033	0.5015660
17	35.36 to 41.72	0.4160228	0.3542994	0.4017148	0.4862591	0.5509873
18	41.72 to 49.23	0.3832128	0.4202471	0.4600829	0.5466157	0.6033436
19	49.23 to 58.1	0.3557318	0.5108560	0.5336360	0.6065340	0.6453736
20	58.1 to 68.56	0.3396438	0.6138534	0.6106350	0.6487582	0.6599454
21	68.56 to 80.91	0.3216544	0.7359984	0.6989955	0.6839927	0.6395492
22	80.91 to 95.48	0.3222108	0.8606838	0.7854727	0.6922634	0.5751521
23	95.48 to 112.67	0.3488338	1.0076237	0.8666014	0.6896859	0.5004139
24	112.67 to 132.96	0.3426067	1.0942565	0.8645113	0.6274842	0.3652836
25	132.96 to 156.9	0.4207587	1.2005785	0.8627165	0.6080043	0.2856690
26	156.9 to 185.15	0.4379955	1.1638640	0.7192822	0.4544532	0.1404324
27	185.15 to 218.49	0.6145351	1.2542445	0.6909410	0.4004531	0.1284748
28	218.49 to 257.83	0.6547680	1.2735733	0.5489378	0.2749495	0.0704155
29	257.83 to 304.26	0.5781327	1.2651835	0.3779458	0.2621765	0.0220033
30	304.26 to 359.05	0.3480845	1.0592323	0.1717303	0.2751126	0.0000000
31	359.05 to 423.7	0.2918852	0.8434423	0.0976731	0.2918539	0.0000000
32	423.7 to 500	0.4768862	0.6403077	0.0607690	0.3818117	0.0000000

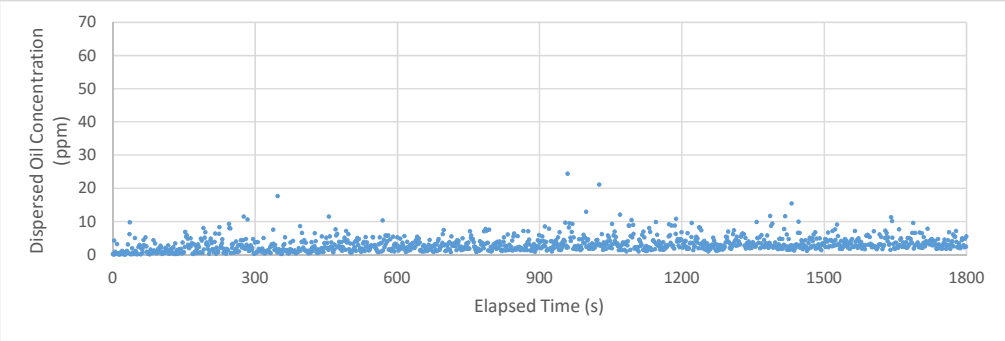
Mean droplet size(µm)	57.7	145.1	78.8	94.7	60.2
90th percentile droplet size (µm)	257.8	359.1	218.5	257.8	112.7
50th percentile droplet size (µm)	30.0	133.0	80.9	68.6	49.2
Volume percent < 75 µm	65%	29%	45%	52%	74%

Test Number 4 **Date** 21/5/2016

Oil and Dispersant	
Oil Type	White Rose
Weathering	10%
Mass Oil Spilled	440.2 (g)
Mass Oil Recovered	294.72 (g)
Dispersion Efficiency	33%
Dispersant	Control
Dispersant:Oil Ratio	N/A

Tank Conditions	
Temperature	12.74 (°C)
Salinity	34 (ppt)
Wave Height	18 (cm)
Wave Period	30 (s)

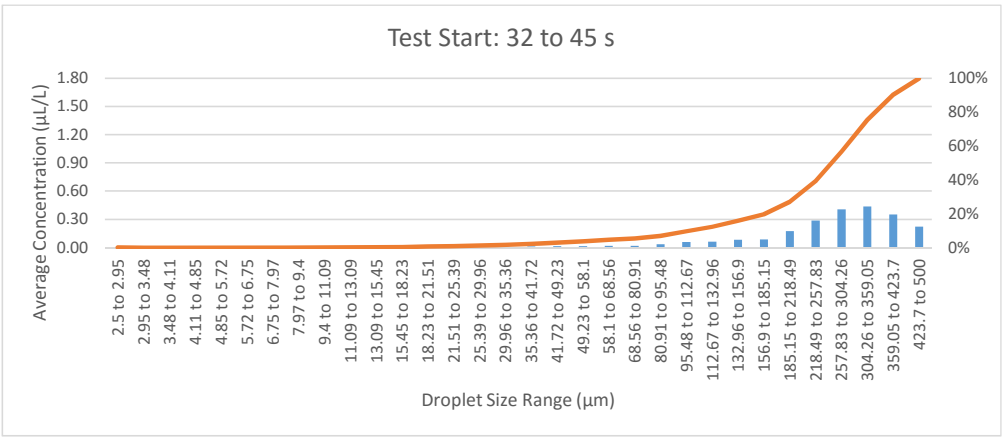
Dispersed Oil Concentration over Time

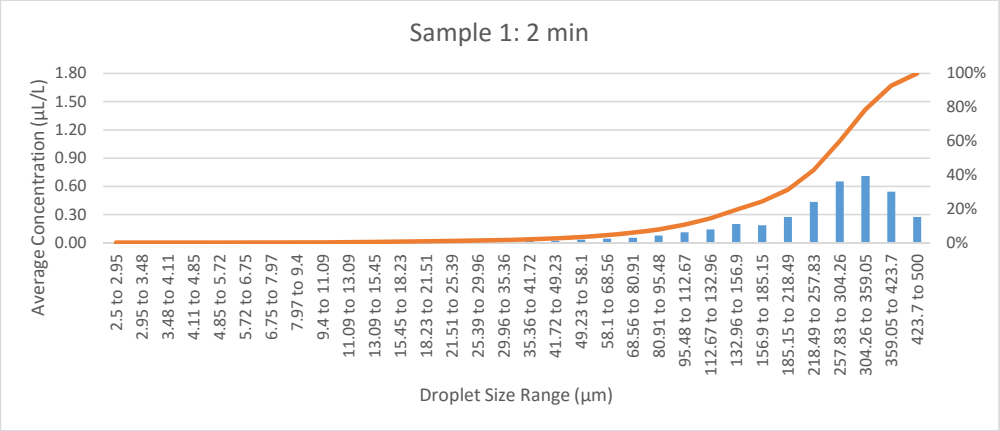


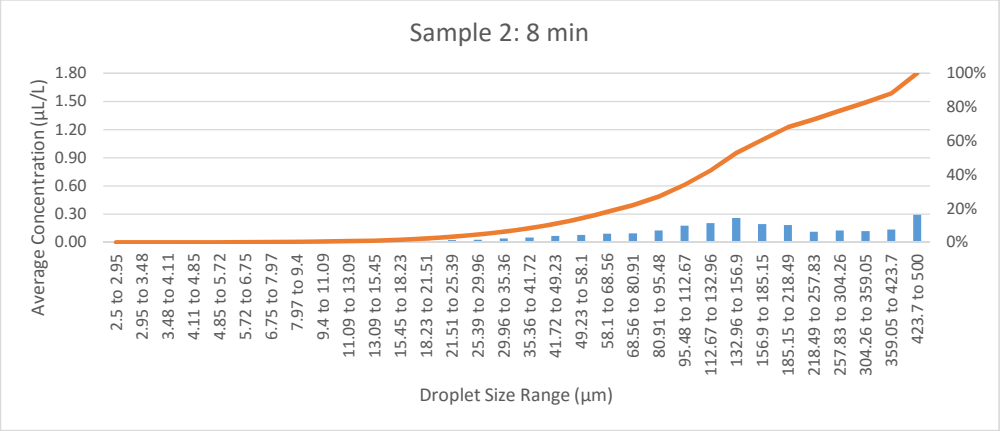
Water Samples

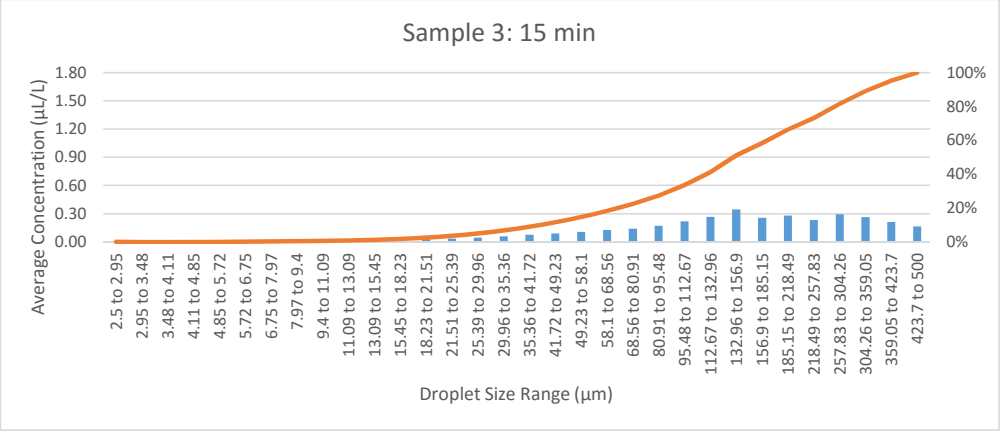
#	Elapsed Time (min)	F1 (µg/L)	F2 (µg/L)	F3 (µg/L)	F4 (µg/L)	B (µg/L)	T (µg/L)	E (µg/L)	X (µg/L)
0	Background	ND	ND	ND	ND	ND	0.0033	ND	ND
1	2	ND	ND	ND	ND	ND	0.0055	ND	ND
2	8	ND	0.04	0.083	0.13	ND	0.0080	0.0014	0.0026
3	15	ND	0.11	0.120	0.19	ND	0.0096	0.0018	0.0065
4	30	ND	0.14	0.160	0.23	ND	0.0095	0.0021	0.0081

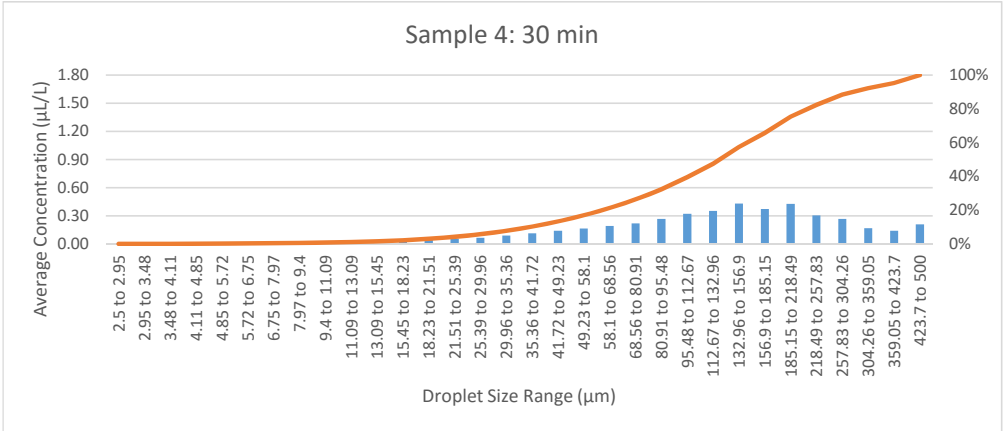
Oil Droplet Distribution




Sample 1: 2 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 2: 8 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 3: 15 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)


Sample 4: 30 min
Average Concentration (µL/L)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
100%
80%
60%
40%
20%
0%
2.5 to 2.95
2.95 to 3.48
3.48 to 4.11
4.11 to 4.85
4.85 to 5.72
5.72 to 6.75
6.75 to 7.97
7.97 to 9.4
9.4 to 11.09
11.09 to 13.09
13.09 to 15.45
15.45 to 18.23
18.23 to 21.51
21.51 to 25.39
25.39 to 29.96
29.96 to 35.36
35.36 to 41.72
41.72 to 49.23
49.23 to 58.1
58.1 to 68.56
68.56 to 80.91
80.91 to 95.48
95.48 to 112.67
112.67 to 132.96
132.96 to 156.9
156.9 to 185.15
185.15 to 218.49
218.49 to 257.83
257.83 to 304.26
304.26 to 359.05
359.05 to 423.7
423.7 to 500
Droplet Size Range (µm)

Average Concentrations by Bin

Bin	Droplet Diameter Range (µm)	Start Waves Average Concentration (µL/L)	Sample 1 Average Concentration (µL/L)	Sample 2 Average Concentration (µL/L)	Sample 3 Average Concentration (µL/L)	Sample 4 Average Concentration (µL/L)
1	2.5 to 2.95	0.0001184	0.0000000	0.0000000	0.0000000	0.0000000
2	2.95 to 3.48	0.0001416	0.0000134	0.0000115	0.0000061	0.0000097
3	3.48 to 4.11	0.0002262	0.0001296	0.0000903	0.0001352	0.0002374
4	4.11 to 4.85	0.0004200	0.0002642	0.0006608	0.0006134	0.0009949
5	4.85 to 5.72	0.0001889	0.0004386	0.0015015	0.0020677	0.0025145
6	5.72 to 6.75	0.0000000	0.0005100	0.0016975	0.0030158	0.0036528
7	6.75 to 7.97	0.0000000	0.0005005	0.0020510	0.0035504	0.0044824
8	7.97 to 9.4	0.0002277	0.0015518	0.0031103	0.0054225	0.0069219
9	9.4 to 11.09	0.0011563	0.0025061	0.0045070	0.0076284	0.0101074
10	11.09 to 13.09	0.0013659	0.0030956	0.0058552	0.0094592	0.0132432
11	13.09 to 15.45	0.0018925	0.0039458	0.0082821	0.0129080	0.0187878
12	15.45 to 18.23	0.0039830	0.0061768	0.0133700	0.0194999	0.0270090
13	18.23 to 21.51	0.0058454	0.0077004	0.0195268	0.0284400	0.0392156
14	21.51 to 25.39	0.0072851	0.0089344	0.0248953	0.0376045	0.0534549
15	25.39 to 29.96	0.0077982	0.0092383	0.0296715	0.0453918	0.0639505
16	29.96 to 35.36	0.0103357	0.0127754	0.0408954	0.0615523	0.0880822
17	35.36 to 41.72	0.0131219	0.0170329	0.0530213	0.0766345	0.1106708
18	41.72 to 49.23	0.0156371	0.0218739	0.0680059	0.0923400	0.1385515
19	49.23 to 58.1	0.0177879	0.0299011	0.0792716	0.1071526	0.1607814
20	58.1 to 68.56	0.0203461	0.0421053	0.0928250	0.1298310	0.1896147
21	68.56 to 80.91	0.0210474	0.0500431	0.0970610	0.1416432	0.2156588
22	80.91 to 95.48	0.0359883	0.0745011	0.1280912	0.1720012	0.2643255
23	95.48 to 112.67	0.0596262	0.1102217	0.1772640	0.2205993	0.3175772
24	112.67 to 132.96	0.0640784	0.1403353	0.2047826	0.2691819	0.3482953
25	132.96 to 156.9	0.0835355	0.1961247	0.2591863	0.3453468	0.4268518
26	156.9 to 185.15	0.0871108	0.1847177	0.1954905	0.2575125	0.3695735
27	185.15 to 218.49	0.1753353	0.2721946	0.1864791	0.2826352	0.4247131
28	218.49 to 257.83	0.2899318	0.4325092	0.1124109	0.2339656	0.3036766
29	257.83 to 304.26	0.4060730	0.6510889	0.1284620	0.2951624	0.2633847
30	304.26 to 359.05	0.4392014	0.7076446	0.1219351	0.2648486	0.1641528
31	359.05 to 423.7	0.3531244	0.5406105	0.1366122	0.2133734	0.1378326
32	423.7 to 500	0.2252709	0.2724617	0.2952065	0.1669884	0.2079769
	Mean droplet size (µm)	270.0	349.6	218.0	199.4	67.0
	90th percentile droplet size (µm)	390.0	390.0	390.0	330.5	170.4
	50th percentile droplet size (µm)	280.1	280.1	144.4	144.4	63.1
	Volume percent < 75 µm	5%	6%	22%	22%	26%

Appendix C

Laboratory Certificates of Analysis

Your P.O. #: 16582
Your C.O.C. #: 58770

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2016/05/03
Report #: R3980405
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B684013
Received: 2016/04/27, 16:10

Sample Matrix: Water
Samples Received: 10

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (PIRI) (1)	10	2016/05/02	2016/05/03	ATL SOP 00113	Atl. RBCA v3 m
VPH in Water (PIRI) (1)	9	N/A	2016/05/02	ATL SOP 00118	Atl. RBCA v3 m
VPH in Water (PIRI) (1)	1	N/A	2016/05/03	ATL SOP 00118	Atl. RBCA v3 m
ModTPH (T1) Calc. for Water (1)	10	N/A	2016/05/03	N/A	Atl. RBCA v3 m

Reference Method suffix “m” indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.
(1) This test was performed by Maxxam Bedford

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226
==========
This report has been generated and distributed using a secure automated process.
Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

Maxxam Job #: B684013
Report Date: 2016/05/03

Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CGL921	CGL922	CGL923	CGL924	CGL925		
Sampling Date		2016/04/21 11:00	2016/04/21 11:00	2016/04/21 11:00	2016/04/21 11:00	2016/04/21 11:00		
COC Number		58770	58770	58770	58770	58770		
	UNITS	T1 0	T1 2	T1 8	T1 15	T1 30	RDL	QC Batch
Petroleum Hydrocarbons								
Benzene	mg/L	ND	ND	ND	ND	ND	0.0010	4480477
Toluene	mg/L	0.0053	0.0056	0.0059	0.0063	0.0064	0.0010	4480477
Ethylbenzene	mg/L	ND	ND	ND	ND	ND	0.0010	4480477
Total Xylenes	mg/L	ND	ND	ND	0.0020	0.0031	0.0020	4480477
C6 - C10 (less BTEX)	mg/L	ND	ND	ND	ND	ND	0.010	4480477
>C10-C16 Hydrocarbons	mg/L	0.062	0.055	0.060	0.061	0.062	0.050	4480411
>C16-C21 Hydrocarbons	mg/L	0.070	0.071	0.082	0.092	0.093	0.050	4480411
>C21-<C32 Hydrocarbons	mg/L	0.18	0.19	0.22	0.24	0.24	0.10	4480411
Modified TPH (Tier1)	mg/L	0.31	0.31	0.36	0.38	0.38	0.10	4476348
Reached Baseline at C32	mg/L	Yes	Yes	Yes	Yes	Yes	N/A	4480411
Hydrocarbon Resemblance	mg/L	COMMENT (1)	COMMENT (1)	COMMENT (1)	COMMENT (1)	COMMENT (1)	N/A	4480411
Surrogate Recovery (%)								
Isobutylbenzene - Extractable	%	114	73	106	108	108		4480411
n-Dotriacontane - Extractable	%	113	71	100	105	109		4480411
Isobutylbenzene - Volatile	%	95	96	96	96	95		4480477

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) One product in fuel oil range. Lube oil fraction.

Maxxam Analytics International Corporation o/a Maxxam Analytics 32 Colonnade Rd, Unit #1000, Nepean, ON K2E 7J6 Phone: 613 274-0573 Fax: 613 274-0574 Website: www.maxxam.ca

Maxxam Job #: B684013
Report Date: 2016/05/03

Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CGL926	CGL927	CGL928	CGL929	CGL934		
Sampling Date		2016/04/25 13:30	2016/04/25 13:30	2016/04/25 13:30	2016/04/25 13:30	2016/04/25 13:30		
COC Number		58770	58770	58770	58770			
	UNITS	T2 0	T2 2	T2 8	T2 15	T2 30	RDL	QC Batch
Petroleum Hydrocarbons								
Benzene	mg/L	ND	ND	ND	ND	ND	0.0010	4480477
Toluene	mg/L	0.0034	0.0092	0.0099	0.012	0.013	0.0010	4480477
Ethylbenzene	mg/L	ND	0.0020	0.0022	0.0028	0.0031	0.0010	4480477
Total Xylenes	mg/L	ND	0.011	0.013	0.016	0.018	0.0020	4480477
C6 - C10 (less BTEX)	mg/L	ND	0.022	0.030	0.034	0.038	0.010	4480477
>C10-C16 Hydrocarbons	mg/L	ND	0.71	0.68	0.85	0.77	0.050	4480411
>C16-C21 Hydrocarbons	mg/L	ND	0.34	0.42	0.61	0.56	0.050	4480411
>C21-<C32 Hydrocarbons	mg/L	ND	0.55	0.67	0.99	0.86	0.10	4480411
Modified TPH (Tier1)	mg/L	ND	1.6	1.8	2.5	2.2	0.10	4476348
Reached Baseline at C32	mg/L	NA	Yes	Yes	Yes	Yes	N/A	4480411
Hydrocarbon Resemblance	mg/L	NA	COMMENT (1)	COMMENT (1)	COMMENT (1)	COMMENT (1)	N/A	4480411
Surrogate Recovery (%)								
Isobutylbenzene - Extractable	%	102	106	104	103	106		4480411
n-Dotriacontane - Extractable	%	99	113	110	106	89		4480411
Isobutylbenzene - Volatile	%	90	90	89	87	87		4480477

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) Weathered fuel oil fraction. One product in fuel / lube range.

Maxxam Analytics International Corporation o/a Maxxam Analytics 32 Colonnade Rd, Unit #1000, Nepean, ON K2E 7J6 Phone: 613 274-0573 Fax: 613 274-0574 Website: www.maxxam.ca

Maxxam Job #: B684013
Report Date: 2016/05/03

Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

GENERAL COMMENTS

Results relate only to the items tested.

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Maxxam Job #: B684013
Report Date: 2016/05/03

Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4480411	BHR	Matrix Spike	Isobutylbenzene - Extractable	2016/05/02		93	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/02		96	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/02		102	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/02		100	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/02		105	%	70 - 130
4480411	BHR	Spiked Blank	Isobutylbenzene - Extractable	2016/05/02		71	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/02		100	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/02		109	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/02		104	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/02		109	%	70 - 130
4480411	BHR	Method Blank	Isobutylbenzene - Extractable	2016/05/02		75	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/02		94	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/02	ND, RDL=0.050		mg/L	
			>C16-C21 Hydrocarbons	2016/05/02	ND, RDL=0.050		mg/L	
			>C21-<C32 Hydrocarbons	2016/05/02	ND, RDL=0.10		mg/L	
4480411	BHR	RPD	>C10-C16 Hydrocarbons	2016/05/02	NC		%	40
			>C16-C21 Hydrocarbons	2016/05/02	NC		%	40
			>C21-<C32 Hydrocarbons	2016/05/02	NC		%	40
4480477	ASL	Matrix Spike	Isobutylbenzene - Volatile	2016/05/02		100	%	70 - 130
			Benzene	2016/05/02		99	%	70 - 130
			Toluene	2016/05/02		105	%	70 - 130
			Ethylbenzene	2016/05/02		105	%	70 - 130
			Total Xylenes	2016/05/02		108	%	70 - 130
4480477	ASL	Spiked Blank	Isobutylbenzene - Volatile	2016/05/02		100	%	70 - 130
			Benzene	2016/05/02		102	%	70 - 130
			Toluene	2016/05/02		106	%	70 - 130
			Ethylbenzene	2016/05/02		107	%	70 - 130
			Total Xylenes	2016/05/02		110	%	70 - 130
4480477	ASL	Method Blank	Isobutylbenzene - Volatile	2016/05/02		102	%	70 - 130
			Benzene	2016/05/02	ND, RDL=0.0010		mg/L	
			Toluene	2016/05/02	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2016/05/02	ND, RDL=0.0010		mg/L	
			Total Xylenes	2016/05/02	ND, RDL=0.0020		mg/L	
			C6 - C10 (less BTEX)	2016/05/02	ND, RDL=0.010		mg/L	
4480477	ASL	RPD	Benzene	2016/05/02	NC		%	40
			Toluene	2016/05/02	NC		%	40
			Ethylbenzene	2016/05/02	NC		%	40
			Total Xylenes	2016/05/02	NC		%	40

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Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

QUALITY ASSURANCE REPORT(CONT'D)

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
			C6 - C10 (less BTEX)	2016/05/02	NC		%	40

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Matrix Spike: A sample to which a known amount of the analyte of interest has been added. Used to evaluate sample matrix interference.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (one or both samples < 5x RDL).

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Maxxam Job #: B684013
Report Date: 2016/05/03

Huntsman Ocean Sciences
Your P.O. #: 16582
Sampler Initials: JM

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

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Maxxam
A Bureau Veritas Group Company

Your P.O. #: 16582
Your Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your C.O.C. #: 58774

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2016/05/13
Report #: R3992147
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B691252
Received: 2016/05/06, 11:50

Sample Matrix: Water
Samples Received: 10

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (PIRI) (1)	6	2016/05/10	2016/05/10	ATL SOP 00113	Atl. RBCA v3 m
TEH in Water (PIRI) (1)	4	2016/05/10	2016/05/11	ATL SOP 00113	Atl. RBCA v3 m
VPH in Water (PIRI) (1)	10	N/A	2016/05/13	ATL SOP 00118	Atl. RBCA v3 m
ModTPH (T1) Calc. for Water (1)	10	N/A	2016/05/13	N/A	Atl. RBCA v3 m

Reference Method suffix “m” indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.
(1) This test was performed by Maxxam Bedford

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226

==========

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Maxxam Job #: B691252
Report Date: 2016/05/13

Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CHS921		CHS922			CHS923	CHS924		
Sampling Date		2016/05/04		2016/05/04			2016/05/04	2016/05/04		
COC Number		58774		58774			58774	58774		
	UNITS	T3-0	RDL	T3-2	RDL	QC Batch	T3-8	T3-15	RDL	QC Batch
Petroleum Hydrocarbons										
Benzene	mg/L	ND	0.0010	0.0025	0.0013	4493135	0.0015	0.0012	0.0010	4493135
Toluene	mg/L	0.0035	0.0010	0.065	0.0013	4493135	0.031	0.029	0.0010	4493135
Ethylbenzene	mg/L	ND	0.0010	0.021	0.0013	4493135	0.0071	0.0068	0.0010	4493135
Total Xylenes	mg/L	ND	0.0020	0.12	0.0026	4493135	0.041	0.040	0.0020	4493135
C6 - C10 (less BTEX)	mg/L	ND	0.010	0.23	0.013	4493135	0.076	0.070	0.010	4493135
>C10-C16 Hydrocarbons	mg/L	ND	0.050	4.3	0.050	4491431	1.3	1.2	0.050	4491434
>C16-C21 Hydrocarbons	mg/L	ND	0.050	3.2	0.050	4491431	1.0	0.97	0.050	4491434
>C21-<C32 Hydrocarbons	mg/L	ND	0.10	4.5	0.10	4491431	1.4	1.4	0.10	4491434
Modified TPH (Tier1)	mg/L	ND	0.10	12	0.10	4487527	3.8	3.7	0.10	4487527
Reached Baseline at C32	mg/L	NA	N/A	Yes	N/A	4491431	Yes	Yes	N/A	4491434
Hydrocarbon Resemblance	mg/L	NA	N/A	COMMENT (1)	N/A	4491431	COMMENT (2)	COMMENT (2)	N/A	4491434
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	106		109		4491431	113	112		4491434
n-Dotriacontane - Extractable	%	119		117		4491431	108	110		4491434
Isobutylbenzene - Volatile	%	95		76 (3)		4493135	82	82		4493135

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) Fuel oil fraction. One product in fuel / lube range. Possible lube oil fraction. Unidentified compound(s) in fuel / lube range.
(2) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.
(3) VPH analysis performed on previously opened vial.

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Maxxam Job #: B691252
Report Date: 2016/05/13

Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CHS925	CHS926	CHS927	CHS928	CHS929		
Sampling Date		2016/05/04	2016/05/05	2016/05/05	2016/05/05	2016/05/05		
COC Number		58774	58774	58774	58774	58774		
	UNITS	T3-30	T4-0	T4-1	T4-2	T4-3	RDL	QC Batch
Petroleum Hydrocarbons								
Benzene	mg/L	0.0011	ND	ND	ND	ND	0.0010	4493135
Toluene	mg/L	0.026	0.0033	0.0055	0.0080	0.0096	0.0010	4493135
Ethylbenzene	mg/L	0.0067	ND	ND	0.0014	0.0018	0.0010	4493135
Total Xylenes	mg/L	0.038	ND	ND	0.0026	0.0065	0.0020	4493135
C6 - C10 (less BTEX)	mg/L	0.066	ND	ND	ND	ND	0.010	4493135
>C10-C16 Hydrocarbons	mg/L	1.3	ND	ND	0.070	0.11	0.050	4491434
>C16-C21 Hydrocarbons	mg/L	1.1	ND	ND	0.083	0.12	0.050	4491434
>C21-<C32 Hydrocarbons	mg/L	1.6	ND	ND	0.13	0.19	0.10	4491434
Modified TPH (Tier1)	mg/L	4.0	ND	ND	0.29	0.43	0.10	4487527
Reached Baseline at C32	mg/L	Yes	NA	NA	Yes	Yes	N/A	4491434
Hydrocarbon Resemblance	mg/L	COMMENT (1)	NA	NA	COMMENT (2)	COMMENT (2)	N/A	4491434
Surrogate Recovery (%)								
Isobutylbenzene - Extractable	%	112	105	105	104	100		4491434
n-Dotriacontane - Extractable	%	112	113	110	108	105		4491434
Isobutylbenzene - Volatile	%	82	92	94	92	92		4493135

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.
(2) One product in fuel / lube range.

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Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CHS930		
Sampling Date		2016/05/05		
COC Number		58774		
	UNITS	T4-4	RDL	QC Batch
Petroleum Hydrocarbons				
Benzene	mg/L	ND	0.0010	4493135
Toluene	mg/L	0.0095	0.0010	4493135
Ethylbenzene	mg/L	0.0021	0.0010	4493135
Total Xylenes	mg/L	0.0081	0.0020	4493135
C6 - C10 (less BTEX)	mg/L	ND	0.010	4493135
>C10-C16 Hydrocarbons	mg/L	0.14	0.050	4491434
>C16-C21 Hydrocarbons	mg/L	0.16	0.050	4491434
>C21-<C32 Hydrocarbons	mg/L	0.23	0.10	4491434
Modified TPH (Tier1)	mg/L	0.54	0.10	4487527
Reached Baseline at C32	mg/L	Yes	N/A	4491434
Hydrocarbon Resemblance	mg/L	COMMENT (1)	N/A	4491434
Surrogate Recovery (%)				
Isobutylbenzene - Extractable	%	99		4491434
n-Dotriacontane - Extractable	%	106		4491434
Isobutylbenzene - Volatile	%	90		4493135

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) One product in fuel / lube range.

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Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
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GENERAL COMMENTS

Results relate only to the items tested.

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Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4491431	BHR	Matrix Spike	Isobutylbenzene - Extractable	2016/05/10		107	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/10		122	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/10		92	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/10		89	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/10		105	%	70 - 130
4491431	BHR	Spiked Blank	Isobutylbenzene - Extractable	2016/05/10		100	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/10		108	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/10		89	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/10		85	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/10		99	%	70 - 130
4491431	BHR	Method Blank	Isobutylbenzene - Extractable	2016/05/10		91	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/10		98	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/10	ND, RDL=0.050		mg/L	
			>C16-C21 Hydrocarbons	2016/05/10	ND, RDL=0.050		mg/L	
			>C21-<C32 Hydrocarbons	2016/05/10	ND, RDL=0.10		mg/L	
4491431	BHR	RPD	>C10-C16 Hydrocarbons	2016/05/10	NC		%	40
			>C16-C21 Hydrocarbons	2016/05/10	NC		%	40
			>C21-<C32 Hydrocarbons	2016/05/10	NC		%	40
4491434	KCR	Matrix Spike	Isobutylbenzene - Extractable	2016/05/10		104	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/10		122	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/10		105	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/10		102	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/10		117	%	70 - 130
4491434	KCR	Spiked Blank	Isobutylbenzene - Extractable	2016/05/11		106	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/11		112	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/11		99	%	70 - 130
			>C16-C21 Hydrocarbons	2016/05/11		94	%	70 - 130
			>C21-<C32 Hydrocarbons	2016/05/11		106	%	70 - 130
4491434	KCR	Method Blank	Isobutylbenzene - Extractable	2016/05/10		77	%	30 - 130
			n-Dotriacontane - Extractable	2016/05/10		107	%	30 - 130
			>C10-C16 Hydrocarbons	2016/05/10	ND, RDL=0.050		mg/L	
			>C16-C21 Hydrocarbons	2016/05/10	ND, RDL=0.050		mg/L	
			>C21-<C32 Hydrocarbons	2016/05/10	ND, RDL=0.10		mg/L	
4491434	KCR	RPD	>C10-C16 Hydrocarbons	2016/05/10	NC		%	40
			>C16-C21 Hydrocarbons	2016/05/10	NC		%	40
			>C21-<C32 Hydrocarbons	2016/05/10	NC		%	40
4493135	ASL	Matrix Spike	Isobutylbenzene - Volatile	2016/05/13		94	%	70 - 130
			Benzene	2016/05/13		104	%	70 - 130
			Toluene	2016/05/13		105	%	70 - 130
			Ethylbenzene	2016/05/13		104	%	70 - 130
			Total Xylenes	2016/05/13		107	%	70 - 130
4493135	ASL	Spiked Blank	Isobutylbenzene - Volatile	2016/05/13		102	%	70 - 130
			Benzene	2016/05/13		103	%	70 - 130
			Toluene	2016/05/13		103	%	70 - 130
			Ethylbenzene	2016/05/13		105	%	70 - 130

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Maxxam Job #: B691252
Report Date: 2016/05/13

Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

QUALITY ASSURANCE REPORT(CONT'D)

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
			Total Xylenes	2016/05/13		108	%	70 - 130
4493135	ASL	Method Blank	Isobutylbenzene - Volatile	2016/05/13		103	%	70 - 130
			Benzene	2016/05/13	ND, RDL=0.0010		mg/L	
			Toluene	2016/05/13	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2016/05/13	ND, RDL=0.0010		mg/L	
			Total Xylenes	2016/05/13	ND, RDL=0.0020		mg/L	
			C6 - C10 (less BTEX)	2016/05/13	ND, RDL=0.010		mg/L	
4493135	ASL	RPD	Benzene	2016/05/13	NC		%	40
			Toluene	2016/05/13	NC		%	40
			Ethylbenzene	2016/05/13	NC		%	40
			Total Xylenes	2016/05/13	NC		%	40
			C6 - C10 (less BTEX)	2016/05/13	NC		%	40

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Matrix Spike: A sample to which a known amount of the analyte of interest has been added. Used to evaluate sample matrix interference.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (one or both samples < 5x RDL).

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Maxxam Job #: B691252
Report Date: 2016/05/13

Huntsman Ocean Sciences
Client Project #: WHITE ROSE
Site Location: OTTAWA, SLR
Your P.O. #: 16582
Sampler Initials: JMC

VALIDATION SIGNATURE PAGE

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Rosemarie MacDonald, Scientific Specialist (Organics)

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Appendix 2 Analytical Reports

2016 Maxxam Variable Loading

B6H0117V1-R2016-08-23_10-49-44_N001 = Hydrocarbon analysis

- CWI725: 0 mg/L loading
- CWI726: 56.88 mg/L loading
- CWI727: 283.75 mg/L loading
- CWI728: 1417.5 mg/L loading
- CWI729: 7086.25 mg/L loading

Huntsman Ocean Sciences

Maxxam Job Number: B6H0117
Report Date: 2016/08/23

Your P.O. #: 16582

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CWI725	CWI726		CWI727		CWI728	CWI729		
Sampling Date		05/08/2016	05/08/2016		05/08/2016		05/08/2016	05/08/2016		
COC Number		D15174	D15174		D15174		D15174	D15174		
	UNITS	**040-T1A**	**040-T2A**	**RDL**	**040-T3A**	**RDL**	**040-T4A**	**040-T5A**	**RDL**	**QC Batch**
Petroleum Hydrocarbons										
Benzene	mg/L	ND	0.0031	0.0010	0.015	0.010	0.068	0.19	0.025	4620867
Toluene	mg/L	ND	0.094	0.0010	0.46	0.010	1.5	2.4	0.025	4620867
Ethylbenzene	mg/L	ND	0.038	0.0010	0.14	0.010	0.28	0.33	0.025	4620867
Total Xylenes	mg/L	ND	0.21	0.0020	0.78	0.020	1.4	1.7	0.050	4620867
Aliphatic >C6-C8	mg/L	ND (1)	ND (1)	0.020	ND (1)	0.20	ND (1)	ND (1)	0.50	4620867
Aliphatic >C8-C10	mg/L	ND	0.040	0.010	0.11	0.10	ND	ND	0.25	4620867
>C8-C10 Aromatics (-EX)	mg/L	ND	0.17	0.010	0.41	0.10	0.56	0.59	0.25	4620867
Aliphatic >C10-C12	mg/L	ND	0.15	0.010	0.59	0.010	0.99	0.57	0.010	4622920
Aliphatic >C12-C16	mg/L	ND	0.48	0.050	1.6	0.050	2.6	1.6	0.050	4622920
Aliphatic >C16-C21	mg/L	ND	0.56	0.050	1.6	0.050	2.7	1.7	0.050	4622920
Aliphatic >C21-<C32	mg/L	ND	0.69	0.10	1.8	0.10	3.1	2.0	0.10	4622920
Aromatic >C10-C12	mg/L	ND	0.24	0.010	0.49	0.010	0.69	0.57	0.010	4622920
Aromatic >C12-C16	mg/L	ND	0.34	0.050	0.77	0.050	1.1	0.81	0.050	4622920
Aromatic >C16-C21	mg/L	ND	0.26	0.050	0.78	0.050	1.2	0.71	0.050	4622920
Aromatic >C21-<C32	mg/L	ND	0.51	0.10	1.4	0.10	2.2	1.4	0.10	4622920
Modified TPH (Tier 2)	mg/L	ND	3.4	0.10	9.6	0.20	15	9.9	0.50	4617253
Reached Baseline at C32	mg/L	NA	Yes	N/A	Yes	N/A	Yes	Yes	N/A	4622920
Hydrocarbon Resemblance	mg/L	NA	COMMENT (2)	N/A	COMMENT (3)	N/A	COMMENT (3)	COMMENT (3)	N/A	4622920
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	79	95		89		86	86		4622920
n-Dotriacontane - Extractable	%	96	97		98		133 (6)	120		4622920
Isobutylbenzene - Volatile	%	78	65 (4)		96 (5)		97 (5)	100 (5)		4620867

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) Elevated VPH RDL(s) due to detected levels in the method blank.
(2) One product in fuel / lube range.
(3) One product in fuel / lube range. Lube oil fraction.
(4) VPH sample contained headspace. VPH surrogate not within acceptance limits. Insufficient sample to repeat.
(5) Elevated VPH RDL(s) due to sample dilution.
(6) TEH surrogate(s) not within acceptance limits due to product interference.

Results relate only to the items tested.

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Vials for sample 040-T2A contain headspace.

Results relate only to the items tested.

Each temper	Each temper	Each temper	Each temperature is the average of up to three cooler temperatures
Package 1	4.0°C	#N/A	#N/A

Huntsman Ocean Sciences
Attention: Chris Bridger

Report Date: 2016/08/23

Your P.O. #:16582

Quality Assurance Report
Maxxam Job Number: B6H0117

QA/QC Ba	Init	QC Type	Parameter	Date Analy	Value	Recovery	UNITS	QC Limits
4620867	ASL	Spiked Blank	Isobutylbenzene - Volatile	17/08/2016		100	%	70 - 130
			Benzene	17/08/2016		96	%	70 - 130
			Toluene	17/08/2016		94	%	70 - 130
			Ethylbenzene	17/08/2016		96	%	70 - 130
			Total Xylenes	17/08/2016		97	%	70 - 130
4620867	ASL	Method Blank	Isobutylbenzene - Volatile	17/08/2016		104	%	70 - 130
			Benzene	17/08/2016	ND, RDL=0.0010		mg/L	
			Toluene	17/08/2016	ND, RDL=0.0010		mg/L	
			Ethylbenzene	17/08/2016	ND, RDL=0.0010		mg/L	
			Total Xylenes	17/08/2016	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	17/08/2016	0.010, RDL=0.010		mg/L	
			Aliphatic >C8-C10	17/08/2016	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	17/08/2016	ND, RDL=0.010		mg/L	
4622920	SHF	Spiked Blank	Aliphatic >C10-C12	19/08/2016		94	%	30 - 130
			Aliphatic >C12-C16	19/08/2016		95	%	30 - 130
			Aliphatic >C16-C21	19/08/2016		104	%	30 - 130
			Aliphatic >C21-<C32	19/08/2016		107	%	30 - 130
			Aromatic >C10-C12	19/08/2016		112	%	30 - 130
			Aromatic >C12-C16	19/08/2016		103	%	30 - 130
			Aromatic >C16-C21	19/08/2016		103	%	30 - 130
			Aromatic >C21-<C32	19/08/2016		106	%	30 - 130
4622920	SHF	Method Blank	Isobutylbenzene - Extractable	19/08/2016		57 (1)		30 - 130
			n-Dotriacontane - Extractable	19/08/2016		108	%	30 - 130
			Aliphatic >C10-C12	19/08/2016	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	19/08/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	19/08/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	19/08/2016	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	19/08/2016	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	19/08/2016	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	19/08/2016	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	19/08/2016	ND, RDL=0.10		mg/L	
4622920	SHF	RPD	Aliphatic >C10-C12	19/08/2016	NC		%	40
			Aliphatic >C12-C16	19/08/2016	NC		%	40
			Aliphatic >C16-C21	19/08/2016	NC		%	40
			Aliphatic >C21-<C32	19/08/2016	NC		%	40
			Aromatic >C10-C12	19/08/2016	NC		%	40
			Aromatic >C12-C16	19/08/2016	NC		%	40
			Aromatic >C16-C21	19/08/2016	NC		%	40
			Aromatic >C21-<C32	19/08/2016	NC		%	40

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement
Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method ac

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contaminatior
Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency
NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculat
(1) TEH surrogate(s) not within acceptance limits. Insufficient sample to repeat

B6H5112V1-R2016-09-01_17-19-50_N001 = Hydrocarbon analysis

- CXG341: 0 mg/L loading
- CXG342: 56.88 mg/L loading
- CXG343: 283.75 mg/L loading
- CXG344: 1417.5 mg/L loading
- CXG345: 7086.25 mg/L loading

Huntsman Ocean Sciences

Maxxam Job Number: B6H5112
Client Project #: HMSC-040

Report Date: 2016/09/01

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CXG341		CXG342	CXG343		CXG344	CXG345		
Sampling Date		16/08/2016		16/08/2016	16/08/2016		16/08/2016	16/08/2016		
COC Number		D15175		D15175	D15175		D15175	D15175		
	UNITS	**040-T1B**	**RDL**	**040-T2B**	**040-T3B**	**RDL**	**040-T4B**	**040-T5B**	**RDL**	**QC Batch**
Petroleum Hydrocarbons										
Benzene	mg/L	ND	0.0010	ND	0.014	0.010	0.062	0.17	0.025	4634371
Toluene	mg/L	ND	0.0010	0.11	0.41	0.010	1.3	2.0	0.025	4634371
Ethylbenzene	mg/L	ND	0.0010	0.044	0.12	0.010	0.26	0.29	0.025	4634371
Total Xylenes	mg/L	ND	0.0020	0.25	0.65	0.020	1.3	1.5	0.050	4634371
Aliphatic >C6-C8	mg/L	ND	0.010	ND	ND	0.10	ND	0.27	0.25	4634371
Aliphatic >C8-C10	mg/L	ND	0.010	ND	0.13	0.10	ND	ND	0.25	4634371
>C8-C10 Aromatics (-EX)	mg/L	ND	0.010	0.14	0.31	0.10	0.37	0.39	0.25	4634371
Aliphatic >C10-C12	mg/L	ND	0.010	0.11	0.38	0.010	0.82	0.26	0.010	4629593
Aliphatic >C12-C16	mg/L	ND	0.050	0.40	1.1	0.050	2.1	0.77	0.050	4629593
Aliphatic >C16-C21	mg/L	ND	0.050	0.47	1.3	0.050	2.2	0.86	0.050	4629593
Aliphatic >C21-<C32	mg/L	ND	0.10	0.57	1.6	0.10	2.7	1.0	0.10	4629593
Aromatic >C10-C12	mg/L	0.036	0.010	0.25	0.49	0.010	0.77	0.49	0.010	4629593
Aromatic >C12-C16	mg/L	ND	0.050	0.36	0.81	0.050	1.3	0.59	0.050	4629593
Aromatic >C16-C21	mg/L	ND	0.050	0.30	0.82	0.050	1.3	0.43	0.050	4629593
Aromatic >C21-<C32	mg/L	ND	0.10	0.58	1.5	0.10	2.4	0.73	0.10	4629593
Modified TPH (Tier 2)	mg/L	ND	0.10	3.2	8.4	0.10	14	5.8	0.25	4625181
Reached Baseline at C32	mg/L	NA	N/A	Yes	Yes	N/A	Yes	Yes	N/A	4629593
Hydrocarbon Resemblance	mg/L	NA	N/A	COMMENT (1)	COMMENT (1)	N/A	COMMENT (1)	COMMENT (1)	N/A	4629593
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	44 (2)		98	104		105	88		4629593
n-Dotriacontane - Extractable	%	94		99 (3)	95 (3)		110 (3)	91 (3)		4629593
Isobutylbenzene - Volatile	%	97		90 (4)	98 (4)		98 (4)	100 (4)		4634371

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.
(2) TEH surrogate(s) not within acceptance limits. Analysis was repeated with similar results.
(3) TEH sample decanted from 2 x 500 mL bottles into a 1L bottle.
(4) Elevated VPH RDL(s) due to sample dilution.

Results relate only to the items tested.

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Each temperature is the average of up to three cooler temperatures

Each temper	Each temper	Each temper	Each temperature is the average of up to three cooler temperatures
Package 1	3.3°C	#N/A	#N/A

Sample 040-T1B received in unpreserved bottles. Sample poured into proper containers prior to analysis as per client request.

Results relate only to the items tested.

Huntsman Ocean Sciences
Attention: Chris Bridger
Report Date: 2016/09/01 Client Project #: HMSC-040

Quality Assurance Report
Maxxam Job Number: B6H5112

QA/QC Ba	Init	QC Type	Parameter	Date Anal	Value	Recovery	UNITS	QC Limits
4629593	BCD	Spiked Blank	Aliphatic >C10-C12	26/08/2016		82	%	30 - 130
			Aliphatic >C12-C16	26/08/2016		82	%	30 - 130
			Aliphatic >C16-C21	26/08/2016		88	%	30 - 130
			Aliphatic >C21-<C32	26/08/2016		94	%	30 - 130
			Aromatic >C10-C12	26/08/2016		123	%	30 - 130
			Aromatic >C12-C16	26/08/2016		108	%	30 - 130
			Aromatic >C16-C21	26/08/2016		107	%	30 - 130
			Aromatic >C21-<C32	26/08/2016		107	%	30 - 130
4629593	BCD	Method Blank	Isobutylbenzene - Extractable	26/08/2016		43 (1)		30 - 130
			n-Dotriacontane - Extractable	26/08/2016		89	%	30 - 130
			Aliphatic >C10-C12	26/08/2016	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	26/08/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	26/08/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	26/08/2016	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	26/08/2016	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	26/08/2016	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	26/08/2016	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	26/08/2016	ND, RDL=0.10		mg/L	
4629593	BCD	RPD [CXG341-01]	Aliphatic >C10-C12	26/08/2016	NC (2)		%	40
			Aliphatic >C12-C16	26/08/2016	NC (2)		%	40
			Aliphatic >C16-C21	26/08/2016	NC (2)		%	40
			Aliphatic >C21-<C32	26/08/2016	NC (2)		%	40
			Aromatic >C10-C12	26/08/2016	NC (2)		%	40
			Aromatic >C12-C16	26/08/2016	NC (2)		%	40
			Aromatic >C16-C21	26/08/2016	NC (2)		%	40
			Aromatic >C21-<C32	26/08/2016	NC (2)		%	40
4634371	ASL	Spiked Blank	Isobutylbenzene - Volatile	26/08/2016		96	%	70 - 130
			Benzene	26/08/2016		76	%	70 - 130
			Toluene	26/08/2016		81	%	70 - 130
			Ethylbenzene	26/08/2016		83	%	70 - 130
			Total Xylenes	26/08/2016		94	%	70 - 130
4634371	ASL	Method Blank	Isobutylbenzene - Volatile	26/08/2016		99	%	70 - 130
			Benzene	26/08/2016	ND, RDL=0.0010		mg/L	
			Toluene	26/08/2016	ND, RDL=0.0010		mg/L	
			Ethylbenzene	26/08/2016	ND, RDL=0.0010		mg/L	
			Total Xylenes	26/08/2016	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	26/08/2016	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	26/08/2016	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	26/08/2016	ND, RDL=0.010		mg/L	

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement
Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method ac

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contaminatior
Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficienc
NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculat
(1) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run.
(2) Elevated TEH RDL(s) due to limited sample.

B6I3491V1-R2016-09-12_16-07-58_N001 = Hydrocarbon analysis

- CYU623: 0 mg/L loading
- CYU624: 56.88 mg/L loading
- CYU625: 283.75 mg/L loading
- CYU626: 1417.5 mg/L loading
- CYU627: 7086.25 mg/L loading

Huntsman Ocean Sciences

Maxxam Job Number: B6I3491
Report Date: 2016/09/12

Your P.O. #: 16582

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		CYU623		CYU624		CYU625		CYU626	CYU627		
Sampling Date		24/08/2016		24/08/2016		24/08/2016		24/08/2016	24/08/2016		
COC Number		D 15507		D 15507		D 15507		D 15507	D 15507		
	UNITS	**T1-C**	**RDL**	**T2-C**	**RDL**	**T3-C**	**RDL**	**T4-C**	**T5-C**	**RDL**	**QC Batch**
Petroleum Hydrocarbons											
Benzene	mg/L	ND	0.0010	ND (2)	0.013	0.018	0.010	0.080	0.21	0.033	4644139
Toluene	mg/L	ND	0.0010	0.11	0.013	0.54	0.010	1.5	2.4	0.033	4644139
Ethylbenzene	mg/L	ND	0.0010	0.040	0.013	0.15	0.010	0.26	0.33	0.033	4644139
Total Xylenes	mg/L	ND	0.0020	0.23	0.026	0.81	0.020	1.4	1.7	0.065	4644139
Aliphatic >C6-C8	mg/L	ND (1)	0.020	ND (3)	0.26	ND (1)	0.20	ND (3)	ND (3)	0.65	4644139
Aliphatic >C8-C10	mg/L	ND	0.010	ND (2)	0.13	0.18	0.10	ND (2)	ND (2)	0.33	4644139
>C8-C10 Aromatics (-EX)	mg/L	ND	0.010	0.18	0.13	0.38	0.10	0.42	0.43	0.33	4644139
Aliphatic >C10-C12	mg/L	ND	0.010	0.079	0.010	0.21	0.010	0.47	0.10	0.010	4642103
Aliphatic >C12-C16	mg/L	ND	0.050	0.25	0.050	0.58	0.050	1.2	0.30	0.050	4642103
Aliphatic >C16-C21	mg/L	ND	0.050	0.30	0.050	0.67	0.050	1.4	0.34	0.050	4642103
Aliphatic >C21-<C32	mg/L	ND	0.10	0.38	0.10	0.84	0.10	1.7	0.47	0.10	4642103
Aromatic >C10-C12	mg/L	ND	0.010	0.20	0.010	0.35	0.010	0.50	0.49	0.010	4642103
Aromatic >C12-C16	mg/L	ND	0.050	0.27	0.050	0.47	0.050	0.65	0.48	0.050	4642103
Aromatic >C16-C21	mg/L	ND	0.050	0.18	0.050	0.37	0.050	0.56	0.25	0.050	4642103
Aromatic >C21-<C32	mg/L	ND	0.10	0.32	0.10	0.67	0.10	1.1	0.38	0.10	4642103
Modified TPH (Tier 2)	mg/L	ND	0.10	2.2	0.26	4.7	0.20	8.0	3.3	0.65	4639004
Reached Baseline at C32	mg/L	NA	N/A	Yes	N/A	Yes	N/A	Yes	Yes	N/A	4642103
Hydrocarbon Resemblance	mg/L	NA	N/A	COMMENT (4)	N/A	COMMENT (4)	N/A	COMMENT (4)	COMMENT (4)	N/A	4642103
Surrogate Recovery (%)											
Isobutylbenzene - Extractable	%	75		100		91		89	97		4642103
n-Dotriacontane - Extractable	%	95 (5)		109 (5)		127 (5)		113 (5)	127 (5)		4642103
Isobutylbenzene - Volatile	%	82		101 (6)		105		96 (6)	92 (6)		4644139

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) Elevated VPH RDL(s) due to detected levels in the method blank.
(2) Elevated VPH RDL(s) due to sample dilution.
(3) Elevated VPH RDL(s) due to detected levels in the method blank. Elevated VPH RDL(s) due to sample dilution.
(4) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.
(5) TEH sample tranferred from 2 x 500 mL bottles into a 1L bottle.
(6) VPH analysis performed on previously opened vial.

Results relate only to the items tested.

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Results relate only to the items tested.

Each temper	Each temper	Each temper	Each temperature is the average of up to three cooler temperatures
Package 1	7.7°C	#N/A	#N/A

Huntsman Ocean Sciences
Attention: Chris Bridger

Report Date: 2016/09/12

Your P.O. #:16582

Quality Assurance Report
Maxxam Job Number: B6I3491

QA/QC Ba	Init	QC Type	Parameter	Date Analy	Value	Recovery	UNITS	QC Limits
4642103	BCD	Spiked Blank	Aliphatic >C10-C12	07/09/2016		85	%	30 - 130
			Aliphatic >C12-C16	07/09/2016		85	%	30 - 130
			Aliphatic >C16-C21	07/09/2016		93	%	30 - 130
			Aliphatic >C21-<C32	07/09/2016		98	%	30 - 130
			Aromatic >C10-C12	07/09/2016		123	%	30 - 130
			Aromatic >C12-C16	07/09/2016		106	%	30 - 130
			Aromatic >C16-C21	07/09/2016		105	%	30 - 130
			Aromatic >C21-<C32	07/09/2016		105	%	30 - 130
4642103	BCD	Method Blank	Isobutylbenzene - Extractable	07/09/2016		52 (1)		30 - 130
			n-Dotriacontane - Extractable	07/09/2016		98	%	30 - 130
			Aliphatic >C10-C12	07/09/2016	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	07/09/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	07/09/2016	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	07/09/2016	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	07/09/2016	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	07/09/2016	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	07/09/2016	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	07/09/2016	ND, RDL=0.10		mg/L	
4644139	ASL	Spiked Blank	Isobutylbenzene - Volatile	02/09/2016		83	%	70 - 130
			Benzene	02/09/2016		104	%	70 - 130
			Toluene	02/09/2016		106	%	70 - 130
			Ethylbenzene	02/09/2016		112	%	70 - 130
			Total Xylenes	02/09/2016		113	%	70 - 130
4644139	ASL	Method Blank	Isobutylbenzene - Volatile	02/09/2016		92	%	70 - 130
			Benzene	02/09/2016	ND, RDL=0.0010		mg/L	
			Toluene	02/09/2016	ND, RDL=0.0010		mg/L	
			Ethylbenzene	02/09/2016	ND, RDL=0.0010		mg/L	
			Total Xylenes	02/09/2016	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	02/09/2016	ND, RDL=0.020 (2)		mg/L	
			Aliphatic >C8-C10	02/09/2016	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	02/09/2016	ND, RDL=0.010		mg/L	

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method ac
Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contaminatior
Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficienc
(1) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run. No impact on data quality
(2) Elevated VPH RDL(s) due to detected levels in the method blank

2017 Maxxam

B718348V2R-R2017-02-17_16-43-48_R006 = Hydrocarbon Analysis

- 17024-5: 50% WAF (24-hr mix)
- 17025-2: 6.25% WAF
- 17025-3: 12.5% WAF
- 17025-4: 25% WAF
- 17025-5: 50% WAF

Your P.O. #: 16582
Your C.O.C. #: 552304-02-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/02/17
Report #: R4362339
Version: 2 - Revision

CERTIFICATE OF ANALYSIS – REVISED REPORT

MAXXAM JOB #: B718348
Received: 2017/01/27, 10:57

Sample Matrix: Water
Samples Received: 5

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (AA PIRI)	5	2017/01/30	2017/02/02	ATL SOP 00116	Atl. RBCA v3 m
VPH in Water (PIRI2)	5	2017/01/30	2017/01/31	ATL SOP 00120	Atl. RBCA v3 m
ModTPH (T2) Calc. for Water	5	N/A	2017/02/03	N/A	Atl. RBCA v3 m

Remarks:

Maxxam Analytics' laboratories are accredited to ISO/IEC 17025:2005 for specific parameters on scopes of accreditation. Unless otherwise noted, procedures used by Maxxam are based upon recognized Provincial, Federal or US method compendia such as CCME, MDDELCC, EPA, APHA.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported: unless indicated otherwise, associated sample data are not blank corrected.

Maxxam Analytics' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Maxxam has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Maxxam, unless otherwise agreed in writing.

Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods. Results relate to samples tested.

Reference Method suffix "m" indicates test methods incorporate validated modifications from specific reference methods to improve performance.

* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226

==========

Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		DVB218		DVB219		DVB220		DVB221		
Sampling Date		2017/01/25		2017/01/25		2017/01/25		2017/01/25		
COC Number		552304-02-01		552304-02-01		552304-02-01		552304-02-01		
	UNITS	17024-5	RDL	17025-2	RDL	17025-3	RDL	17025-4	RDL	QC Batch
Petroleum Hydrocarbons										
Benzene	mg/L	0.041	0.013	0.0055	0.0010	0.011	0.0010	0.021	0.013	4843668
Toluene	mg/L	0.81	0.013	0.094	0.0010	0.21 (1)	0.013	0.41	0.013	4843668
Ethylbenzene	mg/L	0.14	0.013	0.015	0.0010	0.034	0.0010	0.064	0.013	4843668
Total Xylenes	mg/L	0.74	0.026	0.078	0.0020	0.17	0.0020	0.34	0.026	4843668
Aliphatic >C6-C8	mg/L	ND (2)	0.13	0.024	0.010	ND (1)	0.13	ND (1)	0.13	4843668
Aliphatic >C8-C10	mg/L	ND (2)	0.13	ND	0.010	ND (1)	0.13	ND (1)	0.13	4843668
>C8-C10 Aromatics (-EX)	mg/L	0.24 (2)	0.13	0.028	0.010	ND (1)	0.13	ND (1)	0.13	4843668
Aliphatic >C10-C12	mg/L	ND (3)	0.023	ND (3)	0.022	0.026 (3)	0.021	0.041 (3)	0.022	4843875
Aliphatic >C12-C16	mg/L	ND (3)	0.11	ND (3)	0.11	ND (3)	0.10	0.12 (3)	0.11	4843875
Aliphatic >C16-C21	mg/L	ND (3)	0.11	ND (3)	0.11	ND (3)	0.10	0.14 (3)	0.11	4843875
Aliphatic >C21-<C32	mg/L	ND (3)	0.23	ND (3)	0.22	ND (3)	0.21	ND (3)	0.22	4843875
Aromatic >C10-C12	mg/L	0.19 (3)	0.023	0.055 (3)	0.022	0.070 (3)	0.021	0.17 (3)	0.022	4843875
Aromatic >C12-C16	mg/L	0.16 (3)	0.11	ND (3)	0.11	ND (3)	0.10	0.21 (3)	0.11	4843875
Aromatic >C16-C21	mg/L	ND (3)	0.11	ND (3)	0.11	ND (3)	0.10	0.13 (3)	0.11	4843875
Aromatic >C21-<C32	mg/L	ND (3)	0.23	ND (3)	0.22	ND (3)	0.21	0.24 (3)	0.22	4843875
Modified TPH (Tier 2)	mg/L	0.59	0.23	ND	0.22	ND	0.21	1.2	0.22	4842053
Reached Baseline at C32	mg/L	Yes	N/A	NA	N/A	NA	N/A	Yes	N/A	4843875
Hydrocarbon Resemblance	mg/L	COMMENT (4)	N/A	NA	N/A	NA	N/A	COMMENT (5)	N/A	4843875
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	85		87		87		91		4843875
n-Dotriacontane - Extractable	%	124		100		113		112		4843875
Isobutylbenzene - Volatile	%	96 (6)		88		90		98 (6)		4843668

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(2) Elevated VPH RDL(s) due to sample dilution.
(3) Elevated TEH RDL(s) due to limited sample.
(4) One product in the gas/fuel oil range. Unidentified compound(s) in fuel oil range.
(5) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range. One product in the gas/fuel oil range.
(6) VPH analysis performed on previously opened vial.

Maxxam Analytics International Corporation o/a Maxxam Analytics 200 Bluewater Rd, Suite 105, Bedford, Nova Scotia Canada B4B 1G9 Tel: 902-420-0203 Toll-free: 800-565-7227 Fax: 902-420-8612 www.maxxamanalytics.com

Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		DVB222		
Sampling Date		2017/01/25		
COC Number		552304-02-01		
	UNITS	17025-5	RDL	QC Batch
Petroleum Hydrocarbons				
Benzene	mg/L	0.061	0.013	4843668
Toluene	mg/L	1.1	0.013	4843668
Ethylbenzene	mg/L	0.18	0.013	4843668
Total Xylenes	mg/L	0.92	0.026	4843668
Aliphatic >C6-C8	mg/L	ND (1)	0.13	4843668
Aliphatic >C8-C10	mg/L	ND (1)	0.13	4843668
>C8-C10 Aromatics (-EX)	mg/L	0.29	0.13	4843668
Aliphatic >C10-C12	mg/L	0.058 (2)	0.021	4843875
Aliphatic >C12-C16	mg/L	0.16 (2)	0.11	4843875
Aliphatic >C16-C21	mg/L	0.20 (2)	0.11	4843875
Aliphatic >C21-<C32	mg/L	0.31 (2)	0.21	4843875
Aromatic >C10-C12	mg/L	0.23 (2)	0.021	4843875
Aromatic >C12-C16	mg/L	0.23 (2)	0.11	4843875
Aromatic >C16-C21	mg/L	ND (2)	0.11	4843875
Aromatic >C21-<C32	mg/L	ND (2)	0.21	4843875
Modified TPH (Tier 2)	mg/L	1.6	0.21	4842053
Reached Baseline at C32	mg/L	Yes	N/A	4843875
Hydrocarbon Resemblance	mg/L	COMMENT (3)	N/A	4843875
Surrogate Recovery (%)				
Isobutylbenzene - Extractable	%	90		4843875
n-Dotriacontane - Extractable	%	124		4843875
Isobutylbenzene - Volatile	%	98 (4)		4843668

RDL = Reportable Detection Limit

QC Batch = Quality Control Batch

N/A = Not Applicable

(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.

(2) Elevated TEH RDL(s) due to limited sample.

(3) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range. One product in the gas/fuel oil range.

(4) VPH analysis performed on previously opened vial.

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Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	0.7°C

Revised report - reissued report with revised results for sample 17025-2 due to lab transcription error.HM Feb 17/17

Results relate only to the items tested.

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Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4843668	ASL	Spiked Blank	Isobutylbenzene - Volatile	2017/01/30		96	%	70 - 130
			Benzene	2017/01/30		105	%	70 - 130
			Toluene	2017/01/30		110	%	70 - 130
			Ethylbenzene	2017/01/30		111	%	70 - 130
			Total Xylenes	2017/01/30		110	%	70 - 130
4843668	ASL	Method Blank	Isobutylbenzene - Volatile	2017/01/30		94	%	70 - 130
			Benzene	2017/01/30	ND, RDL=0.0010		mg/L	
			Toluene	2017/01/30	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2017/01/30	ND, RDL=0.0010		mg/L	
			Total Xylenes	2017/01/30	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	2017/01/30	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	2017/01/30	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	2017/01/30	ND, RDL=0.010		mg/L	
4843875	BCD	Spiked Blank	Aliphatic >C10-C12	2017/02/02		82	%	30 - 130
			Aliphatic >C12-C16	2017/02/02		84	%	30 - 130
			Aliphatic >C16-C21	2017/02/02		87	%	30 - 130
			Aliphatic >C21-<C32	2017/02/02		111	%	30 - 130
			Aromatic >C10-C12	2017/02/02		109	%	30 - 130
			Aromatic >C12-C16	2017/02/02		98	%	30 - 130
			Aromatic >C16-C21	2017/02/02		97	%	30 - 130
			Aromatic >C21-<C32	2017/02/02		118	%	30 - 130
4843875	BCD	Method Blank	Isobutylbenzene - Extractable	2017/02/02		51 (1)		30 - 130
			n-Dotriacontane - Extractable	2017/02/02		81	%	30 - 130
			Aliphatic >C10-C12	2017/02/02	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	2017/02/02	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	2017/02/02	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	2017/02/02	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	2017/02/02	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	2017/02/02	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	2017/02/02	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	2017/02/02	ND, RDL=0.10		mg/L	
4843875	BCD	RPD	Aliphatic >C10-C12	2017/02/02	NC		%	40
			Aliphatic >C12-C16	2017/02/02	NC		%	40
			Aliphatic >C16-C21	2017/02/02	NC		%	40
			Aliphatic >C21-<C32	2017/02/02	NC		%	40
			Aromatic >C10-C12	2017/02/02	NC		%	40
			Aromatic >C12-C16	2017/02/02	NC		%	40
			Aromatic >C16-C21	2017/02/02	NC		%	40

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Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

QUALITY ASSURANCE REPORT(CONT'D)

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
			Aromatic >C21-<C32	2017/02/02	NC		%	40

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (one or both samples < 5x RDL).

(1) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run.

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Maxxam Job #: B718348
Report Date: 2017/02/17

Huntsman Ocean Sciences
Your P.O. #: 16582

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

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B757178V1-R2017-03-30_11-20-36_R006 = Hydrocarbon Analysis

- 17070-A5: 10% WAF
- 17070-B4: 10% CEWAF

Maxxam
A Bureau Veritas Group Company

Your P.O. #: 16582
Your C.O.C. #: N/A

Attention:Ben de Jourdan

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/03/30
Report #: R4407549
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B757178
Received: 2017/03/22, 10:49

Sample Matrix: Water
Samples Received: 2

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (AA PIRI)	2	2017/03/24	2017/03/28	ATL SOP 00116	Atl. RBCA v3 m
VPH in Water (PIRI2)	2	2017/03/23	2017/03/23	ATL SOP 00120	Atl. RBCA v3 m
ModTPH (T2) Calc. for Water	2	N/A	2017/03/29	N/A	Atl. RBCA v3 m

Remarks:

Maxxam Analytics' laboratories are accredited to ISO/IEC 17025:2005 for specific parameters on scopes of accreditation. Unless otherwise noted, procedures used by Maxxam are based upon recognized Provincial, Federal or US method compendia such as CCME, MDDELCC, EPA, APHA.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported: unless indicated otherwise, associated sample data are not blank corrected.

Maxxam Analytics' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Maxxam has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Maxxam, unless otherwise agreed in writing.

Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods.
Results relate to samples tested.

Reference Method suffix "m" indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

Your P.O. #: 16582
Your C.O.C. #: N/A

Attention:Ben de Jourdan

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/03/30
Report #: R4407549
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B757178
Received: 2017/03/22, 10:49

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226
==========

This report has been generated and distributed using a secure automated process.
Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

Maxxam Job #: B757178
Report Date: 2017/03/30

Huntsman Ocean Sciences
Your P.O. #: 16582

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		ECC811	ECC812		
Sampling Date		2017/03/11	2017/03/11		
COC Number		N/A	N/A		
	UNITS	17070-A5	17070-B4	RDL	QC Batch
Petroleum Hydrocarbons					
Benzene	mg/L	0.0051	0.0065	0.0010	4910570
Toluene	mg/L	0.11 (1)	0.17 (1)	0.013	4910570
Ethylbenzene	mg/L	0.023	0.051	0.0010	4910570
Total Xylenes	mg/L	0.12	0.26	0.0020	4910570
Aliphatic >C6-C8	mg/L	ND (2)	ND (2)	0.13	4910570
Aliphatic >C8-C10	mg/L	ND (2)	ND (2)	0.13	4910570
>C8-C10 Aromatics (-EX)	mg/L	ND (2)	0.16 (2)	0.13	4910570
Aliphatic >C10-C12	mg/L	ND	0.29	0.011	4912260
Aliphatic >C12-C16	mg/L	ND	0.74	0.054	4912260
Aliphatic >C16-C21	mg/L	ND	0.86	0.054	4912260
Aliphatic >C21-<C32	mg/L	ND	1.1	0.11	4912260
Aromatic >C10-C12	mg/L	0.046	0.20	0.011	4912260
Aromatic >C12-C16	mg/L	ND	0.39	0.054	4912260
Aromatic >C16-C21	mg/L	ND	0.36	0.054	4912260
Aromatic >C21-<C32	mg/L	ND	0.74	0.11	4912260
Modified TPH (Tier 2)	mg/L	ND	4.8	0.13	4909725
Reached Baseline at C32	mg/L	NA	Yes	N/A	4912260
Hydrocarbon Resemblance	mg/L	NA	COMMENT (3)	N/A	4912260
Surrogate Recovery (%)					
Isobutylbenzene - Extractable	%	71	89		4912260
n-Dotriacontane - Extractable	%	98	99		4912260
Isobutylbenzene - Volatile	%	102	86		4910570

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial.
(2) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(3) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.

Maxxam Analytics International Corporation o/a Maxxam Analytics 200 Bluewater Rd, Suite 105, Bedford, Nova Scotia Canada B4B 1G9 Tel: 902-420-0203 Toll-free: 800-565-7227 Fax: 902-420-8612 www.maxxamanalytics.com

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	6.7°C

Results relate only to the items tested.

Maxxam Analytics International Corporation o/a Maxxam Analytics 200 Bluewater Rd, Suite 105, Bedford, Nova Scotia Canada B4B 1G9 Tel: 902-420-0203 Toll-free: 800-565-7227 Fax: 902-420-8612 www.maxxamanalytics.com

Maxxam Job #: B757178
Report Date: 2017/03/30

Huntsman Ocean Sciences
Your P.O. #: 16582

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4910570	ASL	Spiked Blank	Isobutylbenzene - Volatile	2017/03/22		96	%	70 - 130
			Benzene	2017/03/22		109	%	70 - 130
			Toluene	2017/03/22		112	%	70 - 130
			Ethylbenzene	2017/03/22		110	%	70 - 130
			Total Xylenes	2017/03/22		112	%	70 - 130
4910570	ASL	Method Blank	Isobutylbenzene - Volatile	2017/03/22		98	%	70 - 130
			Benzene	2017/03/22	ND, RDL=0.0010		mg/L	
			Toluene	2017/03/22	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2017/03/22	ND, RDL=0.0010		mg/L	
			Total Xylenes	2017/03/22	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	2017/03/22	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	2017/03/22	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	2017/03/22	ND, RDL=0.010		mg/L	
4912260	BCD	Spiked Blank	Aliphatic >C10-C12	2017/03/28		90	%	30 - 130
			Aliphatic >C12-C16	2017/03/28		86	%	30 - 130
			Aliphatic >C16-C21	2017/03/28		97	%	30 - 130
			Aliphatic >C21-<C32	2017/03/28		95	%	30 - 130
			Aromatic >C10-C12	2017/03/28		110	%	30 - 130
			Aromatic >C12-C16	2017/03/28		107	%	30 - 130
			Aromatic >C16-C21	2017/03/28		107	%	30 - 130
			Aromatic >C21-<C32	2017/03/28		114	%	30 - 130
4912260	BCD	Method Blank	Isobutylbenzene - Extractable	2017/03/28		76	%	30 - 130
			n-Dotriacontane - Extractable	2017/03/28		96	%	30 - 130
			Aliphatic >C10-C12	2017/03/28	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	2017/03/28	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	2017/03/28	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	2017/03/28	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	2017/03/28	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	2017/03/28	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	2017/03/28	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	2017/03/28	ND, RDL=0.10		mg/L	

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

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Maxxam Job #: B757178
Report Date: 2017/03/30

Huntsman Ocean Sciences
Your P.O. #: 16582

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

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B7A2778V1-R2017-06-02_14-52-59_R006 = Hydrocarbon Analysis

- 17135-A4: 10% WAF
- 17135-B4: 3.2% CEWAF

Maxxam
A Bureau Veritas Group Company

Your P.O. #: 17545
Your C.O.C. #: 552304-05-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496528
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2778
Received: 2017/05/19, 10:42

Sample Matrix: Water
Samples Received: 2

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (AA PIRI)	2	2017/05/23	2017/05/26	ATL SOP 00116	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	1	2017/05/26	2017/05/26	ATL SOP 00120	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	1	2017/05/26	2017/05/30	ATL SOP 00120	Atl. RBCA v3.1 m
ModTPH (T2) Calc. for Water	2	N/A	2017/06/02	N/A	Atl. RBCA v3 m

Remarks:

Maxxam Analytics' laboratories are accredited to ISO/IEC 17025:2005 for specific parameters on scopes of accreditation. Unless otherwise noted, procedures used by Maxxam are based upon recognized Provincial, Federal or US method compendia such as CCME, MDDELCC, EPA, APHA.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported: unless indicated otherwise, associated sample data are not blank corrected.

Maxxam Analytics' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Maxxam has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Maxxam, unless otherwise agreed in writing.

Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods.
Results relate to samples tested.

Reference Method suffix "m" indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

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Your P.O. #: 17545
Your C.O.C. #: 552304-05-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496528
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2778
Received: 2017/05/19, 10:42

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226
==========

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Maxxam Job #: B7A2778
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		EKI527		EKI528		
Sampling Date		2017/05/15		2017/05/15		
COC Number		552304-05-01		552304-05-01		
	UNITS	17135-A4	RDL	17135-B4	RDL	QC Batch
Petroleum Hydrocarbons						
Benzene	mg/L	0.0047	0.0010	0.0014	0.0010	5000127
Toluene	mg/L	0.10 (1)	0.013	0.044	0.0010	5000127
Ethylbenzene	mg/L	0.020	0.0010	0.013	0.0010	5000127
Total Xylenes	mg/L	0.11	0.0020	0.075	0.0020	5000127
Aliphatic >C6-C8	mg/L	ND	0.010	ND	0.010	5000127
Aliphatic >C8-C10	mg/L	0.019	0.010	0.033	0.010	5000127
>C8-C10 Aromatics (-EX)	mg/L	0.050	0.010	0.060	0.010	5000127
Aliphatic >C10-C12	mg/L	ND	0.020	0.063	0.020	4994101
Aliphatic >C12-C16	mg/L	ND	0.098	0.16	0.099	4994101
Aliphatic >C16-C21	mg/L	ND	0.098	0.22	0.099	4994101
Aliphatic >C21-<C32	mg/L	ND	0.20	0.33	0.20	4994101
Aromatic >C10-C12	mg/L	0.079	0.020	0.16	0.020	4994101
Aromatic >C12-C16	mg/L	ND	0.098	0.24	0.099	4994101
Aromatic >C16-C21	mg/L	ND	0.098	0.18	0.099	4994101
Aromatic >C21-<C32	mg/L	ND	0.20	0.31	0.20	4994101
Modified TPH (Tier 2)	mg/L	ND	0.20	1.7	0.20	4990740
Reached Baseline at C32	mg/L	NA	N/A	Yes	N/A	4994101
Hydrocarbon Resemblance	mg/L	NA	N/A	COMMENT (2)	N/A	4994101
Surrogate Recovery (%)						
Isobutylbenzene - Extractable	%	92		110		4994101
n-Dotriacontane - Extractable	%	126 (3)		125 (3)		4994101
Isobutylbenzene - Volatile	%	87		78		5000127

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution. VPH dilution analysed past recommended hold time as per client request.
(2) One product in fuel / lube range. Unidentified compound(s) in fuel oil range.
(3) Elevated TEH RDL(s) due to limited sample.

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Maxxam Job #: B7A2778
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	0.7°C

Results relate only to the items tested.

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Maxxam Job #: B7A2778
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4994101	BCD	Spiked Blank	Aliphatic >C10-C12	2017/05/25		102	%	30 - 130
			Aliphatic >C12-C16	2017/05/25		100	%	30 - 130
			Aliphatic >C16-C21	2017/05/25		102	%	30 - 130
			Aliphatic >C21-<C32	2017/05/25		98	%	30 - 130
			Aromatic >C10-C12	2017/05/25		93	%	30 - 130
			Aromatic >C12-C16	2017/05/25		91	%	30 - 130
			Aromatic >C16-C21	2017/05/25		90	%	30 - 130
			Aromatic >C21-<C32	2017/05/25		90	%	30 - 130
4994101	BCD	Method Blank	Isobutylbenzene - Extractable	2017/05/25		89	%	30 - 130
			n-Dotriacontane - Extractable	2017/05/25		110	%	30 - 130
			Aliphatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
5000127	MS3	Spiked Blank	Isobutylbenzene - Volatile	2017/05/26		91	%	70 - 130
			Benzene	2017/05/26		97	%	70 - 130
			Toluene	2017/05/26		105	%	70 - 130
			Ethylbenzene	2017/05/26		105	%	70 - 130
			Total Xylenes	2017/05/26		106	%	70 - 130
5000127	MS3	Method Blank	Isobutylbenzene - Volatile	2017/05/26		90	%	70 - 130
			Benzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Toluene	2017/05/26	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Total Xylenes	2017/05/26	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	2017/05/26	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	2017/05/26	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	2017/05/26	ND, RDL=0.010		mg/L	

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

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Maxxam Job #: B7A2778
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

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B7A2270V1-R2017-06-02_14-15-42_R006 = Hydrocarbon Analysis (regression relationship used for estimating concentrations in 2017 studies)

- 17136-A1: Control seawater
- 17136-A2: 1% WAF
- 17136-A3: 3.2% WAF
- 17136-A4: 10% WAF
- 17136-A5: 32% WAF
- 17136-A6: 100% WAF
- 17136-B1: Corexit Control (nominal =15 mg/L)
- 17136-B2: 0.32% CEWAF
- 17136-B3: 1% CEWAF
- 17136-B4: 3.2% CEWAF
- 17136-B5: 10% CEWAF
- 17136-B6: 32% CEWAF

Your P.O. #: 17545
Your C.O.C. #: 552304-03-01, 552304-04-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496460
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2270
Received: 2017/05/18, 10:32

Sample Matrix: Water
Samples Received: 12

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (AA PIRI)	3	2017/05/23	2017/05/25	ATL SOP 00116	Atl. RBCA v3.1 m
TEH in Water (AA PIRI)	4	2017/05/23	2017/05/26	ATL SOP 00116	Atl. RBCA v3.1 m
TEH in Water (AA PIRI)	5	2017/05/23	2017/05/31	ATL SOP 00116	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	6	2017/05/26	2017/05/26	ATL SOP 00120	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	1	2017/05/26	2017/05/27	ATL SOP 00120	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	5	2017/05/26	2017/05/30	ATL SOP 00120	Atl. RBCA v3.1 m
ModTPH (T2) Calc. for Water	12	N/A	2017/06/02	N/A	Atl. RBCA v3 m

Remarks:

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All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported: unless indicated otherwise, associated sample data are not blank corrected.

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Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods.
Results relate to samples tested.

Reference Method suffix "m" indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

Your P.O. #: 17545
Your C.O.C. #: 552304-03-01, 552304-04-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496460
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2270
Received: 2017/05/18, 10:32

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Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226
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Maxxam Job #: B7A2270
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		EKF933		EKF934		EKF935		EKF936		
Sampling Date		2017/05/16		2017/05/16		2017/05/16		2017/05/16		
COC Number		552304-03-01		552304-03-01		552304-03-01		552304-03-01		
	UNITS	17136-A1	RDL	17136-A2	RDL	17136-A3	RDL	17136-A4	RDL	QC Batch
Petroleum Hydrocarbons										
Benzene	mg/L	ND	0.0010	ND	0.0010	0.0013	0.0010	0.0047	0.0010	5000127
Toluene	mg/L	ND	0.0010	0.0092	0.0010	0.029	0.0010	0.10 (1)	0.013	5000127
Ethylbenzene	mg/L	ND	0.0010	0.0016	0.0010	0.0052	0.0010	0.020	0.0010	5000127
Total Xylenes	mg/L	ND	0.0020	0.0088	0.0020	0.029	0.0020	0.10	0.0020	5000127
Aliphatic >C6-C8	mg/L	ND	0.010	ND	0.010	ND	0.010	ND	0.010	5000127
Aliphatic >C8-C10	mg/L	ND	0.010	ND	0.010	ND	0.010	0.019	0.010	5000127
>C8-C10 Aromatics (-EX)	mg/L	ND	0.010	ND	0.010	0.013	0.010	0.051	0.010	5000127
Aliphatic >C10-C12	mg/L	ND	0.020	ND	0.019	ND	0.020	ND	0.020	4994101
Aliphatic >C12-C16	mg/L	ND	0.10	ND	0.095	ND	0.099	ND	0.099	4994101
Aliphatic >C16-C21	mg/L	ND	0.10	ND	0.095	ND	0.099	ND	0.099	4994101
Aliphatic >C21-<C32	mg/L	ND	0.20	ND	0.19	ND	0.20	ND	0.20	4994101
Aromatic >C10-C12	mg/L	ND	0.020	ND	0.019	ND	0.020	0.078	0.020	4994101
Aromatic >C12-C16	mg/L	ND	0.10	ND	0.095	ND	0.099	ND	0.099	4994101
Aromatic >C16-C21	mg/L	ND	0.10	ND	0.095	ND	0.099	ND	0.099	4994101
Aromatic >C21-<C32	mg/L	ND	0.20	ND	0.19	ND	0.20	ND	0.20	4994101
Modified TPH (Tier 2)	mg/L	ND	0.20	ND	0.19	ND	0.20	ND	0.20	4990740
Reached Baseline at C32	mg/L	NA	N/A	NA	N/A	NA	N/A	NA	N/A	4994101
Hydrocarbon Resemblance	mg/L	NA	N/A	NA	N/A	NA	N/A	NA	N/A	4994101
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	88		86		88		88		4994101
n-Dotriacontane - Extractable	%	130 (2)		108 (2)		133 (3)		122 (2)		4994101
Isobutylbenzene - Volatile	%	90		90		87		87		5000127

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(2) Elevated TEH RDL(s) due to limited sample.
(3) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run. Elevated TEH RDL(s) due to limited sample.

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Maxxam Job #: B7A2270
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		EKF937		EKF938		EKF939		EKF940		
Sampling Date		2017/05/16		2017/05/16		2017/05/16		2017/05/16		
COC Number		552304-03-01		552304-03-01		552304-03-01		552304-03-01		
	UNITS	17136-A5	RDL	17136-A6	RDL	17136-B1	RDL	17136-B2	RDL	QC Batch
Petroleum Hydrocarbons										
Benzene	mg/L	0.015	0.0010	0.056	0.010	ND	0.0010	ND	0.0010	5000127
Toluene	mg/L	0.32 (1)	0.013	1.2 (2)	0.033	ND	0.0010	0.0036	0.0010	5000127
Ethylbenzene	mg/L	0.061	0.0010	0.23	0.010	ND	0.0010	0.0011	0.0010	5000127
Total Xylenes	mg/L	0.31	0.0020	1.2	0.020	ND	0.0020	0.0060	0.0020	5000127
Aliphatic >C6-C8	mg/L	ND	0.010	ND (3)	0.10	ND	0.010	ND	0.010	5000127
Aliphatic >C8-C10	mg/L	0.046	0.010	0.15 (3)	0.10	ND	0.010	ND	0.010	5000127
>C8-C10 Aromatics (-EX)	mg/L	0.14	0.010	0.52	0.10	ND	0.010	ND	0.010	5000127
Aliphatic >C10-C12	mg/L	ND	0.019	ND	0.020	ND	0.019	ND	0.020	4994101
Aliphatic >C12-C16	mg/L	ND	0.097	ND	0.10	ND	0.097	ND	0.099	4994101
Aliphatic >C16-C21	mg/L	ND	0.097	ND	0.10	ND	0.097	ND	0.099	4994101
Aliphatic >C21-<C32	mg/L	ND	0.19	ND	0.20	ND	0.19	ND	0.20	4994101
Aromatic >C10-C12	mg/L	0.16	0.019	0.52	0.020	ND	0.019	ND	0.020	4994101
Aromatic >C12-C16	mg/L	0.15	0.097	0.43	0.10	ND	0.097	ND	0.099	4994101
Aromatic >C16-C21	mg/L	ND	0.097	0.14	0.10	ND	0.097	ND	0.099	4994101
Aromatic >C21-<C32	mg/L	ND	0.19	ND	0.20	ND	0.19	ND	0.20	4994101
Modified TPH (Tier 2)	mg/L	0.50	0.19	1.8	0.20	ND	0.19	ND	0.20	4990740
Reached Baseline at C32	mg/L	Yes	N/A	Yes	N/A	NA	N/A	NA	N/A	4994101
Hydrocarbon Resemblance	mg/L	COMMENT (4)	N/A	COMMENT (5)	N/A	NA	N/A	NA	N/A	4994101
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	84		92		99		109		4994101
n-Dotriacontane - Extractable	%	125 (6)		125 (6)		141 (7)		129 (6)		4994101
Isobutylbenzene - Volatile	%	89		96 (3)		85		88		5000127

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(2) VPH analysis performed on previously opened vial.
(3) Elevated VPH RDL(s) due to sample dilution.
(4) Unidentified compound(s) in fuel oil range.
(5) One product in fuel oil range. Unidentified compound(s) in fuel oil range.
(6) Elevated TEH RDL(s) due to limited sample.
(7) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run. Elevated TEH RDL(s) due to limited sample.

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Maxxam Job #: B7A2270
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		EKF941		EKF942		EKF943		EKF944		
Sampling Date		2017/05/16		2017/05/16		2017/05/16		2017/05/16		
COC Number		552304-03-01		552304-03-01		552304-04-01		552304-04-01		
	UNITS	17136-B3	RDL	17136-B4	RDL	17136-B5	RDL	17136-B6	RDL	QC Batch
Petroleum Hydrocarbons										
Benzene	mg/L	ND	0.0010	0.0016	0.0010	0.0054	0.0010	0.019	0.010	5000127
Toluene	mg/L	0.012	0.0010	0.045	0.0010	0.15 (1)	0.013	0.50	0.010	5000127
Ethylbenzene	mg/L	0.0037	0.0010	0.013	0.0010	0.042	0.0010	0.12	0.010	5000127
Total Xylenes	mg/L	0.021	0.0020	0.075	0.0020	0.23	0.0020	0.67	0.020	5000127
Aliphatic >C6-C8	mg/L	0.018	0.010	ND	0.010	ND	0.010	ND	0.10	5000127
Aliphatic >C8-C10	mg/L	0.015	0.010	0.036	0.010	0.083	0.010	0.14	0.10	5000127
>C8-C10 Aromatics (-EX)	mg/L	0.019	0.010	0.060	0.010	0.17	0.010	0.40	0.10	5000127
Aliphatic >C10-C12	mg/L	ND	0.020	0.079	0.020	0.35	0.020	2.0	0.019	4994101
Aliphatic >C12-C16	mg/L	ND	0.098	0.24	0.099	1.2	0.099	5.5	0.097	4994101
Aliphatic >C16-C21	mg/L	ND	0.098	0.30	0.099	1.4	0.099	5.6	0.097	4994101
Aliphatic >C21-<C32	mg/L	ND	0.20	0.46	0.20	1.8	0.20	6.6	0.19	4994101
Aromatic >C10-C12	mg/L	0.083	0.020	0.16	0.020	0.37	0.020	0.95	0.019	4994101
Aromatic >C12-C16	mg/L	0.13	0.098	0.29	0.099	0.80	0.099	2.3	0.097	4994101
Aromatic >C16-C21	mg/L	0.10	0.098	0.26	0.099	0.85	0.099	2.6	0.097	4994101
Aromatic >C21-<C32	mg/L	ND	0.20	0.47	0.20	1.5	0.20	4.6	0.19	4994101
Modified TPH (Tier 2)	mg/L	0.37	0.20	2.3	0.20	8.5	0.20	31	0.19	4990740
Reached Baseline at C32	mg/L	Yes	N/A	Yes	N/A	No	N/A	No	N/A	4994101
Hydrocarbon Resemblance	mg/L	COMMENT (2)	N/A	COMMENT (3)	N/A	COMMENT (4)	N/A	COMMENT (5)	N/A	4994101
Surrogate Recovery (%)										
Isobutylbenzene - Extractable	%	101		105		105		91		4994101
n-Dotriacontane - Extractable	%	128 (6)		118 (7)		120 (7)		162 (8)		4994101
Isobutylbenzene - Volatile	%	85		83		71		97 (9)		5000127

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(2) One product in fuel oil range. Unidentified compound(s) in fuel oil range.
(3) One product in fuel / lube range. Unidentified compound(s) in fuel oil range.
(4) One product in fuel / lube range. Unidentified compound(s) in fuel / lube range.
(5) One product in fuel / lube range. Lube oil fraction. Unidentified compound(s) in fuel / lube range.
(6) TEH surrogate(s) not within acceptance limits. Samples tested had insufficient volume to repeat the analytical run. Elevated TEH RDL(s) due to limited sample.
(7) Elevated TEH RDL(s) due to limited sample.
(8) TEH surrogate(s) not within acceptance limits due to sample dilution / product interference. Elevated TEH RDL(s) due to limited sample.
(9) Elevated VPH RDL(s) due to sample dilution.

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GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	4.0°C

Results relate only to the items tested.

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Maxxam Job #: B7A2270
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4994101	BCD	Spiked Blank	Aliphatic >C10-C12	2017/05/25		102	%	30 - 130
			Aliphatic >C12-C16	2017/05/25		100	%	30 - 130
			Aliphatic >C16-C21	2017/05/25		102	%	30 - 130
			Aliphatic >C21-<C32	2017/05/25		98	%	30 - 130
			Aromatic >C10-C12	2017/05/25		93	%	30 - 130
			Aromatic >C12-C16	2017/05/25		91	%	30 - 130
			Aromatic >C16-C21	2017/05/25		90	%	30 - 130
			Aromatic >C21-<C32	2017/05/25		90	%	30 - 130
4994101	BCD	Method Blank	Isobutylbenzene - Extractable	2017/05/25		89	%	30 - 130
			n-Dotriacontane - Extractable	2017/05/25		110	%	30 - 130
			Aliphatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
5000127	MS3	Spiked Blank	Isobutylbenzene - Volatile	2017/05/26		91	%	70 - 130
			Benzene	2017/05/26		97	%	70 - 130
			Toluene	2017/05/26		105	%	70 - 130
			Ethylbenzene	2017/05/26		105	%	70 - 130
			Total Xylenes	2017/05/26		106	%	70 - 130
5000127	MS3	Method Blank	Isobutylbenzene - Volatile	2017/05/26		90	%	70 - 130
			Benzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Toluene	2017/05/26	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Total Xylenes	2017/05/26	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	2017/05/26	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	2017/05/26	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	2017/05/26	ND, RDL=0.010		mg/L	

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

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Maxxam Job #: B7A2270
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

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B7A2276V1-R2017-06-02_14-21-35_R006 = Hydrocarbon Analysis

- 17137-A4: 10% WAF
- 17137-B4: 3.17% CEWAF

Your P.O. #: 17545
Your C.O.C. #: 552304-06-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496467
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2276
Received: 2017/05/18, 10:27

Sample Matrix: Water
Samples Received: 2

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
TEH in Water (AA PIRI)	1	2017/05/23	2017/05/26	ATL SOP 00116	Atl. RBCA v3.1 m
TEH in Water (AA PIRI)	1	2017/05/23	2017/05/31	ATL SOP 00116	Atl. RBCA v3.1 m
VPH in Water (PIRI2)	2	2017/05/26	2017/05/30	ATL SOP 00120	Atl. RBCA v3.1 m
ModTPH (T2) Calc. for Water	2	N/A	2017/06/02	N/A	Atl. RBCA v3 m

Remarks:

Maxxam Analytics' laboratories are accredited to ISO/IEC 17025:2005 for specific parameters on scopes of accreditation. Unless otherwise noted, procedures used by Maxxam are based upon recognized Provincial, Federal or US method compendia such as CCME, MDDELCC, EPA, APHA.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported: unless indicated otherwise, associated sample data are not blank corrected.

Maxxam Analytics' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Maxxam has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Maxxam, unless otherwise agreed in writing.

Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods.
Results relate to samples tested.

Reference Method suffix "m" indicates test methods incorporate validated modifications from specific reference methods to improve performance.
* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

Your P.O. #: 17545
Your C.O.C. #: 552304-06-01

Attention:Chris Bridger

Huntsman Ocean Sciences
1 Lower Campus Road
St. Andrews, NB
CANADA E5B 2L7

Report Date: 2017/06/02
Report #: R4496467
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B7A2276
Received: 2017/05/18, 10:27

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Heather Macumber, Project Manager
Email: HMacumber@maxxam.ca
Phone# (902)420-0203 Ext:226
==========

This report has been generated and distributed using a secure automated process.
Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

Maxxam Job #: B7A2276
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

ATLANTIC RBCA HYDROCARBONS (WATER)

Maxxam ID		EKF958		EKF959		
Sampling Date		2017/05/17 12:45		2017/05/17 12:47		
COC Number		552304-06-01		552304-06-01		
	UNITS	17137-A4	RDL	17137-B4	RDL	QC Batch
Petroleum Hydrocarbons						
Benzene	mg/L	0.0050	0.0010	0.0015	0.0013	5000127
Toluene	mg/L	0.099 (1)	0.013	0.040	0.0013	5000127
Ethylbenzene	mg/L	0.022	0.0010	0.012	0.0013	5000127
Total Xylenes	mg/L	0.12	0.0020	0.063	0.0026	5000127
Aliphatic >C6-C8	mg/L	ND	0.010	ND	0.013	5000127
Aliphatic >C8-C10	mg/L	0.022	0.010	0.025	0.013	5000127
>C8-C10 Aromatics (-EX)	mg/L	0.057	0.010	0.049	0.013	5000127
Aliphatic >C10-C12	mg/L	ND	0.020	0.069	0.020	4994101
Aliphatic >C12-C16	mg/L	ND	0.10	0.25	0.098	4994101
Aliphatic >C16-C21	mg/L	ND	0.10	0.34	0.098	4994101
Aliphatic >C21-<C32	mg/L	ND	0.20	0.49	0.20	4994101
Aromatic >C10-C12	mg/L	0.078	0.020	0.16	0.020	4994101
Aromatic >C12-C16	mg/L	ND	0.10	0.26	0.098	4994101
Aromatic >C16-C21	mg/L	ND	0.10	0.25	0.098	4994101
Aromatic >C21-<C32	mg/L	ND	0.20	0.45	0.20	4994101
Modified TPH (Tier 2)	mg/L	ND	0.20	2.3	0.20	4990740
Reached Baseline at C32	mg/L	NA	N/A	Yes	N/A	4994101
Hydrocarbon Resemblance	mg/L	NA	N/A	COMMENT (2)	N/A	4994101
Surrogate Recovery (%)						
Isobutylbenzene - Extractable	%	87		104		4994101
n-Dotriacontane - Extractable	%	124 (3)		127 (3)		4994101
Isobutylbenzene - Volatile	%	88		98 (4)		5000127

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
ND = Not detected
N/A = Not Applicable
(1) VPH analysis performed on previously opened vial. Elevated VPH RDL(s) due to sample dilution.
(2) One product in fuel / lube range. Unidentified compound(s) in fuel oil range.
(3) Elevated TEH RDL(s) due to limited sample.
(4) VPH analysis performed on previously opened vial.

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Maxxam Job #: B7A2276
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	7.0°C

Results relate only to the items tested.

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Maxxam Job #: B7A2276
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

QUALITY ASSURANCE REPORT

QA/QC Batch	Init	QC Type	Parameter	Date Analyzed	Value	Recovery	UNITS	QC Limits
4994101	BCD	Spiked Blank	Aliphatic >C10-C12	2017/05/25		102	%	30 - 130
			Aliphatic >C12-C16	2017/05/25		100	%	30 - 130
			Aliphatic >C16-C21	2017/05/25		102	%	30 - 130
			Aliphatic >C21-<C32	2017/05/25		98	%	30 - 130
			Aromatic >C10-C12	2017/05/25		93	%	30 - 130
			Aromatic >C12-C16	2017/05/25		91	%	30 - 130
			Aromatic >C16-C21	2017/05/25		90	%	30 - 130
			Aromatic >C21-<C32	2017/05/25		90	%	30 - 130
4994101	BCD	Method Blank	Isobutylbenzene - Extractable	2017/05/25		89	%	30 - 130
			n-Dotriacontane - Extractable	2017/05/25		110	%	30 - 130
			Aliphatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aliphatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aliphatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
			Aromatic >C10-C12	2017/05/25	ND, RDL=0.010		mg/L	
			Aromatic >C12-C16	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C16-C21	2017/05/25	ND, RDL=0.050		mg/L	
			Aromatic >C21-<C32	2017/05/25	ND, RDL=0.10		mg/L	
5000127	MS3	Spiked Blank	Isobutylbenzene - Volatile	2017/05/26		91	%	70 - 130
			Benzene	2017/05/26		97	%	70 - 130
			Toluene	2017/05/26		105	%	70 - 130
			Ethylbenzene	2017/05/26		105	%	70 - 130
			Total Xylenes	2017/05/26		106	%	70 - 130
5000127	MS3	Method Blank	Isobutylbenzene - Volatile	2017/05/26		90	%	70 - 130
			Benzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Toluene	2017/05/26	ND, RDL=0.0010		mg/L	
			Ethylbenzene	2017/05/26	ND, RDL=0.0010		mg/L	
			Total Xylenes	2017/05/26	ND, RDL=0.0020		mg/L	
			Aliphatic >C6-C8	2017/05/26	ND, RDL=0.010		mg/L	
			Aliphatic >C8-C10	2017/05/26	ND, RDL=0.010		mg/L	
			>C8-C10 Aromatics (-EX)	2017/05/26	ND, RDL=0.010		mg/L	

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

Surrogate: A pure or isotopically labeled compound whose behavior mirrors the analytes of interest. Used to evaluate extraction efficiency.

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Maxxam Job #: B7A2276
Report Date: 2017/06/02

Huntsman Ocean Sciences
Your P.O. #: 17545

VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Rosemarie MacDonald, Scientific Specialist (Organics)

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2018 RPC

267249-OAS = Hydrocarbon Analysis (regression relationship used for estimating concentrations in 2018 studies)

- Bottle #4 = Whole Oil
- A1 = Control Seawater
- AL = Low WAF 32%
- AM = Medium WAF 56%
- AH = High WAF 100%
- B1 = Corexit Control (nominal = 15 mg/L)
- BL = Low CEWAF 18%
- BM = Medium CEWAF 32%
- BH = High CEWAF 56%

Report ID: 267249-OAS
Report Date: 20-Apr-18
Date Received: 28-Mar-18

CERTIFICATE OF ANALYSIS

for

Huntsman Marine Science Centre
1 Lower Campus Road
St. Andrews, NB E5B 2L7

rpc
921 College Hill Rd
Fredericton NB
Canada E3B 6Z9
Tel: 506.452.1212
Fax: 506.452.0594
www.rpc.ca

Attention: Ben De Jourdan
Project #: HMSC-040-GM-050

Hydrocarbon Analysis in Soil (Atlantic MUST)

RPC Sample ID:			267249-9
Client Sample ID:			Bottle #4
Date Sampled:			27-Mar-18
Matrix:			oil
Analytes	Units	RL	
Benzene	mg/kg	0.005	51
Toluene	mg/kg	0.05	2500
Ethylbenzene	mg/kg	0.01	1200
Xylenes	mg/kg	0.05	6100
VPH C6-C10 (Less BTEX)	mg/kg	2.5	69000
EPH >C10-C16	mg/kg	12	180000
EPH >C16-C21	mg/kg	12	130000
EPH >C21-C32	mg/kg	12	180000
EPH (>C16-C32)	mg/kg	12	310000
Modified TPH Tier 1	mg/kg	21	560000
VPH Surrogate (IBB)	%		comment
EPH Surrogate (IBB)	%		comment
EPH Surrogate (C32)	%		97
Resemblance			Crude Oil
Return to Baseline at C32			Yes
Moisture Content	%		comment

This report relates only to the sample(s) and information provided to the laboratory.
RL = Reporting Limit; Soil results are expressed on a dry weight basis.

Bruce Phillips
Department Head
Organic Analytical Services

Angela Colford
Lab Supervisor
Organic Analytical Services

Report ID: 267249-OAS
Report Date: 20-Apr-18
Date Received: 28-Mar-18

CERTIFICATE OF ANALYSIS

for
Huntsman Marine Science Centre
1 Lower Campus Road
St. Andrews, NB E5B 2L7

rpc
921 College Hill Rd
Fredericton NB
Canada E3B 6Z9
Tel: 506.452.1212
Fax: 506.452.0594
www.rpc.ca

Attention: Ben De Jourdan
Project #: HMSC-040-GM-050

Hydrocarbon Analysis in Water (Atlantic MUST)

RPC Sample ID:			267249-1	267249-2	267249-3	267249-4	267249-5	267249-6
Client Sample ID:			A1	AL	AM	AH	B1	BL
Date Sampled:			27-Mar-18	27-Mar-18	27-Mar-18	27-Mar-18	27-Mar-18	27-Mar-18
Matrix:			water	water	water	water	water	water
Analytes	Units	RL						
Benzene	mg/L	0.001	< 0.001	0.017	0.030	0.057	< 0.001	0.010
Toluene	mg/L	0.001	< 0.001	0.27	0.46	1.1	< 0.001	0.20
Ethylbenzene	mg/L	0.001	< 0.001	0.052	0.089	0.21	< 0.001	0.046
Xylenes	mg/L	0.001	< 0.001	0.27	0.47	1.1	< 0.001	0.25
VPH C6-C10 (Less BTEX)	mg/L	0.01	< 0.01	0.44	0.74	1.7	0.02	0.58
EPH >C10 - C16	mg/L	0.05	< 0.05	0.27	0.46	0.85	< 0.05	2.0
EPH >C16 - C21	mg/L	0.05	< 0.05	0.06	0.09	0.18	0.10	1.3
EPH >C21-C32	mg/L	0.1	< 0.1	< 0.1	< 0.1	0.2	0.5	1.9
Modified TPH Tier 1	mg/L	0.1	< 0.1	0.8	1.3	2.9	0.6	5.8
VPH Surrogate (IBB)	%		101	104	101	106	104	108
EPH Surrogate (IBB)	%		110	111	113	111	110	112
EPH Surrogate (C32)	%		114	113	112	119	115	111
Resemblance			ND	PAH	PAH	PAH	UP	Crude Oil
Return to Baseline at C32			Yes	Yes	Yes	Yes	Yes	Yes

This report relates only to the sample(s) and information provided to the laboratory.
RL = Reporting Limit

Bruce Phillips
Department Head
Organic Analytical Services

Angela Colford
Lab Supervisor
Organic Analytical Services

Report ID: 267249-OAS
Report Date: 20-Apr-18
Date Received: 28-Mar-18

CERTIFICATE OF ANALYSIS

for
Huntsman Marine Science
Centre
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St. Andrews, NB E5B 2L7

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921 College Hill Rd
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Canada E3B 6Z9
Tel: 506.452.1212
Fax: 506.452.0594
www.rpc.ca

Attention: Ben De Jourdan
Project #: HMSC-040-GM-050

Hydrocarbon Analysis in Water (Atlantic MUST)

RPC Sample ID:			267249-7	267249-8
Client Sample ID:			BM	BH
Date Sampled:			27-Mar-18	27-Mar-18
Matrix:			water	water
Analytes	Units	RL		
Benzene	mg/L	0.001	0.018	0.032
Toluene	mg/L	0.001	0.47	0.76
Ethylbenzene	mg/L	0.001	0.11	0.16
Xylenes	mg/L	0.001	0.54	0.86
VPH C6-C10 (Less BTEX)	mg/L	0.01	1.3	2.1
EPH >C10 - C16	mg/L	0.05	3.3	6.3
EPH >C16 - C21	mg/L	0.05	2.2	4.2
EPH >C21-C32	mg/L	0.1	3.1	6.0
Modified TPH Tier 1	mg/L	0.1	9.9	19
VPH Surrogate (IBB)	%		108	107
EPH Surrogate (IBB)	%		117	117
EPH Surrogate (C32)	%		112	114
Resemblance			Crude Oil	Crude Oil
Return to Baseline at C32			Yes	Yes

CERTIFICATE OF ANALYSIS

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Method Summary

OAS-HC03:The Determination of Petroleum Hydrocarbons (Atlantic MUST) in Soil (VPH)
OAS-HC03: Determination of Petroleum Hydrocarbons (Atlantic MUST) in Soil (EPH)
OAS-HC04: The Determination of Petroleum Hydrocarbons (Atlantic MUST) in Water(VPH)
OAS-HC04: Determination of Petroleum Hydrocarbons (Atlantic MUST) in Water (EPH)

Resemblance Legend

Resemblance Code	Resemblance
AG	Aviation Gasoline
COMMENT	See General Report Comments
FO	Fuel Oil Fraction
FO.LO	Fuel Oil and Lube Oil Fraction
G	Gasoline Fraction
LO	Lube Oil Fraction
ND	Not Detected
NR	No Resemblance (not-petrogenic in origin)
NRLR	No Resemblance in the lube oil range (>C21-C32).
OP	One Product (unidentified)

Resemblance Code	Resemblance
PAH	Possible PAHs Detected
PG	Possible Gasoline Fraction
PLO	Possible Lube Oil Fraction
PWFO	Possible Weathered Fuel Oil Fraction
PWG	Possible Weathered Gasoline Fraction
TO	Tranformer Oil
UP	Unknown Peaks
WFO	Weathered Fuel Oil Fraction
WG	Weathered Gasoline Fraction

General Report Comments

VPH / EPH surrogate(s) unavailable due to product interference/sample dilution.
267249-9 - Moisture content not applicable due to sample matrix (oil).
Return to Baseline: Samples are considered to have returned to baseline if the area from C32-C36 is less than 10% of the area from C10-C32.

Report ID: 267249-OAS
Report Date: 20-Apr-18
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Project #: HMSC-040-GM-050

QA/QC Report

RPC Sample ID:			BLANKC2473	BLANKC2508	SPIKEC2473	SPIKEC2508
Type:			VPH	EPH	VPH	EPH
Matrix:			soil	soil	soil	soil
Analytes	Units	RL			% Recovery	% Recovery
Benzene	mg/kg	0.005	< 0.005	-	100%	-
Toluene	mg/kg	0.05	< 0.05	-	106%	-
Ethylbenzene	mg/kg	0.01	< 0.01	-	112%	-
Xylenes	mg/kg	0.05	< 0.05	-	108%	-
VPH C6-C10 (Less BTEX)	mg/kg	2.5	< 2.5	-	102%	-
EPH >C10-C16	mg/kg	12	-	< 12	-	-
EPH >C16-C21	mg/kg	12	-	< 12	-	-
EPH >C21-C32	mg/kg	12	-	< 12	-	-
EPH >C10-C32	mg/kg	21	-	-	-	99%

RL = Reporting Limit

Report ID: 267249-OAS
Report Date: 20-Apr-18
Date Received: 28-Mar-18

CERTIFICATE OF ANALYSIS

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Project #: HMSC-040-GM-050

QA/QC Report

RPC Sample ID:			BLANKC2472	BLANKC2480	BLANKC2484	SPIKEC2472	SPIKEC2480	SPIKEC2484
Type:			VPH	EPH	EPH	VPH	EPH	EPH
Matrix:			water	water	water	water	water	water
Analytes	Units	RL				% Recovery	% Recovery	% Recovery
Benzene	mg/L	0.001	< 0.001	-	-	109%	-	-
Toluene	mg/L	0.001	< 0.001	-	-	109%	-	-
Ethylbenzene	mg/L	0.001	< 0.001	-	-	107%	-	-
Xylenes	mg/L	0.001	< 0.001	-	-	107%	-	-
VPH C6-C10 (Less BTEX)	mg/L	0.01	< 0.01	-	-	103%	-	-
EPH >C10 - C16	mg/L	0.05	-	< 0.05	< 0.05	-	-	-
EPH >C16 - C21	mg/L	0.05	-	< 0.05	< 0.05	-	-	-
EPH >C21-C32	mg/L	0.1	-	< 0.1	< 0.1	-	-	-
EPH >C10 - C32	mg/L		-	-	-	-	111%	104%

RL = Reporting Limit

Report ID: 267249-OAS
Report Date: 20-Apr-18
Date Received: 28-Mar-18

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Project #: HMSC-040-GM-050

Summary of Date Analyzed

	VPH		EPH	
RPC Sample ID	**Extracted**	**Analyzed**	**Extracted**	**Analyzed**
267249-1	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-2	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-3	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-4	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-5	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-6	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-7	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-8	29-Mar-18	29-Mar-18	29-Mar-18	3-Apr-18
267249-9	29-Mar-18	29-Mar-18	9-Apr-18	10-Apr-18

267249-OAS-B = PAH and Alkyl PAH Analysis (regression relationship used for estimating concentrations in 2018 studies)

- Bottle #4 = Whole Oil
- A1 = Control Seawater
- AL = Low WAF 32%
- AM = Medium WAF 56%
- AH = High WAF 100%
- B1 = Corexit Control (nominal = 15 mg/L)
- BL = Low CEWAF 18%
- BM = Medium CEWAF 32%
- BH = High CEWAF 56%

Report ID: 267249-OAS-B
Report Date: 20-Apr-18
Date Received: 28-Mar-18

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Attention: Ben De Jourdan
Fax #:
benjamin.dejourdan@huntsmanmarine.ca

Project #: HMSC-040-GM-050

PAH and Alkyl PAH Analysis in Water

RPC Sample ID:			267249-1	267249-2	267249-3	267249-4
Client Sample ID:			A1	AL	AM	AH
Date Sampled:			27-Mar-18	27-Mar-18	27-Mar-18	27-Mar-18
Date Extracted:			05-Apr-18	05-Apr-18	05-Apr-18	05-Apr-18
Date Analyzed:			10-Apr-18	10-Apr-18	10-Apr-18	10-Apr-18
Matrix:			water	water	water	water
Analytes	Units	RL				
Naphthalene	µg/L	0.05	< 0.05	46	80	150
Acenaphthylene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.5
Acenaphthene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.5
Fluorene	µg/L	0.01	< 0.01	1.0	1.6	2.9
Phenanthrene	µg/L	0.01	< 0.01	1.0	1.7	2.8
Anthracene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Fluoranthene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Pyrene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(a)anthracene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Chrysene/Triphenylene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(b)fluoranthene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(k)fluoranthene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(e)pyrene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(a)pyrene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Indenopyrene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Bz(g,h,i)perylene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Dibz(a,h)anthracene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
C1-Naphthalenes	µg/L	0.10	< 0.10	25	42	78
C2-Naphthalenes	µg/L	0.10	< 0.10	8.0	13	25
C3-Naphthalenes	µg/L	0.10	< 0.10	1.5	2.2	4.0
C1-Phenanthrenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
C2-Phenanthrenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
C3-Phenanthrenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
Dibenzothiophene	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
C1-Dibenzothiophenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
C2-Dibenzothiophenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
C3-Dibenzothiophenes	µg/L	0.10	< 0.10	< 1.0	< 2.0	< 4.0
1-methylnaphthalene	µg/L	0.05	< 0.05	22	37	66
2-methylnaphthalene	µg/L	0.05	< 0.05	19	33	60
Perylene	µg/L	0.01	< 0.01	< 0.1	< 0.2	< 0.4
Biphenyl	µg/L	0.05	< 0.05	3.1	5.1	9.3
Surrogate Recoveries						
2-fluorobiphenyl	%	-	88	94	96	91
p-terphenyl-d14	%	-	104	106	109	111

This report relates only to the sample(s) and information provided to the laboratory.
Method: Solvent extraction followed by GC/MS analysis; based on USEPA 3510C/8270C.
RL = Reporting Limit
Elevated RL's due to sample dilution.

Identification of alkyl PAHs and dibenzothiophenes was based on GC-MSD mass spectral library match. Quantification for alkyl PAHs is based on the response of the parent molecule. Quantitation for Dibenzothiophenes is based on the response of phenanthrene.

Bruce Phillips
Dept. Head
Organic Analytical Services

Angela Colford
Lab Supervisor
Organic Analytical Services

Report ID: 267249-OAS-B
Report Date: 20-Apr-18
Date Received: 28-Mar-18

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Attention: Ben De Jourdan
Fax #:
benjamin.dejourdan@huntsmanmarine.ca

Project #: HMSC-040-GM-050

PAH and Alkyl PAH Analysis in Water

RPC Sample ID:			267249-5	267249-6	267249-7	267249-8	Blank	Spike Rec. (%)
Client Sample ID:			B1	BL	BM	BH		
Date Sampled:			27-Mar-18	27-Mar-18	27-Mar-18	27-Mar-18		
Date Extracted:			05-Apr-18	05-Apr-18	05-Apr-18	05-Apr-18	05-Apr-18	05-Apr-18
Date Analyzed:			10-Apr-18	10-Apr-18	10-Apr-18	10-Apr-18	10-Apr-18	10-Apr-18
Matrix:			water	water	water	water	water	water
Analytes	Units	RL						
Naphthalene	µg/L	0.05	< 0.05	39	67	120	< 0.05	86
Acenaphthylene	µg/L	0.01	< 0.01	0.37	0.6	1.1	< 0.01	94
Acenaphthene	µg/L	0.01	< 0.01	0.45	0.7	1.3	< 0.01	87
Fluorene	µg/L	0.01	< 0.01	4.1	6.8	12	< 0.01	92
Phenanthrene	µg/L	0.01	< 0.01	8.2	14	24	< 0.01	99
Anthracene	µg/L	0.01	< 0.01	< 0.05	< 0.1	< 0.1	< 0.01	91
Fluoranthene	µg/L	0.01	< 0.01	0.25	0.4	0.7	< 0.01	94
Pyrene	µg/L	0.01	< 0.01	0.38	0.7	1.2	< 0.01	90
Bz(a)anthracene	µg/L	0.01	< 0.01	< 0.05	< 0.1	< 0.1	< 0.01	95
Chrysene/Triphenylene	µg/L	0.01	< 0.01	< 0.05	< 0.1	< 0.1	< 0.01	105
Bz(b)fluoranthene	µg/L	0.01	< 0.01	0.10	0.2	0.3	< 0.01	105
Bz(k)fluoranthene	µg/L	0.01	< 0.01	0.10	0.2	0.3	< 0.01	105
Bz(e)pyrene	µg/L	0.01	< 0.01	0.60	1.0	1.8	< 0.01	100
Bz(a)pyrene	µg/L	0.01	< 0.01	0.06	0.1	0.1	< 0.01	101
Indenopyrene	µg/L	0.01	< 0.01	< 0.05	< 0.1	< 0.1	< 0.01	99
Bz(g,h,i)perylene	µg/L	0.01	< 0.01	0.08	0.2	0.3	< 0.01	109
Dibz(a,h)anthracene	µg/L	0.01	< 0.01	0.05	< 0.1	0.2	< 0.01	112
C1-Naphthalenes	µg/L	0.10	< 0.10	47	90	160	< 0.10	-
C2-Naphthalenes	µg/L	0.10	< 0.10	47	88	160	< 0.10	-
C3-Naphthalenes	µg/L	0.10	< 0.10	23	41	79	< 0.10	-
C1-Phenanthrenes	µg/L	0.10	< 0.10	9.5	18	32	< 0.10	-
C2-Phenanthrenes	µg/L	0.10	< 0.10	10	19	32	< 0.10	-
C3-Phenanthrenes	µg/L	0.10	< 0.10	4.3	7.9	13	< 0.10	-
Dibenzothiophene	µg/L	0.10	< 0.10	< 0.5	< 0.1	< 1.0	< 0.10	-
C1-Dibenzothiophenes	µg/L	0.10	< 0.10	1.7	3.1	5.8	< 0.10	-
C2-Dibenzothiophenes	µg/L	0.10	< 0.10	1.1	2.0	3.6	< 0.10	-
C3-Dibenzothiophenes	µg/L	0.10	< 0.10	< 0.5	< 1.0	< 1.0	< 0.10	-
1-methylnaphthalene	µg/L	0.05	< 0.05	39	67	120	< 0.05	80
2-methylnaphthalene	µg/L	0.05	< 0.05	33	57	100	< 0.05	83
Perylene	µg/L	0.01	< 0.01	< 0.05	< 0.1	< 0.1	< 0.01	109
Biphenyl	µg/L	0.05	< 0.05	7.9	15	27	< 0.05	87
Surrogate Recoveries								
2-fluorobiphenyl	%	-	94	84	81	84	93	91
p-terphenyl-d14	%	-	100	120	120	96	107	98

Report ID: 267249-OAS-B
Report Date: 20-Apr-18
Date Received: 28-Mar-18

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Attention: Ben De Jourdan
Fax #:
benjamin.dejourdan@huntsmanmarine.ca

Project #: HMSC-040-GM-050

PAH and Alkyl PAH Analysis in Oil

RPC Sample ID:			267249-9	Blank	Spike Rec. (%)
Client Sample ID:			Bottle #4		
Date Sampled:			27-Mar-18		
Date Extracted:			09-Apr-18	09-Apr-18	09-Apr-18
Date Analyzed:			17-Apr-18	17-Apr-18	17-Apr-18
Matrix:			oil	other	other
Analytes	Units	RL			
Naphthalene	µg/g	0.10	730	< 0.10	96
Acenaphthylene	µg/g	0.10	13	< 0.10	85
Acenaphthene	µg/g	0.10	15	< 0.10	92
Fluorene	µg/g	0.10	130	< 0.10	94
Phenanthrene	µg/g	0.10	240	< 0.10	96
Anthracene	µg/g	0.10	< 2.0	< 0.10	96
Fluoranthene	µg/g	0.10	5.4	< 0.10	100
Pyrene	µg/g	0.10	12	< 0.10	102
Bz(a)anthracene	µg/g	0.10	< 2.0	< 0.10	110
Chrysene/Triphenylene	µg/g	0.10	< 2.0	< 0.10	105
Bz(b)fluoranthene	µg/g	0.10	3.7	< 0.10	103
Bz(k)fluoranthene	µg/g	0.10	2.0	< 0.10	103
Bz(e)pyrene	µg/g	0.10	13	< 0.10	107
Bz(a)pyrene	µg/g	0.10	< 2.0	< 0.10	107
Indenopyrene	µg/g	0.10	< 2.0	< 0.10	107
Bz(g,h,i)perylene	µg/g	0.10	2.5	< 0.10	86
Dibz(a,h)anthracene	µg/g	0.10	< 2.0	< 0.10	126
C1-Naphthalenes	µg/g	0.10	1300	< 0.10	-
C2-Naphthalenes	µg/g	0.10	1500	< 0.10	-
C3-Naphthalenes	µg/g	0.10	750	< 0.10	-
C1-Phenanthrenes	µg/g	0.10	280	< 0.10	-
C2-Phenanthrenes	µg/g	0.10	300	< 0.10	-
C3-Phenanthrenes	µg/g	0.10	130	< 0.10	-
Dibenzothiophene	µg/g	0.10	< 2.0	< 0.10	-
C1-Dibenzothiophenes	µg/g	0.10	50	< 0.10	-
C2-Dibenzothiophenes	µg/g	0.10	32	< 0.10	-
C3-Dibenzothiophenes	µg/g	0.10	< 2.0	< 0.10	-
1-methylnaphthalene	µg/g	0.10	1200	< 0.10	-
2-methylnaphthalene	µg/g	0.10	1000	< 0.10	-
Perylene	µg/g	0.10	< 2.0	< 0.10	-
Biphenyl	µg/g	0.10	230	< 0.10	-
Surrogate Recoveries					
2-fluorobiphenyl	%	-	99	80	85
p-terphenyl-d14	%	-	77	87	86

This report relates only to the sample(s) and information provided to the laboratory.
Method: Solvent extraction followed by GC/MS analysis; based on USEPA 3510C/8270C.
RL = Reporting Limit
Raised RL's due to sample dilution.
Identification of alkyl PAHs and dibenzothiophenes was based on GC-MSD mass spectral library match. Quantification for alkyl PAHs is based on the response of the parent molecule. Quantitation for Dibenzothiophenes is based on the response of phenanthrene.

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Organic Analytical Services

Angela Colford
Lab Supervisor
Organic Analytical Services

2018 EMBSI BE-SPME

VERIFIED 22 May 2018

Study **Huntsman Marine Science Center**

Apr-18

BE-SPME

[µmol as 2,3 dimethylnaphthalene / mL PDMS]

EMBSI ID	Sample	analyzed 25 Jan 2018				
	Description	rep-1	rep-2	rep-3	MEAN	StDev
-						
MRD-18-621	HMSC Filtered Seawater (dilution water) (D)	2.70	nd			
MRD-18-621	HMSC Corexit Control (H)	3.35	nd			
MRD-18-621	HMSC 32% WAF (C)	14.4	13.6		**14.0**	**0.6**
MRD-18-621	HMSC 18% CEWAF (G)	32.8	26.3		**29.6**	**4.6**
MRD-18-621	HMSC 56% WAF (B)	22.7	22.5		**22.6**	**0.1**
MRD-18-621	HMSC 32% CEWAF (F)	33.0	31.3		**32.2**	**1.2**
MRD-18-621	HMSC 100% WAF (A)	38.5	34.1		**36.3**	**3.1**
MRD-18-621	HMSC 56% CEWAF (E)	36.2	44.5		**40.4**	**5.9**
MRD-18-621	HMSC 100% CEWAF	61.2	40.3		51	10

note: Each sample vial preserved with 0.2 g od Sodium Bisulfate Monohydrate in seawater samples

practical quantitation limit (PQL) ~ 0.5 µmol as 2,3 dimethylnaphthalene / mL PDMS

nd = not detected

calculated by/date:

verified by/date: