PROJECT INFORMATION

Project Director's Name*	Christoph Aeppli
Organization*	Bigelow Laboratory for Ocean Sciences
Project Title*	Assessing Toxicity of Oil Weathered on the Sea Surface: The Importance of Oil Photo- Products
Reporting Period*	1/15/2018 - 4/15/2022

Note to Grantees: In sections 1 to 5, we ask you to highlight your accomplishments (including outputs and outcomes) through this grant award. These sections of the final grant report will be made available to the public.

1. GOALS AND ACCOMPLISHMENTS

1.1 Please restate the goals and objectives of your project.*

This project tested the hypothesis that oil photo-products lead to significant health risks and organism injury. Three critical aspects of their toxicity were investigated: (i) What are the formation rates and environmental concentrations of oil photo-products? (ii) Which products can be taken up by organisms and to what extent? (iii) What are their acute and latent sub-lethal effects? To answer these questions, this project had five parts:

PART I: DETERMINE THE PHOTO-OXIDATION OF OILS
The first goal of this part was to better understand the dependence of oil photooxidation on the intensity and spectrum of sunlight. This wavelength- and intensity-dependence of oil photo-oxidation is necessary information for the incorporation of photooxidation into oil spill models (Part IV). The second goal of this part was to develop methods for controlled photooxidation of oil to be used for toxicity tests.

PART II: CHARACTERIZE OIL PHOTO-PRODUCTS AND THEIR BIOAVAILABILITY
The goal of this part was to investigate and quantify the sunlight-induced degree of oil photooxidation (bulk-level measurements) and of oil photoproduct formation (molecular measurements). Second, the bioavailability of oil photoproducts was investigated.

PART III: DETERMINE THE TOXIC RESPONSE OF PHOTOOXIDIZED OIL ON MODEL AQUATIC ORGANISMS

The overarching goals of Part III were to investigate the biological effects of Macondo oil in the Gulf killifish. These biological effects included: a) assessing the impacts of photo-oxidized oil on embryo development and hatch, and b) characterizing the latent effects of oil exposure on Gulf killifish later in life or to fish in the next generation, including assessing the ability of the oil to impair cardiovascular and craniofacial development and swimming behavior, and c) determine the gene expression response of Gulf killifish to exposure of photooxidized oil.

PART IV: INTEGRATE PHOTO-PRODUCTS FORMATION AND EFFECTS INTO OIL SPILL MODELS

The existing oil trajectory and fate model, SIMAP (Spill Impact Model Application Package) used for this effort included major transport and fate processes: spreading, evaporation of volatile oil components from surface oil, transport on the surface and in the water column, turbulent diffusion, emulsification, entrainment of oil as droplets into the water column due to waves (either without or facilitated by dispersant application), dissolution of soluble and semi-soluble hydrocarbon components, volatilization of dissolved hydrocarbons from the surface water, adherence of oil droplets to suspended particulate matter (SPM), adsorption of semi-soluble hydrocarbons to SPM, sedimentation, stranding on shorelines, and biodegradation (based on component-specific biodegradation rates). The oil fate model computes concentrations over space and time. The SIMAP model’s predictions of floating oil amounts and in-water concentrations have been validated by comparison to field observations and measurements after oil spills. The time-varying exposures of aquatic organisms to oil components are estimated using the organism behavior-based exposure model in the SIMAP biological effects model system. A summed toxic unit approach is used to address dynamic exposures that exhibit spatially and temporally changing compositions of the dissolved oil component mixture to which biota are exposed. The goal was to update and test the model to include photo-oxidation in the oil fate model and photoproducts in the exposure and biological effects (acute toxicity) model.

PART V: ESTABLISH ANALYTICAL METHODS FOR CHARACTERIZATION OF PHOTOOXIDZED OIL

The goal of this part was to advance the development and standardization of chemical analysis of oil photoproducts. Both bulk and molecular measures of oil photooxidation were included.

1.2 Describe the accomplishments of your project. You should include both the anticipated accomplishments that you outlined in your project proposal as well as any *unanticipated* accomplishments that have since occurred. Describe any activities you have conducted, programmatic progress made, or project benchmarks and milestones met.*

PART I: OIL PHOTOOXIDATION

- ACHIEVEMENT 1: A method was developed for the production of a large volume of photooxidized oil under controlled conditions. This method was used to produce a large volume of oil to be used in toxicity incubations.

- ACHIEVEMENT 2: An approach was validated to characterize wavelength dependence of photochemical reactions using light-emitting diodes. This approach was then used to characterize the production of water-soluble products from the photochemical oxidation of Macondo Well oil.

- ACHIEVEMENT 3: The team determined how sunlight influences the rate of oil photochemical oxidation. Furthermore, it was determined how the dose and spectrum of sunlight influenced the rate of oil photochemical oxidation. The results indicate that over long periods of time (> weeks) the apparent quantum yield (AQY) decreases by up to a factor of two. This result is more relevant for photochemical oxidation of stranded oil, rather than floating oil, and might contribute to the persistence of oil residues washed up on shore. These data were then used in Part IV to incorporate oil photooxidation in oil spill modeling.

PART II: OIL PHOTOPRODUCTS

- ACHIEVEMENT 1: Quantifying bioaccumulative products using biomimetic extraction. We found that the use of solid-phase microextraction (SPME) is suitable to estimate the bioaccumulation of compounds. This technique is also referred to as ‘biomimetic extraction’ (BE). We, therefore, started using this method for analyzing water samples used for the killifish embryo exposure (analysis still ongoing). Furthermore, we also analyzed embryos produced in Part III to quantify bioaccumulative oil in killifish embryos when oil droplets are present; our results are part of the manuscript to be submitted shortly (see next section, Part III report).

- ACHIEVEMENT 2: Preparative isolation of photoproduct-containing fractions from photooxidized oil. We performed the fractionation of photo-oxidized oil into hydrocarbon and photo-products fractions, that were used in Part III for embryo exposures.

- ACHIEVEMENT 3: Physico-Chemical Properties Of Oil Photoproducts: The physico-chemical properties of approx. 400 model photo-products have been calculated using computational tools. These data are used for estimating the fate of photooxidized oil for oil spill modeling. The data and results of these efforts will be published in a forthcoming manuscript.

- ACHIEVEMENT 4: Synthesis: A review and a book chapter summarizing the current knowledge of oil photoproducts formation, characterization, and fate were published (Aeppli 2022a, Aeppli 2022b).

PART III: TOXICITY OF OIL PHOTO-PRODUCTS

- ACHIEVEMENT 1: Exposure System. Before we could perform these embryonic fish exposures, we needed to develop a reliable method to produce water-soluble fractions of each oil type, including the a) dark control oil derived from evaporation of Macondo oil under dark conditions; b) total photo-oxidized oil following exposure of the dark control oil to a sunlight simulator; c) a non-polar fraction and d) a polar fraction derived from the total photo-oxidized oil described in (b). As the project developed, it became apparent that the viscosity of total photo-oxidized oil and the polar fraction made it difficult to create water-soluble fractions. One of the issues is that we wanted to solubilize the oil without mechanical agitation to prevent the production of oil droplets within the mixture. It was felt that oil droplets would compromise the utility of our data for use in the oil-spill fate models being developed in Part IV. As such, we spent the first eighteen months of the project consulting with the team and testing multiple procedures. Extensive work was also performed to ensure that the concentrations of polyaromatic hydrocarbons and other constituents in the oil fractions remained constant throughout embryo exposures. It was this latter issue that necessitated the development of the two-stage dosing system.

- ACHIEVEMENT 2: Determine The Toxicity Of Photooxidized Oil On Gulf Killifish. In 2020, we conducted a large-scale embryo exposure to four different oil types (i.e., dark control; total photo-oxidized oil, the non-polar fraction oil of photo-oxidized oil; and the polar fraction oil of photo-oxidized at four different concentrations per oil type plus a clean control (referred herein as Experiment 6). This exposure was meant to repeat an experiment conducted in September 2019 (referred to herein as Experiment 5), which was cut short due to equipment malfunction. In experiment 6, each of the seventeen treatments had five replicate containers receiving 0.5 mL per minute of the water-soluble fraction of each oil type. One important note is that experiment 6 used the original water-soluble oil fractions of each oil type created for experiment 5 and held long-term at 4 °C. Embryos were exposed for 21 days and monitored daily for mortality, hatch, and developmental abnormalities.

- ACHIEVEMENT 3: In 2020 and 2021, we spent considerable time characterizing the physiological effects of parental crude oil exposure on adult offspring for two generations using an assessment of swimming performance and geometric morphometric (GM) techniques to quantify variation in body shape changes in the Gulf killifish. Male and female Gulf killifish were exposed previously for 36-44 days to sublethal concentrations of Macondo oil like that described for the dark control treatment. These fish were then used to generate one of four lineages of F1 fish using a full-matrix mating design with gametes from control and oil-exposed F0 adults. The F1 generation was not exposed to oil directly at any time following fertilization. This F1 generation was then used to create an F2 generation of fish that also was never exposed directly to oil. Although the oil exposures were performed before 2020, all analyses were performed predominantly during the reporting period of this annual report.

- ACHIEVEMENT 4: This activity assessed the impacts of parental and grandparental oiling on craniofacial development and the impacts of these deformities on feeding behavior and fitness. This activity addressed our goal of assessing the latent effects of exposure of Gulf killifish to weathered Macondo oil. Using the same animals described in activity 2, we assessed the influence of parental dark control oil exposure on the jaw structure and prey capture ability of two generations of Gulf killifish progeny. A novel feeding assay was developed, involving the presentation of F1 and F2 generation adult killifish, with a fixed number of live mysid shrimp, and enumerating the number of predatory strikes towards prey, the distance swam by killifish during feeding and the percent prey capture success during 60-second feeding trials.

- ACHIEVEMENT 5: As with activity 3, activity 4 assessed the impacts of parental and grandparental oiling on development. However, we focused this study on assessing the effects of parental and grandparental oiling on spinal deformation and its impact on swim performance. This activity addressed our goal of assessing the latent effects of exposure of Gulf killifish to weathered Macondo oil.

- ACHIEVEMENT 6: We developed a high throughput method to quantify the diameter and density of oil droplets suspended in water and adsorbed to the surface of fish eggs. This method employs a custom macro developed in an open-source imaging program.

- ACHIEVEMENT 7: Improve RNAseq Protocol. A new protocol for RNA-seq (transcriptomics) library preparation was optimized. This protocol significantly improved throughput by decreasing costs by 60% while maintaining depth of coverage and sequencing quality. These substantial improvements reduce library preparation time, reduce consumable costs, produce sequence quality comparable to traditional methods, and increase data quantity by ~20%. A manuscript describing and comparing our RNA-sequencing method to alternative methods has been developed.

- ACHIEVEMENT 8: Determine Gene Response Of Gulf Killifish To Photooxidized Oil. For the 2020 21-day incubation experiments of Gulf killifish, pooled killifish embryo samples were collected. These samples were processed for RNA extraction and preparation of RNA sequencing libraries. The libraries were been pooled and sent for sequencing. The results were evaluated, and the results will be described in a forthcoming manuscript.

PART IV: INTEGRATE PHOTO-PRODUCTS FORMATION INTO OIL SPILL MODELS

- ACHIEVEMENT 1: Developing Photooxidation Algorithm for Oil Spill Model. The oil composition is simulated using pseudo-components, i.e., groups of compounds with similar properties, each treated as if a single chemical with characteristics typical of the group. The oil compounds were binned into 18 pseudo-components: nine volatile soluble and semi-soluble (S&SS), eight volatile, insoluble, and one non-volatile, insoluble residual oil component. The S&SS pseudo-components included volatile and soluble monoaromatic hydrocarbons (MAHs, such as benzene, toluene, ethylbenzene, and xylene; BTEX), semi-volatile polynuclear aromatic compounds (PACs, which include (primarily) polycyclic aromatic hydrocarbons, PAHs, and related heterocyclic compounds that contain S, N, or O), and soluble alkanes (which along with BTEX are volatile organic compounds, VOCs). Sublots of the spilled oil are represented by Lagrangian Elements (LEs), each characterized by location, state (floating, droplet in water, sedimented, ashore), the mass of the various hydrocarbon components, water content, thickness, diameter, and density, viscosity, and associated SPM mass. A separate set of LEs is used to track the mass and movements of the dissolved hydrocarbons. The inclusion of photoproducts was accomplished by creating additional S&SS pseudo-components in the LEs to track their fate, as well as exposure of and toxicity to aquatic biota.
Model scenarios were run with and without photo-oxidation. The inclusion of photoproducts altered the predicted oil component exposures such that the effects of photoproducts could be assessed. As test cases, and to examine the potential importance of photo-oxidation, the scenarios were 2-hour and 20-hour releases of MC252 (Deepwater Horizon spill; properties and composition as defined in French-McCay et al. 2021b) Louisiana crude oil at the water surface during June 2010 in the Gulf of Mexico. Winds were assumed light (5 knots) or moderate (15 knots), which allowed us to assess the importance of oil entrainment into the water after photo-oxidation (under 15-knot winds) to exposure and acute toxicity, as opposed to the dissolution of the original S&SS components and photoproducts from floating oil (under 5-knot winds). Results were summarized as the volume of surface water where sensitive aquatic organisms (5th percentile in sensitivity from a species sensitivity distribution of acute lethal levels) would be killed. The fractional losses in water parcels were summed to an equivalent volume of 100% kill as a summary metric.

- ACHIEVEMENT 2: Deepwater Horizon Model Run: The model matrix, run both with and without photo-oxidation included, was as in Table IV.1. The spill location (88.35695°W, 28.73814°N) was assumed at the water surface at the site of the Deepwater Horizon spill. Winds and currents were assumed constant and unidirectional: winds from the north and currents 5 cm/s towards the east. The water temperature and salinity were assumed 27.8oC and 34.7 psu, typical for June (based on climatic data) at the spill site. The pycnocline depth was assumed 40 m, and concentrations were calculated for 8 vertical layers to that 40 m depth. Suspended sediment concentration was assumed 3 mg/l, resulting in little interaction with SPM and sinking of the oil. Horizontal and vertical turbulent dispersion coefficients in the surface mixed layer were assumed 2.0 and 0.001 m2/s, respectively. The horizontal turbulent dispersion coefficient for floating oil was assumed 100 m2/s. The model simulations were run for 120 hours.

For testing ultraviolet (UV) light data at four wavelengths were obtained from US NASA's Earth Observing System (EOS), Aura satellite data product: the Level-3 daily global gridded Aura-OMI Spectral Surface UVB Irradiance and Erythemal Dose, 1.0 degree x 1.0 degree V3 (OMUVBd) product. This product provided irradiances at four wavelengths (305, 310, 324, 380 nm) at local solar noon in 1.0 degree by 1.0 degree grids for each day of 2010. Data for 18-23 June 2010 were used. The Lambert-Beer law was used to calculate light as a function of depth into the oil and into the seawater. Extinction coefficients in oil were 1.15, 0.969, 0.665, and 0.198 per micron for 305, 310, 324, 380 nm, respectively. Extinction coefficients in offshore seawater were assumed 0.17, 0.16, 0.14, and 0.04 per meter for 305, 310, 324, 380 nm, respectively.

Part I provided the apparent quantum yield (AQY) on pre-weathered oil-containing compounds with greater than about 16 carbons (Figure I.1). Thus, only oil components containing >16 carbons were modeled as photo-oxidizing using these AQY model-estimated production rates. Model runs were performed assuming only the floating oil was photo-oxidized and both floating oil and subsurface droplets were photo-oxidized.

The literature indicates that many compounds can be photo-oxidized to varying degrees. Thus, several assumptions were made regarding the sources of photoproducts and the properties of the products. Model cases were run assuming all the AQY applied to PACs (in proportion to their relative concentrations in the oil), all the AQY applied to both PACs and C17-C20 alkanes (in proportion to their relative concentrations in the oil), and all the AQY applied to the total residual fraction (as an extreme case).

Of the aromatics, the literature showed that C16 to C24 compounds accounted for most of the photo-oxidized products measured in studies using three western Canadian oil blends, Accessed Western Blend (AWB), Cold Lake Bitumen (CLB), and Alberta Sweet Mixed Blend (ASMB). Other studies also found that most of the photo-oxidized PAHs in the aqueous phase of a sunlight system with floating MC252 oil were C16-C24. Biomarkers are mainly C23+ aromatics, and so would be unlikely to photo-degrade significantly at short time scales. Of the SIMAP pseudo-components, AR8 consists mostly of C16 to C18 aromatics (3-ring alkyl-substituted PACs). C19 to C24 compounds are included in the residual non-soluble component. The most abundant oxygen species following solar irradiation shifted from C24 to C16 for AWB, C21 to C16 for CLB, and C20 to C15-C16 for ASMB. Thus, the solubility and other properties of the photoproducts from the PACs in AR8 and the residuals were assumed to be like the more soluble PAC pseudo-components AR6 and AR7, respectively.

Photo-oxidation of aliphatics that can become soluble is likely limited to C16 to at most C24 compounds. However, many photo-oxidized aliphatic compounds containing 15 to 25 carbon atoms were found to be hydrophobic, with log(kow)>6, so would remain in the floating oil. Thus, it was assumed that C17-C20 alkanes (pseudo-components AL6 to AL7) photo-oxidize to photoproducts with properties like pseudo-component AR8.

- ACHIEVEMENT 3: Incorporating Toxicity of Oil Photoproducts. Model development to include photo-oxidation and contributions of photoproducts to exposure and acute toxicity have been completed. Test cases were performed and analyzed, as reported here. This work leveraged a parallel study modeling exposure and acute toxicity of representative spills.
Overall, the results suggest that photo-oxidation of C17-C20 alkanes could be substantial, and their photoproducts could be important contributors to the acute toxic effects of oil on aquatic biota in surface waters. Additional information on the properties of these and other photo-oxidized aliphatic compounds would help narrow down the contributors and physical-chemical behavior of photoproducts that solubilize from floating oil. Assessment of lower light conditions would be an appropriate next step to evaluate the potential contributions of photo-oxidized products to aquatic toxicity. Test spills in mesoscale tanks could be used to validate model findings.

PART IV: ANALYTICAL METHODS FOR CHARACTERIZATION OF PHOTOOXIDIZED OIL
- ACHIEVEMENT 1: Method For Quantification of Oil Photoproducts. We developed and optimized analytical methods to quantify oil photooxidation using bulk-level and molecular-level analyses. The target analytes were a series of aliphatic oxygenated compounds (ketones, carboxylic acids) and oxygenated PAHs. We also showed that bulk-level measures of oil photooxidation (oxygen content, carbonyl index, polar fraction) correlate with PAH depletion for oil photooxidation. The methods and results have been published (Katz et al, 2022).

- ACHIEVEMENT 2: Biomimetic extraction (BE) interlaboratory calibration exercise.
Through outreach activity, we learned about an industry effort to coordinate an inter-laboratory comparison of BE measurements, and were able to join this group. The results of this effort showed that BE is a suitable method for the quantification of total bioaccumulation compounds, without the need for analytical standards of each of the possible compounds. Therefore, the BE method was then widely applied to characterize the mass of bioavailable compounds for PART II of this project.

2. Outputs

Before the form is completed, you may click "Save & Continue Editing" at the bottom of the page at any time to save your work or "Next" to move onto the next page of this form.

When the form is completed, you may click "Mark as Complete" at the bottom of the page to save your work and return to the dashboard.

** denotes required fields*

2. OUTPUTS

Outputs are tangible or measurable deliverables, products, data, or publications produced during the project period.

2.1. Please indicate the number of students (K-12, undergraduate, or graduate), postdoctoral scholars, citizen scientists, or other trainees involved in the project. *

Please enter 0 if none were involved.

K-12 students	0
Undergraduate students	4
Graduate students	3
Postdoctoral scholars	1
Citizen Scientists	0
Other Trainees	0

2.2. Has your project generated any data and/or information products? *

Generation of data includes transformations of existing data sets and generation of data from existing resources (e.g., maps and images). Information products include publications, models, software, code, curricula, and digital resources.

(Check all that apply.)

Responses Selected:

Information Products

2.3. Briefly describe how you fulfilled the approved Data Management Plan and, if applicable, any changes from the approved plan. *

The main outputs of this project are peer-reviewed scientific publications. Some of the publications are still in review or are forthcoming. All data associated with these publications will be publicly available at the time of publication through electronic supplemental information associated with the publication and/or data or pre-print repositories.

If your project has produced publications, websites or data portals, GIS applications, models or simulations, software packages or digital tools, code, curricula, or other interactive media, please download the Excel worksheet entitled GRP Information Management Reporting. Use the "Information Products Report" tab in the worksheet to create an inventory of these products and to verify deposit in a curation facility. Upon completion, please upload the worksheet to your task list. If you need guidance on how to complete the Information Products Report, please e-mail gulfgrants@nas.edu. A member of GRP's data management staff will reach out to you.

2.4. Aside from data and information products, what other tangible or measurable deliverables or products (e.g., workshops, trainings, and outreach events) were produced during the project period? *

Upon completion of this form, you may upload supplemental material that represent the tangible or measurable deliverables or products to complement this narrative report.

- A technical advisory group consisting of industry or agency oil spill professionals was engaged through webinars to help define the design of some of the experiments and investigations and to disseminate the results.
- The investigators of this project participated in oil spill conferences and workshops to disseminate the results of this project and to strengthen connections between oil spill practitioners and researchers.

4. Information Products

Before the form is completed, you may click "Save & Continue Editing" at the bottom of the page at any time to save your work or "Next" to move onto the next page of this form.

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4. INFORMATION PRODUCTS

In this section, please provide a response to each question to complement the **Information Products Report** in the **GRP Information Products Management** Excel worksheet.

4.1. Please select the type(s) of information products that your project produced. *

Responses Selected:

1. Scholarly publications, reports or monographs, workshop summaries, or conference proceedings

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Scholarly publications, reports or monographs, workshop summaries, or conference

proceedings *

Please provide a list of citations for project publication, reports and monographs, workshop summaries, and conference proceedings.

Published Manuscripts:

- Ward, C. P.,Reddy, C. M., and Overton, E. B. (2020), Why sunlight matters for marine oil spills, Eos, 101, https://doi.org/10.1029/2020EO143427.
- Katz SD, Chen H, Fields DM, Beirne EC, Keyes P, Drozd GT, Aeppli C. (2022). Changes in chemical composition and copepod toxicity during photo-oxidization of oil. Environ Sci Technol. 56:5552–5562, doi: 10.1021/acs.est.2c00251
- Aeppli C (2022) 11 Photooxidation of crude oil: formation, characterization, and fate of oil photoproducts in the environment. In: The Chemistry of Oil and Petroleum Products. De Gruyter, pp 519–546
- Aeppli C (2022) Recent advance in understanding photooxidation of hydrocarbons after oil spills. Curr Opin Chem Eng 36:100763.

Submitted Manuscripts

- Hess, C.D., Little, L., Brown, C., Kaller, M., and Galvez, F. (2022) Transgenerational effects of parental crude oil exposure on the morphology of adult Fundulus grandis. Submitted
- Brown C., Aeppli C., Galvez F (2022) Characterization of weathered Macondo oil droplets in suspended WAF solutions and adsorbed to Gulf killifish chorions. Submitted.
- Roman-Hubers AT, Aeppli C, Dodds JN, Baker ES, McFarlin KM, Letinski DJ, Zhao L, Mitchell DA, Parkerton TF, Prince RC, Nedwed T, Rusyn I (2022) Role of Photooxidation in Modulating Temporal Chemical Composition Changes in Water Below a Crude Oil Slick Irradiated with Natural Sunlight. Submitted

Forthcoming Manuscripts

- Aeppli C. and Nabi D. (2022), Estimation of the fate of oil photoproducts based on calculated physico-chemical properties
- McNabb D., Galvez F., Aeppli C., Whitehead A (2022) Physiological and genetic effect of photooxidized oil Fundulus grandis embryos
- Galvez F., Brown C., Aeppli C (2022), Characterization of a flow-through exposure system for determining the aquatic toxicity of photooxidized oil.
- French McCay D., et al (2022), Incorporating oil photooxidation in the fate modeling of the Deepwater Horizon oil

- Hess, C.D., Little, L., Brown, C., Kaller, M., and Galvez, F. (2022), Parental oil exposure alters spinal development and impairs swimming in Fundulus grandis across multiple generations
- Hess, C.D., Little, L., Brown, C., Kaller, M., and Galvez, F. (2022), Parental crude oil exposure causes transgenerational jaw deformities and impaired prey capture in adult gulf killifish (Fundulus grandis)

4.2. Beyond depositing information products in a repository, what other activities have you undertaken or will undertake to ensure that others (e.g., researchers, decision makers, and the public) can easily discover and access the listed information products? *

Publication in peer-reviewed scientific journals.

4.3. Are any of the information products you produced confidential, proprietary, or subject to special license agreements? *

No

5. Project Outcomes

Before the form is completed, you may click "Save & Continue Editing" at the bottom of the page at any time to save your work or "Next" to move onto the next page of this form.

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** denotes required fields*

5. PROJECT OUTCOMES

Outcomes refer to the **impact(s), consequence(s), result(s), or effect(s)** that occur from carrying out the activities or outputs of the project. Outcomes may be environmental, behavioral, health-related, or programmatic. Example outcomes include, but are not limited to: increased learning, knowledge, skills, and motivation; policy changes; actions taken by a group as a result of information generated by your project.

5.1. Please describe the outcomes achieved during your project and how they were assessed. For this question, we are interested in learning about the immediate short-term outcomes that have already occurred during or as a result of your project. Do not include long-term outcomes you foresee your work contributing to beyond the end of the project. *

PART I: OIL PHOTOOXIDATION
- OUTCOME 1: The developed method for controlled production of photooxidized oil using a solar simulator and a defined oil film thickness will be very useful for further studies investigating the fate and effect of photooxidized oil in the environment.

- OUTCOME 2: The determined apparent quantum yield of oil photooxidation and its dependence on wavelength, irradiance, and oil film thickness is essential information for incorporating into oil spill models. Furthermore, this method can be used for further research, investigating a large range of petroleum products.

PART II: OIL PHOTOPRODUCTS
- OUTCOME 1: A set of oil photoproducts have been quantified for a series of increasingly irradiated oils. Although aliphatic photoproducts are relatively abundant, our data suggest that aromatic compounds are more important as precursors of oil photoproducts than aliphatic compounds. The results of this investigation have been published in Katz et al. (2022).

- OUTCOME 2: Biomimetic extraction (BE) was successfully used to quantify bioavailable products in photooxidized oil. The results show that organic acids formed by photooxidation comprise a large part of bioaccumulation compounds in photooxidized oil, demonstrating that PAHs might not be the most toxic compounds. The results of this investigation have been published in Katz et al. (2022). The BE method has also been applied for the effluents used in Part III, and the results are described in a forthcoming manuscript.

- OUTCOME 3: Physicochemical properties of approx. 400 photoproducts have been calculated using quantum-chemical-based methods. The availability of this information is useful for determining the fate and effects of photooxidized oil in oil spill modeling. The results of this investigation are described in a forthcoming manuscript.

- OUTCOME 4: A book chapter has been published describing the current knowledge of the formation, characterization, and fate of oil photoproducts (Aeppli 2022a). Furthermore, a review has been published describing recent advances in the research of the fate and effects of oil photoproducts (Aeppli 2022b).

PART III: TOXICITY OF PHOTOOXIDZED OIL

- OUTCOME 1: A novel exposure system for oil toxicity testing was developed. The system is a two-phase system. Thereby, each oil type was adsorbed to gravel and loaded into custom-made glass desorption columns. In the first phase, oiled-water effluents were prepared by passing brackish water through the oiled gravel contained in a glass desorption column at 4 °C. These columns were flushed for 2 days during which the 6 g/kg effluents were collected and stored at 4 °C. The preparation and storage of the water-soluble fractions at 4 °C decreased oil degradation. In the second phase, the oiled effluents from the glass columns were then used as stocks to create serially diluted concentrations for use in toxicity testing on Gulf killifish embryos. The original stocks and the diluted stocks were held at 4 °C before being delivered using a peristaltic pump to fish embryos. By performing most of these operations in chilled conditions, we were able to ensure that the oil residue composition of the oil types remained relatively constant during the exposure period.

- OUTCOME 2: The toxicity of photooxidized oil on Gulf Killifish was determined. The first set of incubations was assessed on day 3, when each exposed embryo was assessed for impaired growth (Figure III.1). These data suggest that treatments exposed to the highest concentration of the polar oil fraction were slightly underdeveloped at day 3 of exposure compared to the exposure to the other oil types. Embryos were then also sampled on day 7 of exposure for deformities (Figure III.2) and heart rate (Figure III.3). Figure III.2 shows the extent of developmental abnormalities based on a phenotypic score ranging from 0 to 4 based on the presence or absence of four measures of deformity including hemorrhage, pericardial edema, underdevelopment, and tube heart (all measures of oil effect in embryos). Embryos with any of these abnormalities received a score of 1 for each abnormality or received a 0 if they did not display any deformity. These data suggest that treatments exposed to the highest concentration of the polar oil fraction had at least a six-fold higher frequency of abnormality at day 7 of exposure compared to the control treatment. However, oil effects were modest. Figure III.3 suggests there is a biphasic response following exposure to dark control and perhaps polar oil on heart rate. These data demonstrate a slight increase in heart rate of about 15% above control levels at intermediate oil concentrations. However, at higher concentrations of oil, this increase in heart rate disappears. Interestingly, there appears to be no biphasic response in the heart to oil in embryos at 4 days of age. This represents the time the heart first starts beating. Also worth noting is that a similar biphasic response was seen in experiment 5 following only a two-day exposure to these similar treatments of oil. As part of experiment 6, we also sampled embryos at 7 days for genome expression analysis using RNA-seq and for hatch and mortality analyses. The results of these investigations are described in a forthcoming manuscript.

- OUTCOME 3: Transgenerational Toxic Oil Effects: In our transgenerational studies, we found no differences in embryonic fate outcome (% dead, % hatched, and % unhatched) in any of the four lineages of F1 and F2 generation fish. However, as adults, F1 and F2 fish derived from the oil-exposed males of the F0 generation had significantly lower critical swimming speeds (Ucrit) than both the control and maternally oil-exposed lineages. Additionally, the progeny of oil-exposed fish had altered body shape based on the statistical analysis of two-dimensional landmark-based geometric morphometrics. Fish from oil-exposed lineages showed changes in increased body depth, altered spinal curvature, and changes in the upward angle of projection of the head. Both generations had a significant main effect of maternal and paternal oil exposure on shape; however, the F0 paternal oil exposure explained more of the variance in shape across both generations relative to F0 maternal exposure. Our findings demonstrate that parental exposure to oil can impact the shape and aerobic swimming capacity of offspring for at least two generations after the original paternal oiling. A manuscript describing this study has been submitted and is currently under review.

- OUTCOME 4: Novel Sublethal Effect Assay: Based on the results of the feeding assay, a novel vision assay was developed to quantify the visual responses to a virtual visual cue in the absence and presence of a chemical stimulus. After completing the fitness assessments, the same fish were further utilized to assess jaw morphology using 3-dimensional geometric morphometric techniques. These data suggest that jaw morphology and strike success are impaired in F1- and F2-generation fish derived from oiled fish, particularly oiled fathers and grandfathers. Despite this, there were no significant differences in their visual responses when presented with a virtual prey item. These studies suggest the potential for sublethal oil exposure in adult killifish can impact progeny feeding efficiency for at least two generations. A manuscript describing the outcomes of this study will be submitted for publication by July 2022.

- OUTCOME 5: Transgenerational Effects: Morphology: We found that both F1 and F2 fish with (grand)paternal oiling displayed significantly higher incidences of spinal deformation than the control lineage; however, fish with only (grand)maternal oiling were no different than control fish. In general, fish that were (grand)paternally exposed displayed higher incidences of kyphosis, lordosis, and scoliosis relative to the control. (Grand)paternally exposed lineages also presented a higher percentage of fish with deformities in both abdominal and caudal regions than control fish across generations. The F1 and F2 fish with the spinal deformities present also performed significantly worse in anaerobic swimming challenges than non-exposed fish. Based on these findings, we emphasize the need for more long-term, multigenerational studies of the effects of crude oil exposure and the importance of not limiting investigations to larval and juvenile endpoints. A manuscript describing the outcomes of this study will be submitted for publication by August 2022.

- OUTCOME 6: Mechanism of Oil Uptake Through Droplets: We found that removal of an outer-extrachorionic layer from killifish embryos results in increased absorption of mechanically dispersed oil droplets leading to significantly reduced hatch success. This study demonstrates the need for investigating the interaction of droplets with animals. Finally, we were able to utilize this method to demonstrate that the water-soluble fractions of each of our four oil types described in activity 1 possess few oil droplets. It suggests that one of our goals of producing a water-soluble fraction with few oil droplets was achieved. A manuscript describing the outcomes of this study has been submitted and is currently under review.

- OUTCOME 7: Optimizing Transcriptomics Protocols: A new protocol for RNAseq library preparation has been developed. A manuscript describing and comparing our RNA-sequencing method to alternative methods has been written and will be submitted shortly.

PART IV: OIL SPILL MODELING

- OUTCOME 1: Including Oil Photooxidation in Oil Spill Models: The existing the SIMAP model was updated to assess photo-oxidation (Figure IV.1). This included (1) input of irradiance data incident at the water surface as a function of location and time at a range of wavelengths known to photo-oxidize oil; (2) inclusion of a photon absorbance algorithm that accounts for light extinction by wavelength as it penetrates an oil layer and through water prior to reaching subsurface oil droplets; (3) calculation of photoproduct production as a function of absorbed photons using an Apparent Quantum Yield (AQY) equation (i.e., calculating moles of photoproduct per mole of photons absorbed at a specific wavelength) developed in Part I. (4) creation of new pseudo-components in the model to track the fate and concentrations of the photo-products along with the initial components of the oil; (5) characterization of the physical and chemical properties (e.g., molecular weight, vapor pressure, solubility, octanol-water partitioning coefficient) of photoproduct pseudo-components; (6) calculation of biodegradation of photoproducts using pseudo-component specific rates input to the model; (7) inclusion of photoproducts in the exposure and aquatic toxicity models for calculation of effects. A manuscript describing these results is forthcoming.

- OUTCOME 2: Including Toxicity Of Oil Photoproducts In Oil Spill Model: Table IV.2 summarizes the results for the model test cases. Note that under light winds and with no photo-oxidation, the volumes killed were negligible. This is because evaporation from floating oil was faster than dissolution, and entrainment was negligible in light winds so there was no dissolution from subsea droplets. In moderate winds, with no photo-oxidation included, the volumes killed increased with spill volume. Including photo-oxidation on the polyaromatic compounds (PACs) made only a small difference in the results, which was not significant as compared to the uncertainty of the model calculations. This was because the available source PAC mass was rapidly (within hours) depleted by photo-oxidation, i.e., the amount of photoproduct was source-limited. These calculations only accounted for the photo-oxidation by the four wavelength bands of UV in the irradiance data set used. The inclusion of the full light spectrum that was found to produce measurable photoproducts would not result in significantly different results because of this substrate-limitation. This suggests that at full sunlight in the northern Gulf of Mexico in June, PACs would be rapidly photo-oxidized to leave negligible amounts in floating oil. However, with the dilution occurring in the surface mixed layer, the contribution of photoproducts from PACs to acute toxic effects is likely negligible.

Including photo-oxidation of C17-C20 alkanes in the model increased the volume killed by an order of magnitude. Including photo-oxidation of all the aliphatics in the residual increased the volume killed even more substantially (Table 2). In both these model scenarios, the amount of photoproduct was substrate-limited, as were the PACs. The differences from the cases assuming only PACs photo-oxidize are due to the much higher concentrations of the aliphatic substrates in the oil. The scenario assuming PACs + C16-20 alkanes photo-oxidize is the most realistic scenario, based on current information. These results suggest that the contribution of photoproducts from C16-C20 alkanes to acute toxicity could be substantial. A manuscript describing these results is forthcoming.

PART V: ANALYTICAL METHODS FOR OIL PHOTOPRODUCTS

- OUTCOME 1: The methods for quantification of a set of oil photoproducts have been published in Katz et al, 2022. These methods have also been used to quantify oi photoproducts in the effluent in the toxicity tests in Part III.

- OUTCOME 2: Biomimetic Extraction (BE) Method: A BE interlaboratory comparison we participated in showed that BE is a suitable method to detect bioaccumulation native oil hydrocarbons as well as oil photoproducts. We, therefore, used BE extensively in this project. BE results helped interpret toxicity in the Katz et al (2022) paper, and in the Part III toxicity studies.

5.2. We’re interested in hearing not just the results of your project but what are their implications for or contributions to:

- **offshore energy system safety,**
- **environmental protection and stewardship, and/or**
- **health and community resilience**

Please describe what you consider to be the most remarkable accomplishment or finding of your project. What can others learn from your accomplishment and finding? How do you see it fitting in with your greater field of study or community of practice? *

Although PAHs can be used to explain and predict the toxicity of fresh crude oil pretty accurately, this is not the case when oil has been irradiated, even for just a relatively short time. In fact, a multitude of oil photo-products, along with PAHs, are responsible for the toxicity. Determining the contribution of oil photo-products to observed toxicities is essential information for assessing the risk of an oil spill. What is more, incorporating the formation and toxicity of photo-products into the oil spill model is impactful, since oil spill practitioners will be able to take advantage of this research project and improve their prediction of the fate and effect of oil after spills.

6. Communication

Before the form is completed, you may click "Save & Continue Editing" at the bottom of the page at any time to save your work or "Next" to move onto the next page of this form.

When the form is completed, you may click "Mark as Complete" at the bottom of the page to save your work and return to the dashboard.

** denotes required fields*

Note to Grantees: In Section 6, we seek input from you to help us evaluate the Gulf Research Program’s funding strategy. This section will not be made available to the public.

6. Information to Inform GRP Evaluations

6.1. Sharing the difficulties you encountered helps us learn from your experience. Describe any challenges you encountered in your project and how you addressed or overcame them. Challenges are inherent to conducting any complex project. These may include (but are not limited to): unexpected staffing changes, changes in the community you are working in, appearance of a new technology or dataset in the field you are working in, challenges accessing a field site, policy or regulatory changes that affect the issue you are addressing, low recruitment rates, delays in setting up services, or other problems in implementing and conducting your project. *

A challenge for Part I was the production of a large volume of oil. The experiment ended up requiring twice as much oil as we initially planned. This adjustment to the plan consumed more time and resources than initially planned.

The delay in oil production had a downstream effect on Part III, which led to some delays. A typical 21-day embryo exposure requires 6-30 grams of photo-oxidized product to produce the total photo-oxidized oil and the non-polar and polar fractions. In response to this problem, we began to consider new experimental paradigms. First, we have decided to reduce the length of exposures from 21 days to no more than 1-day windows of exposure at different stages of embryo development. Second, following these oil exposures, we are proposing to expose embryos to ultraviolet (UV) light simulators. There is considerable work, including research performed previously in the Galvez laboratory, demonstrating that some types of bioaccumulated polyaromatic hydrocarbons (PAHs) exert toxic effects if the PAHs are excited by UV radiation. This phenomenon is referred to as photo-enhanced toxicity. In fact, we think that this experimental modification is warranted considering the photo-oxidation of oil and photo-enhanced toxicity of oil are both produced by UV radiation within natural sunlight.

In 2020, we only had a limited amount of photo-oxidized oil that was not sufficient for a full-scaled experiments like that above However, we still had some water-soluble fractions sufficient for a pair of 1-day oil exposures. The first experiment was performed to assess the amount of UV light exposure killifish embryos could tolerate during the first seven days of development. The second experiment exposed killifish for 14 hours to each of the four oil types produced for experiment 5. We then proceeded to expose the killifish to 12 hours of UV radiation. These investigations provided some interesting results, but due to the age of the water-soluble fraction, we were not able to perform final experiments. Our laboratories plan to write another grant application to explore this phenomenon in further detail.

For Part IV, the planned project scope was not changed significantly. However, testing of the model was more limited than initially envisioned. In order to perform the model test cases discussed in this report, the Apparent Quantum Yield (AQY) equation was needed. This information was published in February 2022. Thus, model test cases were performed recently. Model results are still under review before inclusion in a manuscript for publication., which will be forthcoming.

6.2. We like to hear about what you learned from your work and how you feel it affects future work or the work of others. Think back on your project strategies, methods, and activities, what worked and what did not? Is there anything you would do differently in the future? If so, tell us what and why. *

We certainly had to be flexible and nimble during this project in order to manage the disruptions the COVID-19 pandemic caused. Our program managers were helpful and receptive to our proposed changes in scheduling and timing.

6.3. What are the next steps for this work, either for you and your project team or other researchers? Has this project led to other opportunities to work in this area? *

This project led to a good number of scientific publications with valuable data on the importance of oil photooxidation during an oil spill. There are follow-up questions that merit closer investigation within new research projects we will apply for. For example, the question of photosensitized toxicity vs toxicity of photoproducts. Second, while our toxicity tests show the effects of photo-products, the modeling results suggest that dilution will rapidly mitigate this effect. A more in-depth investigation of the spatial and temporal effects of oil photoproducts would be another follow-up project.

6.4. Have you developed new collaborations or partnerships (formal or informal) as a result of this work? If yes, please describe the new collaborations or partnerships. *

The project partners collaborated intensely during this project. These collaborations are expected to continue. Furthermore, the engagement with oil spill professionals from the industry, consulting, and government led to a larger network and new opportunities for research. Lastly, through this project, we started new collaborations with partners from other research networks, such as the Canadian MPRI. We expect that such close collaborations will continue.

6.5. What, if any, positive changes in policy or practice do you foresee as a result of your work? *

The oil spill response community has been very interested in understanding the effects of photooxidation on oil spill response options and is receptive to our results. We expect that the results of this project will have an impact on this community. For example, the 2019 National Academies dispersant report already included a discussion of the potential effects of oil photooxidation on oil spill response. Furthermore, team members of this project are giving input to the current revision of the National Academies "Oil at Sea" report.

6.6. If you could make one recommendation to the Gulf Research Program for how best to build on the work you conducted in this project, what would it be? *

The continuation of Research-Practice project of project, where fundamental research meets the needs of stakeholders, would be ideal for a continuation of this work.

7. Communication and Dissemination

Before the form is completed, you may click "Save & Continue Editing" at the bottom of the page at any time to save your work or "Next" to move onto the next page of this form.

When the form is completed, you may click "Mark as Complete" at the bottom of the page to save your work and return to the dashboard.

** denotes required fields*

Note to Grantees: In Section 7, we ask you to help us communicate the importance, progress, and accomplishments of your work. Information provided in this section will be used by the Gulf Research Program to highlight its funded projects in print and electronic informational and promotional materials. The intended audience for the information provided in this section is different and should be thought of as a general audience. When you return to the dashboard, you may upload images that represent and illustrate the work of your project.

7.1. Please describe the most exciting or surprising thing you have learned while working on this project in a way that is understandable by a general audience. *

Oil “photo-products” are formed at the water surface in large quantities in the early phase of an oil spill by the interaction of floating oil with sunlight. Although these products are persistent and potentially toxic, there is significant uncertainty about translating their environmental concentration to potential toxic effects. Currently, there is insufficient information to account for these products when assessing fishery closures or public health risk, or evaluating the impacts of an oil spill. Instead, practitioners typically focus on the concentrations of polycyclic aromatic hydrocarbons (PAHs), a small fraction of photo-oxidized oil. In fact, there is emerging evidence that PAHs alone fail to explain toxic effects of such oil residues. This proposed project aims to improve the current practice of oil spill risk and damage assessment by producing quantitative data for the formation, exposure, and toxicity of oil photo-products, and by integrating this information into algorithms that can be used for oil spill risk assessment. Although PAHs can be used to explain and predict the toxicity of fresh crude oil pretty accurately, this is not the case anymore when oil has been irradiated, even for just a relatively short time. It appears that a multitude of oil photo-products, along with PAHs, are responsible for the toxicity. Figuring out which photo-products are responsible to what extent is an exciting task.

7.2. Do you have any stories that capture the impact of this project? (optional)

If so, please share one or two. Examples of what we are interested in include stories of people/communities that the project has helped; lives that have changed; work that led to policy change, such as legislation or regulation; and research breakthroughs.

(No response)

7.3. Have any communications, outreach, or dissemination activities occurred in relation to your project?*

Please describe:

- Any press releases issued (other than that issued by the National Academies of Sciences, Engineering, and Medicine) about the project.
- Any media coverage or news stories about the project.
- Any social media accounts, websites, listservs, or other communication vehicles used to communicate information about this project. Please include relevant web addresses if available.

(1) Technical Advisory Group: several meetings with oil spill practitioners.

(2) Presentation at Conferences. Here a list of presentations:

- Ward, C.P. (2019) Synthesis of photochemical transformation of crude oil. Gulf of Mexico Research Initiative Synthesis Workshop. 12 June 2019, Washington D.C. Oral Presentation.

-Ward, C.P., & Overton, E.B. (2019) Synthesis of Photochemical Transformations of Oil in Marine Waters. Gulf of Mexico Oil Spill & Ecosystem Science Conference, New Orleans, LA.

-Aeppli C, Nacci, D.E., Clark, B.W., Beirne, E., McNabb, N.A., Whitehead, A., Baker, G. (2020) The Relative Toxicity to Fish Embryos of PAHs and Photo-Products in Weathered Oil Residues. Gulf of Mexico Oil Spill and Ecosystem Conference, Tampa FL, Feb 3-6 2020; oral presentation.

-Aeppli C. (2020) Shining light on marine oil spills: Photo-oxidation effects on the composition, properties, and dispersibility of oil. ExxonMobil Dispersant Webinar series, Oct 20, 2020; oral presentation (virtual).

-French McCay D. (2020) Oral presentation about work relevant for this project at the 2020 GoMOSES conference, “Modeling Deepwater Horizon Oil Compositional Changes from Weathering and Dispersant Use - Implications for Water Column Exposure and Toxicity”,

-Ward, C.P. (2020) How does oil at sea break down and evolve with exposure to sunlight? Gulf of Mexico Sea Grant Program.

-Freeman D and Ward, C.P. (2020) Dissolving oil in a sunlit sea. Department of Fisheries and Oceans Canada Multi-Partner Research Initiative Virtual Symposium. Oral Presentation.

-French McCay D. (2021) Presentation of model development approach at the 5th Meeting of the National Academy of Sciences, Engineering and Medicine (NASEM) Committee on Oil in the Sea IV, January 15, 2021

Figures and Tables

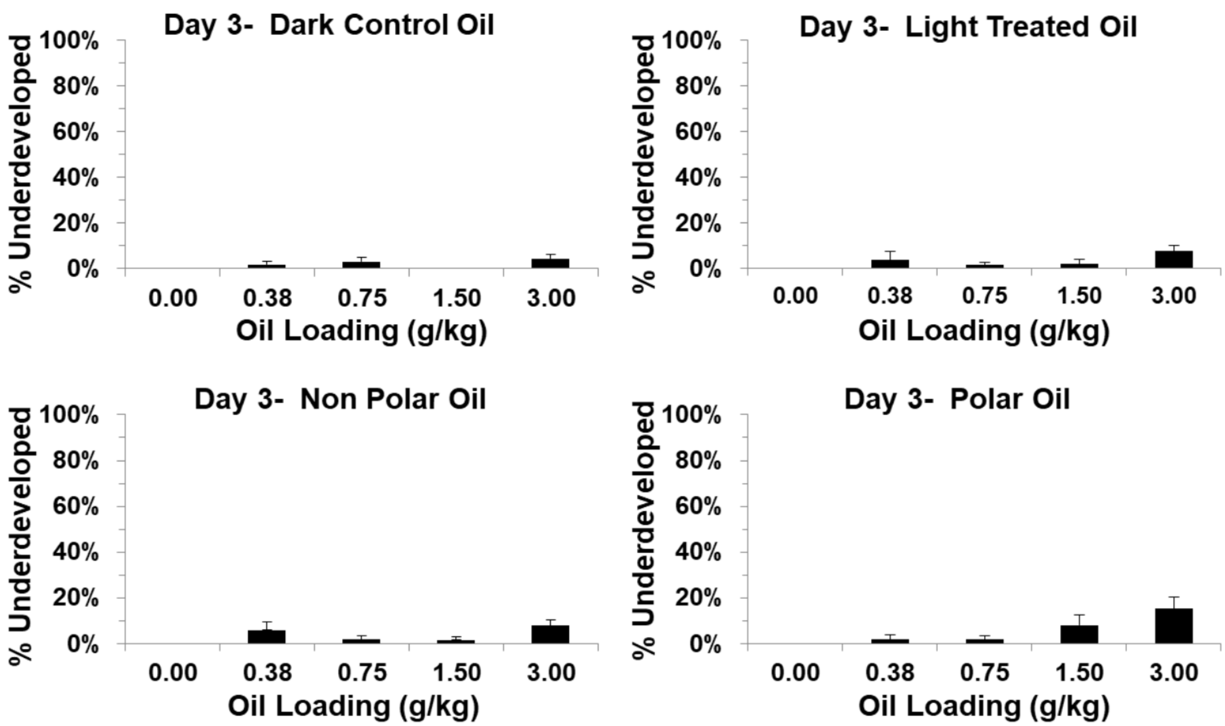

Figure III.1: The percent of embryos showing underdeveloped phenotypes at day 3 of exposure to dark control, light treated, non-polar, or polar oil. Each bar represents the mean ± standard error of the mean of the five replicates.

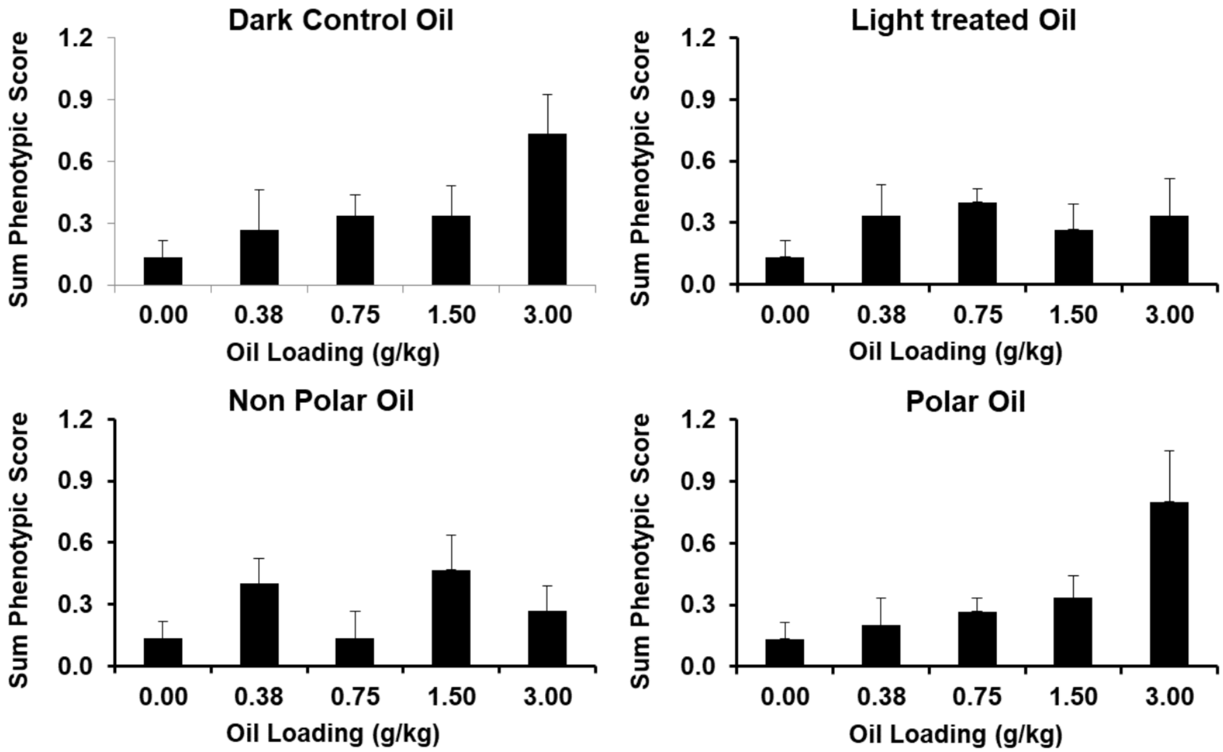

Figure III.2: The sum phenotypic score of embryos at 7 days of exposure to dark control, light treated, non-polar, or polar oil. Each bar represents the mean ± standard error of the mean of the three biological replicates for each of the five technical replicates per treatment.

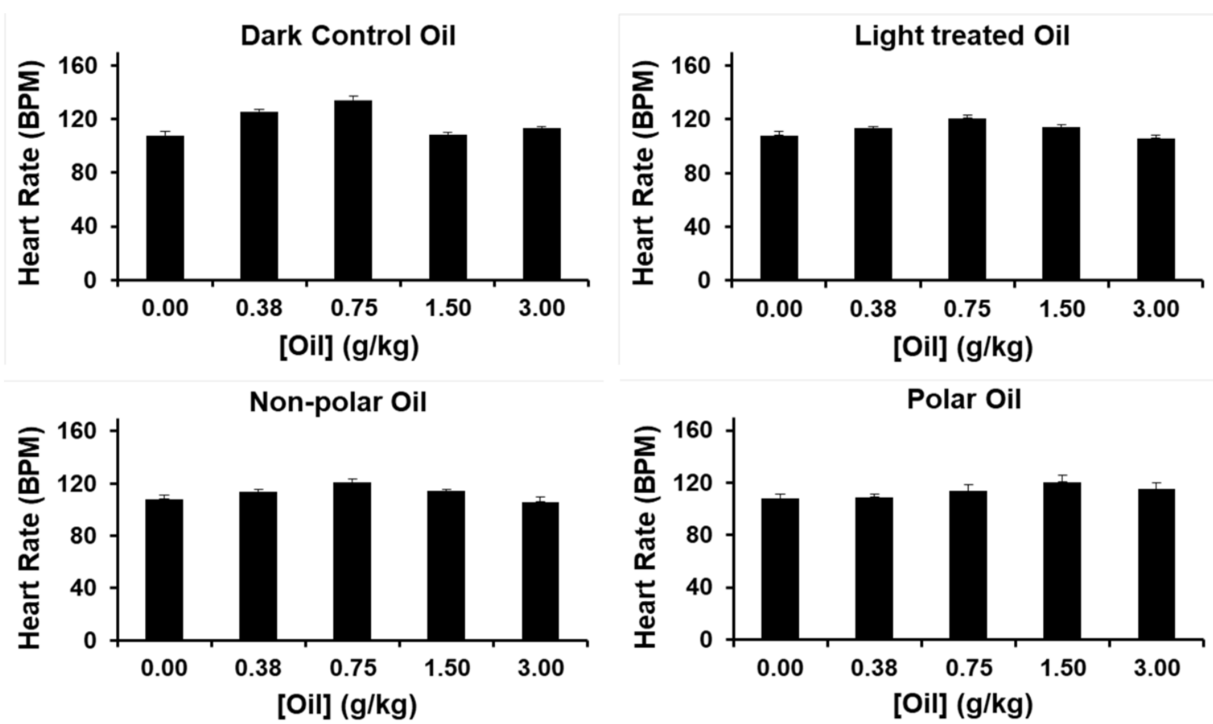

Figure III.3: The heart rate of embryos at 7 days of exposure to dark control, light treated, non-polar, or polar oil. Each bar represents the mean ± standard error of the mean of the three biological replicates for each of the five technical replicates per treatment.

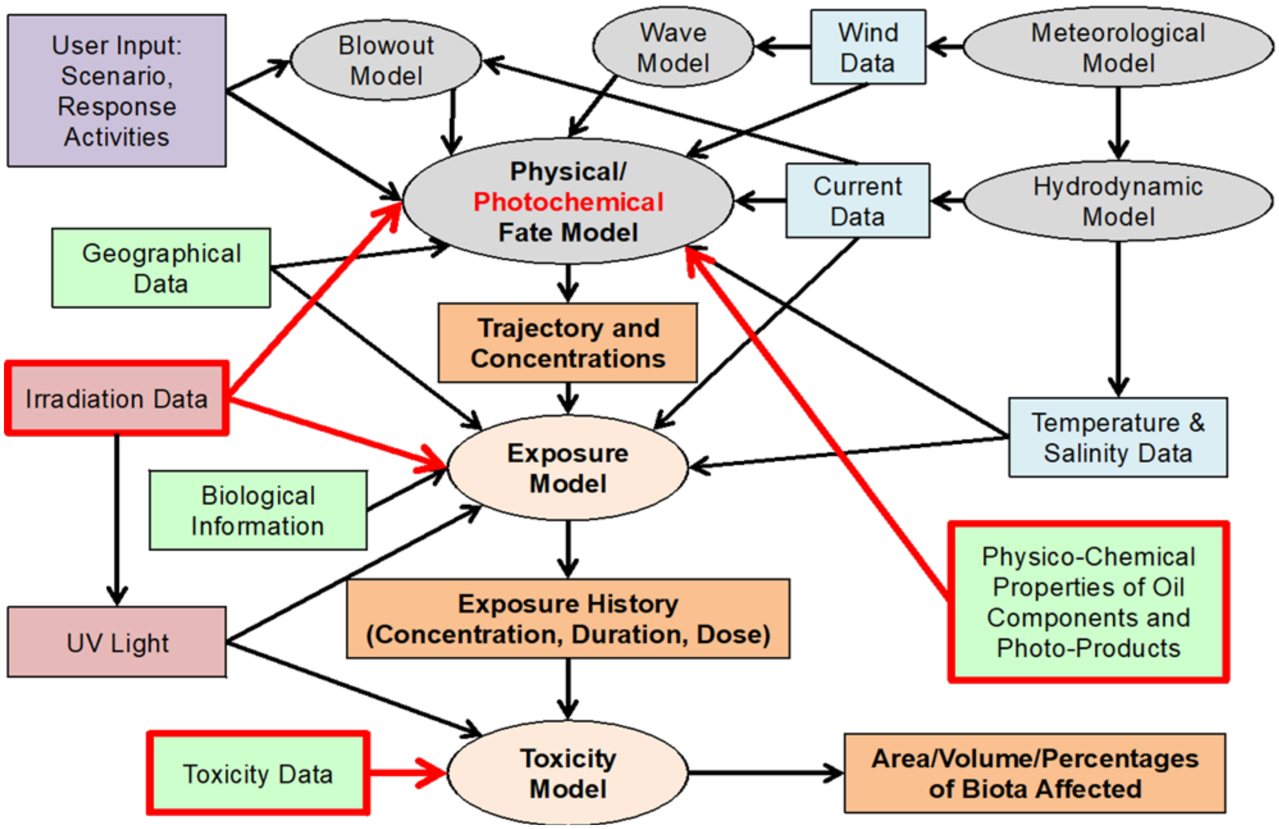

Figure IV.1: Modeling system used for oil fate and biological effects modeling.

Table III.1: Model test scenarios for surface releases in the Gulf of Mexico in June 2010.

Scenario	Wind (kts)	Total bbl	Total MT	Release Duration (hrs)
1,000 bbl Light Wind	5	1000	136.5	2
1,000 bbl Moderate Wind	15	1000	136.5	2
10,000 bbl Light Wind	5	10,000	1,365	20
10,000 bbl Moderate Wind	15	10,000	1,365	20

Table IV.2: Volume (km^3) killed summing over four UV wavelengths only, assuming 5th percentile sensitivity.

Assumed Source(s)	**Source Component Groups**	**1,000 bbl Light Wind**	**1,000 bbl Moderate Wind**	**10,000 bbl Light Wind**	**10,000 bbl Moderate Wind**
Floating + Droplets	none	~0	0.027	~0	0.432
Floating	PACs only	~0	0.028	~0	0.396
Floating + Droplets	PACs only	~0	0.022	~0	0.409
Floating + Droplets	PACs + C16-20 alkanes	~0	0.23	0.063	2.17
Floating + Droplets	Residual	0.00016	1.21	0.266	3.37